9th International Microdialysis Symposium

07 – 09 September 2022

Berlin, Germany

Hosted by:
Freie Universität Berlin, Institute of Pharmacy, Dept. Clinical Pharmacy & Biochemistry

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Word of Welcome:

2020 – 2021 – 2022 …finally: A warm welcome!

After having to cancel and postpone in 2020 and in 2021, it is our great pleasure and honour to welcome you at the 9th International Microdialysis Symposium in Berlin in 2022!

In line with the scientific tradition of the previous events, the goal of this 9th Symposium is to provide a unique exchange platform to discuss the current state-of-the art and future perspectives in various fields of microdialysis, to present latest achievements and to connect with colleagues.

The **9th International Symposium on Microdialysis 2022 – Development, Integration, Translation** focusses on emerging hot topics in the (i) development and application of the microdialysis technique and (ii) integration and translation of microdialysis data from *in vitro* to *in vivo* extrapolation and from pre-clinical species to human. In this context, (iii) the impact of modelling & simulation shall also be emphasised.

We are very happy to welcome 50 participants from 13 countries representing academia, medical hospitals, research institutes, small and big pharma companies. We highly appreciate the diversity in backgrounds and are proud to see keynote talks from world-renowned experts as well as giving young scientists the opportunity to present in oral and poster presentations.

Last but not least, I would like to thank the Scientific Committee and "my Berlin team" as Local Organising Committee for the enthusiastic spirit during the last 2.5 years and last but not least our 'sponsors' M Dialysis, Freie Universität Berlin and the German Pharmaceutical Society (Deutsche Pharmazeutische Gesellschaft, DPhG) for support to realise the Symposium.

Enjoy live and lively scientific discussions and beyond!

Charlotte Kloft
- on behalf of the Local Organising Committee -

Latest version of Symposium booklet

Committees:

Local Organising Committee

Charlotte Kloft	Freie Universität Berlin, Germany
Robin Michelet	Freie Universität Berlin, Germany
Linda Aulin	Freie Universität Berlin, Germany
Felix Müller	Freie Universität Berlin, Germany
Davide Bindellini	Freie Universität Berlin, Germany
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William Couet	University of Poitiers, France
Teresa CT Dalla Costa	Federal University of Rio Grande del Sul, Porto Allegre, Brazil
Elizabeth CM de Lange	Leiden University, The Netherlands
Margareta Hammarlund-Udenaes	University of Uppsala, Uppsala, Sweden
Nathalie Heuze Vourc'h	INSERM U1100 - CEPR, Tours Cedex, France
Charlotte Kloft	Freie Universität Berlin, Germany
Sandrine Marchand	University of Poitiers, France
Robin Michelet	Freie Universität Berlin, Germany
Philipp Simon	Universität Augsburg, Germany
Julie A. Stenken	University of Arkansas, Fayetteville, USA
Hermann Wrigge	BG Klinikum Bergmannstrost Halle, Germany
Markus Zeitlinger	Medical University of Vienna, Austria
in memoriam Hartmut Derendorf	University of Florida, Gainesville, USA

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If you post content on social media, make sure to use **#IMS2022** on Twitter and LinkedIn!

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We kindly invite you to follow our Research Group @CPB_Kloft on LinkedIn and Twitter

Hosted by: Freie Universität Berlin, Institute of Pharmacy, Dept. Clinical Pharmacy & Biochemistry

