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ABSTRACT BOOK 2023

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No more measuring cups and bad taste: personalizing pediatric medication with 3Dprinting

Levent Kocabas, Utrecht University

Acceptance of oral formulations is low in the pediatric population and (dose) personalization of pediatric medication is much needed. Therefore, alternative administration routes like the rectal route, with dosage forms such as suppositories, are common for medicines for children. Since 3Dprinting of medicines is fundamentally different from conventional compounding of suppositories, this novel technique will yield products with tunable properties (such as the drug dose) and tailormade release profiles. In this study, prednisolone suppositories for infants with tunable dose and rapid release are printed. We demonstrate that by using 3D-print technology, promising advances can be made in the compounding of medicine to meet the needs of individual patients, in particular by enabling the development of novel dosage forms and making dose adjustment easy.

Extracellular vesicles and soluble factors secreted by lung fibroblasts have a protective effect on elastase-induced emphysema in mice

Luke van der Koog, Groningen Research Institute of Pharmacy

Chronic Obstructive Pulmonary Disease (CODP) is characterized by a progressive and irreversible airflow limitation, often associated with emphysema. Key mechanisms underlying COPD are enhanced tissue destruction and defective tissue repair. As current therapeutics do not alter disease progression, new therapies that reactivate lung repair are needed. The secretome of lung fibroblasts, composed of extracellular vesicles (EVs) and soluble factors (SFs), has been linked to lung tissue repair.
In this study, we evaluated the effects of lung fibroblast (MRC5)-derived EVs and SFs on repair of elastase-induced lung injury in mice. Elastase treatment altered lung function parameters and increased the degree of tissue injury as measured by the mean linear intercept, indicative of damage to the parenchyma. Prophylactic intratracheal treatment with lung fibroblastderived EVs and SFs attenuated elastase-induced lung tissue destruction and improved lung function parameters. In addition, treatment with EVs and SFs restored gene expression of alveolar epithelial cell markers, suggesting activated regenerative mechanisms. In conclusion, EVs and SFs secreted by lung fibroblasts are an interesting treatment to further pursue for COPD.

Vascular models to study migraine: from human isolated blood vessels to vessel-on-chip

Tessa de Vries, Erasmus University Medical Center

Migraine is a highly disabling neurovascular disorder characterized by a severe headache and activation of the trigeminovascular system, involving the release of the potent vasodilator calcitoningene related peptide (CGRP). Multiple drugs targeting the CGRP pathway have been developed for the acute and prophylactic treatment of migraine, including the monoclonal antibody erenumab and small molecule antagonists called gepants, which both target the CGRP receptor. Surprisingly, despite having the same target, gepants can exert additional effects on top of the monoclonal antibody erenumab in human isolated arteries. Moreover, gepants behave differently in human coronary arteries versus human middle meningeal arteries based on the slope of a Schild plot, and differential expression of subunits of the CGRP receptor family can be observed in the two vascular beds. Furthermore, activation of the CGRP receptor activates multiple intracellular signalling cascades, of which the expected second messenger cAMP, which is generally assumed to be the main signalling molecule, is not the main mediator of vasodilation.

Instead, activation of Gβγ subunits results in relaxation of human coronary arteries.
Interestingly, although CGRP receptor blockade seems an attractive target for the treatment of migraine, treatment is not effective in all migraine patients. Considering the complex pharmacology of CGRP signalling and blockade of its receptor, and its cardioprotective role during ischemia, more in depth analysis of patient-specific responses should be performed. We developed a vessel-on-chip model incorporating induced pluripotent stem cell (iPSC)-derived vascular smooth muscle cells and endothelial cells, allowing to study patient-specific blood vessels. Vascular responses of the cultured blood vessel model were compared to native human blood vessels, and similar responses to CGRP, adrenomedullin and adrenomedullin 2 could be observed in the model compared to human isolated blood vessels. Moreover, CGRP-induced increases in second messenger molecules in the vessel-onchip model could be potently blocked using gepants, showing that the model can be used for pharmacological analyses. Endothelin-1 and angiotensin II, well-known vasoconstrictors, potently increased the intracellular calcium concentration of the 3D cultured blood vessels. This patient-specific blood vessel model can be used to further study the complex pharmacology of CGRP, and possibly aid in identifying the differences between responders and non-responders to this novel class, as well as future classes, of anti-migraine medication.

Long COVID inflammatory phenotypes and potential treatment strategies; results of the Precision Medicine for more Oxygen (P4O2) COVID-19 study.

Nadia Baalbaki, Amsterdam UMC

Long COVID occurs in about 30% of all COVID-19 cases and can be characterized by a broad array of persisting symptoms. We investigated the role of nasal epithelial cells(NECs) and innate lymphoid cells(ILCs) in mediating inflammation in the pathology of long COVID and potential treatment strategies. NECs were cultured at air-liquid-interface and were exposed to viral mimic poly(I:C) and immunomodulatory drug dexamethasone. The phenotype and function of long COVID NECs and blood ILCs was assessed and compared to healthy controls. In Long COVID, the relative gene expression of pro-inflammatory cytokines was increased at baseline and after 24-hour exposure to poly(I:C), while dexamethasone was a potent inhibitor of this inflammatory response. Increased frequencies of IFN - producing ILCs were found in long COVID, that in vitro produced more IFN after exposure to epithelium-derived IL-1 compared to healthy controls. Ex vivo, this inflammatory state resulted in a reduced barrier function and increased regeneration potential of long COVID nasal epithelium. Therefore, long COVID patients displaying increased IL-1 profiles could potentially benefit from Anakinra (anti-IL-1) treatment. While Long COVID is a heterogeneous condition that requires a precision medicine approach, this study demonstrated the potential benefit of immunomodulatory drugs for patients displaying increased local and systemic inflammatory phenotypes.

Tail-flipping nanoliposomes targeting tumor associated macrophages to stimulate tumor immunity

Kunal Pednekar, University of Twente

Introduction

Most solid tumors have suppressed immune microenvironment which allows tumor cells to escape from the immune attack [1]. Tumor-associated macrophages (TAMs), one of the most important cell types in the tumor microenvironment (TME), induces immune suppression by inhibiting adaptive immune cells such as T-cells [2, 3]. TAMs can be divided into M1 type (anti-tumoral) and M2 type (pro-tumoral), while M2-TAMs comprise the major population of a tumor. M2-TAMs secrete factors suppressing proliferation and migration of CD8+ cytotoxic T-cells, which makes immune checkpoint inhibitors such as anti-PD1 ineffective. Re-programming M2-TAMs into M1 phenotype can be an interesting approach to re-activate CD8+ T-cells. Recently, we have developed novel "tailflipping" nanoliposomes that can specifically target M2-TAMs in vivo [2] which could be used to reprogram M2-TAMs into M1-type.

Objectives

The main goal of this study is to target M2-TAMs using our "tail-flipping" nanoliposomes and convert them into anti-tumoral phenotype in vivo and study the effect on the efficacy of immune checkpoint inhibitor (Fig. 1a).

Methods: "Tail-flipping" nanoliposomes containing 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine (PAPC), hydrogenated soybean phosphatidylcholine (HSPC) and cholesterol were prepared using thin-film hydration method [2]. These nanoliposomes (PAPC-L) were characterized for their size using DLS, surface charge and M2-TAM uptake using fluorescence microscopy and flow cytometry. Muramyl tripeptide (MTP-PE), a bacterial wall component, was incorporated into the PAPC-L forming the MTP-loaded nanoliposomes (MTP-PAPC-L) (Fig. 1b). These nanoliposomes were used in vitro to study their effect on murine macrophages (RAW264.7 cell line) using qPCR and ELISA. Colon carcinoma model was induced by injecting CT26 tumor cells subcutaneously in balb/c mice. When tumors reached 50-100 mm3, MTP-PAPC-L (10 g/mouse) were administered intravenously 2x per week in combination with mouse anti-PD1 (200 g/mouse).

Results

Nanoliposomes with a size of 116nm and a zeta potential of -18mV showed specific uptake by the M2-differentiated macrophages. In vitro, MTP-PAPC-L induced the expression of inflammatory cytokines like TNFα and IL-1β in the M2-macrophages (Fig. 1c), confirming biologically activity. In vivo, MTP-PAPC-L alone regressed only 15% tumor inhibition, but in combination of antiPD1 antibody there was a regression of >50% compared to PBS group (Fig. 1d). Interestingly, histological analysis revealed that the mice treated with MTP-PAPC-L had an induced infiltration of macrophages (F4/80+ cells), enhanced expression of M1 macrophages (MHC-II) and reduced expression of M2-TAMs (CD206) (Fig. 1e). With combination therapy, we also found induced expression of CD8+ cytotoxic T-cells and CD4+ helper T-cells (Fig. 1f).

Conclusion

This study reveal that our "tail-flipping" nanoliposomes can deliver MTP to M2-TAMs, thereby re-programming immunosuppressive M2-TAMs into anti-tumoral M1-type in vivo. This shift in the TAM phenotype leads to enhanced infiltration of CD8+ T-cells and improves the efficacy of immune checkpoint inhibitor. This strategy is in potential to be further developed to enhance the therapeutic efficacy of immune checkpoint inhibitors in view of future clinical translation.

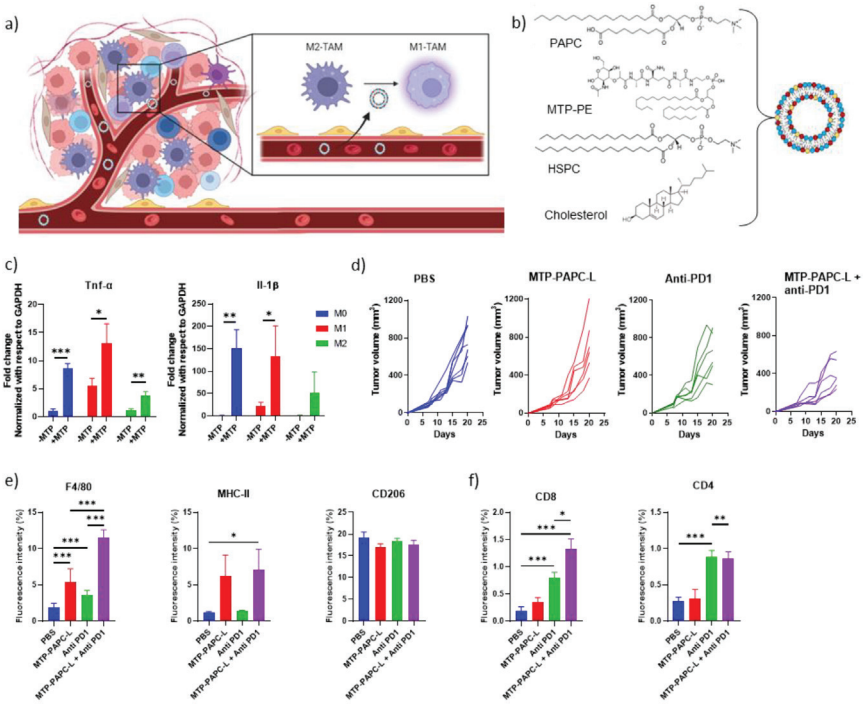

Figure 1: Reprogramming of TAMs using "tail-flipping" nanoliposomes: a) Graphical representation of 'tail-flipping' nanoliposomes converting the M2-TAMs into M1 phenotype, b) An illustration showing composition of lipids present in the 'tail-flipping' nanoliposomes, c) In vitro effect of MTP-PAPC-L in RAW264.7 murine macrophages showing increased inflammatory cytokines (TNFα and IL-1β), d) Individual tumor growth curves of 6-7 animals treated either with PBS, MTPPAPC-L (0.5mg/kg MTP-PE), anti PD1 (200μg/mouse) and the combination of MTP-PAPC-L and anti-PD1, e) Quantification of immunofluorescence staining for F4/80 (total macrophages), MHC-II (M1 macrophages), and CD206 (M2 macrophages) in tumor tissues. f) Quantification of immunofluorescence staining for the expression of CD8 (cytotoxic Tcells) and CD4 (T-helper cells).

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Hypoxic oligodendrocyte precursor cell-derived VEGFA is associated with blood-brain barrier impairment

Narek Manukjan, Maastricht University

Cerebral small vessel disease is characterised by decreased cerebral blood flow and blood-brain barrier impairments which play a key role in the development of white matter lesions. We hypothesised that cerebral hypoperfusion causes local hypoxia, affecting oligodendrocyte precursor cell – endothelial cell signalling leading to blood-brain barrier dysfunction as an early mechanism for the development of white matter lesions. Bilateral carotid artery stenosis was used as a mouse model for cerebral hypoperfusion. Pimonidazole, a hypoxic cell marker, was injected prior to humane sacrifice at day 7. Myelin content, vascular density, blood-brain barrier leakages, and hypoxic cell density were quantified. Primary mouse oligodendrocyte precursor cells were exposed to hypoxia and RNA sequencing was performed. Vegfa gene expression and protein secretion was examined in an oligodendrocyte precursor cell line exposed to hypoxia. Additionally, human blood plasma VEGFA levels were measured and correlated to blood-brain barrier permeability in normalappearing white matter and white matter lesions of cerebral small vessel disease patients and controls.
Cerebral blood flow was reduced in the stenosis mice, with an increase in hypoxic cell number and blood-brain barrier leakages in the cortical areas but no changes in myelin content or vascular density. Vegfa upregulation was identified in hypoxic oligodendrocyte precursor cells, which was mediated via Hif1α and Epas1. In humans, VEGFA plasma levels were increased in patients versus controls. VEGFA plasma levels were associated with increased blood-brain barrier permeability in normal appearing white matter of patients. Cerebral hypoperfusion mediates hypoxia induced VEGFA expression in oligodendrocyte precursor cells through Hif1α/Epas1 signalling leading to increased BBB permeability. In humans, increased VEGFA plasma levels in cerebral small vessel disease patients were associated with increased blood-brain barrier permeability in the normal appearing white matter. Our results support a role of VEGFA expression in cerebral hypoperfusion as seen in cerebral small vessel disease.

Apolipoprotein-based delivery platform for gene silencing in hematopoietic stem and progenitor cells

Stijn Hofstraat, TU Eindhoven

Over the last decade, targeting the adaptive immune system has been a highly successful immunotherapy approach. Similarly, modulating the innate immune system holds great therapeutic potential. Our strategy is to deliver small interfering RNA (siRNA) to the hematopoietic stem and progenitor cells in the bone marrow. Therefore, we have developed a novel RNA delivery platform based on apolipoprotein A1 (ApoA1). ApoA1 acts as a structural stabilizer for lipid-based nanoparticles, and it has inherent affinity for myeloid cells and their progenitors. Using ApoA1, siRNA, and fatty molecules, we developed a library of nanoparticles for siRNA delivery, called siRNA-aNPs. The library nanoparticles were screened for physicochemical properties and in vitro silencing. From the library, we selected 6

representative compositions for in vivo evaluation. Using radiolabeling of the siRNA, we identified several siRNA-aNP formulations that preferentially accumulate in the bone marrow. Furthermore, via flow cytometry we demonstrated significantly higher uptake of our lead siRNA-aNP in hematopoietic stem and progenitors cells compared to standard LNPs for siRNA delivery. In future experiments, we will evaluate the silencing efficacy of the lead candidate in vivo. Finally, we will set-up treatment studies in various disease models to show siRNA-aNPs' full therapeutic potential.

Reduce and reuse: waste-minimising strategies to establish sustainable use of novel drug therapies

Lisa-Marie Smale, Radboudumc

Sustainable and affordable access to novel drug therapies is increasingly at stake, while simultaneously, substantial drug quantities are being wasted after remaining unused by patients. Given the economic and environmental impacts of this waste, new strategies targeting waste of expensive therapies, such as oral anticancer drugs, are required.
My PhD research contributed to the design and evaluation of two waste-minimising strategies, aiming to 1) reduce waste by individualising dispensed quantities, and 2) redispense (i.e., reuse) unused drugs to other patients after quality assurance. Feasibility of individualising dispensed quantities was demonstrated among 50 patients initiating oral anticancer drugs. It reduced wasted tablets/capsules quantities by one-third and was highly accepted by patients and pharmacy employees, yet left room for further waste-minimisation. A redispensing strategy for unused oral anticancer drugs was created collaboratively with patients with cancer and tested in a multicentre intervention study including > 1,000 patients. Wasted packages were reduced by two-third, equalling mean annual cost-savings up to €1,348 (95% 1,039 – 1,697) per patient and mean annual environmental benefits up to 1 kg CO2per patient.
In conclusion, we showed that reduce and reuse strategies contribute to sustainable use of novel drug therapies by improving their affordability and environmental impact.

A step forward for LRRK2 inhibitors in Parkinson's disease

Maurits F.J.M. Vissers, Leiden Academic Centre for Drug Research, Leiden University

Increased leucine-rich repeat kinase 2 (LRRK2) kinase activity is an established risk factor for Parkinson's disease (PD), making LRRK2-kinase inhibition a promising therapeutic approach for slowing down disease progression. To try and develop a safe and effective treatment for PD, three early-stage clinical studies were conducted to translate preclinical discoveries into clinical applications using the potent, selective, CNS-penetrant LRRK2 inhibitor BIIB122 (DNL151). The first study aimed to characterize multiple LRRK2 pathway biomarkers, including total LRRK2 (tLRRK2), phosphorylation of Ser935 on LRRK2 (pS935), phosphorylation of threonine 73 on Rab10 (pRab10), and total Rab10 (tRab10), in different biological sources (whole blood, peripheral blood mononuclear cells [PBMCs], and neutrophils) as candidate human target engagement and pharmacodynamic biomarkers. The subsequent two phase 1 and phase 1b studies were randomized, double-blind, and placebo-controlled, involving a total of 186 healthy participants (146 randomized to BIIB122 and 40 randomized to placebo) and 36 patients with mild to moderate PD (26 randomized to BIIB122 and 10 randomized to placebo), respectively.
Both studies found that BIIB122 was generally well tolerated, with a cerebrospinal fluid (CSF)-to-unbound plasma concentration ratio of approximately 1 (range, 0.7-1.8). Dose-dependent median reductions from baseline were observed in whole-blood pS935 (≤98%), PBMC pRab10 (≤93%), CSF tLRRK2 (≤50%), and urine bis (monoacylglycerol) phosphate (≤74%). These findings demonstrate substantial peripheral LRRK2 kinase inhibition and modulation of lysosomal pathways downstream of LRRK2, with evidence of CNS distribution and target inhibition by BIIB122. Overall, these results support continued investigation of LRRK2 inhibition with BIIB122 in (currently ongoing) late-stage phase 2 and 3 studies for the treatment of PD.

Sequence engineering to de-immunize mRNA to expand its therapeutic window

Dr. Lotte Tholen, **Sander van Asbeck**

Biography

Sander is a molecular biology expert, founder of Mercurna (targeted mRNA therapeutics developer) and founder & CEO of RiboPro, a premier CDMO and technology provider specialized in mRNA and mRNA delivery. As CEO he enjoys working together with all stakeholders to find the best scientific, clinical and/or commercial solutions, to advance his dream of a medical revolution through (m)RNA. Sander previously worked on: mRNA-based drug-development @ Mercurna; Polynucleotide delivery & targeting @ Radboudumc; Oncology & Tissue-engineering.

Introduction

In vitro transcription (IVT) mRNA is emerging as a new class of therapeutic agents. Although structurally similar to naturally occurring mRNA, exogenous IVT mRNA, is immunostimulatory unless strategies for reducing the immune stimulation are applied. This poses a significant challenge for therapeutic applications, as immune activation inhibits protein synthesis, cellular toxicity and pro-inflammatory cytokine release. In our previous studies, we discovered that reducing the cytidine (C), and confirmed that reducing uridine (U), content of mRNA can be used to decrease immunogenicity and consequently enhance translation of the mRNA. However, strongly biased nucleotide usage can provide problems during synthesis of mRNA and translation, for example via the secondary structure.

Objectives

Therefore, we aimed to develop a more sophisticated sequence engineering strategy based on reducing C and/or U content while keeping codon usage and secondary structure in mind to improve safety and protein yield, thereby widening the therapeutic window of mRNA.

Methods

We developed a sequence optimization strategy in which at least 10% of the C and/or optionally U nucleotides of the mRNA sequence of the wildtype mRNA are replaced by a canonical nucleotide that is not cytidine (C-depletion) and/or uridine respectively (U- and UC-depletion). This strategy was applied to secreted nanoluciferase (secNLuc), eGFP and murine EPO (mEPO) WT mRNA sequences. Original codons were replaced by codons encoding the same amino acids but having no or less C and/or U nucleotides. When selecting alternative codons, the relative frequency of the codon in the exome of interest was close to the relative frequency of the original codon. Alternatively, for codons for which no alternative existed alternative codons with a higher relative frequency were selected. Expression of WT- and modified-mRNA was investigated in HeLa cells and in mice at 24h post transfection or 6 hours post injection, respectively.

Results

UC- and C-depleted mRNA achieved over 2x to 15x the protein expression of WT mRNA, respectively. U-depletion alone did have a significantly smaller effect on protein translation. In vivo, secreted mEPO protein levels induced by U-depleted (14x) and UC-depleted (18x) mRNA were significantly higher than the corresponding WT mRNA.

Conclusion

Here, we have developed a sequence optimization strategy to reduce the immunogenicity of the mRNA by reducing C and/or U content of the mRNA. We found that depending on the mRNA sequence, C- or UC-depletion resulted in the highest protein levels from the modified mRNA. Especially in vivo, C or UC-depletion was relevant as protein expression from the WT mEPO sequence was neglectable, demonstrating the potential of this strategy based on canonical nucleotides for increasing efficacy of an mRNA therapeutic. This approach is a valuable alternative for the use of chemically modified nucleotides in IVT mRNA.

Drug waste of ready-to-administer syringes in the Intensive Care Unit: aseptic syringes versus prefilled sterilized syringes

MSc Thomas Van Gelder ,PharmD, PhD Arief Lalmohamed, PharmD, PhD Kim Dorst-Mooiman, PharmD, MBA Jan Dekker, MSc, MBM Marcel Schinkel, MD, PhD Maaike Sikma, Professor, PharmD, PhD Toine Egberts

Biography

Thomas van Gelder studied pharmacy (BSc and MSc) in Utrecht from 2012 to 2019, where he actively participated in student organizations. Thomas did his MSc research on Health Technology Assessment in Sydney, Australia in 2018. In 2020 he started working in the University Medical Center Utrecht (UMCU) as a hospital pharmacist in training. Thomas combines his work as a hospital pharmacy trainee with PhD research, which focuses on clinical pharmacology and pharmaco-epidemiology in the intensive care unit. Thomas developed a special interest in pharmaceutical technology and drug compounding during his work in UMCU and expanded his field of research. He also followed an internship at Apotheek A15, a large-scale compounding pharmacy in Gorinchem. Thomas hopes to contribute to a more sustainable use of drugs by his translational research.

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Declaration of interest

none

Background

The availability of ready-to-administer (RTA) syringes for intravenous (IV) drugs facilitates rapid and safe administration in emergency and intensive care situations. Hospital pharmacies can prepare RTA syringes through aseptic batchwise filling. Due to excess production of these RTA syringes and their limited shelf-life, a significant amount is wasted, which contributes to environmental pollution. RTA prefilled sterilized syringes (PFSSs) contain sterilized IV drugs which have much longer shelf-lives than non-sterilized RTA syringes and might contribute to reducing drug waste.

Aim

This study aimed to evaluate the difference in drug waste between RTA syringes that were prepared through aseptic batchwise filling and RTA PFSSs in the Intensive Care Unit (ICU).

Methods

We measured drug waste of RTA syringes over an 8 year time period from August 2015 to May 2023 in the 32-bed ICU of the University Medical Center Utrecht. We distinguished between RTA syringes prepared

through aseptic batchwise filling by our hospital pharmacy ("RTA aseptic syringes", shelf-life of 31 days) and RTA PFSSs (shelf-life of 18 months). We defined a control (five drug products) and intervention (three drug products) group for the drug products that were not and that were replaced during the study period by PFSSs, respectively. We then defined four different periods within the total study period, based on quarantine time of the RTA aseptic syringes and time of PFSS introduction: 1) no quarantine, 2) 3-day quarantine, 3) 7-day quarantine and 4) PFSS introduction. Our primary endpoint was the number of RTA syringes that was wasted, expressed as the percentage of the total number of syringes dispensed to the ICU in each of these four periods. We used a Kruskall-Wallis test to test if waste percentages differed between time periods in the control and intervention groups, with a post-hoc Dunn's test for pairwise comparisons. Furthermore, we applied two interrupted time series (ITS) analyses to visualize and test the effect of introducing different quarantine times and the PFSSs on waste percentage.

Results

Introduction of PFSSs significantly decreased drug waste of RTA syringes irrespective of drug type in the intervention group, from 31% during the 7-day quarantine period to 5% after introduction of the PFSS ($p<0.001$). The control group showed no significant decrease in drug waste over the same time periods (from 20% to 16%; $p = 0.726$). We observed a significant difference in the total drug waste of RTA aseptic syringes between time periods, which may be attributed to the implementation of different quality control quarantine procedures. The ITS model of the intervention group showed a direct decrease of 17.7% in waste percentage after the introduction of PFSSs ($p = 0.083$).

Conclusion

Drug waste of RTA syringes for the ICU can be significantly decreased by introducing PFSSs, supporting hospitals to enhance environmental sustainability. Furthermore, the waste percentage of RTA syringes prepared through aseptic batchwise filling is significantly impacted on by duration of quarantine time.

Biocatalytic Synthesis of S-adenosyl-L-methionine Analogs as Novel Methylation-Free Allosteric Activators of Cystathionine-β-synthase

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Biography

Dalibor Nakládal is a researcher at the Comenius University Science Park, pharmacist by education and experimental pharmacologist by training. He completed his doctoral studies in 2019 in the field of pharmacology at the Faculty of Pharmacy of the Comenius University with the thesis topic: "Pre-clinical characterization of SUL compounds: a novel 6-chromanol based drug class derived from hibernation". After obtaining his PhD, he worked for two years as a postdoc at the University Medical Center Groningen, the Netherlands, on an academic-industrial project in the field of drug discovery. He recently received his second PhD, from the University of Groningen, by defending his dissertation "Drug development and new targets for redox disturbances in experimental vascular disease". He is currently executing a Marie Skłodowska-Curie Actions Seal of Excellence project in the field of target-based drug discovery at the Comenius University Science Park in Slovakia.

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Abstract

Cystathionine-beta-synthase (CBS) is the rate-limiting enzyme in the transsulfuration pathway and converts homocysteine to cystathionine, which subsequently facilitates the synthesis of several components involved in redox defense. CBS deficiency can arise in the context of diabetes, leading to hyperhomocysteinaemia and oxidative stress to the kidney. Boosting CBS activity could therefore be a novel strategy for restoring antioxidant defense in diabetes.

Boosting CBS activity is feasible, as S-adenosylmethionine (SAM) has been described as an endogenous allosteric activator of CBS. However, SAM is also a universal methyl-donor, seriously limiting its use as a therapeutic agent. To overcome these problems, this project aims for the development of new methylation-free CBS activators.

SAM can be synthesized from methionine and ATP by the enzyme SAM-synthetase. Interestingly, SAM synthetase is not very selective and also accepts substrates that resemble methionine and ATP. We therefore utilized a SAM synthetase-based biocatalysis approach to generate new SAM-like molecules using analogs of methionine and ATP.

Rational design of SAM analogs was guided by published experimental data on SAM-CBS contacts. In silico docking simulations using the smina scoring function were performed on 50 analogs, leading to the identification of promising candidates. Subsequently, 13 commercially available analogs of ATP or

methionine were selected and purchased. SAM synthetase (metK gene) was expressed in E. coli BL21 and isolated using affinity chromatography. Biocatalysis reactions were performed in Tris-HCl buffer (pH 8.2) enriched with ion cofactors at 30°C for 2 hours. Catalysis of SAM analogs was quantified by measuring phosphate production using the Malachite Green assay. Effect on CBS activity was assessed using crude biocatalysis mixtures. Methylation capacity of SAM analogs was tested by Lambda DNA methylation using EcoRI methyltransferase. Out of the 13 SAM analog mixtures tested, four demonstrated CBS activation but these still showed methylation activity.

In summary, rational drug design coupled with biocatalysis represents an effective approach for developing novel allosteric activators of CBS. Further optimization is required to eliminate the methylation capacity of CBS-activating SAM analogs.

Development of new strategies for the delivery of bioactive proteins to Fibroblasts

Zhiyi Huo

Biography:

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Development of new strategies for the delivery of bioactive proteins to Fibroblasts.
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Introduction

Liver fibrosis refers to the diffuse excessive deposition and abnormal distribution of liver extracellular matrix which is a pathological repair response of the liver to chronic injury. The key step in this process is the activation of Hepatic Stellate Cells, or myofibroblasts.

Objective

We aim to deliver therapeutic proteins to these cells, which also are able to reach the nucleus. For that purpose we use a peptide that binds to the PDGF-β receptor on fibroblasts (PPB), and coupled this to the carier protein human serum albumin.

Methods

Binding and uptake by fibroblasts: 3T3cells were seeded in 96 well plates (50000 cells per well) and co-cultured with human serum albumin (HSA)-AF488, PPB-HSA-AF488, or medium alone (negative control) at 4 and 37oC . Fluorescence staining was analyzed by Flow Cytometry and fluorescense microscopy.
Nuclear binding studies: 3T3 cells were cultured in 6 well plates (500000 cells per well) until 90% confluency. After adherence, cells were co-cultured with HSA-AF488, ppb-HSA-AF488 or medium (negative control) for two hours at 37oC. After digestion and centrifugation, the pellet was resuspended in isolation buffer, and nuclei were isolated using a nitrogen bomb, applying a pressure of 250 Psi for 10 mins. DAPI was added as indicator for nuclei and samples were analysed by Flow Cytometry. The effects of sodium azide (4 mM) were also studied.

Results

The results show that at 4oC, no HSA-AF488 was bound to cells, as compared to medium. PPB-HSA-AF488 induced a shift in staining intensity compared to the other two treatments. At 37oC, again no increased flu-staining was seen in cells treated with medium or HSA-AF488, but for PPB-HSA-AF488 there has a clear shift. This means that PPB-HSA-AF488 has been taken up by 3T3 cells. These results were confirmed by Fluorescene microscopy.
Nuclear binding:
Nuclei were identified using DAPI in the Flow Cytometer and AF488 MFI staining was analysed. Results show that compared to medium and HSA-AF488, PPB-HSA-AF488 induced a high MFI for AF488, reflecting binding of PPB-HSA-AF488 to these nuclei. Nuclear binding was reduced by Sodium-azide.

Conclusions

Fibroblast-like cells are the main inducers of liver fibrosis. In the experiments described above, we verified that PPB-HSA can bind to the 3T3 fibroblasts. PpB- has been shown to bind to the PDGF-receptor, highly expressed on 3T3 cells. We have used a new bombing method to isolate nuclei, and results indicate that PPB-HSA can bind to the nucleus whereas HSA does not. Further studies are needed to examine whether this nuclear binding is specific.

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The impact of inflamation on the drug metabolizing enzymes is isoform dependent

Ms. Laura de Jong, Mr. Chandan Harpal, Mr. Dirk-Jan van den Berg, Prof. Jesse Swen, Dr. Martijn Manson

Biography

Laura de Jong is a PhD researcher at the Division of Systems Pharmacology and Pharmacy at the Leiden Academic Centre for Drug Research. She obtained both her bachelor and master degree in Biopharmaceutical sciences at the University of Leiden. Directly after completing her master she started working as a PhD student, engaging in a project to better understand inter-individual variability in drug response through the use of in-vitro liver models. This project will take place in close contact with collaborators (J. Swen) of the hospital pharmacy of the Leiden University Medical Center (LUMC) to facilitate translation of these findings to- and from the clinic.

Authors

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Introduction

Pro-inflammatory cytokine release during inflammation is associated with compromised metabolism of drugs in the liver. Inhibition of cytochrome P450 (CYP450) enzymes is believed to be the primary cause of inflammation-driven changes in metabolic capacity. However, it remains unclear to what degree inflammation affects other classes of drug-metabolizing enzymes (DMEs), such as the flavin monooxygenases (FMOs) and uridine 5'-diphospho-glucuronosyltransferases (UGTs).

Objectives

To address this issue, our study aims to quantify changes in drug-metabolizing enzymes at both the transcriptional and activity levels following treatment with physiologically relevant concentrations of pro-inflammatory cytokines IL-6 and IL-1β.

Methods

Differentiated human HepaRG hepatocarcinoma cells were treated with either IL-6 or IL-1β in concentrations ranging from 0.001 to 10000 pg/ml. After 24 hours, cells were lysed to examine the changes in mRNA of the different DMEs by RT-qPCR. Relative expression values were calculated with the ΔΔCT method. To investigate changes on activity level, differentiated cells were treated (IL-6/IL-1β) for 72h. Afterwards, CYP2C19 and FMO3 activities were determined in cell supernatants using a liquid chromatography with tandem mass spectrometry–based substrate assay. Non-linear regression analysis was used to determine the potency (pIC50) and maximal decrease (Emax) of DMEs upon inflammatory treatment. One-way ANOVA followed by a Dunnett's multiple comparison test was used to compare pIC50 and Emax values between CYP3A4 and other DME isoforms.