07 September 2022 – Development

08:45 to 12:30	**Pre-conference course** [Institute of Pharmacy, Seminar Room 3] **"Clinical Microdialysis Metabolic Monitoring in Peripheral Tissues"** **(organised by M Dialysis and Charité-Universitätsmedizin Berlin)**	
Chairs:	Katarina Åsberg (M Dialysis AB, Sweden) and Michael Boschmann (Charité Universitätsmedizin Berlin, Germany)	
09:00-09:30	Coffee, registration and welcome	
09:30-10:00	History & principles of clinical microdialysis	
10:00-11:15	Physiological and pharmacological applications.	
11:15-12:15	Practical demonstrations in smaller groups: - Implantation of microdialysis catheters in adipose tissue - Demonstration of ISCUSflex microdialysis analyzers	
12:15-13:15	General discussion and sandwiches	
12:30 – 13:45 (Desk open until 14:30)	**Registration to IMS2022** [Institute of Pharmacy]	
13:45 to 14:00	**Welcome and Opening** [Lecture Hall Zoology] Charlotte Kloft (Freie Universität Berlin, Germany)	
14:00 to 15:30	**Session 1** [Lecture Hall Zoology] **"Characterisation and optimisation of microdialysis techniques"**	
Chairs:	Sandrine Marchand (Université de Poitiers, France) and Robin Michelet (Freie Universität Berlin, Germany)	
		Speaker
14:00 - 14:30	Coupling microdialysis to electrophoresis	Susan Lunte (University of Kansas, KS, USA)
14:30 - 15:00	Two-Photon lithography method to create 3D printed microdialysis probes	Julie Stenken (University of Arkansas, AR, USA)
15:00 - 15:30	Abstract talk: Continuous cortisol monitoring by combining microdialysis with Biosensing by Particle Mobility	Laura van Smeden (Eindhoven University of Technology, the Netherlands)
15:30 - 16:30	**Coffee break with poster walk and exhibition** [Institute of Pharmacy]	

16:30 to 18:00	**Session 2** [Lecture Hall Zoology] **"Development and optimisation of microdialysis during the disease state"**	
Chairs:	Teresa Dalla Costa (Federal University of Rio Grande do Sul, Brazil) and Philipp Simon (Augsburg University, Germany)	
		Speaker
16:30 - 17:00	Anti-Infectives drug distribution on infected animals	Bibiana Verlindo de Araújo (Federal University of Rio Grande do Sul, Brazil)
17:00 - 17:30	Antimicrobials distribution in infected animals and humans	Sandrine Marchand (Université de Poitiers, France)
17:30 - 18:00	Abstract talk: A piglet model of pediatric sepsis to investigate tissue penetration of antibiotics in children: a microdialysis study on piperacillin-tazobactam	Eline Hermans (Ghent University, Belgium)
18:00 - 19:00	**Break**	
19:00 - 23:00	**Welcome Reception at Alter Krug**	

Lecture Hall Zoology, Königin-Luise-Straße 1-3, 14195 Berlin
Institute of Pharmacy, Königin-Luise-Straße 2-4, 14195 Berlin
Alter Krug, Königin-Luise-Straße 52, 14195 Berlin

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08 September 2022 – Translation

08:30 to 10:00	**Session 3** [Lecture Hall Zoology] **"Application of microdialysis in highly perfused organs"**	
Chairs:	Elizabeth de Lange (Leiden University, The Netherlands) and Michael Boschmann (Charité-Universitätsmedizin Berlin, Germany)	
		Speaker
08:30 - 09:10	**Keynote:** Microdialysis of the brain	Margareta Hammarlund-Udenaes (Uppsala University, Sweden)
09:10 - 09:35	*Ex vivo* microdialysis of meropenem in patients´ lung undergoing lung transplantation	Christina Scharf (Ludwig-Maximilians-University of Munich, Germany)
09:35 - 10:00	Abstract talk: Continuous real time monitoring of cerebral metabolites using microdialysis-spectroscopic sensor in traumatic brain injury (TBI)	Chisomo Zimphango (University of Cambridge, UK)
10:00 - 11:00	**Coffee break with poster walk and exhibition** [Institute of Pharmacy]	