Results

The CYP3A4 mRNA expression was decreased by 97% with a potency of 0.14 ng/ml for IL-6 and 0.35 pg/ml for IL-1β. The different CYP isoforms were equally sensitive to the effects of IL-6 or IL-1β, as comparable pIC50 and Emax values between CYP3A4, CYP2C9, CYP2C19 and CYP1A2 were observed. In contrast, expression of FMO3 and FMO4 were less sensitive to the effects of inflammation, since FMO3 and FMO4 were only decreased by ~55% (IL-6) and ~82% (IL-1β). A 4 to 7-fold higher concentrations of IL-6 were necessary to elicit 50% of the maximal effect as compared to CYP3A4, and for IL-1B this was even a 17 to 28-fold higher concentration. Similarly, the UGT1A4, UGT2B4 and UGT2B7 isoforms were also less sensitive towards IL-6 and IL-1β as compared to CYP3A4, as ~5-fold (IL-6) and 5 to 14-fold (IL-1β) higher

pIC50 values were observed. The observed distinct sensitivities for inflammation on a transcriptional level between DME classes were also reflected in differential sensitivities on an activity level.

Conclusions

This present study emphasizes that inflammation differentially impacts the various DME families CYPs, FMOs and UGTs. Compared to CYP3A4, enzymes from both the FMO family (FMO3/FMO4) and from the UGT family (UGT2B4/UGT2B7) are less sensitivity towards IL-6 and IL-1β induced downregulation. These results implicate that risk assessment on under- or overexposure during inflammation should consider the metabolic route of a drug.

Disease-drug-drug interaction of intravenous imatinib in repurposing for COVID-19 ARDS

Medhat Said

Biography
Medhat Said is a PhD candidate Pharmacometrics at Amsterdam UMC, location VUmc and the Cancer Center Amsterdam in the Netherlands. He obtained his research master in Bio-Pharmaceutical Sciences at Leiden University and now focuses on using quantitative pharmacology to improve treatment outcomes.

Authors
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Category
Clinical Trials

Introduction
Infections may affect pharmacokinetics of a drug by changing its metabolism, binding to plasma proteins and tissue distribution [1]. In the CounterCOVID study, COVID-19-infected patients hospitalized on a general ward, received imatinib orally and exhibited an increased total imatinib exposure due to upregulated alpha-1-acid glycoprotein (AAG) levels in comparison with CML/GIST patients [2]. In the InventCOVID study, ventilated critically ill COVID-19-infected patients received intravenous administration of imatinib. In the latter study patients also received interleukin-6-receptor (IL-6R) inhibitors upon ICU admittance [3]. IL-6R inhibitors have been reported to reverse IL-6 mediated CYP enzyme suppression and reduce signaling to acute phase proteins [4, 5]. In this population pharmacokinetic (PK) study, we aimed to assess the influence of critical illness and concomitant treatment with IL-6R inhibitors on the PK of imatinib in COVID-19-infected patients.

Objectives
To evaluate the predictive performance of a previously developed AAG-PK model of imatinib on data from ventilated critically ill COVID-19-infected patients participating in the InventCOVID study.
To identify additional covariates on imatinib PK if model evaluation suggested that the prediction of the InventCOVID data was insufficient.

Methods
The published AAG-PK model was based on data from hospitalized patients from the CounterCOVID study and CML/GIST outpatients receiving 100-800 mg oral imatinib once daily. The model described the PK of total imatinib, unbound imatinib and total desmethyl-imatinib plasma concentration and its dependency on AAG levels. The performance of the model was evaluated by performing prediction-corrected visual predictive checks (pcVPC) and by calculation of prediction errors (PE). Further model development was performed if the mean prediction error was > 30%. Covariates examined included body weight, age, gender, albumin, alanine transaminase, aspartate aminotransferase, estimated glomerular filtration rate (eGFR), IL-6, concomitant treatment and disease severity (WHO ordinal Scale for

Clinical Improvement) [6].

Results

InventCOVID patients had higher baseline WHO score than CounterCOVID patients (7 vs. 4, P=<0.0001) and 100% vs 19.8% received mechanical ventilation. InventCOVID patients had significantly higher IL-6 (P=<0.001) and lower AAG levels (P=0.007) than CounterCOVID patients. In InventCOVID 90.6% of the patients received an IL-6R inhibitor. Median free fraction of imatinib at steady state in InventCOVID was significantly increased (5.7%) compared to CounterCOVID (2.7%, P=<0.0001) and previous CML/GIST patients (4.0%, P=<0.0001). The AAG-PK model overpredicted total steady state imatinib concentrations (Css) in InventCOVID with a PE of 84% ± 49% (mean ± SD) and underpredicted unbound imatinib concentrations with a PE of -11% ± 32%. In the newly developed population PK model the dissociation constant of AAG and imatinib (KD) was significantly higher in InventCOVID patients compared to CounterCOVID/CML/GIST patients with respective values of 702 ng/ml and 336 ng/mL. Age, body weight, disease state and the presence of an IL-6R inhibitor were found predictive of imatinib clearance and exposure.

Conclusions

Our study suggests that ventilated critically ill COVID-patients may require other imatinib dosing regimens due to pathophysiological changes and possible disease-drug-drug interactions. Binding of imatinib to AAG may be different in critically-ill patients. Our study emphasizes the need to determine both total and unbound drug levels early in ICU patients when evaluating the dose response association of highly protein bound drugs, as unbound drug exposure may drive the effect.

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Physiologically-based pharmacokinetic modelling of recombinant factor IX Fc fusion protein (rFIXFc) and recombinant FIX (rFIX) to characterize extravasation and binding to type 4 collagen

Michael Cloesmeijer, Assoc. Prof Erik Sjögren, Dr. Peter Lenting, Dr. Marjon Cnossen, Prof. Ron Mathôt

Biography

Michael Cloesmeijer is a current PhD student at Amsterdam UMC, focusing on research in bleeding disorders such as von Willebrand disease and haemophilia. His primary area of expertise lies in pharmacometrics, specifically population pharmacokinetic-pharmacodynamic (PK-PD) and physiological-based pharmacokinetic modeling.
Their work holds potential in improving the treatment of bleeding disorders. By utilizing population PK modeling, they aim to personalize and optimize treatment approaches, enhancing the quality of care for patients.

Authors

Michael Cloesmeijer[1], Erik Sjögren[2,3], Peter Lenting [4], Marjon Cnossen[5] and Ron Mathôt[1] for the OPTI-CLOT study group and SYMPHONY consortium

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Introduction

Patients with severe haemophilia B (HB) are prophylactically treated with factor IX concentrates to prevent bleeding. Recently, the extended half-life (EHL) concentrate recombinant factor IX Fc fusion protein (rFIXFc) have become available that allow less frequent administration than recombinant FIX (rFIX)1-2. rFIXFc exhibits a rapid distribution phase, which is possibly due to binding to type IV collagen (Col4) in the extravascular space3. rFIXFc activity levels in the extravascular space seem crucial, because its haemostatic effect is present even with no measurable FIX activity in plasma. The ratio of the amount FIX bound extravascular and the amount in plasma is approximately 17-20 3,4. Currently, empirical population pharmacokinetic models have been developed for FIX concentrates5. These models describe only plasma FIX levels over time, but not in the extravascular space.

Objectives

» Develop a physiologically-based pharmacokinetic (PBPK) model of rFIXFc in adults HB patients
» Quantify the binding of rFIXFc to COL4
» Investigate the predictive performance of the PBPK model of rFIXFc for rFIX

Methods

The PBPK model for therapeutic proteins in PK-Sim (version 11 – build 150, Open Systems Pharmacology) was used to develop the rFIXFc PBPK model. Physicochemical properties and clinical observations were obtained from literature. Clinical rFIXFc observations were obtained from the EMA assessment report. Patients received an intravenous dose of 50 IU/kg rFIXFc. The binding of rFIXFc to COL4 in the extravascular space was quantified to account for the rapid distribution phase and to describe the levels of rFIXFc to Col4 in the extravascular space. An additional binding site of rFIXFc in the vascular endothelial site was included. To model the degradation of FIX in the plasma, a generic enzyme was added to the model to account for the protease activity. FcRn recycling was calibrated on the observational data by estimating the FcRn dissociation constant (Kd). After developing the model for rFIXFc, the FcRn recycling pathway was disabled to investigate the predictive performance of the rFIXFc model for rFIX in patients receiving 55 IU/kg rFIX by comparing to observed rFIX plasma levels by Suzuki et al.5

Results

Our PBPK model for rFIXFc was able to accurately predict the observed plasma levels of rFIXFc (total residual error = 0.08) and to quantify the binding of rFIXFc to Col4 in the extravascular space in a male population. The total level of rFIXFc to Col4 in the extravascular space was approximately 14 times higher compared to the FIX plasma level. The final PBPK model for rFIXFc could be modified to adequately describe the PK for rFIX (total residual error = 0.18), which demonstrates the adequacy of the developed model.

Conclusion

The developed PBPK model adequately predicts the plasma profiles of rFIXFc and rFIX and the binding of both concentrates to Col4 is quantified. Model predictions display higher levels of FIX to Col4 in the extravascular space when compared to the plasma, which may highlight the importance of FIX in the extravascular space to prevent bleedings in the presence of lower FIX plasma levels.

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PET-Tracer and Target Site Physiologically-based Pharmacokinetics to enable Tyrosine Kinase Inhibitor Precision Oncology & Personalized Dosing

Dr. Imke Bartelink, Dr Maqsood Yaqub, Drs. Robbin Grijseels, Drs. Evelien de Langen, Drs. Zahra Ahmadian, Drs. Daan van Valkengoed, Drs Suzanne van der Gaag, Dr. Idris Bahce, Dr. Mathilde Kouwenhoven, Dr. Myra van Linde, Dr. J.A. Koos Dijkstra, Dr. Bart Westerman, Prof. Dr. N. Harry Hendrikse

Biography

Dr Imke H. Bartelink is a Hospital pharmacist, clinical pharmacologist & pharmacometric researcher at Amsterdam UMC, location VUmc and the Cancer Center Amsterdam in the Netherlands. She completed her PharmD and PhD at the Utrecht Medical Center in Utrecht, the Netherlands. She performed Post-doctoral studies in Integrative Pharmacology from the University of California, San Francisco (UCSF, USA) and a clinical pharmacology fellowship at Department of Medicine, Early Phase Clinical Trials Unit at UCSF. Furthermore, she has prior experience as a senior pharmacometrician at the Department of Clinical Pharmacology & DMPK of Medimmune (now AstraZeneca) in California. She authored more than 50 manuscripts in the field of target site pharmacokinetic-pharmacodynamic modeling to optimize cancer treatment.

Introduction

The tyrosine kinase inhibitor (TKI) osimertinib is indicated for treatment of patients with epidermal growth factor receptor (EGFR) mutated non-small cell lung cancer (NSCLC). TKIs have a relatively small therapeutic window, suboptimal therapy may therefore lead to toxicity or nonresponse. Spatial tumor heterogeneity results in sanctuary sites with some neoplastic cells receiving insufficient TKI concentrations during the treatment period. Personalized dosing may refine the current "one dose fits all" paradigm that currently dominates the field. Imaging by positron emission tomography (PET) with micro-dosed inertly radioactively labeled drugs can be used to identify these sanctuary sites and quantify drug uptake at the site of action in oncological patients. However, a major challenge in using microdosed TKIs to guide precision dosing is that kinetics, including tumor uptake (penetration) of a low dose of radiolabeled TKIs may differ from that at the therapeutic dose.

Objectives

To study whether PET imaged kinetics of TKIs may predict therapeutic kinetics in order to optimize precision medicine, we applied physiologically-based pharmacokinetic modeling (PBPK) for ([11C]) osimertinib.

Methods

Relevant physicochemical, drug specific properties and key hallmarks of NSCLC were included: immune tumor deprivation, unaltered tumor perfusion compared to lung tissue and an acidic tumor environment [1]. To predict therapeutic PK, saturable EGFR binding, target affinity, abundance and tumor volume were included. Rate of drug absorption, metabolism and elimination were included using a published PBPK model of osimertinib [2 & Figure]. For evaluations, Observed mean therapeutic PK in blood after therapeutic oral dose in ten healthy volunteers was included [4]. Furthermore, the time activity curves from blood samples and PET-images of dynamic scans of lung, lung tumor, blood, and of static scans in spleen, liver, bone, brain, skin and muscle were quantified after an IV microdose [11C] osimertinib in four NSCLC patients [3]. The model was considered accurate when the predicted plasma and tissue concentrations fell within 2-fold of observed plasma and tissue concentrations, described as a prediction error (PE) between -100 and 100%.

Results

Using the PBPK-model we adequately predicted compound absorption, distribution, metabolism and elimination (ADME) in lung, lung tumor, blood and other tissues at most time points after micro dose and after therapeutic oral dose: Nearly all predictions fell within a 100% PE.

Conclusion

The developed PBPK model enables prediction of whole body drug distribution after micro- and therapeutic dose levels within twofold of observed static and dynamic PET images, and/or plasma PK samples after microdosing and at therapeutic dose levels. After evaluation of variability in drug penentrance, the PET-based PBPK-model of tumor TKI-uptake may be used to select the optimal dose to enhance TKI-treatment efficacy as well as limit side effects for precision medicine. This strategy may revolutionize the way we practice precision medicine.

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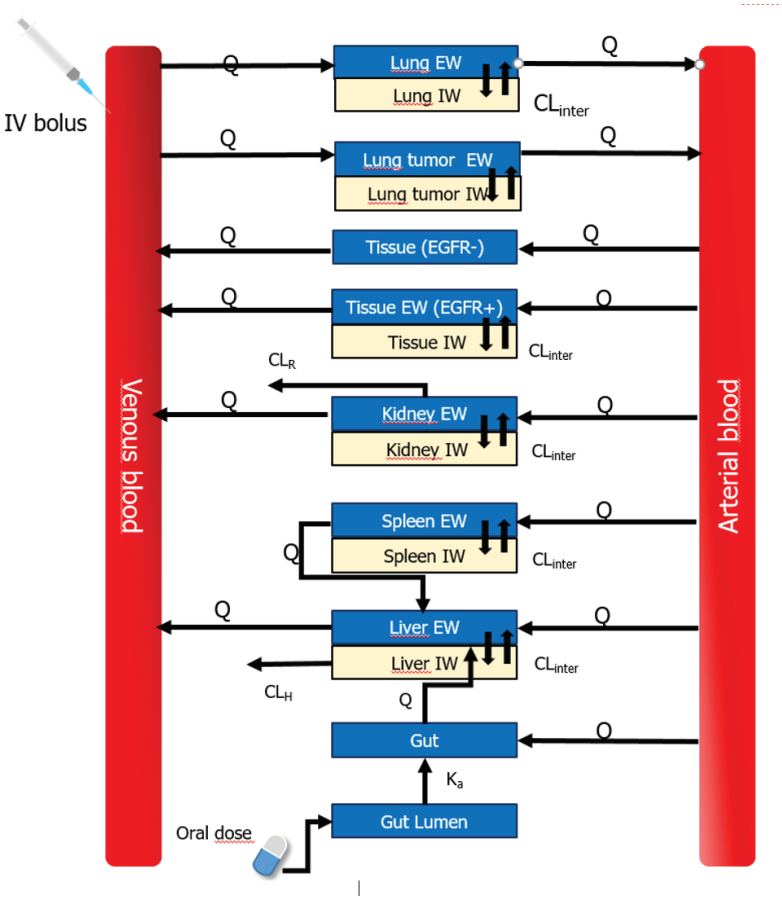

Figure: Schematic overview of the mechanistic PBPK model of osimertinib

Personalised Therapeutics @ Leiden University Medical Center to personalise treatment

Sylvia Klomp, Romy Mosch, Wessel van der Zande, Nina Lute, Dr. Kim Gombert-Handoko, Prof. Dr. Teun van Gelder, Prof. Dr. Henk-Jan Guchelaar, Prof. Dr. Jesse Swen

Biography

Sylvia Klomp, MSc, is currently a PhD student in the department of Clinical Pharmacy & Toxicology at Leiden Medical Center. She earned her MSc in Biopharmaceutical Sciences at the University of Leiden. Her research is in the field of pharmacogenetics and focusses on phenoconversion. Where she tries to unravel the mechanisms of non-genetic factors contributing to the variability in drug response and the impact on patient care.

Authors

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Theme

Personalized Medicine

Introduction

Pharmacogenomics (PGx) is the study of genetic variability affecting an individual's response to a drug. PGx is a critical component of personalised medicine. Currently, PGx is applied for individual drugs and/or individual genetic variants. With a panel of 12 genes, for which the Dutch Pharmacogenetic Working Group (DPWG) has issued evidence based drug dosing guidelines, a 30 % reduction in the risk for severe adverse drug reactions (ADRs) was demonstrated in the PREPARE study [1]. Based upon national prescription data we estimate that 5.6% of all first prescriptions would require an individualisation of the dose or drug [2]. However, in current clinical practice the potential of PGx testing is not fully exploited. Therefore, a prospective implementation study on pre-emptive PGx testing is being performed for patients in the LUMC. In this study, 1,000 patients with a planned surgery will be randomised to PGx-guided dosing or standard of care.
Objectives: To implement pre-emptive panel based PGx testing in LUMC and to determine patient benefit of PGx guided drug prescription and dispensing.

Methods

A prospective, open, randomised implementation study in 1,000 patients with a duration of 2 years. Patients with a planned surgery recruited after a medication reconciliation interview are 1:1 randomised to PGx-guided dosing or standard of care. The PGx-guided group receives pre-emptive PGx testing for a panel of 14 genes (including 227 PGx variants, genotyped with the Global Diversity Array with enhanced PGx-8 V1.0) followed by personalised drug and dose recommendations for newly prescribed drugs. Recommendations are based on the guidelines of the DPWG. Patients in the control group will receive usual drug prescriptions, without PGx-guided drug or dose selection. Ethical approval of the study protocol was obtained in April 2022 and recruitment was started in December 2022. In June 2023, 190 participants have been recruited.

Outcome

The primary outcome is the incidence of ADRs in the first 12 months after inclusion in the study. Side effects collected via the for the study developed pharmacovigilance app, namely the Pharma.Sensor app. Following the collected side effects will be scored as ADR, with regard to their relationship to the drug and severity (≥ grade 3 CTC-AE).

Conclusion

The Personalised Therapeutics @LUMC study was started to implement pre-emptive panel based PGx testing for patients in the LUMC and to determine patient benefit of PGx guided drug prescription and dispensing.

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Label-free detection of prostaglandin transporter (SLCO2A1) activity using a TRACT assay

Dr Tamara Mocking, Luc Mulder, Prof.Dr. Laura H. Heitman, Prof. Dr. Adriaan P. IJzerman

Biography

Dr. Tamara Mocking is a postdoc at Leiden university. She obtained a PhD in molecular pharmacology at Vrije Universiteit Amsterdam studying GPCR photopharmacology. Currently, her research focuses on label-free assay to study Solute carrier transporters in which she uses GPCR activation as a measure for SLC transport activity as part of the ReSolute consortium (https://re-solute.eu/).

Introduction & Aim

The prostaglandin transporter (PGT, SLCO2A1) mediates transport of prostanoids (a.o. prostaglandin E2 (PGE2)) into the cells to promote their degradation. Overexpression of PGT leads to low extracellular PGE2 levels and has been linked to impaired wound healing of diabetic foot ulcers1. Inhibition of PGT would be beneficial, however, there are currently no high-through screening assays for this transporter. Here we developed a label-free impedance-based assay for PGT that measures transport activity through receptor activation (TRACT).

Methods

Label-free impedance-based TRACT measurements were recorded on intact cells using the xCELLigence real-time cell analyzer. Here, activation of prostanoid receptors EP3 or EP4 with PGE2 leads to changes in cell morphology. Uptake of PGE2 by PGT will reduce extracellular PGE¬2 levels and thereby attenuated the response of co-expressed EP3 or EP4 receptor. Thus, PGT activity is detected as a change in receptor activity. To this end, HEK293-JumpIn-SLCO2A1 cells with doxycycline (dox)-inducible SLCO2A1 expression were transfected to express prostanoid receptors EP3 or EP4 and induced or non-induced cells were pretreated with inhibitor or vehicle prior to stimulation.

Results

Induction of PGT expression on EP3 or EP4 expressing HEK293-JumpIN-SLCO2A1 cells results in over 10-fold reduction in potency of PGE2 (Figure 1). Potency was recovered upon inhibition of the PGT-mediated PGE2 uptake with PGT inhibitor Olmesartan and T26A. The results confirm that prostanoid receptor activity can be used as a measure of PGT activity.

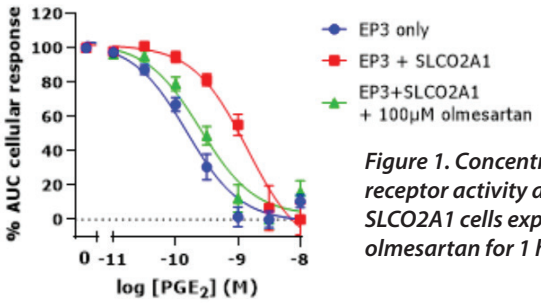

Figure 1. Concentration response curves of the influence of PGT co-expression on EP3 receptor activity as measured by xCELLigence. Induced and non-induced HEK293-JumpIn-SLCO2A1 cells expressing EP3 receptor were pretreated with vehicle or PGT inhibitor olmesartan for 1 hr prior to stimulation with PGE2.

Conclusion

An impedance-based TRACT assay was established that measures prostaglandin transporter (SLCO2A1) activity through prostanoid receptor signaling. This will enable a novel way to better study the wound healing capacity of SLCO2A1 inhibition on a cellular level.

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Development of an Affinity-Based Probe to profile endogenous Human Adenosine A3 Receptor Expression

Bert Beerkens, Inge Snijders, Joep Snoeck, Dr. Rongfang Liu, Dr. Anton Tool, Dr. Sylvia Le Dévédec, Dr. Willem Jespers, Prof. Taco Kuijpers, Prof. Gerard van Westen, Prof. Laura Heitman, Prof. Adriaan IJzerman, Dr. Daan van der Es

Biography

Bert Beerkens is a PhD student in the medicinal chemistry/molecular pharmacology group of prof. Adriaan IJzerman and prof. Laura Heitman (University of Leiden), working under the supervision of Dr. Daan van der Es. In his PhD studies, Bert is developing chemical tool compounds (probes) to target and label the four different subtypes of adenosine receptors. Here, he works on the verge of chemistry, pharmacology and biology: being responsible for the development, chemical synthesis, pharmacological characterization and biological evaluation of the chemical probes.

Introduction

The adenosine A3 receptor (A^3AR) is a G protein-coupled receptor (GPCR) that exerts immunomodulatory effects in physio- and pathological conditions such as inflammation and cancer. Thus far, studies towards the downstream effects of A^3AR activation have yielded contradictory results, i.e. both proliferative and antiproliferative effects have been observed upon A^3AR activation in cancerous cell lines, tissues and animal models. This urges the need for further investigations towards the A^3AR and all of the involved signaling pathways. Various chemical and biological probes have been developed for this purpose, ranging from fluorescent ligands to antibodies. Nevertheless, these probes are limited by their reversible mode of binding, relatively large size and often low specificity.

Objectives

The goal of this study was to develop, synthesize and biologically evaluate an affinity-based probe (AfBP) as an alternative tool compound for the detection of the A^3AR. Such an AfBP must bind the A^3AR with a high affinity, (subtype-)selectivity and an irreversible mode of binding. Most important, the AfBP must allow detection of the A^3AR in a wide variety of biochemical assays.

Methods

AfBP design was based on computational docking studies and the probe was chemically synthesized in-house. Affinity and mode of binding were determined in molecular pharmacological assays; Labeling and detection of the A^3AR were investigated through SDS-PAGE, confocal microscopy and flow cytometry experiments.

Results

The synthesized AfBP showed a high affinity, good selectivity and irreversible mode of binding towards the A^3AR. Specific labeling of the A^3AR on an overexpressing cell line was confirmed in SDS-PAGE and confocal microscopy experiments. Most interestingly, utilization of the AfBP in flow cytometry experiments allowed detection of endogenous A^3AR expression.

Conclusion

The herein developed AfBP can be used as a tool compound for the detection of the A^3AR in a multitude of biochemical assays, examples from this work being SDS-PAGE, confocal microscopy and flow cytometry. The AfBP thereby allows a thorough investigation of A^3AR expression and functionality, on both overexpressing model cell lines and native cells. Ultimately, this tool compound will aid future studies towards the expression and function of the A^3AR in a wide variety of pathologies.

Tuning liposome rigidity to modulate cellular uptake by cells

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Biography

Xinyu Ma is a first year PhD student in Nanomedicine at the University of Groningen under the supervision of Prof. dr. Anna Salvati. Her research focuses on the effect of nanoparticle rigidity on nanoparticle targeting and clearance. Her current projects utilize liposomes with different composition to modulate rigidity and study cellular endocytic behavior and related mechanism. This program is sponsored by Chinese Scholarship Council. Prior to this program, Xinyu completed her master in Medicinal Chemistry at Sichuan University in 2022. She received her BS in Pharmaceutical Engineering from Jiangnan University in 2019.

Introduction

Nano-sized materials have been extensively investigated over the recent decades for their potential application in delivering cancer therapeutics. Numerous studies are trying to elucidate how nanoparticle properties, such as size, charge, material etc., affect nanoparticle behavior in organisms and at the cellular level. Among these properties, nanoparticle rigidity, the ability of the object to resist deformation upon an applied force, is emerging as another parameter which can be used to modulate nanoparticle interactions with cells. However, a clear understanding of how rigidity affects cellular uptake behavior and uptake mechanism is still missing.

Objectives

Within this context, liposomes of different composition are used as a model system to study how rigidity affects cellular uptake behavior and mechanism. Lipids with higher transition temperature (Tm) typically form more rigid lipid bilayers. Thus, by changing their content in a lipid mixture, they can be used to prepare a series of liposomes of same size and surface chemistry but with different rigidity. The liposomes are then used to explore in depth how nanoparticle rigidity affects the interaction with cells and cellular uptake mechanism.

Methods

Lipids with different transition temperature such as DSPC, DPPC and DOPC are mixed in different ratios to form liposomes; Dil is incorporated as a fluorescent label. Liposome size and charge are determined by dynamic light scattering and zeta potential measurements.
Flow cytometry is used to measure cellular uptake.

Results

Liposomes have been prepared by thin-layer hydration, and their size distribution, zeta potential and stability in serum have been determined. The procedure to prepare the liposomes has been optimized in order to allow the inclusion of the lipids with higher transition temperature in the bilayer. Homogenous liposome dispersions could be obtained in water and PBS, however when lipids at higher T^m are included, dispersion in serum led to agglomeration. Preliminary experiments with HeLa cells showed that uptake is higher for the liposomes made with lipids at higher T^m.

Conclusion

Further studies are ongoing to improve the stability in serum when lipids at higher T^m are included and to characterize the mechanism of uptake for the different samples.

Preparation and characterization of lipid nanoparticles for the delivery of RNA

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Biography

Heba is a PhD student in Department of Nanomedicine and Drug Targeting, Groningen Research Institute of Pharmacy (GRIP), University of Groningen, Netherlands, who is supported by a full PhD scholarship from the Cultural Affairs and Mission Sector of the Arab Republic of Egypt. She started her Ph.D. project on September 2022 under the supervision of Prof. Dr. Anna Salvati and Prof. Dr. Klass Poelstra. Heba completed her undergraduate degree in Pharmacy and her master's degree in Pharmaceutical Sciences (Pharmaceutics) at Alexandria University, Egypt. Before moving to Groningen, she was working as a teaching assistant of Pharmaceutics for six years at the Faculty of Pharmacy, Alexandria University, Egypt.

Introduction

Among multiple types of nanoparticles, lipid nanoparticles (LNPs), which have recently arrived in clinical use with the RNA vaccines against COVID-19, have pushed the boundaries of the pharmaceutical industry [1]. They have become a leading non-viral vector for the delivery of nucleic acids, bringing both gene therapy and vaccination into a new rapidly-growing era. In order to be effective, after uptake into cells by endocytosis, the LNPs need to release their RNA load into the cytosol. The currently approved LNP formulations have been optimized to achieve this, however their transfection efficacy is still relatively low and many aspects of how after uptake the LNPs are processed by cells are not fully clarified.

Objectives

In order to study these questions, in the first months of my project, the objective has been to test different methods for the preparation of LNPs. Using poly-adenylic acid **(Poly A)** as a model RNA molecule and a messenger RNA to produce the green fluorescent protein GFP, we compared the properties and cell behaviour (uptake and transfection efficacy) of the nanoparticles prepared under different conditions in HeLa cells.

Methods

LNPs are prepared using the ionizable lipid D-Lin MC3 DMA, the helper lipid DSPC, the PEGylated lipid DMG-PEG2000 and cholesterol. The fluorescently labelled diL lipid is included in order to be able to quantify LNP uptake by cells. LNPs with different polyA amounts are prepared using either vortexing or microfluidic mixing. Then, the LNPs obtained using the two methods are characterized by dynamic light scattering. This is done for LNPs dispersed in water, PBS, as well as in the cell culture medium supplemented with serum in order to test their stability in biological conditions. Poly A entrapment efficiency is determined using a Quant-it Ribogreen mRNA Assay kit. Finally, flow cytometry is used to quantify uptake of the LNPs by cells.

Results

The results obtained so far indicate that LNPs with comparable size and low polydispersity are obtained using both mixing techniques. In addition, a high poly A encapsulation efficiency is achieved, coupled with high loading capacity and the LNPs form homogenous dispersion in medium with serum. First studies by flow cytometry show that the cell fluorescence increases at increasing LNP concentration and exposure time, suggesting efficient internalization. First tests with LNPs containing mRNA confirm a high transfection efficacy on HeLa cells.

Conclusion

These preliminary studies confirm that LNPs encapsulating poly A or mRNA are obtained and show narrow size distribution and stability in serum. The particles are efficiently internalized by cells and show

good transfection efficacy. The next steps will be to study in more detail their mechanism of uptake and intracellular trafficking.

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Antifungal azoles for lung transplant recipients: is standard dose adjustment of tacrolimus sufficient?

Drs. Tanja Zijp, Ms Fadia Hadi, prof.dr. Stephan Bakker, a/prof.dr. Job van Boven, dr. Tji Gan, prof.dr. Daan Touw

Biography

Tanja Zijp is a 4-th year PhD candidate and trial pharmacist at the department of Clinical Pharmacy and Pharmacology of the University Medical Center Groningen. She earned her Bachelor of Pharmacy (2015), Honours College Bachelor (2015) and Master of Pharmacy degrees (2019) at the University of Groningen.

Her research focuses on optimising treatment with tacrolimus in solid organ transplant recipients. Her research includes a pharmacokinetic observational drug-drug interaction study of tacrolimus with antifungal drugs, a literature review on alternative sampling methods, analytical method development using LC-MS/MS, usability studies for smart monitoring devices to study adherence, and retrospective and literature research on drug-drug interactions with immunosuppressants

Authors

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Background

Lung transplant recipients (LTR) are at high risk for opportunistic fungal infections (FI). Yet, treatment with azoles results in drug-drug interactions with tacrolimus, requiring dose adjustment (voriconazole: 67%, fluconazole: 40%). We assessed the interaction magnitude, defined by change in tacrolimus dose-corrected trough (C/D) and its influencing factors.

Methods

In this retrospective study, LTR using tacrolimus p.o. and azoles for FI in 2018-2022 were included. Demographics, drug formulation, doses, concentrations, and blood tests (albumin, ALAT, CRP, eGFR, Hct) were extracted from patient records. Change in C/D before, during and after azole treatment were calculated. Spearman's correlations of C/D during treatment with demographics and lab parameters were assessed using SPSS.

Results

In total, 33 LTR were included with 523 tacrolimus concentrations. C/D increased after azole start, median [IQR] 4.1 times [2.7-6.1] for voriconazole (n=22), and 2.1 times [1.4-3.7] for fluconazole (n=11), suggesting median dose reductions of 75% and 50% respectively. Median first tacrolimus concentrations after azole start were 13.8 µg/L (range: 5.7-46.1 µg/L) for voriconazole and 11.1 µg/L (2.8-28.9) for fluconazole, compared to 9.2 µg/L (5.2-14.9) before start. C/D during treatment was correlated with C/D before treatment ($r=0.60$, $p<.001$), BMI ($r=.36$, $p<.05$), and Hct ($r=-.46$, $p<.01$). After the 3rd sample after azole discontinuation, median C/D was comparable with before treatment.

Conclusion

The drug-drug interaction magnitude between tacrolimus and azoles is variable, and current guidelines for precautionary dose adjustment may not be sufficient.

The Effectiveness of Antiseizure Medication Triple-Therapy in Glioma Patients With Refractory Epilepsy: An Observational Cohort Study

MD Pim van der Meer

Biography

Pim van der Meer is a medical doctor, neuropsychologist, and currently a PhD-candidate at the department of neuro-oncology. The topic of his PhD is the pharmacological treatment of epilepsy in glioma patients. His main interest is the comparative effectiveness of antiseizure medications.

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Background

About 10% of glioma patients with epilepsy need antiseizure medication (ASM) triple-therapy due to refractory epilepsy. Aim of this study was to evaluate whether levetiracetam combined with valproic acid and clobazam (LEV+VPA+CLB), a frequently prescribed triple-therapy, has favorable effectiveness compared to other triple-therapy combinations in glioma patients.

Methods

This was a multicenter retrospective observational cohort study, with as primary outcome the cumulative incidence of time to treatment failure for any reason, from start of ASM triple-therapy treatment. Secondary outcomes included cumulative incidences of: 1) time to treatment failure due to uncontrolled seizures; 2) time to treatment failure due to adverse effects; and 3) time to recurrent seizure. Patients were followed for a max. duration of 36 months.

Results

Out of n=1435 patients in the original cohort, n=90 patients received ASM triple-therapy after second-line ASM treatment failure due to uncontrolled seizures. LEV+VPA+CLB was prescribed to 48% (43/90) and other ASM triple-therapy to 52% (47/90) patients. The cumulative incidence of treatment failure for any reason of LEV+VPA+CLB did not significantly differ from other ASM triple-therapy combinations (12 months: 47% [95%CI, 31-62%] versus 42% [95%CI, 27-56%], p=0.892). No statistical significant differences for treatment failure due to uncontrolled seizures (12 months: 12% [95%CI, 4-25%] versus 18% [95%CI, 8-30%], p=0.445), due to adverse effects (12 months: 22% [95%CI, 11-36%] versus 15% [95%CI, 7-27%], p=0.446), or recurrent seizure (1 month: 65% [95%CI, 48-78%] versus 63% [95%CI, 47-75%], p=0.911) were found.

Conclusions

LEV+VPA+CLB might show equivalent effectiveness compared to other ASM triple-therapy combinations in glioma patients.

Effectiveness of Genotype-specific Tricyclic Antidepressant Dosing in Patients with Major Depressive Disorder: A Randomized Clinical Trial

Cornelis Vos, PharmD Sophie ter Hark, MD PhD Arnt Schellekens, MD PhD Jan Spijker, MD PhD Annemarie van der Meij, PhD Anne Grotenhuis, MD PhD Raluca Mihaescu, PhD Wietske Kievit, PhD Rogier Donders, PharmD PhD Rob Aarnoutse, PhD Marieke Coenen, MD PhD Joost Janzing

Biography

I am Cornelis (Niels) Vos, 33 years old, and I work as a psychiatrist and PhD candidate at the Radboudumc (Nijmegen). My research is about personalizing treatment with psychotropics for major depression. In addition, I am a resident in clinical pharmacology (Radboudumc). I expect to finalize my PhD thesis at the end of 2023. From May 2023 I will work in GGZ Antes (Rotterdam), where I will set up a polyclinic for specialized psychopharmacology. In addition, I will continue working as a researcher at the department of psychiatry in the Radboudumc.

Introduction

Evidence for the clinical benefit of Pharmacogenetics-Informed Treatment (PIT) with antidepressants is still limited. Especially for Tricyclic Antidepressants (TCAs) pharmacogenetics may be of interest since therapeutic plasma concentrations are well-defined, optimal dose finding can be time consuming and treatment is frequently accompanied by adverse effects.

Objectives

The primary objective was to determine whether PIT results in faster attainment of therapeutic TCA plasma concentrations compared to Treatment As Usual (TAU) in patients with unipolar Major Depressive Disorder (MDD). Secondary objectives were to examine if PIT results in more efficacy and in fewer and less severe adverse effects compared to TAU. Secondary outcome measures were severity of depressive symptoms (measured by the HAM-D 17) and adverse effect scores (measured by the Frequency, Intensity, Burden of Side Effects Rating; FIBSER).

Methods

In the PIT group the initial TCA dosage was based on CYP2D6 and CYP2C19 genotypes. Patients were treated with the TCAs nortriptyline, clomipramine or imipramine. The clinical follow-up was seven weeks. At inclusion, patients had unipolar non-psychotic MDD (Hamilton Rating Scale for Depression (HAM-D 17) ≥ 19), were aged 18 to 65 years and were eligible for TCA treatment. Main exclusion criteria were a bipolar or psychotic disorder, substance use disorder, pregnancy, interacting co-medications and co-use of psychotropics. Patients were included from June 2018 until January 2022 in four centres in the Netherlands.

Results

A total of 111 patients (mean age 41.7 (SD 13.3) years, 69 (62%) females) were evaluated: PIT (n=56) and TAU (n=55). The PIT group reached therapeutic concentrations faster than TAU (mean 17.3 days (SD 11.2) versus mean 22.0 days (SD 10.2), Kaplan Meier X12 = 4.3, p=0.039). We observed no significant difference in reduction of depressive symptoms. Linear mixed model analyses showed that the interaction between group and time differed for frequency (F6,125= 4.03, p=0.001), severity (F6,114 = 3.10, p=0.008) and burden (F6,112 = 2.56, p=0.023) of adverse effects respectively, suggesting that adverse effects decreased relatively more for PIT.

Conclusion

In this first RCT comparing PIT to TAU for dosing of TCAs we found that PIT resulted in a faster attainment of therapeutic TCA concentrations with potentially less adverse effects.

Increasing the bioavailability of oral esketamine by a single gift of cobicistat in a patient with severe, treatment resistant depression

Cornelis Vos

Biography

I am a psychiatrist at the Radboudumc and PhD candidate at the Radboudumc (Nijmegen), investigating personalized treatment with psychotropics, especially for major depression. In addition, I am a resident in clinical pharmacology (Radboudumc, Nijmegen). From May 2023 I will start as a psychiatrist at GGZ Antes (hospital psychiatry, connected to the Maasstad ziekenhuis, Rotterdam), where I will set up a specialized polyclinic for patients experiencing problems with regard to psychopharmacology.

Introduction

Intranasal esketamine has recently been approved as a drug for Treatment Resistant Depression (TRD), however, it is costly, not widely available and may result in adverse effects specific for intranasal administration, such as recurrent intranasal pain, irritation and postnasal drip. In this case study we explored whether oral esketamine can be a suitable alternative since our patient, treated for major depression, experienced recurrent, severe intranasal pain using the intranasal esketamine spray. Because oral esketamine has a low bioavailability, it results in a different ratio between esketamine and its active and primary metabolite norketamine. The relative antidepressant effects of esketamine and norketamine are a topic of scientific debate.

Objectives

As our patient responded well to intranasal esketamine twice weekly previously, we aimed to treat her with similar esketamine and norketamine plasma concentrations as achieved with the regular intranasal treatment. To increase the bioavailability we combined oral esketamine with a single gift of the CYP3A4 inhibitor cobicistat. To increase the bioavailability we combined oral esketamine with a single gift of the CYP3A4 inhibitor cobicistat.

Methods

Firstly, we measured esketamine and norketamine plasma concentrations after administration of the regular treatment with 84 mg intranasal esketamine. Blood samples were collected 15 minutes prior and 0.5, 1, 1.5, 3, 6 and 24 hours after each intranasal or oral esketamine ingestion. In the next treatment sessions we administered oral esketamine 1 mg/kg, 2 mg/kg and 4 mg/kg and measured esketamine and norketamine plasma concentrations at similar time intervals. Thereafter, we combined oral esketamine (1 mg/kg and 2 mg/kg) with a single gift of cobicistat 150 mg and measured plasma concentrations at similar intervals. During each esketamine treatment session the patient stayed at the Psychiatry Department of the Radboudumc and she was monitored continuously.

Results

As expected, administration of oral esketamine 1 mg/kg, 2 mg/kg and 4 mg/kg resulted in lower esketamine but higher norketamine plasma concentrations compared to intranasal treatment. Combined oral esketamine (2 mg/kg) and cobicistat (150 mg once) treatment resulted in similar esketamine and norketamine plasma concentration curves as intranasal esketamine administration (84 mg). The combined treatment was well-tolerated.

Conclusion

Our case study demonstrates that cobicistat can be effectively used to increase the bioavailability of oral esketamine and to generate similar esketamine and norketamine plasma concentrations as the intranasal treatment while maintaining the practical advantages of oral treatment. Future studies are needed to the relative effects of esketamine and norketamine in TRD and to the clinical benefit of combined treatment with oral esketamine and cobicistat.

Real-world dispensing patterns of inhalation medication in young adult asthma: an inception cohort study

Irene Mommers

Biography
I am currently enjoying the 2nd-year of a PhD in pharmacoepidemiology at the University of Groningen, focusing on asthma treatment. I am always open to collaborating or social networking. If you want to learn more about me, have a look at: www.linkedin.com/in/irenemommers. Feel free to contact me.

Previous education
2020 - 2021 Epidemiology at Maastricht University
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Background
The Global Initiative for Asthma (GINA) suggests a step-wise approach for pharmacological treatment of asthma. Valid study of real-world treatment patterns using dispensing databases includes proper measurement of medication adherence. We aimed to explore such patterns by applying a time-varying proportion of days covered (tPDC)-based algorithm.

Methods
We designed a retrospective inception cohort study using the University of Groningen IADB.nl community pharmacy dispensing database. Included were 19,184 young adults who initiated asthma medication anywhere between 1994 and 2021, in the Netherlands. Main treatment steps were defined as: 1 - SABA / ICS-formoterol as needed, 2 - low dose ICS, 3 - low dose ICS + LABA or tiotropium, or intermediate dose ICS, 4 - intermediate to high dose ICS + LABA or tiotropium, triple therapy, or high dose ICS, 5 – treatment prescribed by a specialist. Changes in treatment steps were determined using a time-varying proportion of days covered (tPDC)-based algorithm. Individual drug treatment trajectories were visualized over time using a lasagna plot.

Results
At initiation, of the 19,184 included individuals, 52%, 7%, 15%, 16% and 10% started treatment in steps 1 to 5, respectively. The median (IQR) follow-up time was 3 (1-7) years. Median (IQR) number of switches was 1 (0 – 3). Comparing starting step to last observed step, 37% never switched between treatment steps, 20% of individuals stepped down and 22% stepped up.

Conclusion
The low proportion of treatment switches between steps indicates that tailoring of treatment to patients' needs might be suboptimal. The tPDC-based algorithm functions well in translating dispensing data into continuous drug-utilization data, enabling more granular assessment of treatment patterns among asthma patients.

Comparative effectiveness of anti-hypertensive monotherapies in primary prevention of cardiovascular events - a longitudinal inception cohort study

Xuechun Li, Maarten J Bijlsma, Stijn de Vos, Jens H J Bos, Catharina C. M. Schuiling-Veninga, Eelko Hak

Biography

Xuechun Li is in her 2th year of the Ph.D. program in PharmacoTherapy, -Epidemiology and -Economics, Groningen Research Institute of Pharmacy, University of Groningen. Her research interests are in Pharmacoepidemiology. Her current research focuses on primary prevention of cardiovascular and cerebrovascular diseases in the real world.
Xuechun is funded by the China Scholarship Council (file no: 202106070028). She holds a Master of Medicine degree in Biomedical Engineering, University of Electronic Science and Technology of China and a Bachelor of Medicine in Public Health from Sun Yat-sen University.

Authors

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Introduction

Anti-hypertensive drugs can prevent cardiovascular events. However, real-world comparative drug effectiveness studies taking time-varying adherence and confounding into account are absent.

Objectives

We aim to determine the real-world relative effectiveness of anti-hypertensive monotherapies (ACEIs, ARBs, BBs, CCBs, thiazides) in primary prevention of cardiovascular events in longitudinal cohorts.

Methods

We conducted a retrospective inception cohort study covering a 10-year study period using the University of Groningen IADB.nl database with data from 1996 to 2020. Patients were aged ≥18 years free of any cardiovascular drugs prior to initiation of monotherapy. Cohort 1 included adherent patients with follow-up time greater than one year, and Cohort 2 included all patients independent of adherence. Outcome was the time to first prescription of acute cardiac-drug-therapy (CDT) measured by valid drug proxies to identify a first major cardiovascular event. A per-protocol analysis approach using inverse probability of treatment-weighted time-varying Cox regression analysis and beta-blockers (BBs) as reference category was applied to obtain hazard ratios and 95% confidence intervals.

Results

Among cohort 1 (n=15,823) starters, 1,500 (9.5%) patients experienced an acute CDT with an average follow-up time of 3.5±2.3 years. Thiazide starters had lower hazards compared with BB starters (HR: 0.79, 95% CI: 0.68 to 0.91, p=0.001). Among drug treated diabetes and RA patients, point estimates of HRs were even lower (0.42, 0.19 to 0.94 and 0.15, 0.02 to 0.90, respectively). Calcium Channel Blockers (CCBs) starters had higher hazards compared with BB starters (HR: 1.44, 95% CI: 1.18 to 1.74, p<0.001). Among cohort 2 (n=33,427), main results and sensitivity analysis results were similar to cohort 1.

Conclusion

Although indication bias cannot completely be ruled out, this real world analysis suggest that long-term thiazide monotherapy appeared to be more effective than BBs in the prevention of cardiovascular events, notably among high-risk patients. CCB monotherapy was inferior to BBs while ACEIs and ARBs had similar effectiveness as BBs.

Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies, using a weight-of-evidence approach

Hsiao-Tzu Chien, Dr. Helen Prior, Dr. Fiona Sewell, Dr. Katrin Schutte, Dr. Lucinda Weir, Dr. Peter van Meer

Biography
Hsiao-Tzu Chien is a PhD candidate/nonclinical assessor conducting research and nonclinical assessment at Radboud Universty Medical Center and Medicines Evaluation Board. The ambition of her research is to leverage existing regulatory procedures to achieve a meaningful reduction of animal studies that are not informative.

Introduction
To support registration of monoclonal antibodies (mAbs) for chronic indications, 6-month toxicity studies have historically been conducted as per ICH S6(R1) guidance. Experience with mAb development has shown a relatively benign and well-understood safety profile for this class, with most toxicity findings anticipated based on pharmacology.

Objectives
A consortium of 14 pharmaceutical companies, the Medicines Evaluation Board (MEB) and the NC3Rs conducted an European Partnership for Alternative Approaches to Animal Testing (EPAA)-funded study to evaluate whether a 6-month toxicity study is still necessary to assess the long-term safety of mAbs.

Methods
Companies submitted anonymized data by survey, including product information, species selection and pharmacological relevance, study details and information on findings in First-in-Human (FIH)-enabling (short-term) and chronic studies. The incidence of new toxicities identified in chronic studies, along with impact on mAb development or clinical trial design, was reviewed from data shared by industry participants.

Results
Data on FIH-enabling and chronic toxicity studies were shared for 142 mAbs submitted by 11 companies. Opportunities to further optimize study designs to reduce animal usage were identified. For 71% of mAbs, no toxicities or no new toxicities were noted in chronic studies compared to FIH-enabling study findings. New toxicities related to exaggerated pharmacology or ADA-mediated (not considered of human concern) were identified in 15.3% of cases. New toxicities of potential concern for human safety or that changed trial design were identified in 13.5% of cases, with 7% being considered critical and 2% leading to program termination. A longer dosing duration in the FIH-enabling study, e.g., 3 months vs. 1 month, resulted in fewer new toxicities in the chronic studies.

Conclusion
In retrospect, only a small proportion of chronic studies provided additional safety findings relevant for clinical dosing. An iterative, weight-of-evidence model which considers factors that influence the overall risk for a mAb to cause toxicity was developed, to drive selection of the optimal duration of toxicity study without defaulting to a study of 6 months duration. This model enables an evidence-based justification, suggesting when 3-month toxicity studies are likely sufficient to support late-stage clinical development and registration for some mAbs.

Evaluation of rat and rabbit embryofetal development studies: the added value of a second species

Puck Roos, Hsiao-Tzu Chien, Caroline Anggasta, dr. Peter J.K. van Meer, dr. Peter T. Theunissen

Biography

Puck Roos studied pharmaceutical sciences at Utrecht University. Since 2022 she works as a junior researcher at the Dutch Medicines Evaluation Boards. Her research is focused on the 3Rs (reduction, replacement and refinement of animal studies), more specifically in the field of developmental and reproductive toxicity testing and carcinogenicity testing.

Authors

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Introduction

To detect potential adverse effects of human pharmaceuticals on pregnant women and during embryofetal development, embryofetal development (EFD) toxicity studies are conducted. The ICH S5(R3) guideline states that these studies in general need to be performed in one rodent and one non-rodent species, often rat and rabbit. However, the added value of performing EFD toxicity studies in two species is questionable.

Objectives

EFD toxicity studies in rats and rabbits were evaluated, to analyze the added value of a second species.

Methods

Human pharmaceuticals submitted for marketing authorization application (MAA) to the European Medicines Agency (EMA) between 2004-2022 with EFD studies in both rats and rabbits, as well as known human teratogens with rat and rabbit data, were added to a database. Information on compound characteristics, mechanism of action, study design, EFD toxicity, maternal toxicity and animal-to-human exposure margins was collected from the internal database of CBG-MEB. EFD toxicity was categorized into malformations, embryofetal lethality, growth retardation and variations. Data was analyzed to investigate concordance and discordance between rat and rabbit, and to find possible explanations for the discordance.

Results

In total, 369 compounds were included in the database. For 55.3% of the compounds, similar effects were observed in rats and rabbit EFD toxicity studies. Discordance was observed for 44.7% of the compounds. In most cases discordance could be explained based on the occurrence of maternal toxicity (22.5%) or the compound's mechanism of action (5.7%). In other cases, discordance was considered of limited clinical relevance because of high animal-to-human exposure margins (6.0%) or manifestation of EFD toxicity that were considered of less clinical concern (3.0%). In only 5.7% of the compounds, discordance could not be explained and was considered clinically relevant. Additionally, concordance and discordance were analyzed for different ATC codes. Different patterns of EFD toxicity and maternal toxicity were observed for different ATC codes.

Conclusion

In conclusion, the added value of performing EFD studies in two studies is limited. To identify the need

for EFD studies in a second species and interpret the results, mechanism of action, maternal toxicity, exposure margins, manifestations of EFD toxicity and species-specific biological features could be taken into account. In the future, the current database could be helpful to identify scenarios in which (additional) EFD studies could be replaced by new approach methods or waived, or to create a weight-of-evidence approach to identify the need for EFD studies.

Community Use of Repurposed Drugs Before and During COVID-19 Pandemic in the Netherlands: An Interrupted Time-Series Analysis

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Biography

Guiling Zhou is a passionate Ph.D. student in her second year at the University of Groningen, specializing in drug repurposing strategies for preventing and treating respiratory diseases.
Guiling began her educational pursuit at Sun Yat-Sen University in Guangzhou, China, in 2015. Here, she completed her Bachelor's degree in Pharmaceutical Science in 2019. Her thirst for knowledge and commitment to public health drove her to further studies at the University of Groningen. She pursued a Master's degree in Medical Pharmaceutical Sciences, focusing on Pharmacoepidemiology. Her educational path demonstrates her deep-seated dedication to the field of public health and, specifically, pharmacoepidemiology.

Authors

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Background

Repurposing registered drugs could reduce coronavirus disease (COVID-19) burden before novel drugs are authorized. Little is known about how the pandemic and imposed restrictions changed their dispensing. We aimed to investigate the impact of COVID-19 pandemic on repurposed drugs dispensing in the Netherlands.

Methods

We performed interrupted time-series study using University of Groningen prescription database IADB.nl to evaluate dispensing trends of 24 repurposed drugs before (2017-February 2020) and after (March 2020-2021) the pandemic' start. Primary outcomes were monthly prevalence and incidence rates. An autoregressive integrated moving average model assessed the effect of pandemic and stringency index (measuring strictness of government's restriction policies).

Results

Annual number of IADB.nl population ranged from 919,697 to 952,400. Generally, dispensing of common long-term-used drugs was not significantly affected by pandemic. The prevalence of antibacterials (-4.20 users per 1000 people), antivirals (-0.04), corticosteroids (-1.29), prednisolone (-1.32), calcium channel blocker (-0.41), and diuretics (-1.29) was lower than expected after the pandemic's start, while the prevalence of ivermectin (0.07), sulfonylureas (0.15), sodium-glucose co-transporter-2 (SGLT2) inhibitor (0.17), and anticoagulants (1.95) was higher than expected. The pandemic was associated with statistically significant decreases in the incidence of antibacterials (-1.21), corticosteroids (-0.60), prednisolone (-0.64) and anticoagulants (-0.02), and increases in ivermectin (0.02), aggregated antidiabetic drugs (0.13), and SGLT2 inhibitors (0.06). These trends were positively associated with pandemic and negatively associated with stringency index.

Conclusion

Dispensing of most drugs was not significantly associated with pandemic and government's response. Despite some statistically significant disruptions, these were not necessarily clinically relevant due to small absolute differences observed.

Keywords

COVID-19; drug utilization; repurposed drug; stringency index

Real-world metabolism of mycophenolate mofetil in kidney and liver transplant recipients

MSc Fleur B. Nijdam, prof.dr. Stephan J.L. Bakker, prof.dr. Eelko Hak, dr. Frank Klont

Biography

I am a first year's PhD candidate in pharmacoepidemiology at the University of Groningen. I obtained my master's degree in Medical Pharmaceutical Sciences at the University of Groningen. My current research focuses on the metabolism of immunosuppressants in transplantation patients, where I'm incorporating untargeted metabolomics in pharmacoepidemiologic research. Feel free to contact me for collaborations or social networking.

Authors

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Introduction

Pharmacogenetics, the science that studies the influence of genetic variability on drug treatment response, is gaining momentum in clinical research. This field of research integrates genetic information of individual drug users with a consensus understanding of how a drug is metabolized. This consensus typically relies on studies conducted by pharmaceutical companies on rather small, homogenous and healthy populations. Recent findings in the field of pharmacometabolomics showed that drug metabolization patterns in real-world patients frequently disagree with such consensus. The application of pharmacometabolomics to real-world drug metabolism studies may therefore contribute to a better understanding of pharmacogenetics-based personalized medicine.

Objectives

We aimed to identify possible differences between the real-world and consensus metabolism of mycophenolate mofetil (MMF) among kidney and liver transplant recipients participating in the TransplantLines prospective cohort study [1].

Methods

Untargeted 'SWATH' metabolomics was applied to 24-hour urine samples of >1500 transplantation patients included in the TransplantLines Food and Nutrition Biobank and Cohort Study (NCT02811835) and the TransplantLines Biobank and Cohort Study (NCT03272841) [2,3]. Metabolomics data were used to identify MMF metabolites and their abundances were assessed in the context of various clinical patient data, including sex, kidney function, and self-reported drug use.

Results

The metabolomics data unveiled unanticipated discrepancies in MMF metabolite patterns, particularly the detection of unexpected MMF metabolites according to the concensus. These data also show large variations in metabolite patterns between study participants, implying underlying individual (patho-) physiologic differences and/or potential drug-drug interactions. Research on metabolite pattern variations is still ongoing but already showed pronounced differences between study participants.

Conclusion

Our real-world clinical metabolomics data showed that detected metabolite patterns of MMF are

in disagreement with the current consensus, thereby suggesting that pharmacogenetics-based personalized medicine has possibly not yet reached its full potential. Furthermore, our findings may contribute to individualized dosing. Finally, our results demonstrate that metabolite patterns vary markedly between participants and further research is required to explain such variations.

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External Cross Validation of two Ciprofloxacin Population Pharmacokinetic Models in Intensive Care Patients

Mrs. Irma Rigter, Eleonora Swart, Roger Brüggeman, Tingjie Guo, Paul Elbers, Reinier van Hest

Biography

Irma Rigter is a Hospital Pharmacist at the Amsterdam University Medical Centers. With a broad spectrum of interests and eighteen years of clinical experience from tropical medicine to medicine shortages. She is now shifting focus from intensive care for children to acute and Intensive care clinical pharmacy for adults with the intension of improving care for this critical group of patients.

Introduction

Ciprofloxacin is commonly used in the intensive care unit (ICU) as treatment for serious infections. Due to heterogeneity and changes in pathophysiology in ICU patients, pharmacokinetics (PK), and thus exposure, are highly variable within this patient population.
Population PK (popPK) models can be a tool for individual dose optimization, but external validation of a tailored popPK model is warranted prior to use. Previously developed models are not always valid for use in other populations1. Several ciprofloxacin models have already been developed and indicated high unexplained PK variability2. although these models were internally evaluated, predictive performance in other ICU populations is unknown.

Objectives

The aim of the study was to cross validate two ciprofloxacin popPK models, one newly developed and one already published. The cross-validation was performed using the same data sets that were utilized to develop both models to elucidate whether both models can adequately predict each other's observed data.

Methods

Data were collected from ICU patients of the Amsterdam University Medical Centre, location VUmc (AUMC) and a popPK model for ciprofloxacin was newly developed using Non-Linear Mixed-effects modelling (NONMEM). Data and a published popPK model therewith from the ICU of the Radboud University Medical Centre (RUMC) were used for cross-validation3. The data in the RUMC dataset were used to externally validate the AUMC model and vice versa. The predictive performance of the models was evaluated by comparing the population predicted concentration with the observed concentrations in the dataset. Primary endpoints were bias and accuracy, calculated as the mean error (ME) and mean absolute error (MAE) respectively. Criteria for acceptable predictive performance were: 0 to be included in the 95% confidence interval (CI) of ME and a maximum MAE of 2 mg/L. Visual Predictive Checks (VPC) and Bland Altman plots were created to visualize the predictive performance.

Results

The AUMC dataset consisted of 159 samples from 32 ICU patients and the RUMC dataset of 531 concentration-time-data from 39 ICU patients. A two-compartment linear model with MDRD as a covariate on clearance provided the best fit in both study populations. Tabel 1 shows the patient baseline characteristics. The final AUMC model predicted the RUMC concentration-time-data with a non-significant ME of 0.0577 mg/L (95% CI -0.215 - 0.137) and a MAE of 0.610 mg/L (95% CI 0.551 – 0.670). The final RUMC model predicted the AUMC concentration-time-data with a non-significant ME of -0.0613 mg/L (95% CI -0.303 - 0.180) and a MAE 1.01 mg/L(95% CI 0.829 - 1.19).
The VPC's indicated acceptable predictive performance as the observed data fell within their corresponding 95% prediction intervals although the AUMC model overestimated variability as a wider range of concentrations was predicted than the RUMC data contained (fig.1). The Bland Altman plots indicated that both models were able to estimate the ciprofloxacin concentrations without bias and with sufficient precision, although the RUMC model underestimated some of the higher AUMC concentrations (Fig.2).

Conclusion

Both ciprofloxacin popPK models are unbiased and precise according to the primary endpoints although the AUMC model is predicting more variability.

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Table 1. Patient characteristics at baseline

	AUMC	RUMC
Total number of patients	32	39
Observations	159	531
Sex, male/%	72	72
Age, Yr	69 (57-77)	68 (61-74.5)
Weight, kg	83.4 (68.8-95.6)	80 (66-98.5)
Serum creatinine µmol/L	116 (74-206)	83(66-146.5)
Albumine (g/L)	19 (17-22)	23(18.5-26)

Data are expressed in median (interquartile range), unless stated otherwise

Fig.1a VPC AUMC model with RUMC data

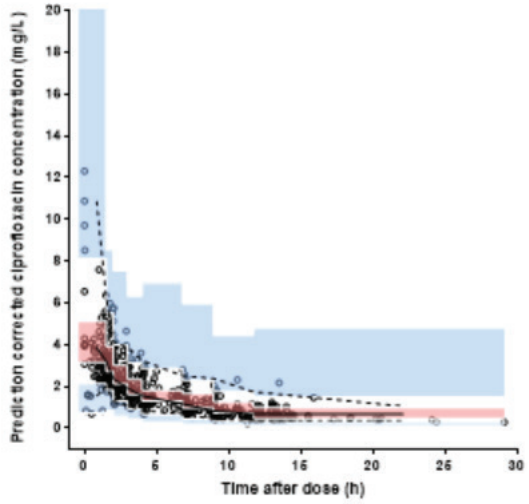
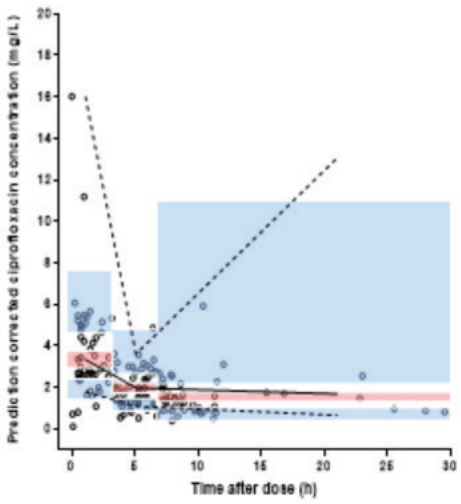
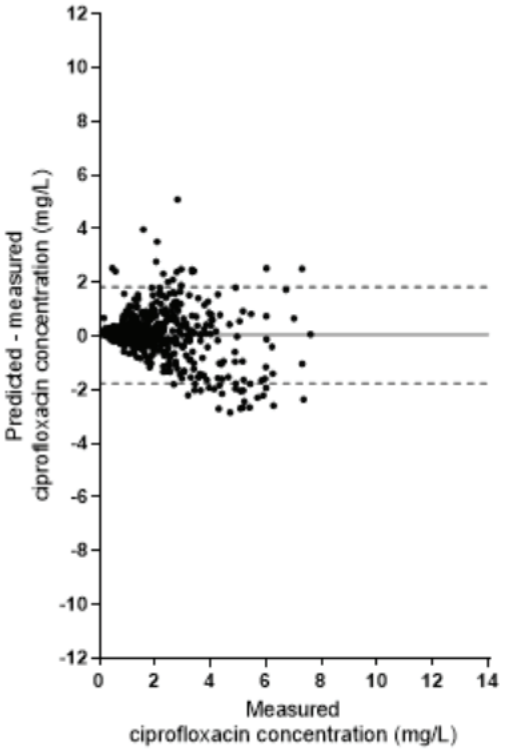


Fig.1b VPC RUMC model with AUMC data





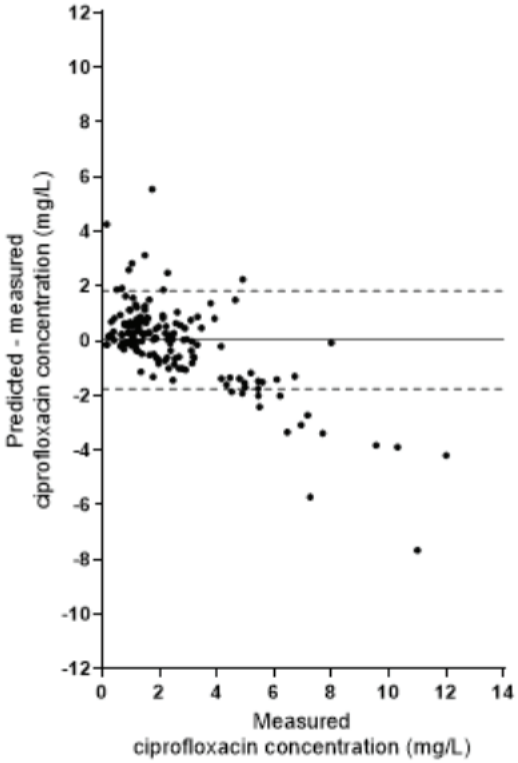


Fig.2a Bland Altman plot AUMC model with RUMC data

Fig.2b Bland Altman plot RUMC model with AUMC data

Authors

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Methods in Dynamic Treatment Regimens using Observational Data: A Systematic Review

David Liang, Animesh Kumar Paul, Daniala Weir, Vera Deneer, Russell Greiner, Arno Siebes, Helga Gardarsdottir

Biography

David Liang is a second year Pharmacoepidemiology PhD student in the Division of Pharmacoepidemiology and Clinical Pharmacology at Utrecht Institute for Pharmaceutical Sciences (Utrecht University) in the Netherlands. He has a Bachelor in Pharmacy and a Masters in Pharmaceutical Science from the University of Copenhagen. Before the start of his PhD project, David gained experience from working with Pharmacovigilance in the pharmaceutical industry.

Introduction

In the management of chronic conditions (e.g., Diabetes, HIV, and Parkinson's disease), clinicians must treat patients at multiple time points for achieving the best long-term outcomes, involving dynamic treatment regimens (DTRs). Different types of methods (e.g., mathematical, statistical, machine learning) have been adapted to build evidence-based tools for helping clinicians personalize the medicine to the specific patient population.

Objectives

The aim of this study is to provide insight into 1) what methods have been applied for calculating DTRs utilizing observational data and, 2) which methods can be to answer different types of questions (e.g, types of medical conditions, short or long-term outcomes, and characteristics of the dataset) for calculating DTRs utilizing observational data.

Methods

A systematic review was conducted including studies using observational data to calculate DTRs published in PubMed or EMBASE between January 1950 and until January 2022. Only peer-reviewed English language full research articles were included. Two independent reviewers (DL, AKP) screened the titles and abstracts using the tool, ASReview, and the relevant articles were full-text screened for inclusion. Additionally, reference lists of the included articles were screened for relevant articles. Information from the articles was extracted in duplicate using a predefined data extraction form. Extracted data included article characteristics as well as the name of the method, therapeutic area, and validation method.