11:00 to 12:30	**Session 4** [Lecture Hall Zoology] **"Quantifying target site concentrations in peripheral tissues"**	
Chairs:	Nathalie Heuzé-Vourc'h (Institut National de la Santé et de la Recherche Médicale (Inserm), France) and Susan Lunte (University of Kansas, KS/USA)	
		Speaker
11:00 - 11:30	Clinical microdialysis for monitoring interactions of hemodynamic, metabolic and neuroendocrine responses in peripheral tissues on healthy volunteers and patients	Michael Boschmann (Charité, Germany)
11:30 - 12:00	Application of hollow microneedles for skin ISF harvesting in models of allergy and chronic urticaria	Nana Shi (Charité/Fraunhofer Institute for Translational Medicine and Pharmacology, Germany)
12:00 - 12:30	Abstract talk: Semi-mechanistic model-based analysis of plasma and target-site cefazolin pharmacokinetics and protein binding in obese and nonobese patients to evaluate current dosing regimens adequacy	Davide Bindellini (Freie Universität Berlin, Germany)
12:30 - 13:30	**Lunch** [Institute of Pharmacy]	
13:30 to 15:00	**Session 5** [Lecture Hall Zoology] **"State of the art applications of microdialysis"**	
Chairs:	Julie Stenken (University of Arkansas, AR/USA) and Margareta Hammarlund-Udenaes (Uppsala University, Sweden)	
		Speaker
13:30 - 14:10	**Keynote:** Exploring the fate of inhaled therapeutic proteins in the lungs by microdialysis	Nathalie Heuzé-Vourc'h (Institut National de la Santé et de la Recherche Médicale (Inserm), France)
14:10 - 14:35	A window to the brain: on-line measurement of biologics concentrations and target engagement using microdialysis and cerebral open-flow microperfusion	Florie Le Prieult (AbbVie, Germany)
14:35 - 15:00	Abstract talk: Proteomic study of brain microdialysis samples from subarachnoid haemorrhage patients with and without cerebral vasospasm	Axel Tingskull (Linköping University Hospital, Sweden)
15:00 - 16:00	**Coffee break with poster walk and exhibition** [Institute of Pharmacy]	

16:00 to 17:30	**Session 6** **"Microdialysis in a clinical setting"**	[Lecture Hall Zoology]
Chairs:	Markus Zeitlinger (Medical University of Vienna, Austria) and Michael Boschmann (Charité-Universitätsmedizin Berlin, Germany)	
		Speaker
16:00 - 16:30	Microdialysis in the obese: Leipzig study	Philipp Simon (Augsburg University, Germany)
16:30 - 17:00	Lung microdialysis during cardiothoracic surgery	Maximilian Edlinger-Stanger (Medical University of Vienna, Austria)
17:00 - 17:30	Abstract talk: Lefamulin exposure in soft tissues: population pharmacokinetics and pharmacokinetic/pharmacodynamic target attainment	Wisse van Os (Medical University of Vienna, Austria)
17:30-17:40	**Voting: best young investigator presentations**	
17:40 - 19:00	**Break**	
19:00 - 23:00	**Conference dinner at Harnack Haus**	

Lecture Hall Zoology, Königin-Luise-Straße 1-3, 14195 Berlin
Institute of Pharmacy, Königin-Luise-Straße 2-4, 14195 Berlin
Harnack Haus, Ihnestraße 16-20, 14195 Berlin

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09 September 2022 – Integration

09:00 to 10:30	**Session 7** **"Modelling and simulation of microdialysis data"**	[Lecture Hall Zoology]
Chairs:	William Couet (Université de Poitiers, France) and Linda Aulin (Freie Universität Berlin, Germany)	
		Speaker
9:00 – 9:40	**Keynote:** Modelling and simulation of microdialysis data	Elizabeth De Lange (Leiden University, the Netherlands)
9:40 - 10:05	Minimal PBPK modeling of antibiotics CNS distribution	Alexia Chauzy (Université de Poitiers, France)
10:05 - 10:30	Model-based integration of target-site drug exposure is key for antibiotic dose-individualisation in obese patients	David Busse (Freie Universität Berlin, Germany)
10:30 - 11:00	**Coffee break with posters and exhibition**	[Institute of Pharmacy]

11:00 to 12:45	**Session 8** [Lecture Hall Zoology] **"Regulatory acceptability and future direction of microdialysis"**	
Chairs:	Charlotte Kloft (Freie Universität Berlin, Germany) and Elizabeth de Lange (Leiden University, The Netherlands)	
		Speaker
11:00 - 11:30	Microdialysis in translational brain exposure and distribution	Mariette Heins (Charles River, the Netherlands)
11:30 - 12:00	Acceptability of microdialysis data for registration agencies	Markus Zeitlinger (Medical University of Vienna, Austria)
12:00 - 12:45	Panel Discussion	
12:45 – 12:55	**Award ceremony best young investigator presentations**	
12:55-13:00	**Wrap-up and closure**	

Lecture Hall Zoology, Königin-Luise-Straße 1-3, 14195 Berlin
Institute of Pharmacy, Königin-Luise-Straße 2-4, 14195 Berlin

Program:

Keynote and invited speakers:

Session 1: Characterisation and optimisation of microdialysis techniques

Susan Lunte

Susan M. Lunte is the Ralph N. Adams Distinguished Professor of Chemistry and Pharmaceutical Chemistry, Director of the Adams Institute for Bioanalytical Chemistry, and Director of the NIH COBRE Center for Molecular Analysis of Disease Pathways at the University of Kansas, Lawrence, KS. She received a B.S. degree in chemistry from Kalamazoo College and a Ph.D. in Analytical Chemistry in 1984 from Purdue University. Dr. Lunte served as an associate editor and then Editor-in-Chief of *Analytical Methods* between 2009-2017 and is currently on the Editorial Board of the *Analyst***.** From 2013-2018 she was a member of the NIH Instrumentation and Systems Development Study Section and served as Chair in 2017-18. Dr. Lunte is a Fellow of the RSC, AAPS, ACS, AAAS and AIMBE. Most recently, she was recipient of the ANACHEM Award in 2018 and the ACS-ANYL Roland F. Hirsch Distinguished Service Award in 2021. In 2019 she was a visiting Faculty Fellow at Paris Tech in France as well as the University of Tasmania in Australia. Dr. Lunte's research interests include the development of new methodologies for separation and detection of peptides, amino acids, neurotransmitters and pharmaceuticals in biological fluids. This includes separation-based sensors for the continuous monitoring of drugs and neurotransmitters in freely roaming animals and new methodologies for the determination of reactive oxygen and nitrogen species in cells.

Coupling Microdialysis to Electrophoresis

Susan M. Lunte [a,b,c]

[a] Ralph N. Adams Institute for Bioanalytical Chemistry, University of Kansas, Lawrence, KS, USA
[b] Department of Chemistry, University of Kansas, Lawrence, KS, USA
[c] Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA

Objectives: The direct coupling of microdialysis (MD) with microchip electrophoresis (ME) yields a separation-based sensor that is capable of continuous in vivo monitoring of drugs and neurotransmitters. Electrochemical detection (EC) is well suited for these sensors due to the possibility of integrating the working and reference electrodes directly into the chip, as well as the availability of a miniaturized isolated potentiostat. Previously, we developed an on-animal MD-ME-EC system for the continuous monitoring of the transdermal delivery of nitroglycerin in an awake freely roaming sheep [1]. This demonstrated the feasibility of on-animal systems for monitoring drug metabolism and behavior, but was limited in scope. In this presentation, recent progress regarding the development of on-line MD-ME-EC systems for the continuous monitoring of catecholamines will be described. Our overall objective is to develop a variety of MD-ME based systems that can be employed for near real-time continuous monitoring of amino acids, catecholamines, and nitric oxide metabolites in awake, freely roaming animals.

Methods: "Double T" chips consisting of both a separation channel and a microdialysis interface are fabricated out of glass and/or PDMS using standard photolithographic methods [1-3]. Electrochemical detection was accomplished using a working electrode consisting of either carbon fiber embedded in PDMS or a pyrolyzed photoresist film (PPF) or metal electrode fabricated on a glass substrate. Injection into the chip was achieved using a gated injection. Electrophoretic separations were optimized for the specific analytes of interest and amperometric detection was accomplished using an electrically isolated potentiostat. A high voltage power supply was used to apply the potentials used for injection and the electrophoretic separation.

Results: A on-animal separation-based sensor was previously developed and demonstrated for the investigation of drug metabolism in a freely moving sheep [1]. This system has been modified for the separation and detection of catecholamines and related neurotransmitters [2] and has been demonstrated through monitoring the release of dopamine following high potassium infusion into the brain [3].

Conclusion: On-line MD-ME-EC can be used for the continuous near real time monitoring of drugs and neurotransmitters *in vivo.* Significantly, the separation-based system can be customized for specific analyte classes through the judicious choice of microdialysis probe, electrophoresis conditions and detector type. Current efforts are focused on improving the limits of detection for dopamine and other catecholamines, as well the development of additional MD-ME based systems for other neuroactive compounds.