Results

In total, 54,341 articles were identified. Of those, 36 were eligible for inclusion after the full-text review. Most articles (72.2%; 26/36) were published after 2018 and involved the therapeutic areas metabolism and endocrinology (30.5%; 11/36; of which ten were on diabetes), and infectious diseases (19.4%; 7/36; of which six were on HIV/AIDS). We found that reinforcement learning (RL) methods (27.8%; 10/36) and g-methods (16.7%; 6/36) were the most common for computing DTRs. Specifically, Q-learning and dynamic programming were the common RL methods, while marginal structural models and g-formula were the common g methods. 72.2% (26/36) of the articles validated their methods, where 61.5% (16/26) were validated based on observational data, and 30.8% (8/26) consulted with clinicians to evaluate the results of their method. In the former validation category, 56.3% (9/16) estimated the error of the model using a variety of measures (e.g., accuracy, root mean square error, square error, absolute error, precision, and area under the curve), and 43.7% (7/16) estimated the patients' expected outcomes.

Conclusions

The use of observational data for calculating DTRs has increased in the past years; RL and g methods are the most commonly used. We found that more than two-thirds of articles validated their methods using three types of evaluation methods. Further research is required to assess which of these algorithms and validation methods are most appropriate in specific research settings.

Reduced quality of life, persistent symptoms and dissatisfaction in LT4-treated hypothyroid patients: A medical need for improved treatment

Dr Ellen Molewijk

Biography
Previously, researcher in pharmaceutical industry and medical/pharmaceutical writer/communications. Currently, teacher/lecturer and researcher at Farmakunde and Research Group Innovations of Pharmaceutical Care, University of Applied Sciences Utrecht

Authors
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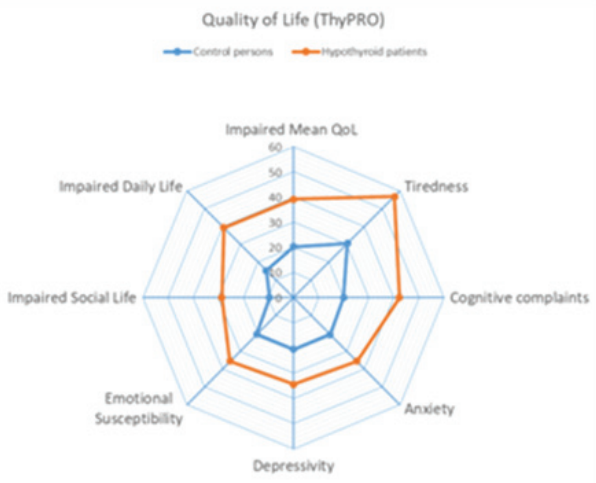
Introduction
Generally, it is believed that standard levothyroxine (LT4) therapy is sufficient to restore euthyroidism and relieve hypothyroid symptoms in hypothyroid patients. However, a considerable proportion of treated patients remains symptomatic despite normal TSH/FT4 serum values.

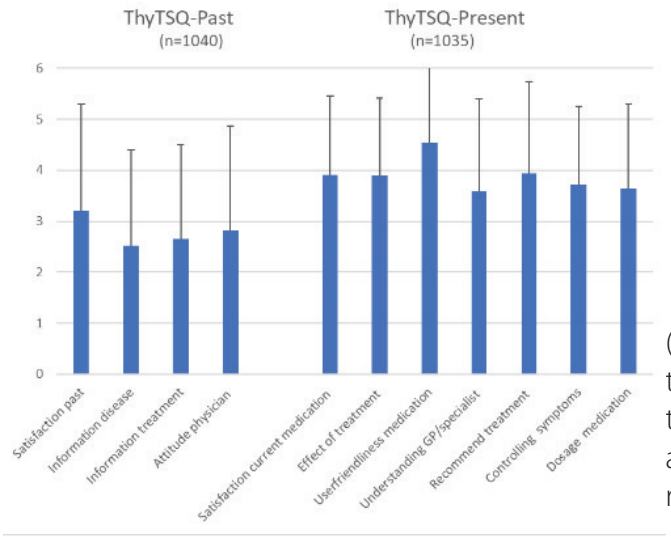
Objectives
We examined the quality of life (QoL), daily functioning, symptoms and satisfaction with treatment and care of hypothyroid patients on thyroid replacement therapy in The Netherlands.

Methods
A digital survey comprised several PROMs, the ThyPRO (QoL), SF-36 items and statements (daily functioning), ThySHI (hypothyroid-related symptoms, past, present) and ThyTSQ and additional questions (satisfaction with treatment and care) in hypothyroid patients and in control persons without thyroid diseases (QoL, daily functioning and present symptoms only). Patients and control persons were recruited through patient organizations, posters/flyers and social media.

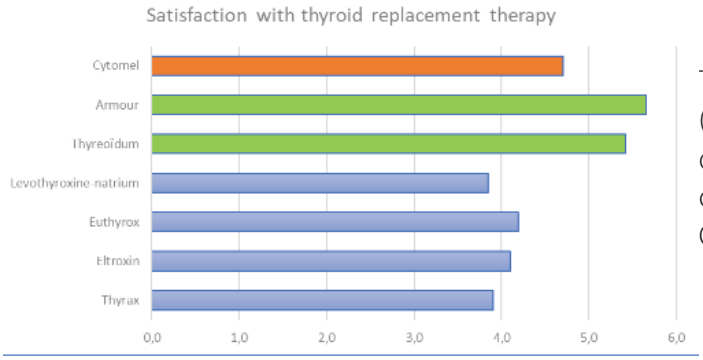
Results
The QoL of hypothyroid patients (n=1195) was significantly (mean +93%) more disrupted in all domains, as compared to controls (n=240) (p<0.001).



TSH, FT4, age, gender and duration of illness did not significantly affect QoL, whereas the M3 comorbidity index (weighted sum of reported comorbidities) did to a minor extent. Hypothyroid patients had significantly more impairment of daily functioning and reported significantly (mean 2.8 times) higher scores for symptoms related to hypothyroidism, as compared to control persons (all p<0.001). The majority of patients (78%) reported not feeling well while their thyroid blood values were within the reference range. Three-quarters would like to have a better treatment for hypothyroidism (75%, n=1194). The mean overall satisfaction score was 3.5 out of 6



(58%). The lowest satisfaction was expressed for the information given about the illness and its treatment, as well as the attitude of the physician, around the time of diagnosis (means 2.5, 2.7, 2.8 resp.).



The satisfaction with the various LT4 treatments (blue) was relatively low (4 out of 6), with desiccated thyroid extracts (DTE, green) high (5.5 out of 6), and moderate with the T3 preparation Cytomel (4.7 out of 6).

Conclusions

In this comprehensive study, treated hypothyroid patients had a significantly higher symptom-load and lower QoL/daily functioning as compared to control persons, despite thyroid replacement therapy and serum TSH/FT4 within the target range values. Furthermore, hypothyroid patients expressed dissatisfaction with care and with thyroid replacement therapy, especially LT4 preparations. As such, we see a clear medical need for improved treatment modalities for a large population of patients with hypothyroidism.

Pedmed-NL - support for paediatric clinical trials

Dr Tessa van der Geest, Fenna Mahler, Prof dr Saskia de Wildt

Biography

Tessa van der Geest is a biomedical scientist with a PhD in nuclear medicine. After her PhD, she started as a project manager on European projects (PedCRIN, EPTRI and conect4children) to build an European infrastructure to facilitate paediatric clinical trials. The overarching goal is to improve medicines for children, since children have the right to have access to safe medication.

Authors

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Objectives

High quality paediatric clinical trials are of utmost importance in the quest to improve medicines for children. Since high quality infrastructure was missing in the Netherlands, we aimed to develop a paediatric clinical trial network: Pedmed-NL. The Pedmed-NL consortium facilitates paediatric clinical trials with the ultimate goal to realise better medicines for children.

Method

First, the academic hospitals were invited to join the Pedmed-NL consortium, followed by general hospitals and patient organizations. Secondly, Pedmed-NL became the Dutch national hub within the pan-European collaborative network conect4children (c4c). Support for both investigator as industry initiated clinical trials regarding feasibilities, site identification, study budgets, IMP, contracting, application to the ethical committee and competent authority, as well as trial coordination has been set up. Pedmed-NL is continuously evolving via regular meetings with consortium members, the set up of working groups and the implementation of needs from the field.

Result

Since 2018, a consortium of 18 partners was formed, including all university medical centres, general hospitals and a patient organization. Pedmed-NL services were opened for pharmaceutical sponsors, academic sponsors and CROs. Pedmed-NL has supported over 130 requests for trial feasibilities, 4 grant applications and 2 applications to the ethical committee and national authority. Additionally, Pedmed-NL has developed a budget tool to support realistic budget estimates for paediatric clinical trial. As c4c national hub, Pedmed-NL supports the proof-of-viability trials to test the c4c infrastructure.

Conclusion

Pedmed-NL has developed into the Dutch network for paediatric clinical trials. By offering support to both industry and academia and acting as match maker between sponsors and sites, Pedmed-NL has contributed to the facilitation of paediatric clinical trials. Pedmed-NL empowers collaborative efforts, promotes harmonization between centres and acts as a direct contact point for researchers and industry with the goal to improve medicines for children.

conect4children: service development and delivery by the pan-European paediatric clinical research network

Drs. Fenna Mahler, Prof. dr. Mark Turner, Heidrun Hildebrand, MD. Katharine Cheng, Sabah Attar, Phd, Pharm D Francesca Rocchi, Rebecca Leary, MD. Phd Ricardo Fernandez, Prof. dr. Saskia de Wildt, Prof. dr. Gilles Vassal, Begonya Nafria, Laura Mangiari, Carlo Giaquinto

Biography

Fenna Mahler is trained as medical scientist at Radboudumc, Nijmegen. She has worked in varoius positions within pharmaceutical companies, CROs and academia in the field of clinical research. She is currently project lead/ head of advice service manager of the a paediatric IMI project conect4children.

Authors

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9. Novartis, Newark NJ, United States of America
10. FSJD, Barcelona, Spain,
11. PENTA, Padova, Italy

Objectives

Paediatric clinical research has specific features that can make research difficult. The conect4children partnership aimed to address some of the difficulties in paediatric clinical research by co-creating several services for academic and industry research.

Methods

A public private partnership was funded by the Innovative Health Initiative involving 10 large pharmaceutical companies and 33 academic organizations. The partnership identified key services, co-developed and tested processes, implemented a suite of standard operating procedures, including quality control, and built business models for each service.

Results

An Expert Advice service was built around over 400 selected experts working in 24 clinical and methodology groups, embedding patient and public involvement. The service has managed 50 requests for advice (12 involving children and young people, 10 academic requests) and 3 multi-stakeholder meetings. Services for site finding and feasibility were built around 20 National Hubs (most of which were developed by the project) involving 250 sites. The services were tested using 3 academic studies and 8 industry studies. Education and Training was built around a Moodle-based platform. A suite of 7 core courses (related to GCP) was supplemented by 25 short courses and an Advanced Course in Paediatric Drug Development. 2243 people have used the Education and Training portal. A paediatric data dictionary was developed and used to create the Paediatric User Guide (PUG) by CDISC, the leading data standards organization for clinical research in drug development. The PUG includes 91 paediatric terms. A non-profit foundation, conect4children Stichting has been incorporated to provide these services to academia and industry in a sustainable manner.

Conclusion

A public private partnership provided a platform for the development and testing of services to facilitate patient-centric paediatric clinical research. These services will be available to academic and industry drug developers.

This project has received funding from the Innovative Medicine initiative 2 joint undertaking under grant agreement number 777389. The Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA

Disease-drug-drug interaction of intravenous imatinib in repurposing for COVID-19 ARDS

Medhat Said

Biography

Medhat Said is a PhD candidate Pharmacometrics at Amsterdam UMC, location VUmc and the Cancer Center Amsterdam in the Netherlands. He obtained his research master in Bio-Pharmaceutical Sciences at Leiden University and now focuses on using quantitative pharmacology to improve treatment outcomes.

Authors

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Category

Clinical Trials

Introduction

Infections may affect pharmacokinetics of a drug by changing its metabolism, binding to plasma proteins and tissue distribution [1]. In the CounterCOVID study, COVID-19-infected patients hospitalized on a general ward, received imatinib orally and exhibited an increased total imatinib exposure due to upregulated alpha-1-acid glycoprotein (AAG) levels in comparison with CML/GIST patients [2]. In the InventCOVID study, ventilated critically ill COVID-19-infected patients received intravenous administration of imatinib. In the latter study patients also received interleukin-6-receptor (IL-6R) inhibitors upon ICU admittance [3]. IL-6R inhibitors have been reported to reverse IL-6 mediated CYP enzyme suppression and reduce signaling to acute phase proteins [4, 5]. In this population pharmacokinetic (PK) study, we aimed to assess the influence of critical illness and concomitant treatment with IL-6R inhibitors on the PK of imatinib in COVID-19-infected patients.

Objectives

To evaluate the predictive performance of a previously developed AAG-PK model of imatinib on data from ventilated critically ill COVID-19-infected patients participating in the InventCOVID study.
To identify additional covariates on imatinib PK if model evaluation suggested that the prediction of the InventCOVID data was insufficient.

Methods

The published AAG-PK model was based on data from hospitalized patients from the CounterCOVID study and CML/GIST outpatients receiving 100-800 mg oral imatinib once daily. The model described the PK of total imatinib, unbound imatinib and total desmethyl-imatinib plasma concentration and its dependency on AAG levels. The performance of the model was evaluated by performing prediction-corrected visual predictive checks (pcVPC) and by calculation of prediction errors (PE). Further model development was performed if the mean prediction error was > 30%. Covariates examined included body weight, age, gender, albumin, alanine transaminase, aspartate aminotransferase, estimated

glomerular filtration rate (eGFR), IL-6, concomitant treatment and disease severity (WHO ordinal Scale for Clinical Improvement) [6].

Results

InventCOVID patients had higher baseline WHO score than CounterCOVID patients (7 vs. 4, P=<0.0001) and 100% vs 19.8% received mechanical ventilation. InventCOVID patients had significantly higher IL-6 (P=<0.001) and lower AAG levels (P=0.007) than CounterCOVID patients. In InventCOVID 90.6% of the patients received an IL-6R inhibitor. Median free fraction of imatinib at steady state in InventCOVID was significantly increased (5.7%) compared to CounterCOVID (2.7%, P=<0.0001) and previous CML/GIST patients (4.0%, P=<0.0001). The AAG-PK model overpredicted total steady state imatinib concentrations (Css) in InventCOVID with a PE of 84% ± 49% (mean ± SD) and underpredicted unbound imatinib concentrations with a PE of -11% ± 32%. In the newly developed population PK model the dissociation constant of AAG and imatinib (KD) was significantly higher in InventCOVID patients compared to CounterCOVID/CML/GIST patients with respective values of 702 ng/ml and 336 ng/mL. Age, body weight, disease state and the presence of an IL-6R inhibitor were found predictive of imatinib clearance and exposure.

Conclusions

Our study suggests that ventilated critically ill COVID-patients may require other imatinib dosing regimens due to pathophysiological changes and possible disease-drug-drug interactions. Binding of imatinib to AAG may be different in critically-ill patients. Our study emphasizes the need to determine both total and unbound drug levels early in ICU patients when evaluating the dose response association of highly protein bound drugs, as unbound drug exposure may drive the effect.

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Personalised Therapeutics @ Leiden University Medical Center to personalise treatment

Sylvia Klomp, Romy Mosch, Wessel van der Zande, Nina Lute, Dr. Kim Gombert-Handoko, Prof. Dr. Teun van Gelder, Prof. Dr. Henk-Jan Guchelaar, Prof. Dr. Jesse Swen

Biography

Sylvia Klomp, MSc, is currently a PhD student in the department of Clinical Pharmacy & Toxicology at Leiden Medical Center. She earned her MSc in Biopharmaceutical Sciences at the University of Leiden. Her research is in the field of pharmacogenetics and focusses on phenoconversion. Where she tries to unravel the mechanisms of non-genetic factors contributing to the variability in drug response and the impact on patient care.

Authors

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Theme

Personalized Medicine

Introduction

Pharmacogenomics (PGx) is the study of genetic variability affecting an individual's response to a drug. PGx is a critical component of personalised medicine. Currently, PGx is applied for individual drugs and/or individual genetic variants. With a panel of 12 genes, for which the Dutch Pharmacogenetic Working Group (DPWG) has issued evidence based drug dosing guidelines, a 30 % reduction in the risk for severe adverse drug reactions (ADRs) was demonstrated in the PREPARE study [1]. Based upon national prescription data we estimate that 5.6% of all first prescriptions would require an individualisation of the dose or drug [2]. However, in current clinical practice the potential of PGx testing is not fully exploited. Therefore, a prospective implementation study on pre-emptive PGx testing is being performed for patients in the LUMC. In this study, 1,000 patients with a planned surgery will be randomised to PGx-guided dosing or standard of care.
Objectives: To implement pre-emptive panel based PGx testing in LUMC and to determine patient benefit of PGx guided drug prescription and dispensing.

Methods

A prospective, open, randomised implementation study in 1,000 patients with a duration of 2 years. Patients with a planned surgery recruited after a medication reconciliation interview are 1:1 randomised to PGx-guided dosing or standard of care. The PGx-guided group receives pre-emptive PGx testing for a panel of 14 genes (including 227 PGx variants, genotyped with the Global Diversity Array with enhanced PGx-8 V1.0) followed by personalised drug and dose recommendations for newly prescribed drugs. Recommendations are based on the guidelines of the DPWG. Patients in the control group will receive usual drug prescriptions, without PGx-guided drug or dose selection. Ethical approval of the study protocol was obtained in April 2022 and recruitment was started in December 2022. In June 2023, 190 participants have been recruited.

Outcome

The primary outcome is the incidence of ADRs in the first 12 months after inclusion in the study. Side effects collected via the for the study developed pharmacovigilance app, namely the Pharma.Sensor app. Following the collected side effects will be scored as ADR, with regard to their relationship to the drug and severity (≥ grade 3 CTC-AE).

Conclusion

The Personalised Therapeutics @LUMC study was started to implement pre-emptive panel based PGx testing for patients in the LUMC and to determine patient benefit of PGx guided drug prescription and dispensing.

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Optimal dosing of oral anticancer drugs in older adults with cancer: a randomized pilot study

Msc Esther Broekman, Prof. dr. An Reyners, Dr. Pauline de Graeff, Prof. dr. Daan Touw, Dr. Thijs Oude Munnink

Biography

Esther Broekman works as a clinical assessor of oncology products at the Medicines Evaluation Board, and as a medical oncologist at the UMCG in Groningen. She is specialised in the treatment of thyroid cancer, and as such a member of the multidisciplinary team at the UMCG, national consortia and the national guideline committee on treatment of thyroid cancer. Currently, she is also training as a clinical pharmacologist, with an interest in optimal use of oncolytics after registration.

Introduction

The prevalence of cancer in the geriatric population is increasing. Efficacy and safety of newly registered therapies in the elderly population are mainly extrapolated from a younger pivotal trial population, because elderly patients are underrepresented in clinical trials. This is also the case for dosing recommendations, with starting doses near or equivalent to the maximum tolerated dose. With elderly patients being more prone to adverse events, it is important to address this evidence gap. It is our hypothesis that a lower starting dose can lead to better treatment tolerability in older patients, with preserved efficacy.

Objectives

The main objective of this pilot study is to investigate if it is feasible to study whether a lower starting dose with step-up approach leads to a better overall treatment utility compared to standard dosing of oral anticancer drugs in older adults with cancer.

Methods

Thirty patients >65 years of age with an indication for starting treatment with pazopanib, olaparib, lenvatinib, sunitinib or palbociclib and without contra-indications for starting at the recommended starting dose will be included. They will be randomized 1:1, stratified by type of anti-cancer treatment to one of two treatment groups. The control group will be treated with standard SmPC dosing. The intervention group will be treated with a lower starting dose with dose-escalation inversely following the dosing steps from the SmPC every 2 weeks in case of good tolerability.

The primary endpoint is the feasibility of the trial protocol, defined by:
» The percentage of patients that are willing to participate, from all eligible patients.
» The percentage of patients that successfully complete the first 12 weeks of the trial.
» The percentage of data points that are successfully collected during the first 12 weeks of the trial.

The key secondary endpoint is the overall treatment utility after 12 weeks of treatment.
Other secondary endpoints are progression-free survival, overall survival, quality of life, adverse events, hospital care use, pharmacokinetic parameters and switch to other medical treatment. Exploratory endpoints are pharmacogenomic parameters.

Study visits are planned every 2 weeks for a total study duration of 12 weeks, the time point for analysis of the primary endpoint. Blood samples for PK analysis are collected every 2 weeks. A baseline blood sample will be collected for pharmacogenomic analysis.

Results

The results of this pilot study will be used to inform the design (primary endpoint, frequency of assessments, sample size calculation, single center or multicenter design) of a larger phase 2 study.

Conclusion

A randomized phase 2 study is planned to investigate whether a lower starting dose with step-up approach leads to a better overall treatment utility compared to standard dosing of oral anticancer drugs in older adults with cancer. To inform the design of this study and optimalize its feasibility, first a pilot study will be performed in 30 patients.

Co-trimoxazole induced hyperkalemia in hospitalized patients using potassium sparing drugs: an observational study

Madelon Butterhoff, Dr. Jeroen Derijks, Dr. Walter Hermens, Dr Paul van der Linden

Biography
Hospital Pharmacist in Tergooi MC

Objective
To investigate the risk of hyperkalemia in hospitalized patients using Co-trimoxazole and a potassium sparing diuretic and/or RAS-inhibitor.

Methods
We conducted a nested case control study within a cohort of hospitalized patients using a potassium sparing diuretic and/or a RAS-inhibitor from the PHARMO Database Network.
We estimated the odds ratio (ORs) and 95% confidence intervals (CI) for the risk of hyperkalemia in patients receiving Co-trimoxazole and a potassium sparing drug (RAS-inhibitor and potassium sparing diuretic) compared to patients only receiving a potassium sparing drug.

Results
Among a cohort of 25.849 patients, we identified 2054 cases of hyperkalemia during hospitalization in patients also using a potassium sparing drug. Using Co-trimoxazole in addition to a potassium sparing drug was associated with an increased risk for hyperkalemia in hospitalized patients (ORadj: 1.65 (95% CI: 1.26 – 2.16) compared to using only a potassium sparing drug. There was a trend of a more pronounced association between hyperkalemia and the co-use of Co-trimaxozole and potassium sparing drugs in patients with an estimated GFR of 15-29 ml/min (ORadj: 3.15 (95% CI: 1.29 – 7.70)).

Conclusion
Using the combination of Co-trimoxazole with a potassium-sparing drug (either a potassium sparing diuretic and/or RAS-inhibitor) in hospitalized patients, increases the risk of hyperkalemia compared to using only a potassium-sparing drug. Physicians should be aware of hyperkalemia and routinely monitor serum potassium levels in hospitalized patients using this combination of drugs.

Health professionals' views on discontinuing antidepressant use: an update of a systematic review of qualitative studies

Dr Ellen Van Leeuwen

Biography
» GP and certified clinical pharmacologist (NVK&FB)
» Post-doc researcher at Ghent University and coordinator of the pharmacotherapy education in the medical curriculum.
» Research into polypharmacy and deprescribing and appropriate use of psychotropics.

Authors
Elle Van Leeuwen, Emma Maund, Catherine Woods, Hannah Bowers, Tony Kendrick, Thierry Christiaens

Background
Long-term antidepressant use, much longer than recommended by guidelines, may cause harms and generate unnecessary costs.

Objective
To explore health professionals' (HPs) views and experiences of antidepressant treatment with a focus on barriers and facilitators to discontinuing.

Design
A systematic review with thematic synthesis. Nine electronic databases were searched until May 2022: MEDLINE, PubMed, Embase, PsycINFO, CINAHL, AMED, Health Management Information Consortium, OpenGrey, and the Networked Digital Library of Theses and Dissertation. Qualitative studies of any HPs' attitudes, beliefs, and perceptions on continuing or discontinuing AD use were included. Updated searches were carried out in July 2023. Study quality was assessed using the Critical Appraisal Skills Programme checklist and COREQ criteria. The review is an update of a qualitative evidence synthesis of HPs' perspectives on discontinuing long-term antidepressants published in 2019 that could not be performed due to insufficient data.

Results
Thirteen studies were included in the review. Nine studies were of general practitioners' perspectives, one study of psychiatrists, and three of a mix of HPs. Barriers and facilitators to discontinuing long-term antidepressants emerged within eight themes, ordered chronologically according to the considerations that HPs make at each step when they review an antidepressant: perception of AD use, fears, HPs' role and responsibility, HPs' perception of antidepressant discontinuation, HPs' confidence regarding their ability to stop an antidepressant, perceived patient readiness to stop, support from trusted people from patients' network and other HPs.

Conclusion
Deprescribing long-term antidepressants is a challenging concept for HPs. The review found evidence that the barriers far outweigh the facilitators, and a main HP barrier was fear of relapse. HP education, reassurance and confidence-building is key to increase the initiation of the discontinuation process. Further research should explore perspectives of other HPs than the GP, as well as the role of the patients' network.

Infliximab in Inflammatory Bowel Disease for Children: A Comprehensive Literature Review and Evaluation of Literature Models

Mrs. Omnia Heikal, dr. P.F. Patrick Van Rheenen, Dr. Daan Touw, Dr. Paola Mian

Biography

Omnia Heikal is a highly motivated individual with a strong background in pharmacy, toxicology, and pharmacokinetics. With a track record of academic excellence, Omnia has demonstrated a commitment to learning and professional development. She graduated with high honors from the Bachelor of Pharmacy program at the German University in Cairo. During this time, Omnia gained a solid foundation in pharmacy and biotechnology related sciences. Upon graduation she pursued a diploma in Clinical Pharmacy alongside working as a Clinical Biologist, further enhancing her knowledge in disease and medicine. She is currently pursuing a Master's degree in Medical Pharmaceutical Sciences at the Rijksuniversiteit Groningen and is also currently an intern at the University Medical Center Groningen (UMCG) clinical pharmacy department.

Introduction

Infliximab (IFX), has transformed autoimmune disorder treatment, especially Inflammatory bowel disease (IBD). However, variability in response among children led to the increasing need for therapeutic drug monitoring (TDM). Population pharmacokinetic modeling is a promising approach for therapy optimization in children but with the increasing number of models in the literature, model validation is essential.

Objectives

This study aims to conduct a literature review describing infliximab's pharmacokinetics in children and externally validate published population pharmacokinetic (PopPK) models in this population.

Methods

A total of 36 relevant studies were included in the literature analysis. Data was retrospectively collected from patients, including patient characteristics, dosing information, clinical scores, and lab values. Population pharmacokinetic models were rebuilt using mrgsolve and other R studios packages. Model validation was performed through graphical analysis such as observed versus predicted plots, residuals against time, eta distributions, and correction predicted visual predictive checks.

Results

73 patients were included in the dataset and seven models were selected for evaluation. Graphical analyses and comparisons were conducted to evaluate model performance. Goodness-of-fit plots indicated a general tendency for overprediction, especially at early time points. Prediction-corrected visual predictive checks further highlighted differences between observed and simulated data, suggesting potential model misspecifications and confirming the pattern of overprediction.

Conclusion

This study validated existing literature PopPK models of infliximab in children. Results revealed that the evaluated models did not fit the data perfectly, emphasizing the need for further research and more robust models that consider the different IBD subtypes and patient characteristics.

Distinct COVID-19 vaccine combinations result in divergent immune responses

Wendy Tan

Biography

Wendy Tan was a hospital pharmacist in the UK and she is currently a PhD candidate at the hospital pharmacy department at Erasmus MC. Wendy is investigating the immunogenicity of COVID vaccines in individuals with different vaccination history and how this knowledge can be used to personalise future vaccination campaigns.

Authors

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Introduction

Waning immune responses and continuous evolution of SARS-CoV-2 contribute to reduced vaccine-induced protection over time. Bivalent vaccines incorporating the Omicron spike (S) protein were introduced to preserve protection against severe COVID-19 on a population level. The SWITCH-ON trial described here aims to assess immunogenicity of Omicron BA.1 and BA.5 bivalent booster vaccinations in individuals primed with either adenovirus-based (Ad26.COV2.S) or mRNA-based vaccine (BNT162b2 or mRNA-1273).

Objectives

To evaluate these bivalent vaccines against different priming regimes, three questions are addressed: (1) How immunogenic are Omicron BA.1 or BA.5 bivalent boosters?, (2) Do bivalent boosters differ in breadth of neutralizing antibody responses, particularly against current circulating variants?, (3) How do the original priming vaccination responses evolve over time and what can we learn for the future?

Methods

A total of 434 hospital workers from four university hospitals in the Netherlands were included and randomised to either a direct boost (DB) or postponed boost group (PPB) to receive an Omicron BA.1 or BA.5 bivalent booster in October or December 2022, respectively. Blood samples were taken on the day of the booster vaccination (pre-boost), 7 days, 28 days and 3 months after booster. S1-specific antibodies, T-cell responses, and SARS-CoV-2-neutralising antibodies were measured. In each group, participants were further divided into four arms combining two priming regimes (Ad26.COV2.S or mRNA-based) with two bivalent boosters (DB: BNT162b2 Omicron BA.1 or mRNA-1273.214; PPB: BNT162b2 Omicron BA.5 or mRNA-1273.222). The data are presented in an observational manner to describe the immunological response following bivalent vaccines. Statistical tests to examine the difference between and within groups were not performed due to protocol deviation.

Results

Regardless of booster timing and the BA.1 or BA.5 antigen, the increases observed for S1-specific antibodies, T-cell responses and neutralising antibodies reached similar levels within the first 28 days for both groups. The largest increase was observed within the first 7 days after the boost. However, at three months, all three parameters against ancestral SARS-CoV-2 wane, especially T-cell responses which almost returned back to baseline. We observed higher levels of S1-specific antibodies detected in mRNA-primed individuals compared to Ad26.COV2.S-primed individuals, but this was not seen for ancestral neutralising antibodies and T-cell responses. Although levels of neutralising antibodies were higher for ancestral SARS-CoV-2 compared to that of BA.1 and BA.5 variants, they all wane at three months with the slowest waning rate observed for Omicron variants. Only low levels of neutralising antibodies against the currently circulating XBB.1.5. variant were detected.

Conclusion

Three immunological parameters achieved similar magnitude within the first 28 days independent of booster timing. Omicron-specific neutralising antibodies in bivalent boosted individuals were more durable than ancestral SARS-CoV-2 neutralising antibodies if participants were had received an mRNA-based priming vaccination regimen. Omicron BA.5 booster induced a broader neutralising response in mRNA-primed individuals compared to Ad26.COV2.S-primed individuals or recipients of Omicron BA.1 booster. Limited XBB.1.5 cross-neutralising antibodies were detected, which should be considered in future vaccination campaigns involving healthy populations. Our data emphasize the importance of continuous surveillance of circulating variants and immune response assessment towards variants of concern.

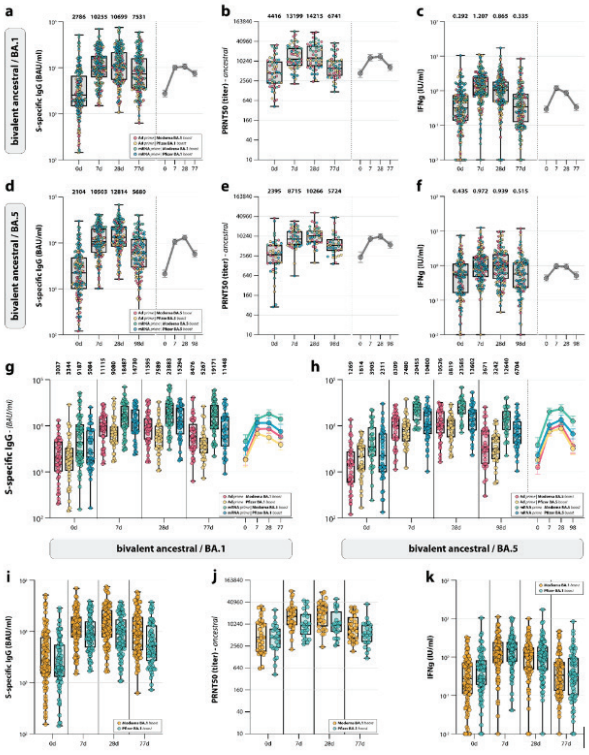


Figure 1. Antibody and T-cell responses after bivalent booster vaccination. a-f, Detection of (ancestral) spike (S)-specific binding IgG antibodies (a,d), ancestral SARS-CoV-2 neutralising antibodies (b,e), and T-cell responses measured by interferon-gamma (IFN-γ) release assay (IGRA) (c,f) after Omicron BA.1 (a-c) or BA.5 (d-f) bivalent booster vaccination at baseline, and 7 days, 28 days, and 3 months post-boost. Colours indicate the specific prime-boost regimen (red = Ad26.COV2.S prime, mRNA-1273.214 or .222 boost; yellow = Ad26.COV2.S prime, BNT162b2 Omicron BA.1 or BA.5 boost; green = mRNA-based prime, mRNA-1273.214 or .222 boost; blue = mRNA-based prime, BNT162b2 Omicron BA.1 or BA.5 boost). g,h, S-specific binding IgG antibody levels in subgroups based on the different combinations of prime and boost. i-k, S-specific binding IgG antibodies (i), ancestral SARS-CoV-2 neutralising antibodies (j), and T-cell responses measured by IGRA (k) in participants boosted with a bivalent Omicron BA.1 vaccine separated by booster vaccine (mRNA-1273.214 [orange] or BNT162b2 Omicron BA.1 [teal]). Data are shown in box-and-whisker plots, with the horizontal lines indicating the median, the bounds of the boxes indicating the IQR, and the whiskers indicating the range. Bold numbers above the plots represent the respective geometric mean (titre) per timepoint. The line graphs next to each panel depict a time course of the respective geometric mean values with 95% confidence intervals.

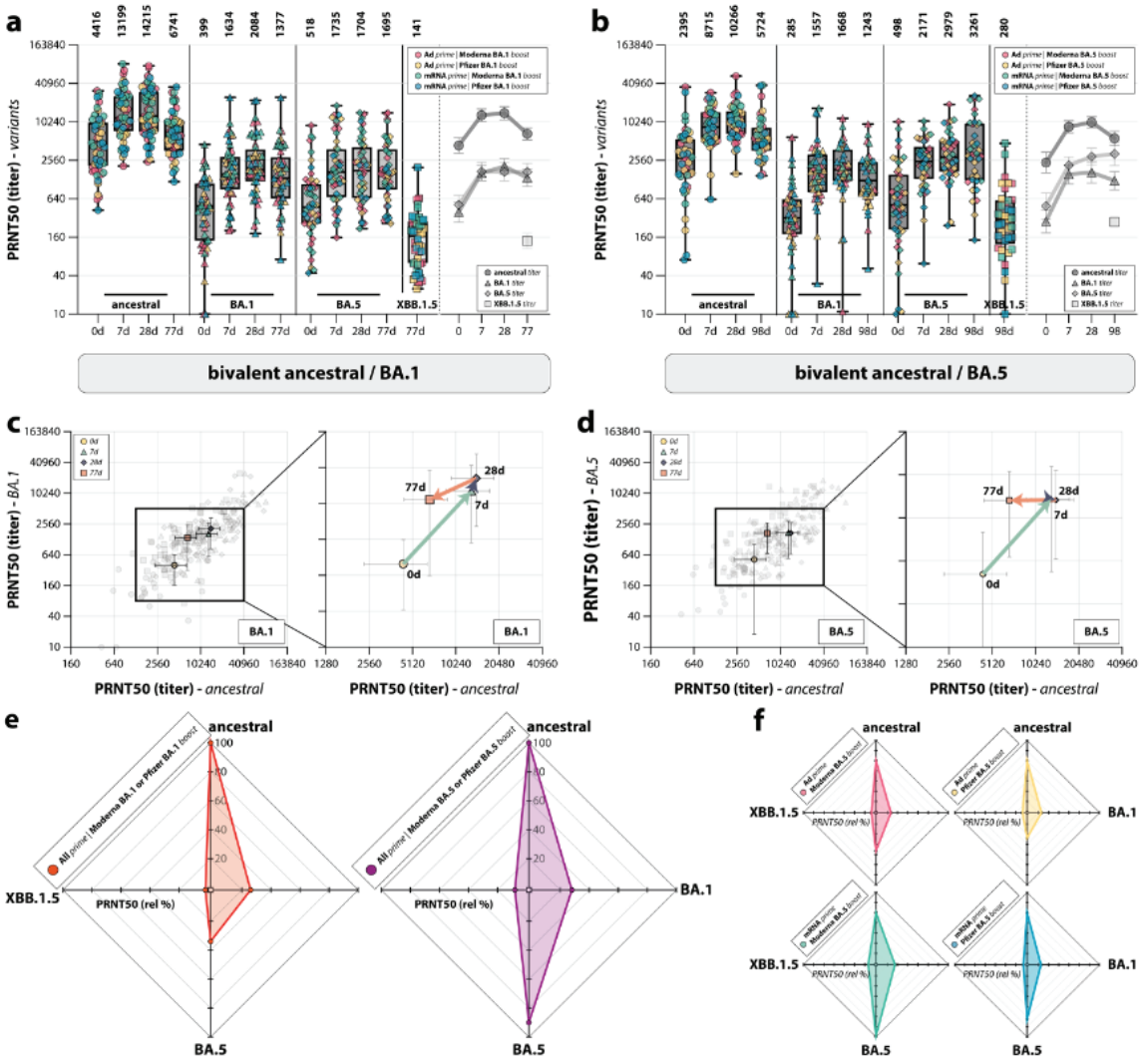


Figure 2. Breadth of the neutralising antibody response after bivalent booster vaccination. a,b, Detection of neutralising antibodies targeting ancestral SARS-CoV-2 and Omicron BA.1, BA.5, and XBB.1.5 variants after Omicron BA.1 (a) or BA.5 (b) bivalent booster vaccination at baseline, and 7 days, 28 days, and 3 months post-boost. Colours indicate the specific prime-boost regimen (red = Ad26. COV2.S prime, mRNA-1273.214 or mRNA-1273.222 boost; yellow = Ad26.COV2.S prime, BNT162b2 Omicron BA.1 or BNT162b2 Omicron BA.5 boost; green = mRNA-based prime, mRNA-1273.214 or mRNA-1273. 222 boost; blue = mRNA-based prime, BNT162b2 Omicron BA.1 or BNT162b2 Omicron BA.5 boost). c,d, Correlation between PRNT50 titres against ancestral SARS-CoV-2 and the Omicron BA.1 (c) or BA.5 (d) variants over time after Omicron BA.1 vaccination. e,f, Spiderweb plots depicting the variant-specific PRNT50 titres relative to ancestral SARS-CoV-2 neutralisation (set to 100%), after vaccination with bivalent Omicron BA.1 (e) or BA.5 (f). Data in panels a,b are shown in box-and-whisker plots, with the horizontal lines indicating the median, the bounds of the boxes indicating the IQR, and the whiskers indicating the range. Bold numbers above the plots represent the respective geometric mean (titre) per timepoint. The line graphs next to each panel depict a time course of the respective geometric mean values with 95% confidence intervals.

Evaluation of fluconazole treatment in critically ill adults on continuous renal replacement therapy

Drs Saskia Coenradie, Drs Tim Smeets, Dr Hilde de Geus, Prof dr Birgit Koch, Dr Henrik Endeman, Dr Laurent Favie, Dr Nicole Hunfeld

Biography

After my pharmacy study in Utrecht, I had my hospital pharmacist training at "Apotheek Haagse Ziekenhuizen" in the Hague. I have been working as a hospital pharmacist in Reinier de Graaf Gasthuis in Delft from 2005 to date. Besides this I am in training for becoming a clinical pharmacologist in Erasmus MC in Rotterdam. With this abstract I would like to explain the research I conducted within this clinical pharmacology course. Over the years I have been active as a hospital pharmacist on many subjects.
This year I also started as chairman of the FSPZ committee of the NVZA , the professional association of hospital pharmacists.
My main interests are amongst others: clinical pharmacology, medication safety, cardiology, intensive care, vascular surgery and anticoagulation therapy.

Introduction

Fluconazole 400 mg or 800 mg once a day (OD) is used for the prevention or treatment of candidiasis in critically ill patients. In Erasmus MC, therapeutic drug monitoring is a standard procedure aiming for a fluconazole target trough level between 10-50 mg/l in critically ill patients.
Critically ill patients often suffer from Acute Kidney Injury (AKI) requiring Continuous Renal Replacement Therapy (CRRT). Due to the lack of tubular reabsorption in anuric patients and because CRRT is highly efficient in fluconazole elimination, lower fluconazole trough levels have been measured in these patients. Based on literature recommendations, critically ill patients on CRRT are therefore treated with double dosages of fluconazole, 400 mg or 800 mg twice a day (BID).
However, literature regarding therapeutic drug monitoring of fluconazole in critically ill patients on CRRT is still limited and there are no clinical follow-up data demonstrating the effect of the recommended double dosages.

Objectives

The primary objective of this study is to establish the prevalence of target attainment in critically ill CRRT patients on fluconazole and relate this to the documented dosing regimens. This study will also provide information whether there are other (CRRT or clinical) factors that can influence the trough level of fluconazole and can therefore influence target attainment.

Methods

We conducted a retrospective cohort study in critically ill adult patients on CRRT, who received intravenous fluconazole between July 2017 and March 2023 at the ICU of Erasmus MC. Patients with at least one fluconazole trough level were included. Patients with interacting medication or unavailable CRRT data were excluded. Data were extracted from the electronic medical records including patient characteristics and fluconazole, CRRT, infection and clinical data. The prevalence of target attainment after the first fluconazole trough level during CRRT was determined and the patients were divided into two groups: target attainment and non-target attainment. Statistical analysis was performed using IBM SPSS 25 for Windows NT (SPSS Inc. 2004, Chicago, USA).

Results

Twenty-seven patients were included in the study. The patients had an median age of 54.0 years [42.0-65.0], a median BMI of 26.2 kg/m2 [23.6-30.5] and 15 (56%) patients were male (p=NS). All patients received CVVHD (one patient with ultrafiltration 200ml/h). Fluconazole, CRRT and clinical characteristics are summarized in table 1. Data are shown for the target- and non-target attainment groups. The target attainment for the different dosages was 0% (n=2) for the OD 200 mg group, 33.3% (n=6) for the OD 400

mg group and 100% for the BID 400 mg (n=14) and BID 800 mg (n=5) group. Analysis of the different factors that can influence the trough level showed that there were no other significant differences between the target attainment and non-target attainment group except for the dosage of fluconazole (p<0.001).

Conclusion

Double dosages of fluconazole of 400 or 800 mg BID is an adequate dose regimen to achieve target attainment in critically ill patients on CRRT. We found no significant CRRT related and clinical factors associated with target attainment.

Table 1. Fluconazole, CCRT and clinical characteristics

	Total	**Target**	**Non-target**	**p-value**
Patient no	27	21	6	n.a.
Fluconazole data				
Prophylaxis (%)	18 (66.7)	14 (66.7)	4 (66.7)	1.00
Treatment (%)	9 (33.3)	7 (33.3)	2 (33.3)	-
Pathogen (n=9)	8	6	2	n.a.
OD 200 mg (%)	2 (7.4)	0 (0)	2 (33.3)	< 0.001 (*)
OD 400mg (%)	6 (22.2)	2 (9.5)	4 (66.7)	-
BID 400 mg (%)	14 (51.9)	14 (66.7)	0 (0)	-
BID 800 mg (%)	5 (18.5)	5 (23.8)	0 (0)	-
OD 200 mg/400 mg (%)	8 (29.6)	2 (9.5)	6	< 0,001 (#)
BID 400mg/800 mg (%)	19 (70.4)	19 (90.5)	0 (0)	-
No fluconazole loading dose (%)	19 (70.4)	14 (66.7)	5 (83.3)	0.70
Duration fluconazole, days	9.0 [7.0-18.0]	9.0 [6.5-22.0]	10.0 [5.8-38.3]	0.84
Fluconazole trough level mg/l	15.9 [10.8-19.6]	17.7 [14.4-24.3]	4.0 [3.2-5.5]	0.001
CRRT data				
Blood flow ml/min	130.0 [110.0-140.0]	130.0 [105.0-140.0]	125.0 [102.5-152.5]	0.78
Dialysate flow ml/h	2600 [2200-2800]	2600 [2200-2800]	2500 [2050-3050]	0.84
Prescribed renal dose ml/kg/h	30.6 [29.0-31.5]	30.6 [29.1-31.8]	30.8 [28.7-31.3]	1.00
Delivered renal dose ml/kg/h	29.3 [28.1-31.1]	29.6 [28.1-31.8]	29.12 [27.1-30.6]	0.67
Urine production>500 ml/day (%)	3 (11.1)	3 (14.3)	0 (0)	n.a. (too low number)
Duration CRRT, days	24.0 [23.0-24.0]	24.0 [22.5-24.0]	23.8 [21.8-24.0]	0.89
Clinical data				
ICU stay, days (IQR)	21.0 [13.0-43.0]	21.0 [12.0-43.0]	29.00 [14.8-90.5]	0.41
APACHE IV score (n=25)	96.0 [67.0-109.0]	99.0 [78.0-112.0]	80.5 [63.8-97.5]	0.14
SOFA score (n=26)	13.0 [9.8-17.0]	15.0. [9.3-17.0]	13.0 [9.3-14.3]	0.57

Variables are presented as median with the interquartile range between brackets. CRRT = continuous renal replacement therapy; OD = once a day; BID = two times a day; ICU = Intensive Care Unit APACHE IV = Acute Physiology and Chronic Health Evaluation; SOFA = Sequential Organ Failure Assessment; (*) p value for the 4 dosages; (#) p value for OD and BID dosages.

Methylphenidate, A Potential Treatment for Psychotherapy-Resistant Posttraumatic Stress Disorder - A Clinical Case Report

Dr Khalid Abou Farha, Mr Ramy Abou Farha

Biography

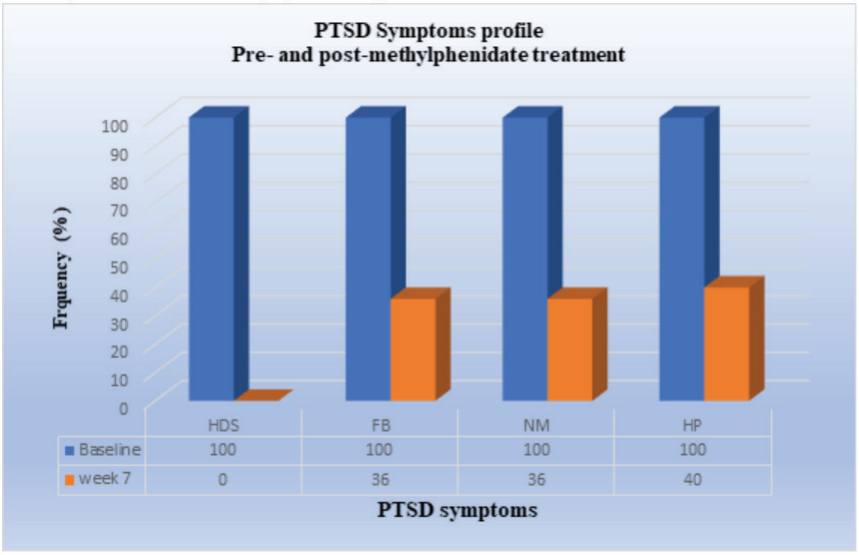
1. Dr Khalid Abou Farha, MBChB, MD, MSC, PhD is a clinical Pharmacologist working at the department of Psychiatry, Aneurin Bevan University Health Board, South Wales, UK.
2. Mr Ramy Abou Farha, Pharmacy MSc graduated from the faculty of Pharmacy, University of Groningen, the Netherlands. He is currently a community Pharmacist at Boots, UK.

Introduction

Chronic hypoarousal and hyperarousal states are 2 key features for the diagnosis of post-traumatic stress disorder (PTSD). They represent an out of tolerance window zones and can negatively affect receiving, processing, and integration of stimuli. They are debilitating clusters of symptoms that reduce quality of life and significantly interfere with the individual's daily functioning. Moreover, chronic significant hyperarousal- hypoarousal shifts can interfere with patient's engagement in psychotherapy. Addressing these out of tolerance window states may reduce patient's distress and improve his/ her quality of life and psychotherapy outcome.

Case presentation

This report describes a clinical case of psychotherapy- resistant PTSD key symptoms in a 32-year-old female patient. Stand-alone psychotherapy in the form of Eye Movement Desensitization and Reprocessing (EMDR) failed to lessen the PTSD symptoms. Persistence of PTSD hypo- and hyper-arousal symptoms significantly interfered with the patient's daily functions and limited the progress and effectiveness psychotherapy. Given the nature and severity of her illness, the patient had been reassessed. Re-assessment included a clinical interview, blood tests, vital signs, ECG, anthropometric characteristics, assessment for attention deficit hyperactivity disorder (ADHD) and borderline personality disorder (BPD). Diagnosis of comorbid ADHD has been confirmed and the patient has been commenced on methylphenidate (MPH) with adjunct propranolol for associated paroxysmal sinus tachycardia. The effect and side effects of the treatment were monitored during a follow-up period of 7 weeks. Main parameters assessed were flashbacks, nightmares, dissociation and fainting, and hypervigilance with paranoid symptoms. Within one week of commencing the treatment the patient reported improvement in all psychotherapy- resistant PTSD symptoms. Remission of symptoms remained until the last follow up review (7 weeks post-treatment) (Figure).



Conclusion

PTSD- related hypoarousal and hyperarousal symptoms reduce patient's quality of life, impair his/ her daily functioning, and could hinder the effective progress of psychotherapy, a corner stone in the treatment of PTSD. Pre-psychotherapy diagnosis and treatment of PTSD comorbidities such as ADHD may decrease PTSD symptoms severity. MPH seems to play a clinically meaningful role in the treatment of PTSD-related hyperarousal- hypoarousal symptoms and thereby reduces patient distress and improves patient's quality of life and may facilitate successful psychotherapy outcome.

Clinical Effectiveness of Methylphenidate and Testosterone in the Treatment of Mixed Depression and Anxiety; A Potential Neuropharmacological Approach in Psychiatry

Dr Khalid Abou Farha, **Mr Ramy Abou Farha**

Biography

1. Dr Khalid Abou Farha, MBChB, MD, MSC, PhD is a clinical Pharmacologist working at the department of Psychiatry, Aneurin Bevan University Health Board, South Wales, UK.
2. Mr Ramy Abou Farha, Pharmacy MSc graduated from the faculty of Pharmacy, University of Groningen, the Netherlands. He is currently a community Pharmacist at Boots, UK.

Background

Depression is a leading cause of disability worldwide. Approximately 85% of patients with depression have significant symptoms of anxiety and demonstrate mixed symptoms of anxiety and depression. The latter often runs a chronic course, becomes treatment-resistant (in up to 30%) and is associated with impairment of quality of life, social functioning, and a higher suicidal rate. Despite the substantial burden of this illness on both patients and society, the currently prescribed antidepressants have limited treatment effectiveness.

Patients and Methods

This report describes two male obese patients, aged 28 (patient A) and 34 (patient B), with established diagnosis of treatment-resistant chronic mixed anxiety and depression, associated with recurrent self-harm, suicidal thoughts, and attempts. Both patients received multiple antidepressants including sertraline (augmented with antipsychotics) with neither subjective nor objective improvement. Given the chronicity and severity of their mental illness, both patients have been reassessed to explore the presence of other potentially treatable etiologic factors. Re-assessment included clinical interviews, blood tests, vital signs, anthropometric characteristics, and self-rating questionnaires to assess for ADHD (ASRSv-1.1) and personality disorders (including borderline personality disorder (BSL-23)). Besides, the Hamilton rating scales for depression (HAM-D17) and anxiety (HAM-A) have been used to quantify the depression and anxiety symptoms severity. Both patients have been treated with a combination of testosterone gel, methylphenidate. The effect and side effects of the treatment were monitored during a follow-up period of 11 and 14 weeks, patient B and A respectively.

Results

Clinical and laboratory reassessment revealed severe degree of anxiety and depression on a background of clinically significant hypogonadism, ADHD- borderline personality disorder comorbidity, and anaemia (in patient B).

Testosterone- methylphenidate (and Iron supplement in patient B) combination improved the subjective symptoms and objective measures in both patients. Clinically meaningful improvement has been reported and observed in both patients at week 7 (patient A) and 11 (patient B).
Patient A reported a 60% improvement in his symptoms at week 7 post-treatment. This has been in line with the improvement observed on Hamilton rating scales (>55% reduction from baseline. The addition of sertraline led to further improvement in the self-reported symptoms (> 90% reduction from baseline) and on the Hamilton rating scales (>80%).

Patient B achieved complete remission in all his signs and symptoms, including suicidal thoughts and self-harm behaviour. At the end of the follow-up period, the patient reported >95% improvement in his symptoms. Clinically significant and meaningful improvement in ADHD (complete remission) and borderline symptoms (91% reduction from baseline). Parallel to this improvement, HAM-D17 and HAM-A demonstrated reduction from baseline respectively -85% and -78%.

Conclusion

The treatment approach for patients with treatment-resistant depression and anxiety needs to be based on a holistic approach and take into account patient's clinical profile including comorbidities, and biological characteristics such as body mass index and hormonal profile. Addressing deficits such as hypotestosteronemia and dopaminergic dysfunctions as encountered in both patients might lay the cornerstone for clinically effective treatment, facilitates antidepressant action, and eventually reduces the suffering of the patient and the burden on the community.

Label-free detection of prostaglandin transporter (SLCO2A1) activity using a TRACT assay

Dr Tamara Mocking, Luc Mulder, Prof.Dr. Laura H. Heitman, Prof. Dr. Adriaan P. IJzerman

Biography

Dr. Tamara Mocking is a postdoc at Leiden university. She obtained a PhD in molecular pharmacology at Vrije Universiteit Amsterdam studying GPCR photopharmacology. Currently, her research focuses on label-free assay to study Solute carrier transporters in which she uses GPCR activation as a measure for SLC transport activity as part of the ReSolute consortium (https://re-solute.eu/).

Introduction & Aim

The prostaglandin transporter (PGT, SLCO2A1) mediates transport of prostanoids (a.o. prostaglandin E2 (PGE2)) into the cells to promote their degradation. Overexpression of PGT leads to low extracellular PGE2 levels and has been linked to impaired wound healing of diabetic foot ulcers1. Inhibition of PGT would be beneficial, however, there are currently no high-through screening assays for this transporter. Here we developed a label-free impedance-based assay for PGT that measures transport activity through receptor activation (TRACT).

Methods

Label-free impedance-based TRACT measurements were recorded on intact cells using the xCELLigence real-time cell analyzer. Here, activation of prostanoid receptors EP3 or EP4 with PGE2 leads to changes in cell morphology. Uptake of PGE2 by PGT will reduce extracellular PGE2 levels and thereby attenuated the response of co-expressed EP3 or EP4 receptor. Thus, PGT activity is detected as a change in receptor activity. To this end, HEK293-JumpIn-SLCO2A1 cells with doxycycline (dox)-inducible SLCO2A1 expression were transfected to express prostanoid receptors EP3 or EP4 and induced or non-induced cells were pretreated with inhibitor or vehicle prior to stimulation.

Results

Induction of PGT expression on EP3 or EP4 expressing HEK293-JumpIN-SLCO2A1 cells results in over 10-fold reduction in potency of PGE2 (Figure 1). Potency was recovered upon inhibition of the PGT-mediated PGE2 uptake with PGT inhibitor Olmesartan and T26A. The results confirm that prostanoid receptor activity can be used as a measure of PGT activity.

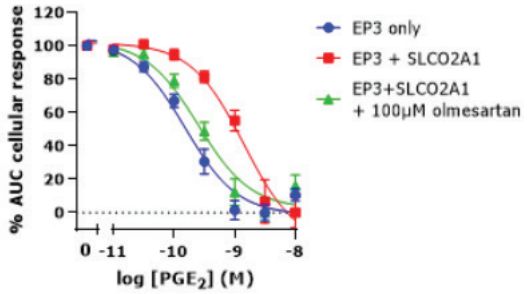


Figure 1. Concentration response curves of the influence of PGT co-expression on EP3 receptor activity as measured by xCELLigence. Induced and non-induced HEK293-JumpIn-SLCO2A1 cells expressing EP3 receptor were pretreated with vehicle or PGT inhibitor olmesartan for 1 hr prior to stimulation with PGE2.

Conclusion

An impedance-based TRACT assay was established that measures prostaglandin transporter (SLCO2A1) activity through prostanoid receptor signaling. This will enable a novel way to better study the wound healing capacity of SLCO2A1 inhibition on a cellular level.

References

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Effect of positive charge on liposome stability, uptake efficacy and impact on cells

Miss Feng Zhao

Biography

Feng Zhao received the B.S. degree in Food Science and Engineering from Southwest University, Chongqing, China, in 2018 and the M.S. degree in Food Science from Northwest A &F University, Shaanxi, China, in 2021. In 2022 she has been awarded a PhD scholarship from the Chinese Scholarship Council to join the University of Groningen, Groningen Research Institute of Pharmacy, for her PhD project under the supervision of Prof. dr. Anna Salvati. Her research project is focused on Nanomedicine and aims at understanding the connection between material properties (size, charge, composition) with the subsequent interactions with cell receptors and mechanisms of uptake. Specifically, she is focusing on the effect of positive charge on liposome stability, uptake efficacy and impact on cells.

Authors

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Introduction

Nanomedicine provides new technologies to improve drug delivery and drug targeting using nano-sized drug carriers. Several nanomedicines are on the market, including recently the RNA and DNA vaccines against Covid19, but a better understanding on how cells interact with and process nano-sized materials is necessary in order to further improve nanomedicine efficacy.

Objectives

In this context, positively charged nanocarriers usually show higher uptake efficiency than neutral or negatively charged ones, but positive charges can also affect nanocarrier stability in serum and in some cases can be toxic to cells. Thus, in this project, using a series of liposomes of same size with increasing amounts of positively charged lipids, we are exploring in detail how the positive charge affects liposome stability, toxicity and uptake efficiency, thus ultimately liposome interactions with cells.

Methods

Positive liposomes were prepared by mixing DOPC and positively charged DC-cholesterol in different ratios. A negative liposome with the same amount of charged lipid was prepared for comparison using DOPC, DOPG and cholesterol. Dil was incorporated in the lipid bilayer as a fluorescent label. Liposome size distribution was determined by dynamic light scattering together with zeta potential measurements. Flow cytometry was used to measure cellular uptake based on fluorescence. Sodium azide was used to deplete cell energy and block active processes in order to test whether liposome uptake is energy dependent. The MTT assay was used to measure cellular metabolic activity as an indicator of cell viability.

Results

Liposomes of 100 nm with increasing amounts of positive charge were obtained. DLS results showed that once dispersed in serum, the more charged liposomes (DOPC: DC-Chol 1:1 and 5:1) aggregated while the less positive liposomes (20:1) were stable. Uptake kinetics indicated that uptake efficiency was higher at increasing positive charge, while when comparing liposomes with the same amount of charged lipid, the positive liposomes showed higher uptake than the negative ones. Energy depletion by sodium azide blocked the uptake of the less positive liposomes (20:1) but had lower effects at increasing positive charge, suggesting that for the most positive liposomes passive mechanisms of uptake are involved (possibly by fusion with the cell membrane). The MTT assay confirmed that the higher the positive charges, the higher the toxicity of the liposomes on cells.

Conclusion

Our results show that the uptake efficiency and toxicity of liposomes increased for liposomes with increasing amounts of positively charged lipids. Further work is ongoing to characterize the mechanism of uptake for the different formulations.

Tuning liposome rigidity to modulate cellular uptake by cells

Xinyu Ma, Prof Anna Salvati

Biography
Xinyu Ma is a first year PhD student in Nanomedicine at the University of Groningen under the supervision of Prof. dr. Anna Salvati. Her research focuses on the effect of nanoparticle rigidity on nanoparticle targeting and clearance. Her current projects utilize liposomes with different composition to modulate rigidity and study cellular endocytic behavior and related mechanism. This program is sponsored by Chinese Scholarship Council. Prior to this program, Xinyu completed her master in Medicinal Chemistry at Sichuan University in 2022. She received her BS in Pharmaceutical Engineering from Jiangnan University in 2019.

Authors
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Introduction
Nano-sized materials have been extensively investigated over the recent decades for their potential application in delivering cancer therapeutics. Numerous studies are trying to elucidate how nanoparticle properties, such as size, charge, material etc., affect nanoparticle behavior in organisms and at the cellular level. Among these properties, nanoparticle rigidity, the ability of the object to resist deformation upon an applied force, is emerging as another parameter which can be used to modulate nanoparticle interactions with cells. However, a clear understanding of how rigidity affects cellular uptake behavior and uptake mechanism is still missing.

Objectives
Within this context, liposomes of different composition are used as a model system to study how rigidity affects cellular uptake behavior and mechanism. Lipids with higher transition temperature (Tm) typically form more rigid lipid bilayers. Thus, by changing their content in a lipid mixture, they can be used to prepare a series of liposomes of same size and surface chemistry but with different rigidity. The liposomes are then used to explore in depth how nanoparticle rigidity affects the interaction with cells and cellular uptake mechanism.

Methods
Lipids with different transition temperature such as DSPC, DPPC and DOPC are mixed in different ratios to form liposomes; Dil is incorporated as a fluorescent label. Liposome size and charge are determined by dynamic light scattering and zeta potential measurements.
Flow cytometry is used to measure cellular uptake.

Results
Liposomes have been prepared by thin-layer hydration, and their size distribution, zeta potential and stability in serum have been determined. The procedure to prepare the liposomes has been optimized in order to allow the inclusion of the lipids with higher transition temperature in the bilayer. Homogenous liposome dispersions could be obtained in water and PBS, however when lipids at higher Tm are included, dispersion in serum led to agglomeration. Preliminary experiments with HeLa cells showed that uptake is higher for the liposomes made with lipids at higher Tm.

Conclusion
Further studies are ongoing to improve the stability in serum when lipids at higher Tm are included and to characterize the mechanism of uptake for the different samples.

Development of a Personalized Tumor Neoantigen Based Vaccine Formulation for the Treatment of Advanced Non-Small Cell Lung Cancer

Drs. ir. Linette Trea Oosting

Biography

Drs. ir. Linette Oosting is a dedicated and inquisitive PhD candidate specialising in biomedical and pharmaceutical engineering. Linette's interests shine brightly on innovative drug development and formulation.

Driven by an insatiable thirst for knowledge, Linette holds a Bachelor of Science in Biology and Medical Laboratory Research, followed by a Master's degree in Medical Pharmaceutical Sciences from the University of Groningen, where she worked on astounding research projects. Linette's first project at the University of Groningen involved the development of novel drug carriers. In her second Master's project, she collaborated with the UMCG and Frame Cancer Therapeutics focusing on a personalized neoantigen vaccine as a novel therapy for Non-Small-Cell Lung Cancer.

Linette is currently working on a PhD in the cutting-edge area of CAR-T Cell Therapy, focussing on novel drug formulations and ensuring stability. Her research unfolds within the Departments of Clinical Pharmacy & Pharmacology and Immunohematology at the UMCG.

Introduction

Stage III–IV non-small cell lung cancer (NSCLC) is a devastating disease characterized by a poor prognosis. NSCLC tumors carry genetic mutations, which can lead to the expression of altered protein sequences. Peptides originating from mutated proteins and bound to MHC molecules on the tumor cell surface are referred to as neoantigens, as they are tumor-specific and not expressed in normal cells. Due to their tumor specificity, neoantigens have a strong potential to induce an anti-tumor immune response and have been investigated for development of personalized therapeutic cancer vaccines. The current study describes the development of a clinical grade neoantigen vaccine formulation (FRAME-001) intended as immunotherapy in advanced NSCLC in combination with the immune checkpoint inhibitor pembrolizumab. The detection of aberrant tumor-specific transcripts as well as an algorithm to select immunogenic neoantigen peptides are described. Subsequently, selected neoantigen peptides were synthesized with a high throughput synthesis platform and aseptically formulated under good manufacturing practice (GMP) conditions into four aqueous peptides mixtures that each contained six neoantigen peptides. A validated stability-indicating analytical method was developed in which we considered the personalized nature of the formulation. An extensive stability study performed either at −25 °C or −80 °C showed that the formulation was stable for up to 32 weeks. The formulation was mixed with the vaccine adjuvant Montanide ISA 51 VG, which yielded the final vaccine emulsion. The stability of the vaccine emulsion was demonstrated using microscopic examination, differential light scattering, and the water-drop test. The presented data show that FRAME-001 is a feasible personalized vaccine formulation for the treatment of stage III–IV NSCLC. The presented data may give guidance in the development of novel personalized therapeutic vaccines since this formulation strategy could be used for any cancer indication.

Development of new strategies for the delivery of bioactive proteins to Fibroblasts

Zhiyi Huo

Biography

PhD candidate of university of Groningen
Development of new strategies for the delivery of bioactive proteins to Fibroblasts.

Authors

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Introduction

Liver fibrosis refers to the diffuse excessive deposition and abnormal distribution of liver extracellular matrix which is a pathological repair response of the liver to chronic injury. The key step in this process is the activation of Hepatic Stellate Cells, or myofibroblasts.

Objective

We aim to deliver therapeutic proteins to these cells, which also are able to reach the nucleus. For that purpose we use a peptide that binds to the PDGF-β receptor on fibroblasts (PPB), and coupled this to the carier protein human serum albumin.

Methods

Binding and uptake by fibroblasts: 3T3cells were seeded in 96 well plates (50000 cells per well) and co-cultured with human serum albumin (HSA)-AF488, PPB-HSA-AF488, or medium alone (negative control) at 4 and 37oC . Fluorescence staining was analyzed by Flow Cytometry and fluorescense microscopy. Nuclear binding studies: 3T3 cells were cultured in 6 well plates (500000 cells per well) until 90% confluency. After adherence, cells were co-cultured with HSA-AF488, ppb-HSA-AF488 or medium (negative control) for two hours at 37oC. After digestion and centrifugation, the pellet was resuspended in isolation buffer, and nuclei were isolated using a nitrogen bomb, applying a pressure of 250 Psi for 10 mins. DAPI was added as indicator for nuclei and samples were analysed by Flow Cytometry. The effects of sodium azide (4 mM) were also studied.