References:

[1] Scott, D.E., et. al., *Analyst*, 140:3820-9 (2015)
[2] Saylor, R.A. and Lunte, S.M., *Electrophoresis* 39:462-469 (2018)
[3] Gunawardhana, S.M. et.al., *Analyst*, 145: 1768-1776 (2020)

Move up so dont block foot note

Julie Stenken

Julie Stenken holds the rank of Professor and 21st Century Chair in Proteomics at the University of Arkansas. She also serves as the Vice Chair for the Department of Chemistry and Biochemistry. Professor Stenken received her B.S. in Chemistry from the University of Akron, Ohio in 1990 and her Ph.D. in Bioanalytical Chemistry from the University of Kansas in 1995 under the direction of Dr. Craig Lunte (d. 2015). During the 1994-1995 academic year, Professor Stenken was a J. William Fulbright Fellow at the Karolinska Institute (KI) in Stockholm, Sweden. She was mentored by Dr. Lars Ståhle in the Department of Clinical Pharmacology, KI. In 1996, she started her first academic appointment at Rensselaer Polytechnic Institute in Troy, NY. In 2007, she moved to the University of Arkansas. Professor Stenken is affiliated with the Programs in Cell and Molecular Biology as well as Materials Science and Engineering at the University of Arkansas.

Research in the Stenken group over the last 25 years has focused on a variety of bioanalytical challenges associated with microdialysis sampling. These have included issues in calibration, needs to improve relative recovery of lipophilic as well as large proteins (i.e., cytokines), as well as non-specific adsorption. We have had significant interest in cytokine and matrix metalloproteinase (MMP) signaling during various stages of wound healing in both the brain and subcutaneous tissue. More recently we have applied microdialysis sampling to understand the localized chemical dynamics of bacterial quorum sensing and the inflammatory response to bacterial biofilms. Our most recent work focuses on utilizing microdialysis sampling to obtain signals from the soil and elucidate localized chemical communication between plants and their localized microenvironment. Finally, we continue to pursue new ideas for improving microdialysis recovery as well as device function with our focus on 3D printing of microdialysis probes.

Two-Photon Lithography Method to Create 3D Printed Microdialysis Probes

Julie A. Stenken[a,b], Patrick M. Pysz[a,b], Julia Hoskins,[b,c] Josh Goss,[c] and Min Zou[b,c]

[a] Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR, 72701, USA
[b] Program in Materials Science and Engineering, University of Arkansas, Fayetteville, AR, 72701, USA
[c] Department of Mechanical Engineering, University of Arkansas, Fayetteville, AR, 72701, USA

Microdialysis sampling performed with commercially-available or home-made probes can only be used to either collect or deliver solutes of interest. These devices exclusively use cylindrical geometry since they incorporate hollow-fiber dialysis membranes. Their ability to perform multi-functional tasks are minimal at the present time. To develop microdialysis probes that may embed various multi-functional capabilities (e.g., optogenetics) or additional on-device sensors requires techniques that are not easily accomplished using standard photolithography techniques. These photolithography techniques have failed to bring novel microdialysis probes to fruition. Additionally, standard photolithography fabrication techniques limit the complexity of the final probe geometry to simple stacked Cartesian slices in a 2D plane. To overcome this limitation, additive manufacturing (AMF) techniques are highly sought after and researched in microfluidic applications. Readily available and low-cost commercial AMF technology is limited to final geometry features larger than 100 µm due to the available voxel resolutions in the 10s to 100s of microns. In contrast, we have demonstrated a combination of two 3D printing technologies to overcome this resolution limitation and fabricate a fully 3D printed microdialysis-based microsampling probe. The Nanoscribe GTgrade two-photon stereolithography 3D printer allows for feature sizes as small as 200 nm, and thus the fabrication of highly complex and difficult to fabricate microfluidic geometries. The Nanoscribe was used to print a complex 4 mm long triangular sampling needle with an array of 3330 x 5 µm pores that is attached to a larger inlet/outlet and structural support section. The downside is print times increase quadratically with this reduction in feature size. To overcome this problem, the low-cost larger section was fabricated using the UV-laser-based stereolithography Peopoly Moai 130 3D Printer, which uses UV-laser-based stereolithography, and is capable of feature sizes ranging from 10 µm to 70 µm features. The main inlet, outlet, and structural support section is fabricated on the Moai 130, while the smaller sampling section comprising of complex geometries is fabricated on the Nanoscribe. This dual printer printing method allowed for the rapid fabrication, characterization, and revision of highly customized, optimized, and complex microsampling probe design that is now in its initial stages of testing.