Results

The results show that at 4oC, no HSA-AF488 was bound to cells, as compared to medium. PPB-HSA-AF488 induced a shift in staining intensity compared to the other two treatments. At 37oC, again no increased flu-staining was seen in cells treated with medium or HSA-AF488, but for PPB-HSA-AF488 there has a clear shift. This means that PPB-HSA-AF488 has been taken up by 3T3 cells. These results were confirmed by Fluorescene microscopy.
Nuclear binding: Nuclei were identified using DAPI in the Flow Cytometer and AF488 MFI staining was analysed. Results show that compared to medium and HSA-AF488, PPB-HSA-AF488 induced a high MFI for AF488, reflecting binding of PPB-HSA-AF488 to these nuclei. Nuclear binding was reduced by Sodium-azide.

Conclusions

Fibroblast-like cells are the main inducers of liver fibrosis. In the experiments described above, we verified that PPB-HSA can bind to the 3T3 fibroblasts. PpB- has been shown to bind to the PDGF-receptor, highly expressed on 3T3 cells. We have used a new bombing method to isolate nuclei, and results indicate that PPB-HSA can bind to the nucleus whereas HSA does not. Further studies are needed to examine whether this nuclear binding is specific.

References

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Development and Optimization of Fluorescent Labeled Lectins in the Treatment of Oncologic and Inflammatory Diseases

Jonathan Shaheen

Biography

Jonathan Shaheen was born on 26 July 1998. In 2021 Jonathan obtained his Bachelor of Science with honors, major in Pharmacy at the University of Groningen. In 2022 Jonathan started his ambitious research career under supervision of prof. Dr W.B.N. Nagengast and Dr. B. Gareb. as a pharmacy graduate student for the development, production and clinical application of lectin-based tracers. The results of the successful development were defended in 2023, and is writing the manuscript for publication. Meanwhile Jonathan started a second research project for the Development and Verification of Physiologically-Based Pharmacokinetic Models for Treatment of Leukemia with TKI's in Various Patient Populations. This project will be further applied for personalized treatment of leukemia patients.

Introduction

During the last decade, huge efforts were invested in the complex and formidable development of optical molecular tracers of which multiple reached late-stage clinical trials. However, despite the recent advances in antibody-based tracers, clinical application is hampered by its costly, labor-intensive, and mostly unvalidated development endeavors in addition to suboptimal clinical outcomes, e.g. signal-to-noise, resulting in technical and molecular limitations.

Objectives

Glycosylation levels seem to be affected in, as far is known now, neurodegenerative disease, autoimmune disease, infectious diseases, inflammatory diseases, cardiovascular diseases, and cancer. Lectins are oligomeric natural proteins functioning as carbohydrate-recognition scanners with exponentially higher binding affinity to longer oligosaccharides. As such, development of a novel lectin-based tracer is a major theme, thought to overcome current molecular and financial shortcomings of antibody-based tracers and will form a milestone in the application of sustainable glycobiology in visualization and treatment of oncologic and inflammatory diseases. IRDye-800CW and IRDye-680LT are two optical indocyanine dyes with distinguishable absorption spectra in the near infrared region, with high extinction coefficients > 200.000 M-1cm-1, ought to produce a bright non-nuclear fluorescent signal.

Methods

The development of optical tracers is divided into 4 unique phases: analysis, labeling, purification, and formulation. For quantitative and qualitative analyses of the individual components as well as the produced tracer size-exclusion high-pressure liquid chromatography method was developed. Secondly, the lectin-IRDye conjugate was obtained via an optimized labelling route regarding dye-to-protein ratio, conjugation time, conjugation buffer, and protein concentrations, availing to establish the tracer signal-to-noise ratio. The product was purified from fragments, aggregates, free-dye, and by-products using validated prepacked desalting columns. Lastly, the development is finalized by the formulation phase, in which an extensive stability study is performed determining the product shelf-life and optimal formulation buffer. To ensure that the final product meets the current Good Manufacturing Practice (cGMP). The product specifications shown in table 1 were set up.

Results

Lectins obtained from Glycomatrix®, Figure 1, showed absent absorbance at 280 nm. Native-PAGE proved a molecular weight 30x lower than the product description value. Adequately sourced lectin was later obtained from Thermo Fischer Scientific® with confirmed product specifications.

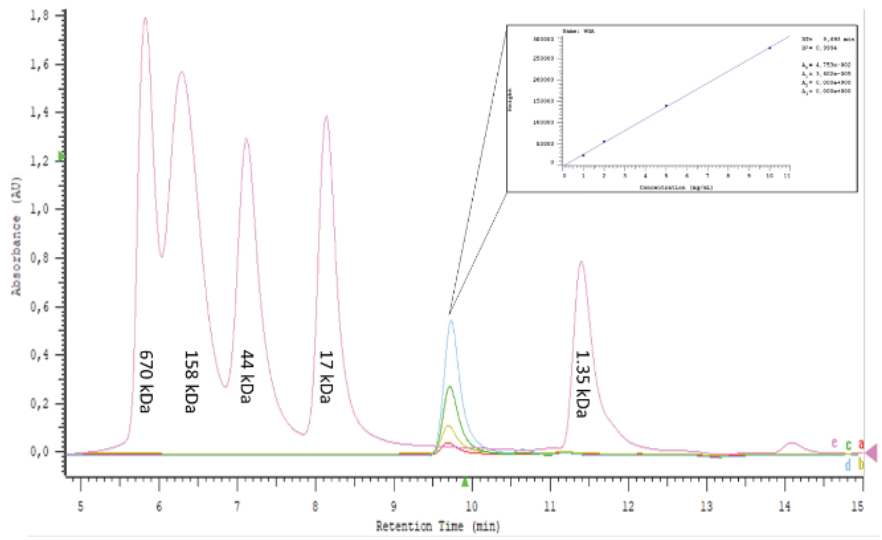

Figure 1, Multi-chromatogram display. (A) in red is WGA-pure 1 mg/mL, (B) in yellow is WGA-pure 2 mg/mL, (C) in green is WGA-pure 5 mg/mL, (D) in blue is WGA-pure 10 mg/mL, (E) in pink is gel filtration standard at 10x dilution

Two tracers were successfully developed, proving the feasibility of in-house production of clinically applicable lectin-based tracers. The tracers are ready for validation of the manufacturing processes, in addition to formulation of the tracer in an adequate, and stable buffer with pre-set acceptance cGMP criteria.

Table 1. product profile of the WGA-IRDye tracer for intravenous administration

Parameter	Requirement
Identity API [a]	Conform unlabeled WGA
Content of API [a]	0.95-1.05 mg/mL
Dye-WGA conjugation ratio [a]	1:2
Content of free-dye [a]	< 3 %
Visible particles [c]	Practically free of visible particles
pH [c]	6.8 - 7.2
Osmolality [c]	270-330 mOsmol/kg
Affinity binding [d,e]	50 % - 200 % compared to unlabeled WGA
Endotoxins [c]	< 5 EU/mL
Bioburden [c]	< 1 CFU/mL
Tertiary protein structure [e]	No apparent deviations compared to WGA-pure
Residual Solvents: DMSO [c]	< 5000 ppm
Product yield [a]	> 75 %
Labelling efficiency [a]	For information only
Filter integrity [c]	< 2mL air at 10mL
Appearance [b]	Clear or slightly opalescent solution
Color [b]	Green for 800CW / Blue for 680LT

[a]: determined with HPLC-SEC. [b]: visual inspection [c]: determined according to Ph. Eur. [d]: determined with immunoassay. [e]: determined with fluorescence spectroscopy

Conclusion

In this thesis we set a quality control guideline for lectin-based tracers with two IRDyes, IRDye-800CW and IRDye-680LT, we drew the lectin-IRDye production details in 4 phases, and provided evidence of a successfully produced WGA-IRDye of unmatched quality.

Redispensing of expensive oral anticancer medicines: A practical application

Msc Kübra Akgöl, MSc Lisanne van Merendonk, MSc Hannerieke Barkman, MSc Dorieke van Balen, Msc Hester van den Hoek, drs. Marjolein Klous, dr. Jeroen Hendrikx, dr. prof. Alwin Huitema, dr. prof. Jos Beijnen, dr. Bastiaan Nuijen

Biography
My name is Kübra Akgöl, and I'm in my last year of my traineeship to become a hospital pharmacist. My interests are product development and sustainable use of drugs.

Introduction
Oral anticancer medicines are widely used in the treatment of solid tumors and are administered orally in cycles that require self-administration at home. However, dose adjustments and discontinuations often lead to leftover medication. Therefore, the increasing use of expensive oral anticancer medicines comes with the downside of a financial and environmental burden, partially caused by unused medication. Returned oral anticancer medicine to the pharmacy could be considered for redispensing to other patients providing guaranteed quality.

Objectives
This study aimed to identify and implement quality aspects and criteria for redispensing oral anticancer medicine in daily pharmacy practice.

Methods
A systematic risk analysis was conducted to determine the eligibility of oral anticancer medicine for redispensing taking relevant guidelines and product information into account. Additionally, an integrity study was performed to validate visual assessment of the physical condition of returned oral anticancer medicines using a vacuum leak-test. Furthermore, over a one-year period, the number of returned oral anticancer medicine accepted for redispensing was quantified, and the reduction in financial waste and environmental burden calculated based on this assessment.

Results
Four categories of quality aspects were identified for determining the eligibility of oral anticancer medicine for redispensing by the risk analysis: Product presentation suitability (stability characteristics, storage requirements), physical condition (unopened or opened secondary or primary packaging, visual appearance), authentication (Falsified Medicines Directive, confirmation of initial dispense, recall), and additional aspects (remaining shelf life, period of storage in uncontrolled conditions). The flowchart assessment for redispensing oral anticancer medicines is shown in Figure 1. The study found that 75% of the OAM dispensed were eligible for redispensing. The integrity study validated the visual assessment of primary blister packaging with 99.9%. A standardized procedure for redispensing was implemented in daily pharmacy practice. During the study period, 10,415 oral anticancer medicine dose units out of 13,210 returns (79%) were accepted for redispensing. The total value of oral anticancer medicine accepted for redispensing was €483,301, accounting for 0.9% of the total value dispensed during this period. Furthermore, the potential reduction in environmental burden was estimated at 1132.1 g of potent active pharmaceutical ingredient.

Conclusion
By implementing strict procedures considering all relevant quality aspects, redispensing of oral anticancer medicine can be successfully implemented into daily pharmacy practice, resulting in a significant reduction in financial waste and environmental burden.

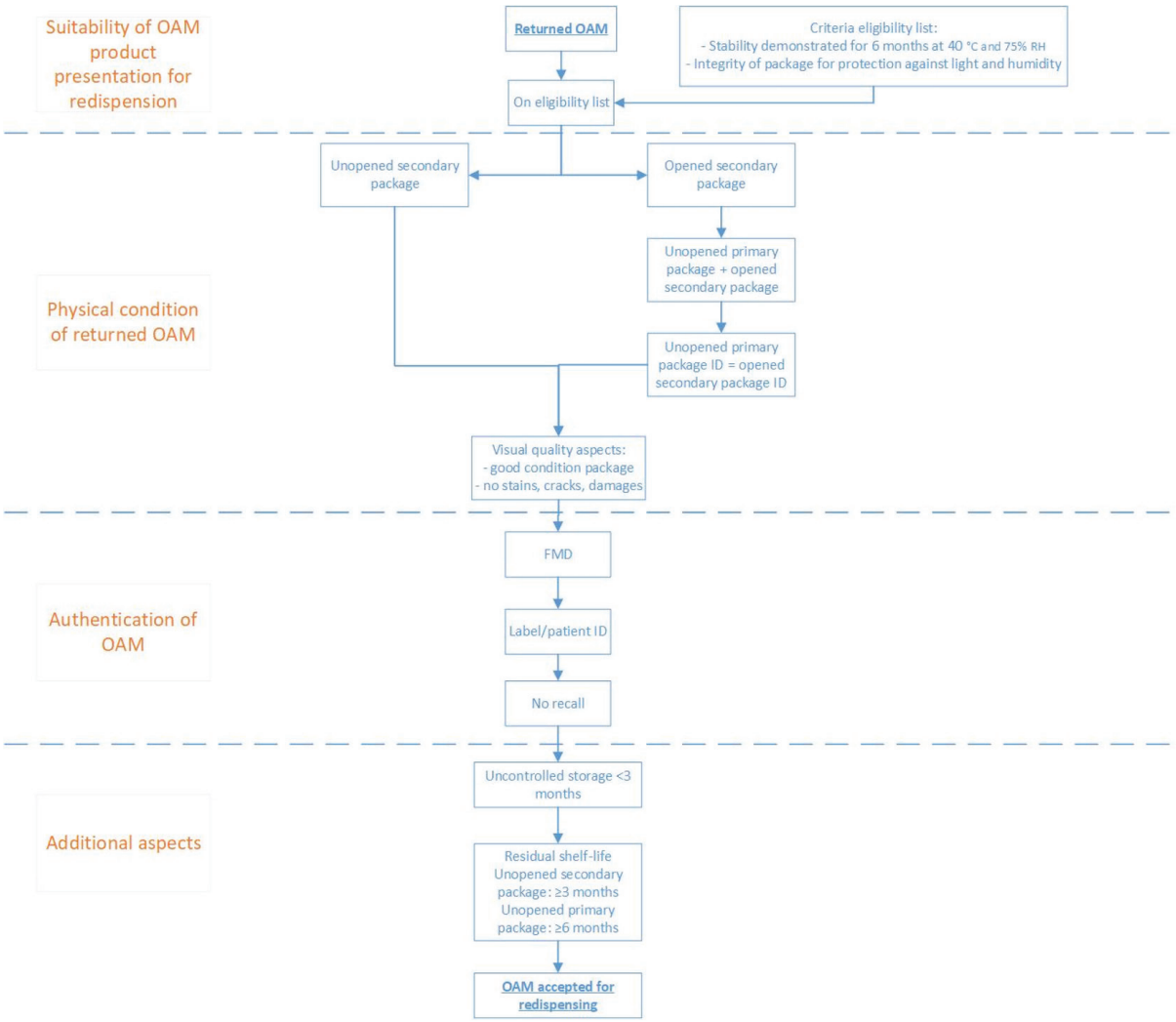

Figure: Flowchart assessment of eligibility for redispensing

Biocatalytic Synthesis of S-adenosyl-L-methionine Analogs as Novel Methylation-Free Allosteric Activators of Cystathionine-β-synthase

dr. Dalibor Nakladal, Rick Oerlemans, Zoe van der Gun, dr. Guido Krenning, Adrianus Cornelis van der Graaf, Christina Yoseif, prof. Matthew Groves, prof. André Heeres, Gabriel Zorkócy, dr. Zdenko Levarski, prof. Ján Kyselovič, prof. Rob Henning, dr. Leo Deelman

Biography

Dalibor Nakládal is a researcher at the Comenius University Science Park, pharmacist by education and experimental pharmacologist by training. He completed his doctoral studies in 2019 in the field of pharmacology at the Faculty of Pharmacy of the Comenius University with the thesis topic: "Pre-clinical characterization of SUL compounds: a novel 6-chromanol based drug class derived from hibernation". After obtaining his PhD, he worked for two years as a postdoc at the University Medical Center Groningen, the Netherlands, on an academic-industrial project in the field of drug discovery. He recently received his second PhD, from the University of Groningen, by defending his dissertation "Drug development and new targets for redox disturbances in experimental vascular disease". He is currently executing a Marie Skłodowska-Curie Actions Seal of Excellence project in the field of target-based drug discovery at the Comenius University Science Park in Slovakia.

Authors

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Abstract

Cystathionine-beta-synthase (CBS) is the rate-limiting enzyme in the transsulfuration pathway and converts homocysteine to cystathionine, which subsequently facilitates the synthesis of several components involved in redox defense. CBS deficiency can arise in the context of diabetes, leading to hyperhomocysteinaemia and oxidative stress to the kidney. Boosting CBS activity could therefore be a novel strategy for restoring antioxidant defense in diabetes.

Boosting CBS activity is feasible, as S-adenosylmethionine (SAM) has been described as an endogenous allosteric activator of CBS. However, SAM is also a universal methyl-donor, seriously limiting its use as a therapeutic agent. To overcome these problems, this project aims for the development of new methylation-free CBS activators.

SAM can be synthesized from methionine and ATP by the enzyme SAM-synthetase. Interestingly, SAM synthetase is not very selective and also accepts substrates that resemble methionine and ATP. We therefore utilized a SAM synthetase-based biocatalysis approach to generate new SAM-like molecules using analogs of methionine and ATP.

Rational design of SAM analogs was guided by published experimental data on SAM-CBS contacts. In silico docking simulations using the smina scoring function were performed on 50 analogs, leading to

the identification of promising candidates. Subsequently, 13 commercially available analogs of ATP or methionine were selected and purchased. SAM synthetase (metK gene) was expressed in E. coli BL21 and isolated using affinity chromatography. Biocatalysis reactions were performed in Tris-HCl buffer (pH 8.2) enriched with ion cofactors at 30°C for 2 hours. Catalysis of SAM analogs was quantified by measuring phosphate production using the Malachite Green assay. Effect on CBS activity was assessed using crude biocatalysis mixtures. Methylation capacity of SAM analogs was tested by Lambda DNA methylation using EcoRI methyltransferase. Out of the 13 SAM analog mixtures tested, four demonstrated CBS activation but these still showed methylation activity.

In summary, rational drug design coupled with biocatalysis represents an effective approach for developing novel allosteric activators of CBS. Further optimization is required to eliminate the methylation capacity of CBS-activating SAM analogs.

Everolimus decreases [U-13C]glucose utilization by pyruvate carboxylase in breast cancer cells in vitro and in vivo

Dr Mathilde Jalving, Drs Jiske Tiersma, Drs Gerke Ariaans, Dr Bernard Evers, prof Daan Touw, dr Albert Gerding, prof Barbara Bakker, prof Steven de Jong

Biography

Dr. M. Jalving is a medical oncologist at the University Medical Centre Groningen, Groningen, the Netherlands. She completed her basic medical and specialist training in Groningen, with training periods at the Royal Marsden Hospital, Sutton, UK and at the Oxford Radcliffe Hospitals Trust, Oxford, UK. She obtained an MSc in Experimental Therapeutics at Oxford University and her PhD on apoptosis in colorectal cancer at the University of Groningen.
She is currently involved in patient care, teaching and research. Her main interests are melanoma and gynaecological cancer and is she active in early phase clinical research. Her research focuses on understanding the altered metabolism of cancer in patients and utilising the altered metabolic profile as a potential treatment target.

Background

Reprogrammed metabolism is a hallmark of cancer cells, but notoriously difficult to target due to metabolic plasticity, especially in response to single metabolic interventions. Previously, we found that the combination of the mTOR inhibitor everolimus and the mitochondrial complex 1 inhibitor metformin resulted in metabolic synergy in in vitro models of triple-negative breast cancer. Here, we investigated whether the effect of this drug combination on tumor size is reflected in changes in tumor metabolism using [U-13C]glucose labeling in a triple negative breast cancer xenograft model.

Methods

The triple-negative breast cancer cell line MDA-MB-231 was treated with everolimus, metformin or a combination, both in vitro and in a SCID/Beige mouse model. Subsequently, the effect on signaling pathway activation, tumor size, plasma and tumor drug levels and mitochondrial and glycolytic function in relation to [U-13C]glucose labeling was determined.

Results

The in vitro effects of everolimus and metformin treatment on oxidative phosphorylation and glycolysis were reflected in the changes in 13C-labeling of various metabolites in MDA-MB-231 cells. Treatment of the MDA-MB-231 xenografts with everolimus resulted in slower tumor growth and reduced both tumor size and tumor viability by 35%. Metformin treatment moderately inhibited tumor growth but did not enhance the everolimus-induced effects. In vivo, everolimus decreased TCA cycle metabolite labeling and inhibited pyruvate carboxylase. Metformin only caused a mild reduction in glycolytic metabolite labeling and did not affect pyruvate carboxylase activity or TCA cycle metabolite labeling. This was related to the relatively high levels of everolimus, and the low levels of metformin achieved ***in vivo***.

Conclusion

Treatment with everolimus, but not metformin, decreased tumor size and tumor viability. Furthermore, the efficacy of everolimus was reflected in reduced 13C-labeling of TCA cycle intermediates and pyruvate carboxylase activity. To optimize metabolic targeted therapies, in-depth analysis of drug-induced changes in glucose metabolism in combination with measurement of drug levels in tumor and plasma is essential.

Keywords

Metformin, everolimus, metabolome, breast cancer, [U-13C]glucose

Sequence engineering to de-immunize mRNA to expand its therapeutic window

Dr. Lotte Tholen, Sander van Asbeck

Biography
Sander is a molecular biology expert, founder of Mercurna (targeted mRNA therapeutics developer) and founder & CEO of RiboPro, a premier CDMO and technology provider specialized in mRNA and mRNA delivery. As CEO he enjoys working together with all stakeholders to find the best scientific, clinical and/or commercial solutions, to advance his dream of a medical revolution through (m)RNA. Sander previously worked on: mRNA-based drug-development @ Mercurna; Polynucleotide delivery & targeting @ Radboudumc; Oncology & Tissue-engineering.

Introduction
In vitro transcription (IVT) mRNA is emerging as a new class of therapeutic agents. Although structurally similar to naturally occurring mRNA, exogenous IVT mRNA, is immunostimulatory unless strategies for reducing the immune stimulation are applied. This poses a significant challenge for therapeutic applications, as immune activation inhibits protein synthesis, cellular toxicity and pro-inflammatory cytokine release. In our previous studies, we discovered that reducing the cytidine (C), and confirmed that reducing uridine (U), content of mRNA can be used to decrease immunogenicity and consequently enhance translation of the mRNA. However, strongly biased nucleotide usage can provide problems during synthesis of mRNA and translation, for example via the secondary structure.

Objectives
Therefore, we aimed to develop a more sophisticated sequence engineering strategy based on reducing C and/or U content while keeping codon usage and secondary structure in mind to improve safety and protein yield, thereby widening the therapeutic window of mRNA.

Methods
We developed a sequence optimization strategy in which at least 10% of the C and/or optionally U nucleotides of the mRNA sequence of the wildtype mRNA are replaced by a canonical nucleotide that is not cytidine (C-depletion) and/or uridine respectively (U- and UC-depletion). This strategy was applied to secreted nanoluciferase (secNLuc), eGFP and murine EPO (mEPO) WT mRNA sequences. Original codons were replaced by codons encoding the same amino acids but having no or less C and/or U nucleotides. When selecting alternative codons, the relative frequency of the codon in the exome of interest was close to the relative frequency of the original codon. Alternatively, for codons for which no alternative existed alternative codons with a higher relative frequency were selected. Expression of WT- and modified-mRNA was investigated in HeLa cells and in mice at 24h post transfection or 6 hours post injection, respectively.

Results
UC- and C-depleted mRNA achieved over 2x to 15x the protein expression of WT mRNA, respectively. U-depletion alone did have a significantly smaller effect on protein translation. In vivo, secreted mEPO protein levels induced by U-depleted (14x) and UC-depleted (18x) mRNA were significantly higher than the corresponding WT mRNA.

Conclusion
Here, we have developed a sequence optimization strategy to reduce the immunogenicity of the mRNA by reducing C and/or U content of the mRNA. We found that depending on the mRNA sequence, C- or UC-depletion resulted in the highest protein levels from the modified mRNA. Especially in vivo, C or UC-depletion was relevant as protein expression from the WT mEPO sequence was neglectable, demonstrating the potential of this strategy based on canonical nucleotides for increasing efficacy of an mRNA therapeutic. This approach is a valuable alternative for the use of chemically modified nucleotides in IVT mRNA.

Physiologically based pharmacokinetic modelling of the PSMA radioligand 18F-DCFPyL to predict the tissue distribution in patients with prostate cancer

Drs. Suzanne Van Der Gaag, Habibe Yilmaz, Jan Amsu, Dr. Daniela Oprea-Lager, Prof. Dr. Harry Hendrikse, Dr. Imke Bartelink

Biography

Drs Suzanne van der Gaag is a hospital pharmacist, PhD-student and clinical pharmacologist in training working at the Amsterdam UMC, location VUmc and Cancer Center Amsterdam in the Netherlands. Suzanne completed her PharmD at the Utrecht University in The Netherlands and hospital pharmacist specialization in the Amsterdam UMC, location VUmc and OLVG, both in Amsterdam. Currently she is working as a hospital pharmacist in the department of Radiology & Nuclear Medicine and as a PhD-student focusing on optimization of PSMA-targeted radioligand therapy.
Physiologically based pharmacokinetic modelling of the PSMA radioligand 18F-DCFPyL to predict the tissue distribution in patients with prostate cancer

Introduction

The most common malignancy in middle and older aged men worldwide is prostate cancer (PCa) [1]. Characteristic for PCa is a high expression of prostate-specific membrane antigen (PSMA) on tumor cells. Recently, the use of PSMA-targeted radioligand therapy for metastatic castration-resistant PCa has been approved by the Food and Drug Administration (FDA) [2,3]. The current fixed-dose-strategy (7400 MBq/cycle) of PSMA-radioligands may lead to inadequate response for a patient due to inter-individual variability in exposure of PSMA receptors. Therefore, personalized treatment, in which tumor volume and PSMA receptor density are taken into account, may lead to better efficacy and less toxicity. Whole body physiological based pharmacokinetic-models (wbPBPK-models) may predict sources of variability in pharmacokinetics among patients. For PBPK modelling positron emission tomography (PET) imaging is a valuable tool to visualize and quantify the tissue distribution for the verification of a wbPBPK-model. For PSMA PET imaging, 18F-DCFPyL is a suitable radiotracer which is in clinical use because of its high affinity for PSMA receptors.

Objectives

The aim of this study was to develop a wbPBPK-model to predict the tissue distribution of 18F-DCFPyL, a 18F-fluorinated diagnostic PET-radiotracer for imaging PSMA expression in patients with metastatic PCa.

Methods

We developed a wbPBPK-model including compartments for blood, tumor, prostate and healthy tissues, based on a previously published PBPK-model (Figure 1) [4]. The model was extended for accurate calculation of free and occupied PSMA receptors and to represent the renal clearance of 18F-DCFPyL. Data from a previously published prospective study of 18F-DCFPyL distribution in eight PCa patients in the Amsterdam UMC was used to validate our results [5]. Iterative fitting was used to estimate individual receptor- and flow-densities for each patient.

Model simulations were performed to determine tissue distribution of 18F-DCFPyL up to 120 minutes after intravenous injection. A prediction error (PE) was calculated to assess the predictive performance of the model to predict concentrations over the full scan window. The model was considered accurate when this performance fell within 2-fold of observed PET-derived data from all tissues of interest.

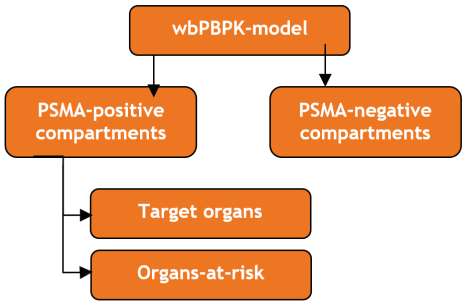

Figure 1. Simplified representation of the whole-body PBPK model. A distinction is made between organs expressing PSMA (PSMA-positive compartments) and organs not expressing PSMA (PSMA-negative compartments). Organs-at-risk are healthy organs expressing PSMA and target organs are affected organs expressing PSMA.

Results

The tissue distribution of 18F-DCFPyL was simulated in arterial blood, total tumor lesion, lungs and muscles. The model showed an accurate prediction of the distribution of 18F-DCFPyL in tumor tissue, arterial blood, lungs and muscles, as shown in Figure 2 for a typical patient. The mean prediction error in tumor tissue and all tissues was 6.7% and 8.9% (CI -38.9 – 56.7%), respectively.

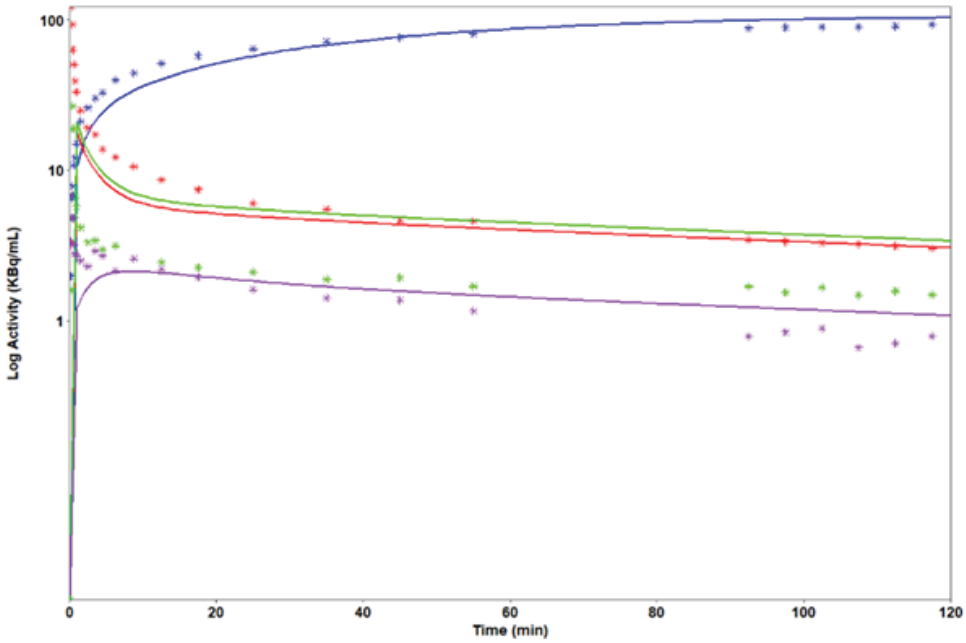

Figure 2. Time-activity curve of 18F-DCFPyL distribution of a typical patient in tumor (blue), blood (red), lungs (green) and muscles (purple). The distribution predicted by the wbPBPK-model is shown by the solid lines. The observed distribution in a typical patient is shown by the stars.

Conclusion

The final wbPBPK-model was able to adequately predict tissue distribution of 18F-DCFPyL when compared to observations from the prospective study [5]. When properly validated and predictive at therapeutic dose levels, the wbPBPK-model is a potential tool to determine an individual dose for therapeutic PSMA-radioligands to treat prostate cancer.

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Inducing receptor degradation as a novel approach to target CC Chemokine Receptor 2 (CCR2)

Dr. Natalia Ortiz Zacarías, BSc. Jeremy D. Broekhuis, Dr. Daan van der Es, Prof. Dr. Laura H. Heitman

Biography

Natalia Ortiz Zacarías completed her PhD in the field of Medicinal Chemistry at the Leiden Academic Centre for Drug Research (LACDR), Leiden University. Her PhD thesis was awarded the 2019-2020 best thesis prize from the KNCV-Medicinal Chemistry & Chemical Biology (MCCB). After her PhD, she continued working as a Postdoctoral Researcher at both the LACDR and Oncode Institute, focusing on novel pharmacological concepts to better target chemokine receptors in cancer. In 2021, she worked as Research Scientist in the startup ARTICA Therapeutics, focusing on the characterization of novel small-molecule drugs. In 2021 she also obtained a Veni grant from the Dutch Research Council (NWO) for her project "Towards illuminating and modulating chemokine receptor fate", which she is currently performing at the group of Prof. Heitman, at the LACDR and Oncode Institute.

Introduction

CC chemokine receptor 2 (CCR2) is a G protein-coupled receptor (GPCR) highly expressed in immune cells. Dysregulation of CCR2 has been linked to many inflammatory or immune diseases, such as atherosclerosis, multiple sclerosis and cancer. Yet, all CCR2 inhibitors developed so far have failed in clinical trials, which warrants the development of novel strategies to target this receptor. A novel approach to inhibit protein function is to induce the degradation of the target protein by harnessing the proteasomal degradation system with the use of proteolysis-targeting chimeras (PROTACs).

Objectives

Before we embark on the development of CCR2 PROTACs, we aim to investigate whether proteasomal degradation can be chemically induced for CCR2, and thus, if this approach can be used therapeutically.

Methods

To measure receptor degradation, we used luminescence-based assays by tagging CCR2 with a HiBiT tag. Complementation of HiBiT with an LgBiT subunit generates a luminescent enzyme, which enables protein quantification. To induce degradation we used the commercially available HaloPROTAC3, which selectively degrades HaloTag® fusion proteins.1 Thus, we designed and characterized a CCR2-HaloTag-HiBiT construct, which was transfected into HEK293T cells or HEK293 cells stably expressing LgBiT. In addition, we used a label free, impedance-based functional assay (xCELLigence) to measure changes in CCR2 activity after inducing receptor degradation.

Results

HiBiT assays show that treatment with HaloPROTAC3 leads to a marked reduction in CCR2 levels, while this is not observed after treatment with negative control or with known CCR2 antagonists (Figure 1). In addition, experiments in the presence of proteasomal or lysosomal inhibitors shed light on the degradation pathways involved in HaloPROTAC3-induced degradation of CCR2. Finally, xCELLigence assays show that degradation of CCR2 results in a reduced response after agonist stimulation.

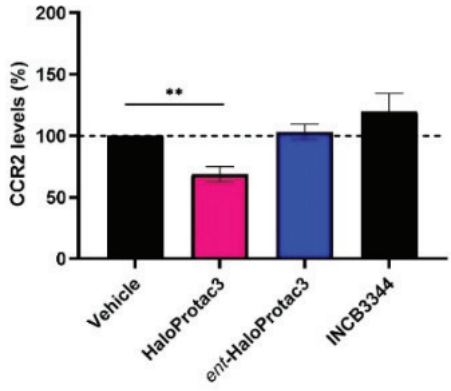

Figure 1. Effect on CCR2-HaloTag-HiBiT levels after 24h treatment with 1 µM of HaloPROTAC3, negative control ent-HaloPROTAC3, or reference CCR2 antagonist INCB3344. Luminescence was measured using the NanoGlo® HiBiT Lytic Detection system (Promega).

Conclusion

We successfully set-up a degradation assay for CCR2, and our data with HaloPROTAC3 show that CCR2 is amenable to targeted degradation. Therefore, the development of CCR2 PROTACs may represent a novel strategy to target this receptor in a variety of diseases.

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Chitosan oligosaccharides are promising agents to reduce the infection potential of the human pathogen Staphylococcus aureus

Mr Amirmohammad Afsharnia

Biography

Amirmohammad Afsharnia (Amir), was born in 1986, studied professional doctorate in veterinary medicine in Iran. He played several roles in poultry industry for 10 years. In 2021 he started his PhD at pharmacology department of Utrecht University. The main focus of his research project is to find anti-pathogenic effects of non-digestible oligosaccharides and human-milk oligosaccharides.

Authors

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Introduction

Staphylococcus aureus (S. aureus), a notorious bacterial pathogen, is becoming increasingly resistant to antibiotics and is a major public health problem. *S. aureus* can cause a range of acute illnesses, including food poisoning and gastroenteritis, and is capable of forming biofilms, which are difficult to eradicate. Staphylococcal infections are difficult to treat due to antimicrobial resistance and therefore, it is critical to identify alternative strategies for controlling and eliminating these infections.
Non-digestible oligosaccharides (NDOs) are complex carbohydrates that resist digestion in the small intestine and are known for their prebiotic properties by promoting the growth of beneficial intestinal bacteria. In addition to their prebiotic properties, NDOs possess several anti-pathogenic functions, and might be potential therapeutic agents to combat infectious diseases.

Objectives

The aim of this study is to evaluate the effects of NDOs, namely chitosan oligosaccharides (COS) on various pathogenic aspects of *S. aureus*. Furthermore, the structure-activity relationship of COS was investigated by including two monomeric building blocks of COS, including glucosamine hydrochloride (GAM) and N-acetyl-D-glucosamine (NGAM).

Methods

The antibacterial effects of COS, GAM and NGAM (0.25% to 8%) were evaluated by determining the minimum inhibitory concentration (MIC) and minimum biofilm inhibitory concentration (MBIC) against *S. aureus*. Additionally, the anti-inflammatory effects of COS, GAM and NGAM were investigated by measuring the cytokine interleukin-8 (IL-8) in human colorectal adenocarcinoma HT-29 cells stimulated with lipoteichoic acid (aLTA), isolated from the cell wall of *S. aureus*.

Results and conclusion

Although none of the NDOs tested (0.25% to 8%) affected the growth of S. aureus in the MIC assay, COS and GAM completely prevented the biofilm formation. Interestingly, GAM (2%) was far more potent than COS (2%). NGAM was hardly effective in the MBIC assay which points to a structure-activity relationship. Surprisingly, a similar structure- activity relationship for these NDOs was found for aLTA-induced IL-8 release from the HT-29 cell line. GAM and COS significantly inhibited the IL-8 release by more than 75%, while NGAM was ineffective.

In conclusion, depending on the structure, NDOs can modify several pathogenic aspects of S. aureus. GAM and COS could represent a novel and effective approach against antibiotic-resistant infections caused by S. aureus. The use of NDOs, as an alternative treatment for Staphylococcal infections, may also have broader consequences and may help to reduce the use of antibiotics, ultimately leading to a decrease in the emergence of antibiotic-resistant bacteria.

Preparation and characterization of lipid nanoparticles for the delivery of RNA

Heba Fayyaz

Biography

Heba is a PhD student in Department of Nanomedicine and Drug Targeting, Groningen Research Institute of Pharmacy (GRIP), University of Groningen, Netherlands, who is supported by a full PhD scholarship from the Cultural Affairs and Mission Sector of the Arab Republic of Egypt. She started her Ph.D. project on September 2022 under the supervision of Prof. Dr. Anna Salvati and Prof. Dr. Klass Poelstra. Heba completed her undergraduate degree in Pharmacy and her master's degree in Pharmaceutical Sciences (Pharmaceutics) at Alexandria University, Egypt. Before moving to Groningen, she was working as a teaching assistant of Pharmaceutics for six years at the Faculty of Pharmacy, Alexandria University, Egypt.

Authors

Heba A. Fayyaz[1], Anna Salvati[1]

1. Department of Nanomedicine and Drug Targeting, Groningen Research Institute of Pharmacy (GRIP), University of Groningen, The Netherlands.

Introduction

Among multiple types of nanoparticles, lipid nanoparticles (LNPs), which have recently arrived in clinical use with the RNA vaccines against COVID-19, have pushed the boundaries of the pharmaceutical industry [1]. They have become a leading non-viral vector for the delivery of nucleic acids, bringing both gene therapy and vaccination into a new rapidly-growing era. In order to be effective, after uptake into cells by endocytosis, the LNPs need to release their RNA load into the cytosol. The currently approved LNP formulations have been optimized to achieve this, however their transfection efficacy is still relatively low and many aspects of how after uptake the LNPs are processed by cells are not fully clarified. .

Objectives

In order to study these questions, in the first months of my project, the objective has been to test different methods for the preparation of LNPs. Using poly-adenylic acid (Poly A) as a model RNA molecule and a messenger RNA to produce the green fluorescent protein GFP, we compared the properties and cell behaviour (uptake and transfection efficacy) of the nanoparticles prepared under different conditions in HeLa cells.

Methods

LNPs are prepared using the ionizable lipid D-Lin MC3 DMA, the helper lipid DSPC, the PEGylated lipid DMG-PEG2000 and cholesterol. The fluorescently labelled diL lipid is included in order to be able to quantify LNP uptake by cells. LNPs with different polyA amounts are prepared using either vortexing or microfluidic mixing. Then, the LNPs obtained using the two methods are characterized by dynamic light scattering. This is done for LNPs dispersed in water, PBS, as well as in the cell culture medium supplemented with serum in order to test their stability in biological conditions. Poly A entrapment efficiency is determined using a Quant-it Ribogreen mRNA Assay kit. Finally, flow cytometry is used to quantify uptake of the LNPs by cells.

Results

The results obtained so far indicate that LNPs with comparable size and low polydispersity are obtained using both mixing techniques. In addition, a high poly A encapsulation efficiency is achieved, coupled with high loading capacity and the LNPs form homogenous dispersion in medium with serum. First studies by flow cytometry show that the cell fluorescence increases at increasing LNP concentration and exposure time, suggesting efficient internalization. First tests with LNPs containing mRNA confirm a high transfection efficacy on HeLa cells.

Conclusion

These preliminary studies confirm that LNPs encapsulating poly A or mRNA are obtained and show narrow size distribution and stability in serum. The particles are efficiently internalized by cells and show good transfection efficacy. The next steps will be to study in more detail their mechanism of uptake and intracellular trafficking.

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Quantification of tacrolimus in scalp hair of solid organ transplant recipients

Stephan Bakker, Tji Gan, Lenneke Junier, Daan Touw, Job van Boven, **Drs. Tanja Zijp**

Biography

Tanja Zijp is a 4-th year PhD candidate and trial pharmacist at the department of Clinical Pharmacy and Pharmacology of the University Medical Center Groningen. She earned her Bachelor of Pharmacy (2015), Honours College Bachelor (2015) and Master of Pharmacy degrees (2019) at the University of Groningen. Her research focuses on optimising treatment with tacrolimus in solid organ transplant recipients. Her research includes a pharmacokinetic observational drug-drug interaction study of tacrolimus with antifungal drugs, a literature review on alternative sampling methods, analytical method development using LC-MS/MS, usability studies for smart monitoring devices to study adherence, and retrospective and literature research on drug-drug interactions with immunosuppressants.

Authors

Zijp, Tanja[1*]; Junier, Lenneke[1]; van Boven, Job[1]; Gan, Tji[1] ; Bakker, Stephan[1]; Touw, Daan[1]

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Introduction

Tacrolimus is an immunosuppressant used to prevent graft rejection in solid organ transplant recipients (SOTr). Tacrolimus blood concentrations are frequently measured for therapeutic drug monitoring, but only provide information on circulating concentrations at a single moment. Hair analysis may be of interest to assess medication adherence and tacrolimus tissue exposure over longer periods.

Aim

To develop a method to quantify tacrolimus concentrations in hair and to demonstrate its feasibility in SOTr.

Material and methods

An LC-MS/MS method was developed and validated following FDA and EMA guidelines, including stability and reproducibility in tacrolimus-positive hair. Approximately 20 mg of hair was washed in dichloromethane and extracted with tacrolimus [13C,2H4] IS in methanol, under pulverisation with stainless steel balls in a mixer mill. Thereafter, the extract was centrifuged and filtered. Subsequently, 20 µL was automatically injected on the LC-MS/MS. As proof-of-concept, SOTr visiting the outpatient clinic were asked to donate a lock of hair for method development. Hair colour and last measured tacrolimus whole-blood concentration were registered. Hair was taken from the occiput, stored at room temperature protected from light, and segmented in sections of 1–3 cm.

Results and discussion

The method was successfully validated, being linear between 0.05–5.0 µg/L hair extract (2.5–250 pg/mg hair). Homogenised hair was stable for 2.5 years at room temperature (bias: -10.47%) and -80°C (bias: -0.56%), with small precision (<5%). Moreover, the method was used to measure 17 hair segments from 6 SOTr, with black (n=1) and grey (n=5) hair. Median [min–max] hair tacrolimus concentration in the most proximal segment was 13.6 [11.7–18.0] pg/mg. Hair concentrations generally declined in the segments further away from the scalp.

Conclusion

Tacrolimus concentrations can be successfully measured in hair. This method will be used to investigate further relationships with tacrolimus exposure and medication adherence in SOTr.

Keywords

hair analysis; transplantation; immunosuppressant; analytical validation; LC-MS/MS

Solvent-free synthesis of hybrid core/shell nanoparticles

Dr. Olivier Lugier, Dr. Stefania Grecea

Biography

Dr. Olivier Lugier obtained his PhD in 2021 from the University of Amsterdam (UvA) and the Advanced Research Centre for Nanolithography (ARCNL) where he worked on new patterning processes for extreme ultraviolet lithography using advanced materials.

Following his PhD he conducted a first postdoctoral project in the photoconversion materials group of the Vrij Universiteit (VU) and a second at the Functional Materials group of the UvA. Both project focused on the design and integration of advanced materials for chemical sensing. During his time at the Functional Materials group, Dr. Lugier also participated in the successful development of a novel and solvent-free synthesis method for hybrid core/shell nanoparticles.

Since the end of 2022, he has been leading a spinoff project to further develop the technology and enable the commercialisation of high quality nanoparticles for applications in biomedicine and other technological fields with high societal impact.

Introduction

Nanomaterials have emerged as a great opportunity to improve performances and fuel innovation in the field of medical sciences and biotech. But to bridge the gap between materials chemistry labs and clinical trials, better synthesis procedures are needed. Indeed, the methods currently used to produce the complex nanomaterials coveted for biomedical applications are based on lengthy processes with many purification steps that lack versatility and often yield low sample homogeneity and purity.

Objectives and method

The Functional Materials group of the University of Amsterdam (UvA) developed an equipment and the associated procedures for the one-step synthesis of a broad range of core/shell nanoparticles in the absence of solvents and surfactants. Many (semi)conductor materials can be used in combination with a wide range of options for the shell (molecules, metal-organic frameworks, polymers, etc.). Performed at ambient or mild conditions with a combination of vapor phase deposition methods, our procedure provides advantages in terms of efficiency, sustainability, costs and simplicity. In addition, the high purity and uniformity of the product saves the resources usually required for purification. 15+ specimens of inorganic (metal/oxide) and hybrid (metal/organic) core/shell nanoparticles with Iron, Nickel or Zinc as core materials were produced as proof-of-concept using this method.

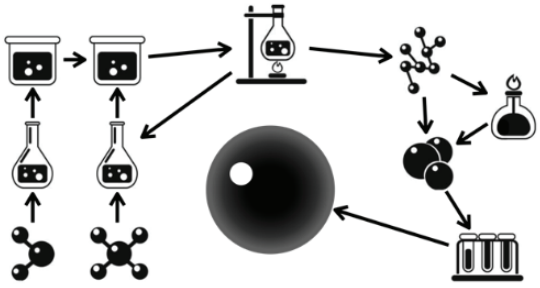
Results

Iron oxide nanoparticles have received a lot of attention for biomedical applications (anaemia medication, thermotherapy, MRI contrast enhancer, fluorescent labelling, etc.) due to their unique physical properties and better biocompatibility. For this reason, we are developing procedures to synthesise iron-based core/shell nanoparticles with relevant physical and chemical functionalities for the biomedical field.

Conclusion

Here, we report on the synthesis of amine-functionalised and maleimide-functionalised Iron core/shell nanoparticles. These specimens were designed to have (i) functional groups at their surfaces that can bind to or react with biomarkers or active molecules, and (ii) a strong core-shell interaction based on covalent bonds to provide high structural integrity. The characterization of the specimens is done with various spectroscopy (ATR, XRD, UV-Vis, PL, etc.) and microscopy (SEM, STEM) techniques.

Common procedures for nanoparticles synthesis

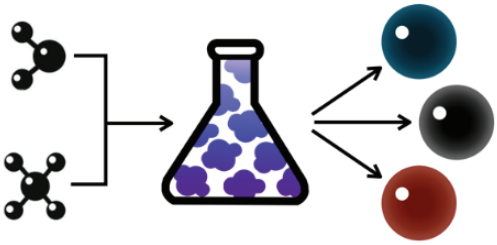


Complex | Lengthy | Wasteful | Limited

VS

NANO HYBRIDS

Solvent-free synthesis of hybrids core/shell nanoparticles



Simple | Direct | Solvent-free | Versatile

2'FL and 3FL prevent HDM-induced cytokine release in vitro and decrease HDM-specific IgE in an allergic asthma murine model

Marit Zuurveld, Annemijn de Kleer, Alinda Berends, Manou Kooy, Nienke Kettelarij, Ing. Ingrid van Ark, Ing. Thea Leusink-Muis, Prof. Dr. Gert Folkerts, Prof. Dr. Johan Garssen, Dr. Belinda van't Land, Dr. Linette E.M. Willemsen

Biography

Marit Zuurveld is a PhD student at the division of Pharmacology at Utrecht University. In her research she focusses on prevention of allergic diseases by specific components in human milk, the human milk oligosaccharides. Furthermore, she develops complex human in vitro models to mimic mucosal tissues, connecting intestinal and airway epithelium to underlying immune cells allowing to study key events in allergic sensitization.

Authors

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Introduction

Allergic asthma is characterized by sensitization to airborne allergens like house dust mite (HDM). Type 2 maturation of dendritic cells (DCs) results in development of T helper 2 immunity contributing to chronic inflammation. Human milk oligosaccharides (HMOS) improve immune maturation and potentially alleviate allergy development.

Objectives

An *in vitro* model for crosstalk between bronchial epithelial cells (BECs), DCs and T cells during HDM exposure and HMOS preincubation was established. Also HMOS were studied in a murine HDM-induced allergic asthma model.

Methods

Calu-3 BECs were cocultured with monocyte-derived DCs (moDCs) during HDM exposure and subsequently cocultured with naïve Th cells. Immunomodulatory effects of the HMOS 2'-fucosyllactose (2'FL) and 3-fucosyllactose (3FL) were studied. 6 Week-old male BALB/cAnNCrl mice were fed 0,1%-0,5% 2'FL or 3FL AIN93G diets two weeks prior to and during HDM sensitization and challenges.

Results

In Calu-3 cells, 10µg/mL HDM enhanced IL33 secretion and decreased barrier resistance. HDM exposure during BEC-DC coculture enhanced type 2 instructing TSLP, while reducing regulatory TGFβ secretion. Coculture of BEC-DCs with naïve Th cells enhanced allergy-related IL4 secretion. These effects were not observed upon direct HDM exposure of DCs. Preincubation of BEC-DCs with 2'FL or 3FL, prevented HDM-induced TSLP and IL8 release. 0,01% 3FL enhanced TGFβ release from BEC-DC, and these primed BEC-DC suppressed IL4 secretion by Th cells, while enhancing the percentage of IFNgamma expressing cells. HDM-allergic mice fed 1% 2'FL or 0,5% 3FL diets showed decreased serum HDM-specific IgE compared to mice fed control diet, and 1% 3FL reduced both IL13 and IFNgamma in lung homogenates.

Conclusion

This *in vitro* coculture model for HDM-induced BEC-DC activation and subsequent type 2 Th cell immune

development allows future in vitro studies on HDM mucosal immune responses. Both HMOS reduced HDM-specific IgE in a murine model for HDM allergic asthma, but did not protect against airway inflammation.

An advanced in vitro human mucosal immune model to predict food sensitizing allergenicity risk: a proof of concept

Marit Zuurveld, Dr. Cristina Bueno Díaz, Dr. Frank Redegeld, Prof. Dr. Gert Folkerts, Prof. Dr. Johan Garssen, Dr. Belinda van't Land, Dr. Linette E. M. Willemsen

Biography

Marit Zuurveld is a PhD student at the division of Pharmacology at Utrecht University. In her research she focusses on prevention of allergic diseases by specific components in human milk, the human milk oligosaccharides. Furthermore, she develops complex human in vitro models to mimic mucosal tissues, connecting intestinal and airway epithelium to underlying immune cells allowing to study key events in allergic sensitization.

Authors

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Introduction

The global demand of sustainable food sources leads to introduction of novel foods on the market, which may pose a risk of inducing allergic sensitization. Currently there are no validated *in vitro* assays mimicking the human mucosal immune system to study allergenicity risk of novel food proteins.

Objectives

The aim of this study was to introduce a series of sequential human epithelial and immune cell cocultures mimicking key immune events after exposure to the common food allergen ovalbumin from intestinal epithelial cell (IEC) activation up to mast cell degranulation.

Methods

This *in vitro* human mucosal food sensitizing allergenicity model combines crosstalk between IEC and monocyte-derived dendritic cells (moDC), followed by coculture of the primed moDCs with allogenic naïve CD4+ T cells. During subsequent coculture of primed CD4+ T cells with naïve B cells, IgE isotype-switching was monitored and supernatants were added to primary human mast cells to investigate degranulation upon IgE crosslinking. Mediator secretion and surface marker expression of immune cells were determined.

Results

Ovalbumin activates IEC and underlying moDCs, both resulting in downstream IgE isotype-switching. However, only direct exposure of moDCs to ovalbumin drives Th2 polarization and a humoral B cell response allowing for IgE mediated mast cell degranulation, IL13 and IL4 release in this sequential DC-T cell-B cell-mast cell model, indicating also an immunomodulatory role for IEC.

Conclusion

This *in vitro* coculture model combines multiple key events involved in allergic sensitization from epithelial cell to mast cell, which can be applied to study the allergic mechanism and sensitizing capacity of proteins.

Identification of areas in greatest need for innovation using the Drug Discovery, Development and Deployment (4D) Map.

MSc Marieke Meulemans, MBA, PharmD Brigitte Drees

Biography

Marieke Meulemans studied Health Sciences at the University of Maastricht. After a career at CROs and Pharma companies, she founded GCP Central in 2012 and still is the driving force behind the company's powerful vision. With 20 years experience in the life science industry, she is involved in many (inter) national initiatives and provides project management, training needs analysis, and process improvement solutions. Since January 2023, Marieke is Project Manager of the GAP analysis Infrastructure and Human Capital at Pivot Park, one of the founding parties of PharmaNL.

Introduction

Drug Development is often visualized as a linear, arrow shaped pipeline. However, this visualisation does not provide a realistic view on the complex process of drug and biologics development. Oversimplification of the process may lead to unrealistic expectations of scientists, investors in life science companies, policy makers and the public.1 The 4D Maps, published by the NIH (USA) in 2017, provide a network view of the development process.

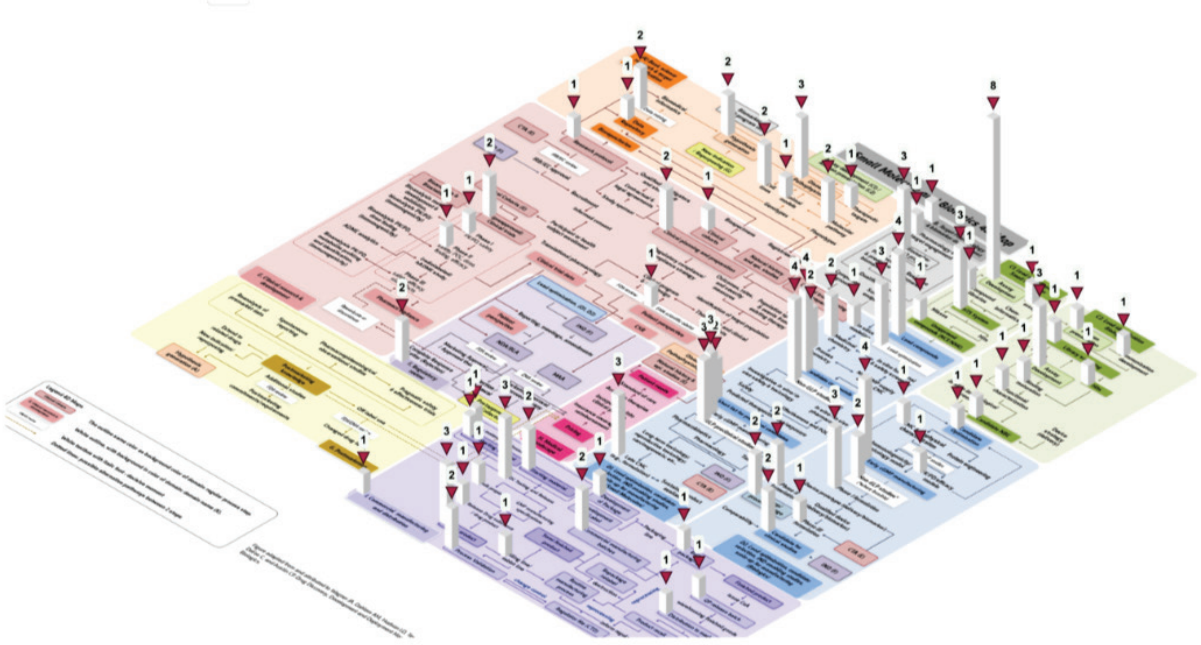
Objectives

PharmaNL, an initiative of Campus Groningen, Pivot Park, LIFE Cooperative, Leiden University / UMC in collaboration with FAST, focuses on strengthening and scaling up the research facilities and stimulating the related human capital. Before every open call, PharmaNL needs to identify areas in greatest need for innovation, by performing gap analysis.
The objective of the Proof of Concept is to investigate if using the 4DM tool to analyze the available and needed machines, equipment, and competencies enables a better prioritization of human capital and infrastructure investments.

Methods

In this Proof of Concept Study at Pivot Park, the 4D Map was discussed and validated by various stakeholders, adjusted and implemented in graph technology software, resulting in a standardized and sustainable online measuring method. Question layers were added for 'infrastructure development' and 'human capital development' showed to the interviewee via a survey per process step in a domain.

In the period March to May 2023, 30 CEOs / CSOs or similar of life science companies in the Oss region were interviewed using the 4D tool to perform a gap analysis of their required infrastructure (machines, equipment) and human capital (competencies and training needs) for innovation. All data was directly added to the tool by the Interviewer, data recorded was provided to the company via a report for review and corrections. Data analysis was done via the software metrics and excel. Feedback provided on the usability and contents of the map was recorded in the 4D Map tool via comments.



3D Visualisation of results: number of companies involved in a process step

Results

4DM provides a template which fit the process activities of biotech and pharmaceutical product development companies, CROs, CMOs and CDMOs. The structured interview process and data capture process leads to a dataset which is standardized and reusable.

Conclusion

Given the rapidly changing environment for drug development and biologics development, the 4D Map and Tool needs adjustment on process steps in certain domain, though provides a usable framework to ongoingly discuss and standardize the Drug and Biologics Development process, enabling conversations between policy makers, regulators, investors, scientists and public.
Implementing the 4DM tool to analyze the available and needed machines, equipment, and competencies may result in a better prioritization of human capital and infrastructure investments by biopharmaceutical campuses, companies, institutes and governments and enables investments to be selected objectively.

Predicting IBD Patient Response to Infliximab Therapy Using Machine Learning

Péter Tőzsér, Luciënne de Vries, Msc David Rojas-Velazquez, Dr. Alejandro Lopez-Rincon, Prof. Dr. Johan Garssen, Sarah Kidwai

Authors
Péter T˝ozsér[1], Luciënne de Vries[1], David Rojas-Velazquez[1,2], Alejandro Lopez-Rincon[1,2], Johan Garssen[1,3], Sarah Kidwai[1]

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Introduction

Inflammatory Bowel Disease (IBD) is a chronic disease encompassing ulcerative colitis (UC) and Crohn's disease (CD) which is increasingly prevalent inWestern societies [1]. Symptoms include abdominal pain, diarrhea, weight loss, constipation, and fatigue [2]. The prevailing biological agents used for treatment is anti-TNF-α agents (e.g. infliximab) and anti-integrin agents (e.g. vedolizumab). Early treatment of IBD is crucial to avoid complications. Unfortunately, utilizing conventional diagnostic techniques, remains a challenge to accurately predict a patient's response to a specific treatment prior to its administration [3]. Therefore, novel biomarkers are needed that can be validated through different mRNA datasets. The early application of effective biologics can minimize corticosteroid use, surgeries, hospitalizations, and enhance quality of life [4].

Objectives

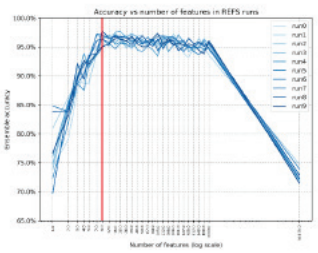
To identify key infliximab response predicting biomarkers in patients with IBD using Recursive Ensemble Feature Selection (REFS). As reproducibility issues are often encountered in these studies, we aim to validate the selected gene signatures in an independent dataset.

Methods

The mucosal mRNA data from Arijs et al [5] underwent analysis via REFS. The dataset consists of 61 mucosal biopsies from 61 IBD patients including untreated CD and UC samples. Employing 8 classifiers from the sci-kit toolbox [6], REFS conducted a nested 10-fold cross-validation, with an additional validationstep involving 5 different classifiers to prevent overfitting. The selected genes were searched in an independent database [7] to validate its effectiveness in predicting therapy response. For determining diagnostic accuracy, we used average AUC [8].

Results

REFS selected 9 genes (206172_at (IL13RA2), 220012_at (ERO1-L(BETA)), 1569830_at (PTPRC), 239233_at (CCDC88A), 1564253_at (N/A), 232834_at (N/A), 1568787_at (N/A), 1563044_at (N/A), 1561144_at (N/A)) from the original 54675 with an average AUC of 0.984. The same 9 genes were found in an independent dataset. After applying the validation step, we obtained an average AUC of 0.666. In figure 2 the best individual AUC for each validation step is shown.



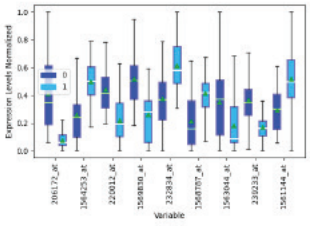
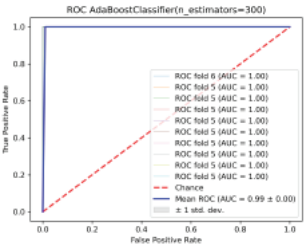


Figure 1: Highest accuracy with a set of 9 genes after 10 runs of REFS (left). Boxplot of the 9 selected genes in responders and non-responders (right).



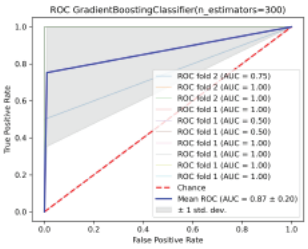


Figure 2: Individual AUC of the best classifier using 9 selected genes on the original dataset (left). Individual AUC of the best classifier using 9 selected genes on the independent dataset (right).

Conclusion

From 9 selected genes that differentiate infliximab responders from non-responders, we identified 4 that were already characterized. 3 of these 4 can be linked to the immune system. The first one, IL13RA2 has been identified as a marker for anti-TNF responsiveness in patients with IBD [7, 9]. Figure 1 (right) shows upregulation in the mucosal mRNA expression of the gene as previously found. It has also been suggested that increased expression of IL13RA2 mRNA reflects the high TNF burden that is associated with non-responders. [9] The last 2 genes that can be directly connected to the immune system are CCDC88A (GIV) [10] and PTPRC (CD45) [11]. By investigating these biomarkers in patients prior to therapy, upon non-responsiveness, an alternative treatment can be used.

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Personalizing IBD: The Search for Genetic Markers Using Machine Learning

Luciënne de Vries, Péter Tőzsér, MSc Sarah Kidwai, Dr. Alejandro Lopez-Rincon, prof. dr. Johan Garssen, MSc David Rojas-Velazquez

Biography

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Introduction

Inflammatory bowel disease (IBD) is the collective name of two major forms, Crohn's disease (CD) and ulcerative Colitis (UC). These conditions are characterized by chronic relapsing inflammation of the gastrointestinal (GI) tract and are caused by: genetic susceptibility, abnormal gut microbiota, immune response dysregulation, and environmental changes. There is currently no treatment for IBD, but it can be managed by using immunosuppressants, amino salicylates and/or biologics and currently, there are 10,000 people living with IBD worldwide. [1]

Objective

To improve IBD diagnostics by identifying new genetic biomarkers.

Materials and methods

The raw data was collected from Gene Expression Omnibus (GEO), from the paper of Burczynski et. al [2], and analyzed using Recursive Ensemble Feature Selection (REFS). REFS is a method to discover biomarkers. The REFS algorithm for the selection phase is composed of 8 classifiers from sci-kit learn [3] in a nested 10-fold cross-validation and the validation was done by taking the genes from another data set and running the same 8 classifiers. The data was divided into two groups, IBD (group 1) and healthy volunteers (group 0). There were 85 IBD samples and 42 healthy controls.

Results

REFS gave us 12 genes from 22,283 with an average AUC of 0.97 which translates to an excellent diagnostic accuracy and validation to an average AUC of 0.79 which gives good diagnostic accuracy [4]. Eight genes were downregulated (bladder cancer associated protein (BLCAP), lysine (K)-specific demethylase 2A (KDM2A), ring finger protein 114 (RNF114), nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 interacting protein (NFATC2IP), KRI1 homolog (S. cerevisiae), uncharacterized LOC100652930 /// aminopeptidase-like 1 /// STX16-NPEPL1 readthrough (NMD candidate), high mobility group box 1 (HMGB1), and ubiquitin-conjugating enzyme E2G 1) and four upregulated (histone cluster 2 H2be, histone cluster 1 H2bh, transmembrane protein 123 (TMEM123), and

amyloid beta (A4) precursor protein) in IBD patients compared to healthy controls.

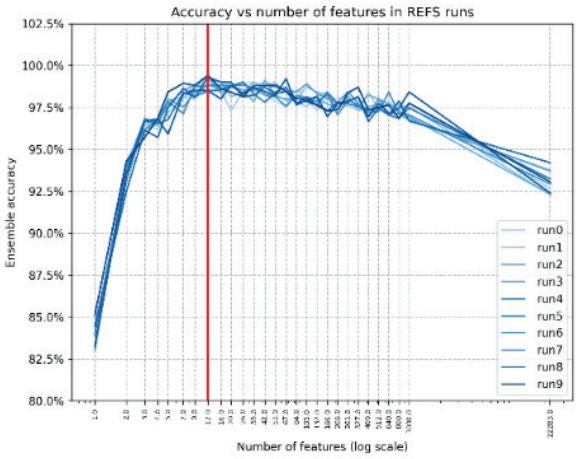

Fig.1: Highest accuracy with 12 genes after ten runs of REFS.

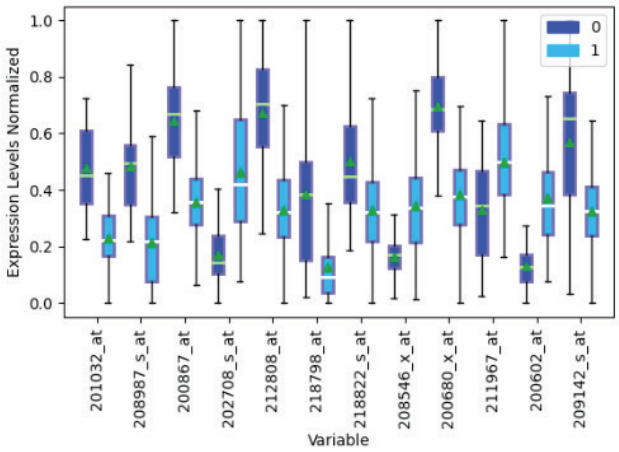

Fig.2: Boxplot of the slected genesin samples and controls

Discussion

Of the 12 genes found by REFS, multiple genes were already found to be diagnostic markers in IBD. However, little information was available regarding functional context of these genes. More information would be beneficial to find new therapeutical targets for IBD.

Firstly, histone cluster 1 and 2 have been found to contribute to the development and function of both intestinal epithelium and T-cells, since differences in chromatin organization have been linked to a difference in gene expression in IBD patients. [5]

Secondly, HMGB1 has been identified as a potential target for IBD treatment, since HMGB1 induces inflammation by inducing IL-6 production. [6]

Lastly, ubiquitin-conjugating enzyme E2G 1 is downregulated in IBD patients. The ubiquitin modifying enzymes work in synergy for optimal ubiquitination of proteins for maintaining intestinal homeostasis. [7]

We hope that by using these biomarkers for diagnostics, the screening of IBD can be improved and will be helpful for future research into treatment options for IBD.

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Semi-mechanistic modeling of temozolomide induced myelosuppression: a missing link in the prediction of severe thrombocytopenia

BSc Daan van Valkengoed, MSc Medhat Said, MSc Nienke Grun, Dr. Imke Bartelink, Dr. Mathile Kouwenhoven

Biography
Daan van Valkengoed is a master student Bio-Pharmaceutical Sciences at Leiden University. He has a keen interest in pharmacometrics and (clinical) pharmacology. This work was part of a 6 month research internship he followed at the Amsterdam UMC - location VUmc, under guidance of Medhat Said and Imke Bartelink.

Authors
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*equally contributed

Introduction
Temozolomide (TMZ) is the gold-standard chemotherapeutic for the treatment of Glioblastoma Multiforme (GBM). TMZ causes morbidity, thereby decreasing the quality of life in most patients. Severe myelotoxicity occurs in approximately 6-11% of patients, causing treatment adjustments and abrogations, which may reduce long term response1. Therefore, understanding which patients are at risk for development of severe myelotoxicity is crucial to optimize treatment.

Objectives
We aimed to identify covariates predictive of severe myelosuppression.

Methods
This study applied a semi-mechanistic thrombocytopenia model to predict platelet count-time curves in 52 GBM patients (24 male, 28 female) treated with TMZ at the Amsterdam UMC. Covariates available were sex, the number of platelets at baseline, BMI, and concomitant drug-use. The developed model was evaluated via diagnostic plots and VPCs.

Results
TMZ concentrations inhibited the proliferation of blood cells precursors through a linear association: drug effect = TMZconcentration x Cslope of 0.208 (1.5% RSE), with a delay of 14.3 days (6.3% RSE) until manifestation on platelet counts. The covariates of interest did not associate with toxicity (Cslope). 23% of all patients needed a platelet transfusion and showed a higher mean Cslope of 0.9 (±0.335 SD) versus 0.175 (±0.105 SD), $p<0.001$, suggesting the presence of unidentified covariate(s) predictive of severe thrombocytopenia. We used mixture models to identify patient groups with different toxicity profiles (Cslopes). With two mixtures, 59% of patients had a low Cslope of 0.1 in mixture one. All 12 transfused patients and 9 non-transfused patients were allocated to the second mixture. Here, the non-transfused patients' Cslope was lower (0.347) than the transfused patients' Cslope (1.02), $p < 0.001$. Women were overrepresented in the transfusion group (75%). Preliminary results with three mixtures to split up mixture two, show that with 4 weeks of data (mean of 5 platelet samples per patient), we were able to re-estimate 69% of the original mixture allocations.

Conclusion
The current study was not able to identify patient characteristics that are predictive of a platelet transfusion during TMZ treatment. With further refinement, the mixture model may be applied to predict severe toxicity based on samples taken early in the treatment. In literature, MGMT promotor methylation has been linked to TMZ toxicity2. The 41% of patients in mixtures two and three match reported MGMT methylation incidence3. This suggests that MGMT status may be the missing covariate of risk, supported by similar sex distribution in the mixtures as found for MGMT methylation4,5. Therefore, inclusion of

pharmacokinetic and pharmacogenomic data in the comprehensive PKPD model could aid in identifying patients at risk for severe myelotoxicity and allow for personalized dosing regimens to explore the optimal balance between efficacy and toxicity.

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Lipopolysaccharide (LPS) induces growth in mouse lung organoids through inflammation and tissue remodeling associated pathways

Dan Li, Kelly B.I. Douglas, Rosa K. Kortekaas, Sophie Bos, Prof. dr. Reinoud Gosens, Prof. dr. Martina Schmidt

Biography

The presenter, Dan Li, was born in China in 1994-4-10. She started her bachelor's in Veterinary Medicine at China Agricultural University (CAU) in the summer of 2012 and continued her master in Veterinary Medicine at CAU in 2017. In 2019, she started her PhD study at the department of Molecular Pharmacology, University of Groningen, under the supervison of Prof. Martina Schmidt, Prof. Reinoud Gosens and Prof. Barbro Melgert. Her research focused on the mechanism of potential novel theraputic target of Chronic Obstructive Pulmonary Disease (COPD) and asthma. One of her research projects is partly showing in this abstract. In 2019, she received the scholarship from China Scholarship Council (CSC). During her study, she participated in several national/ international conferences by oral presentation or poster presentation, such as the Dutch lung congress 2022 (oral presentation), the Lung Science Conference 2023 (poster presentation).

Authors

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Respiratory infections in the lungs are a significant global cause of mortality. While bacterial infections can occur even in healthy individuals, they can exacerbate chronic obstructive pulmonary disease (COPD) and asthma in affected patients. Such infections trigger an inflammatory response and tissue repair process, marked by immune cell infiltration and fibrosis. This study focused on exploring the impact of lipopolysaccharide (LPS), a prominent constituent of Gram-negative bacteria's outer membrane, on murine lung organoids. The aim was to unravel the mechanism through which LPS influences the inflammatory response and tissue remodeling in the lungs.

Mouse lung epithelial cells (identified as CD31-CD45-EpCAM+) were isolated and co-cultured with mouse CCL206 fibroblasts. This co-culture system was then exposed to 100 ng/ml of LPS. Following a 14-day exposure to LPS, the number and size of the organoids were measured. Additionally, after a 3-day exposure to LPS, the organoids were sorted into two groups: EpCAM+ cells and fibroblasts. These groups were subjected to bulk RNA sequencing analysis to examine gene expression profiling patterns. Furthermore, after a 24-hour exposure to LPS on the 14th day of organoid culture, the medium in which the organoids were cultured was collected to measure the secretion of mouse KC cytokine (the homologue of human interleukin-8) using an enzyme-linked immunosorbent assay (ELISA).

LPS had significant effects on mouse lung organoids. It increased the number and size of the organoids, suggesting that it altered the activation of epithelial progenitor cells and promoted organoid proliferation. Additionally, when the organoids were treated with LPS for 24 hours at the 14th day, the secretion of KC (a marker of inflammation) was increased, indicating that LPS also influenced inflammatory responses in the organoids. Furthermore, examining the gene expression patterns through bulk RNA sequencing, both EpCAM+ cells and fibroblasts in the organoids showed inflammatory markers like CXC motif chemokine ligand 3 (Cxcl3), CXC motif chemokine ligand 5 (Cxcl5), C-C motif chemokine ligand 20 (Ccl20) and Lipocalin-2 (Lcn2) were up-regulated (with a significant log-fold change of 5), and fibrosis-related markers such as Matrix Metallopeptidase 13 (Mmp13), Interleukin 33 (Il33) were up-regulated (with a log-fold change of 2), indicating that LPS affected both the inflammatory response

and tissue remodeling processes involving EpCAM+ cells and fibroblasts. Pathway enrichment analysis revealed that LPS treatment affected several pathways, including the TNF-α signaling via NF-κB pathway and the epithelial-mesenchymal transition pathway. These findings suggest that LPS may exert its effects through these pathways, potentially contributing to the observed changes in the organoids.

LPS stimulates epithelial progenitor cells activation and mouse lung organoids proliferation as well as inflammatory cytokine secretion. These effects are linked to the activation of gene expression pathways involved in inflammation and tissue remodeling in both EpCAM+ cells and fibroblast.

Synbiotics, a promising approach to reduce the exacerbated allergic airway immune responses in offspring maternally exposed to cigarette smoke

Ali Dehghani, Dr Lei Wang, Dr Jeroen van Bergenhenegouwen, Prof. Johan Garssen, Prof. Gert Folkerts, Dr. Saskia Braber

Biography

Ali Dehghani is a PhD candidate in Pharmacology at Utrecht University. His research focuses on the potential of dietary intervention to reduce the risk of allergic airway immune responses in pups maternally exposed to air pollution through gut-lung axis.

Authors

Ali Dehghani[1], Lei Wang[1], Jeroen van Bergenhenegouwen[1,2], Johan Garssen[1,2], Gert Folkerts[1], and Saskia Braber[1,2]

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Air pollution remains one of the biggest and most immediate environmental threats to human health. Environmental cigarette smoke (CS) exposure serves as a primary contributor to household air pollution in both developing and developed countries. Exposure to air pollution has detrimental effects on human health, starting during early pregnancy and persisting throughout childhood and adolescence, ultimately contributing to the development of severe respiratory diseases.

Considering the importance of immune development in early life, the present preclinical study investigated the effects of synbiotic supplementation on allergic asthma symptoms in house dust mite (HDM)-sensitized and challenged pups maternally exposed to CS. Pregnant dams were exposed to either CS or air during pregnancy and lactation. After weaning, offspring received a control or synbiotic diet, and were intranasally sensitized and challenged with HDM to induce allergic asthma. After the HDM challenges, the lung function, bronchoalveolar lavage (BAL) cell counts, T cell subsets in the lungs and antigen-specific serum immunoglobulins were measured in the offspring. In addition, the composition of the gut microbiome was analyzed.

In HDM-sensitized and challenged offspring of CS-exposed dams, lung resistance and antigen-specific serum immunoglobulins (IgE and IgG1) were significantly higher compared to the PBS-treated group. Synbiotic supplementation effectively mitigated this increase in lung resistance and elevated immunoglobulin levels. HDM-sensitized and challenged offspring of air-exposed dams showed increased numbers of eosinophils in BAL fluid, while maternal CS exposure further enhanced this inflammatory response in the offspring. Synbiotic supplementation significantly reduced the eosinophil numbers in BAL fluid of HDM-treated offspring from air and CS-exposed dams. Furthermore, Th2 cell activation was higher in HDM-allergic offspring born to CS-exposed mothers, and synbiotic supplementation tended to decrease the infiltration and activation of Th2 cells. Additionally, synbiotic supplementation altered the composition and richness of the gut microbiome, favoring the presence of beneficial microbes, like bifidobacterium and akkermansia.

In conclusion, early life synbiotic supplementation can exert a beneficial influence on allergic asthma by reducing the exacerbated allergic airway responses, and altering the diversity and composition of the gut microbiome in HDM-challenged offspring maternally exposed to CS. These findings suggest the potential of synbiotics as an immediate and safe clinical strategy for the management of allergic asthma in context of maternal air pollution exposure.

Production costs of 3D printed pharmaceutical tablets: a case study with hydrocortisone for adrenal insufficiency.

MSc Sejad Ayyoubi

Biography

Sejad Ayyoubi is a pharmacist by training. He became familiar with 3D printing technology during his research project at the Complutense University of Madrid. After obtaining his master's degree in early 2020 he started working as a production pharmacist at Apotheek Haagse Ziekenhuizen. Around the same time, due to his fascination on 3D printing, Sejad set-up a research project regarding the 3D printing of hydrocortisone. He started looking for an unmet medical need which could not be solved using conventional production techniques. The idea to print hydrocortisone came up in meetings with an endocrinologist who was treating patients with adrenal insufficiency. Sejad's work was noticed among colleagues and a PhD position at the Erasmus university Medical Center followed in 2021. His research varies from formulation development to economic aspects of 3D printed medication. He aspires to make personalized medication a reality for the benefit of patients.

Authors

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Introduction

3D printing (3DP) allows pharmacists to personalize medication by modifying drug dosage, release profile, taste, and shape. While clinical trials for 3D-printed medication are underway, the economic perspective, especially concerning production costs, is lacking. Costs insights are paramount for the technology's progression and its integration within pharmaceutical care.

Objectives

The aim of this study was to construct a framework to quantify the production costs of 3DP. Subsequently, this framework was applied to estimate the production cost of a case study of hydrocortisone in the setting of an academic medical center.

Methods

A micro-costing study was conducted based on fused deposition modeling 3DP. 3D printed hydrocortisone (M3DICORT) was used as case drug. The 3DP process was divided in 3 uniform steps, pre-printing, printing and post-printing, and subdivided in mutually exclusive cost categories (materials, equipment, facility and materials). This framework was used to calculate production costs in different scenarios. Since commercial inks are currently not available, we have included ink production costs in the main analysis, where full production takes place in an academic hospital setting. In other scenario analyses we assumed that commercial parties scale-up production of inks, which can be purchased for printing personalized medication locally. We here present ranges of outcomes instead of fixed numbers, emphasizing the evolving nature of our ongoing research.

Results

Weighing, mixing, ink production, printing, packaging and quality assurance of one tablet translates to 0.74 – 0.98 euro in personnel costs (Fig. 1). Material costs, including raw materials, packaging and disposables are 0.36-0.40 euro per printed tablet. Equipment and facility costs are respectively 0.03-0.06 and 0.12-0.24 euro per printed tablet. Costs for quality control of one tablet are estimated at 0.06 euro and included in the materials category. Total costs of a printed MEDICORT tablet produced in an academic setting is estimated between 1.30-1.80 euro. Several factors impacted the costs. Tablet rate production is an important factor for instance, as new printers contain multiple printing heads, printing times can be reduced by a factor 2—3. Furthermore, costs are also influenced by sharing equipment and facilities instead of dedicating them to one product.

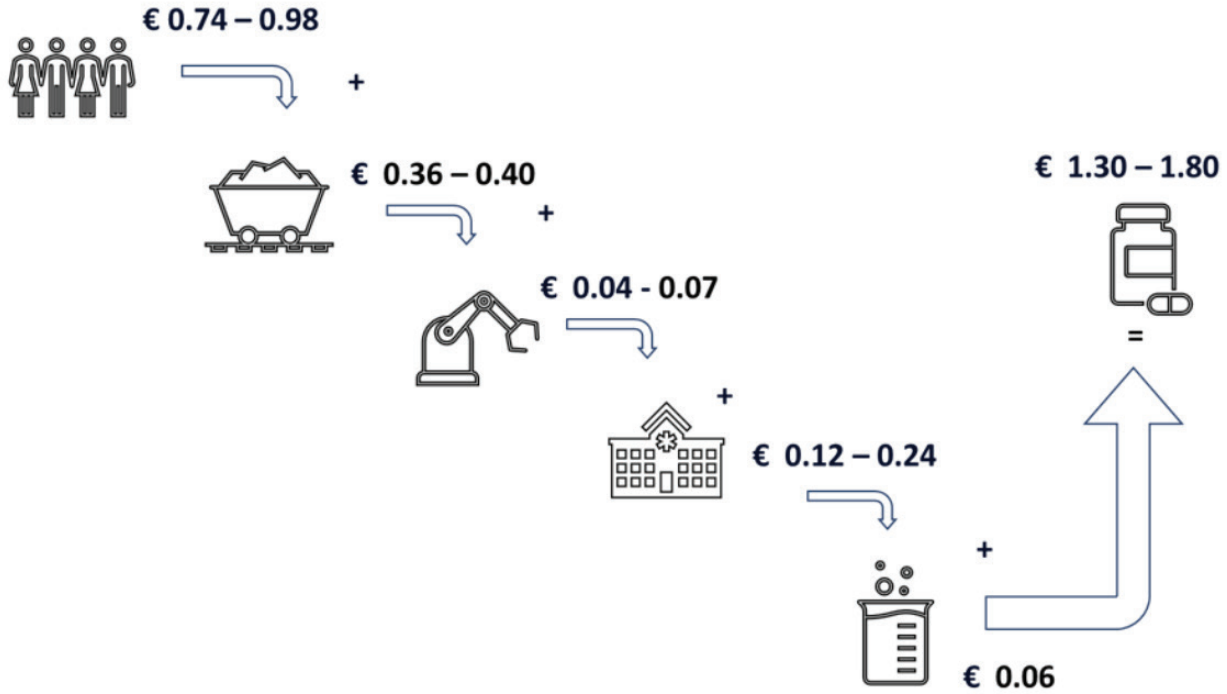

Figure 1. Costs of production of a 3D printed hydrocortisone tablet. From left to right: costs for personnel, materials, equipment, facility, quality control and final product.

Conclusion

This study provides a framework for estimating production costs of 3DP medication. This is useful for pharmaceutical insurers, manufacturers, policy makers and regulators. It can be used to understand and optimize the financial aspects of personalized medication production, which is crucial for the success of this technology in pharmaceutical practice. To demonstrate the framework's utility, we calculated the production costs of a M3DICORT tablet (1.30-1.80 euro). This is a preliminary estimate which needs refinement, further scenario analyses will elucidate other elements that impact production costs. While costs appear high, personalized medication has the potential to lower the disease-burden compared a one-size-fits-all approach and therefore reduce general healthcare costs.

Dietary Influences on the Composition of Breast Milk Microbiome

David Rojas-Velazquez

Biography

David Rojas-Velazquez completed his undergraduate studies in computer science engineering at Benemerita Universidad Autonoma de Puebla. He then pursued a master's degree in computer science at Universidad de las Americas Puebla. Currently, David Rojas-Velazquez is a PhD candidate in the Pharmacology Division at Utrecht University, where he is also affiliated with the data center at Julius Center UMC.
His research focuses on utilizing machine learning techniques to identify biomarkers for the diagnosis of autoimmune diseases. Specifically, David Rojas-Velazquez is currently involved in the development of a methodology for analyzing microbiome 16s rRNA sequences. This methodology has already been applied to conditions such as ASD, IBD, and T2D, showcasing its potential impact.
David's scholarly contributions are evident with an H-index of 3 according to Google Scholar.

Authors

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Introduction

A mother's diet can influence the composition and diversity of the microbiome in her own milk. For instance, the bacteria Staphylococcus and Bifidobacterium are linked to carbohydrate intake, while the Streptococcus genus is associated with the consumption of certain fatty acids. This can affect the baby's health, as research has shown that changes in the intestinal microbiota may increase the risk of allergies, asthma, and eczema [1].

Objectives

Use machine learning algorithms to identify taxa differences in breast milk samples from different diets. This will be a first step towards finding the healthiest diet and proper interventions.

Methods

Data

74 samples from Bioproject PRJNA848748 [2] of 16S rRNA Seq of human breast milk microbiota were processed with DADA2 [3], where 31 subjects had omnivorous diet, 18 vegetarian and 25 vegan.

Feature Selection

From DADA2 with SILVA r138 [4] we get 5,510 taxa after pre-processing. Then, we apply recursive ensemble feature selection (REFS) algorithm [5, 6, 7] to find a reduced set of meaningful taxa than can differentiate among the 3 groups (omnivorous, vegetarian and vegan). REFS uses 8 classifiers to rank features and reduce the feature set by 20%, selecting the best microbiota set in 10 runs. In a 10-fold cross-validation scheme, REFS conducts testing using 5 classifiers that were not included in the biomarker discovery phase.

Results

REFS was applied to the 74 samples identifying 33 taxa that can distinguish among the 3 groups, Fig.

1. The original study reports that there are no significant differences in diversity among the vegan, vegetarian, and omnivore (non-vegetarian) groups. However, by using a machine learning-based multivariate approach, we can distinguish among these groups, where the Multi-layer Perceptron (MLP) classifier has an accuracy of 91.67% and standard deviation of 0.1263 in a 10-fold cross-validation. The MLP's confusion matrix shows class accuracy of 87.10% for non-vegetarian, 88.89% for vegetarian, and 96% for vegan. The most common genus from the 33 taxa is Lactobacillus. The results point that an omnivore diet has more Lactobacillus in the breastmilk microbiota in general

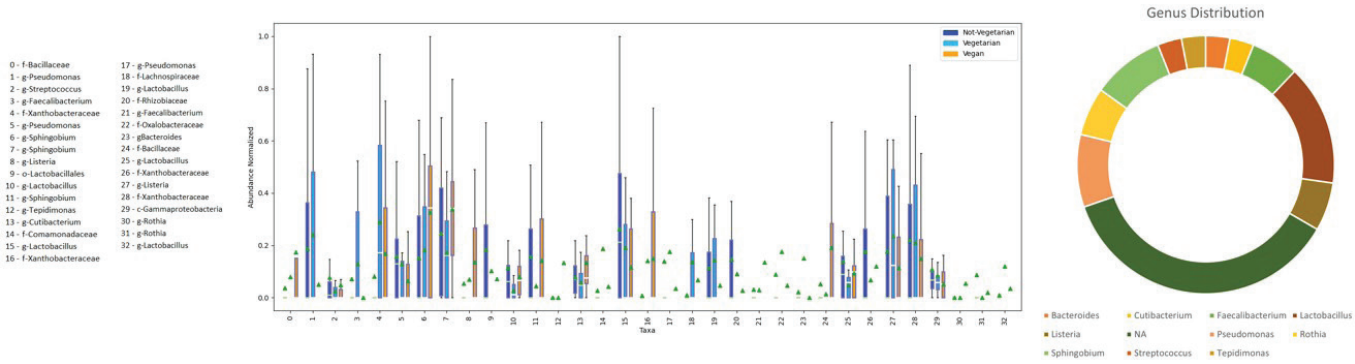

Figure 1: Normalized Abundance of most important microbial taxa and genus distribution.

Conclusion

Maintaining a healthy and diverse balance of microbes in the gut can help to prevent var-ious immune-related diseases. By studying changes in the microbiome, researchers can create specific dietary recommendations to promote a healthy microbiome. As mentioned, the genus Lactobacillus appears as one of the key microbiota to differentiate among groups. Lactobacillus is a type of bacteria that is classified under the Firmicutes phylum and is regarded as one of the key health promoting bacteria in the gut microbiome [8]. Studies have shown that giving Lactobacillus reuteri to breastfed infants can significantly decrease the amount of time they spend crying due to colic [9]. While further research is needed, these findings suggest that a mother's diet can influence the composition of her breast milk. This, in turn, can impact the baby's health by altering the microbiome of the gastrointestinal tract.

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PBPK modelling of azithromycin, clarithromycin and erythromycin exposure during pregnancy for treating chlamydia: towards optimizing macrolide pharmacotherapy in pregnancy

Edine Remmers, Joyce van der Heijden, dr. Joris van Drongelen, prof. dr. Saskia de Wildt, dr. Rick Greupink

Biography

Edine is currently in the final stage of completing her Bachelor's degree at Radboud University. After summer she will start the Toxicology and Drug Safety specialisation of the Biomedical Sciences Master, also at Radboud University. During her Bachelors internship at the division of Pharmacology and Toxicology at the Radboud UMC she focused on developing PBPK models to assess exposure to macrolides during pregnancy.

Authors

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Introduction

The WHO estimates that 129 million new cases of chlamydia trachomatis occur on a yearly basis. Chlamydia poses a risk in pregnancy by increasing the risk of premature rupture of the membranes, intra-amniotic infection and early-onset sepsis, which could eventually lead to adverse pregnancy outcomes. In order for therapy to be effective, a suitable macrolide antibiotic and dose should be selected that results in effective maternal and foetal concentrations. Since pregnancy results in physiological changes that alter maternal pharmacokinetics there is no consensus on which macrolide dose is best used. In addition, there is only limited data on placental transfer, accumulation in the placenta and potential adverse drug effects on placental cells.

Objectives

» To develop pregnancy physiologically-based pharmacokinetic (PBPK) models for azithromycin, clarithromycin and erythromycin to simulate macrolide exposure during pregnancy.
» To study placental transfer of the macrolides in the ex vivo perfused human placenta.
» To study effects of macrolides on placental trophoblast cell viability.

Methods

Simcyp v22 (Certara, UK) was used to develop and verify in silico PBPK models for azithromycin, clarithromycin, and erythromycin, first in non-pregnant individuals and ultimately in pregnant women. Model performance of a single dose was assessed and verified in a virtual healthy adult population for all three pharmaceuticals (intravenous or oral dosing). Ex vivo placenta perfusion experiments provided data for the transplacental transport of the macrolides. To exclude that predicted placental tissue concentrations would have a negative effect on placental function, in vitro BeWo cell exposure assays were performed to study effects on mitochondrial function.

Results

In non-pregnant individuals, PBPK modelling successfully captured known exposures observed in published clinical trials. Predicted-to-observed ratios of PK parameters were generally within 2-fold, which is in line with accepted quality criteria for PBPK modelling. Ex vivo placenta perfusions show that all macrolides cross the placental barrier, but to different extents: 18% for azithromycin, 24% for

clarithromycin and 23% for erythromycin of initial maternal circuit concentration reaches the foetal circuit after 180 minutes of perfusion. This data was used to derive a preliminary set of placental transfer rates, which were incorporated into a pregnancy PBPK model, which also accommodated maternal physiological changes occurring during pregnancy. Subsequent modelling demonstrated that standard macrolide dosages are expected to reach adequate maternal concentrations to treat chlamydia during pregnancy. Foetal exposure appeared to be suboptimal. Predicted maximum placental tissue concentrations were 3.0 mg/L, 3.8 mg/L and 5.8 mg/L for azithromycin, clarithromycin, and erythromycin, respectively. In vitro toxicity studies suggest that erythromycin may reduce trophoblast mitochondrial function at tissue concentrations that follow from a clinically relevant dose.

Conclusion

Our preliminary data indicates that the standard dosages of macrolides reach adequate maternal plasma concentrations to treat chlamydia during pregnancy, while foetal exposure appears suboptimal. Erythromycin may have a negative effect on trophoblast mitochondrial activity after a clinically relevant dose. Future studies will address alternative dosing strategies to optimize pharmacotherapy with macrolides during pregnancy.

Intestinal tissue organoids to study drug transport and metabolism in different regions & pediatric age groups

Eva Streekstra, Dr. Marit Keuper, Dr Rick Greupink, Prof Frans Russel, Dr Evita van de Steeg, Prof Saskia de Wildt

Biography
I studied Molecular Nutrition and Toxicology at Wageningen University. Now my PhD is at the Pharmacy department devision of Pharmacology and Toxicology in collaboration with TNO department of Metabolic Health Research.

Introduction
For the development of drugs, a decent understanding of intestinal absorption, including possible regional differences and age-related effects, is paramount to reach appropriate bioavailability and related drug efficacy. Most drugs are prescribed orally, however, translational preclinical models to investigate oral drug exposure in various age groups are lacking.

Objectives
This study aimed to explore the use of tissue-derived intestinal organoids from adults and children to assess regional drug metabolism and transport differences, as well as the possible impact of age.

Methodology
Organoid lines from fresh adult (jejunum n=3, ileum n=5) and paediatric tissues (ileum n=, age range: 5-56 weeks) were established, cultured and expanded as 3D self-organizing organoids in Matrigel. For studying oral drug absorption, the intestinal organoids were made single-cell and grown as an epithelial monolayer on a permeable membrane. After differentiation, carrier-mediated drug transport by MDR1 and BCRP was determined in bidirectional transport assays and presented as apparent permeability (Papp), the substrates talinolol and rosuvastatin were used, respectively. Transformation of midazolam to 1OH-midazolam was used to assess CYP3A4 metabolic conversion rate (MR). Gene expression of ABCB1/MDR, ABCG2/BCRP and CYP3A4 in fresh intestinal tissue was determined and compared to expression in 3D grown intestinal organoids.

Results
Drug transport studies showed uptake, active efflux transport of talinolol and rosuvastatin and metabolism of midazolam in the intestinal organoid model. The adult epithelial barriers showed region-specific trends in efflux transport, with higher efflux transport (basolateral to apical direction) of talinolol and rosuvastatin by MDR1 and BCRP in ileum (Papp MDR1, b-a: 7.5 ± 0.7; Papp BCRP, b-a: 7.0 ± 0.5 x10-6 cm/s) compared to the jejunum region (Papp MDR1, b-a: 5.3±1.4; Papp BCRP, b-a: 3.0±0.5 x10-6 cm/s). The intestinal organoid monolayers of children and adults both displayed CYP3A4 activity as measured by formation of 1-OH-midazolam, which was undetectable in Caco-2 monolayers. The rate of midazolam metabolism by CYP3A4 showed similar values in adult jejunum (MR: 24.0 ± 9.1 pmol/mg protein/min) compared to adult ileum organoids (MR: 26.5 ± 8.9 pmol/mg protein/min). A peak in metabolic rate was found around 1 year of age compared to adult ileum (MR1-3months: 16.2 ± 14.2 ;MR8-13 months: 68.7 ± 30.7; MRadults: 26.8 ± 9.9 pmol/mg protein/min). No age-related differences in efflux transport were observed (paediatric ileum: Papp MDR1, b-a: 6.2±1.4; Papp BCRP, b-a: 6.8 ± 1.4 x10-6 cm/s) Gene expression in fresh tissue compared to intestinal organoids derived from this tissue showed similar patterns for ABCB1/MDR1, ABCG2/BCRP) and CYP3A4.

Conclusion
Our results show the potential of the application of tissue-derived intestinal organoids to study regional differences in drug transport in adults and children. In adult intestinal organoid monolayers, drug metabolism and transporter functionality appeared to be region-specific and the relative gene expression of ABCB1/MDR1, ABCG2/BCRP was found to be similar in organoids compared to fresh tissue. Future

research will focus on ex vivo transport and metabolism comparison and including more samples from different donors covering a wider age-range.

The gut-hepatobiliary organ perfusion model: demonstrator study showing the absorption, metabolism and excretion of midazolam

Lianne Stevens, Dr. Evita van de Steeg, Dr. Jason Doppenberg, Prof. Ian Alwayn, Prof. Catherijne Knibbe, MD Jeroen Dubbeld

Biography

Bachelor and Master programme at Wageningen university. Master specialisation was Molecular Nutrition and Toxicology. Her PhD focusses on the development of organ perfusion models to study drug pharmacokinetic processes.

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In order to determine the oral bioavailability of a drug, characterization of the absorption, metabolism, distribution and excretion (ADME) is of great importance. To date, complete ADME profiles can only be studied in in vivo models as it remains difficult to recapitulate all organ functions within a single model. The current developments in organ perfusion techniques, opens the possibility to study physiologic processes in an ex vivo environment. Aim of this study was to explore the feasibility of multi-organ perfusion model the first-pass effect and subsequently oral bioavailability of midazolam.

Porcine en-bloc organs were procured from a slaughterhouse (n=4). After obtaining the organs, the lungs and heart were dissected and the aorta was cannulated, ligated and a cold flush (HTK) was initiated. In the meantime the colon was dissected. Thereafter, the organs were flushed with HTK and an additional portal flush was applied. Upon arrival in the laboratory, the stomach was dissected and the small intestine was shortened to ~2 meter. The organs were perfused via the aorta (60-80 mmHg) and portal vein (8-11 mmHg) with a red blood cell based perfusate. After 120 min of normothermic perfusion, 20 mg of midazolam was dosed via the duodenum. Perfusate, portal vein, bile and tissue samples were taken in time. Hourly blood gas analysis was performed to monitor organ viability.

Absorption of midazolam was shown by detection of midazolam in the portal vein with a Cmax of 138.93 ng/mL 90 min after administration. Gut wall metabolism was shown by the formation of 1'-OH midazolam detected in the portal vein as well as the intestinal lumen. The midazolam metabolite 1-'OH midazolam glucuronide was detected in the perfusate 30 min after perfusion. The metabolite rapidly increased throughout the whole perfusion up to 117.99 nM at 240 min. The intestinal gut-wall extraction (EG) of midazolam in the intestinal lumen compartment could be determined and showed to be less than the perfusate 0.051±0.03 vs. 0.16±0.10 respectively. Toghether, a EG of 0.21±0.11 was measured. The hepatic extraction ratio (EH) varied between 0.55-0.74 with an average of 0.65±0.07. The fraction escaping gut wall metabolism (FG) and fraction escaping hepatic extraction (FH) showed a value of 0.78±0.11 and 0.35±0.07 respectively, indicating more extensive hepatic metabolism over intestinal metabolism. Assuming a fraction absorbed of 1, the oral bioavailability showed to be 0.27±0.05. Biliary elimination of midazolam (0.04±0.01%) and its glucuronide (0.02%) from was demonstrated, indicating minimal contribution to the enterohepatic circulation.

We have successfully developed a novel multi-organ model and demonstrated its capabilities. The ability to characterized pre-systemic extraction of midazolam by characterization of intestinal as well as hepatic extraction is very unique. As a result, oral bioavailability could be determined. FH, FG and oral bioavailability findings were in line with pig in vivo data. Ex vivo multi-organ perfusion, complemented with physiologically based pharmacokinetic modelling is a valuable approach to investigate the first-pass effect and oral bioavailability of novel pharmaceutical compounds in human subjects.

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