Session 2: Development and optimisation of microdialysis during the disease state

Bibiana Verlindo de Araújo

Bibiana Verlindo de Araujo is Associate Professor of Pharmacokinetics, Clinical Pharmacokinetics and Pharmacometrics at the Federal University of Rio Grande do Sul, where she teaches in the Faculty of Pharmacy, in the Graduate Program of Pharmaceutical Sciences and in the Graduate Program of Medical Sciences. She has a Pharmacy Bachelor's Degree from the Federal University of Rio Grande do Sul and Ph.D. degree in Pharmaceutical Sciences from the same University. Her researches are focused in Pharmacokinetic/Pharmacodynamic modeling of antifungals, antimalarials and antidiabetics drugs, pharmacokinetics evaluation of new compounds and drug-loaded nanoparticles and microdialysis. In 2012 she was fellow in the University of Florida, improving her skills in population pharmacokinetics in the Departament of Pharmaceutics and in 2019 was visitor professor at the Universidad de Navarra in Spain. Up to now she was advisor of 10 Master of Science Thesis and 05 Doctoral Dissertations. The results of her research were published in different indexed journal such as Antimicrobial Agents and Chemotherapy, European Journal of Pharmaceutical Sciences, International Journal of Antimicrobial Agents and Biomedical Chromatography, with more than 100 abstracts presented at Conferences and Congress. Since 2021 she is a member of the Brazilian Pharmacopeia Technical Thematic Committee on Medicines.

Anti-infective drug distribution in infected animals

Bibiana Verlindo de Araujo[a]

[a] Department of Production and Control of Medicines, Faculty of Pharmacy, Federal University of Rio Grande do Sul, Brazil

Animal models of infection have been used for decades in PK/PD evaluation studies of antimicrobials as they allow an adequate characterization of PK/PD indices and targets associated to in vivo efficacy. Unlike other animal models, in which translation is more difficult due to pharmacodynamic and pathophysiological differences, in infection models, as the target is the microorganism, interactions with the drug can be translated more easily. Another advantage of using these models is the opportunity of evaluating the impact of infections on free tissue levels reached in the biophase, through microdialysis, given that only free concentrations are responsible for the pharmacological effect. In this lecture, it is shown how animal models can be used to assess the impact of infections on the distribution of antimicrobials. In the first example, a model of pneumonia was developed to investigate the impact of biofilm-forming P. aeruginosa infection on tobramycin lung and epithelial lining fluid (ELF) penetration, using microdialysis, and to develop a population pharmacokinetic (popPK) model allowing evaluating the probability of therapeutic target attainment of current dosing regimens employed in fibrocystic and non-fibrocystic patients. Simulations of the recommended treatments for acute and chronic infection achieved > 90% PTA in lung with 4.5 mg/kg q24h and 11 mg/kg q24h, respectively, for the most prevalent P. aeruginosa MIC (0.5 mg/mL), indicating the need to investigate alternative posology [1]. In the second example, the unbound plasma and unbound brain disposition of ceftaroline in healthy and MRSA-infected rats (3 and 5 days after meningitis induction) were evaluated after a single intravenous bolus dose of 20 mg/kg of ceftaroline fosamil. Plasma concentration data was modeled as a one-compartment and brain concentration data was added to the model as a two- compartment model with a bidirectional drug transport between plasma and brain (Q in and Q out). The cardiac output (CO) of the animals showed significant correlation with the relative recovery (RR) of the plasma microdialysis probe. Our results indicate that ceftaroline brain penetration was influenced by an MRSA infection, resulting in mean penetration of about 30% (Q in /Q out) in infected animals [2].

References:

[1] Dias, B.B. et al. Pharmaceutics 14:1237-2022,

[2] Helfer,V.E. (2022). Development of population pharmacokinetic models for ceftaroline viewing the treatment of central nervous system infections [Unpublished doctoral dissertation]. Federal University of Rio Grande do Sul.

Session 2: Development and optimisation of microdialysis during the disease state

Sandrine Marchand

Prof. Dr. Sandrine Marchand (female, born 1972, French) is a professor of Pharmacokinetics at the faculty of Pharmacy of the University of Poitiers since 2012. She studied Pharmacy and obtained her Ph.D. in December 2001 at this same University. She became Assistant Professor in 2001 at the faculty of Pharmacy (University of Poitiers), Professor in 2012 and head of the department of Toxicology and Pharmacokinetics at the University Hospital of Poitiers in 2019. Sandrine Marchand is also member for more than 15 years of the INSERM U1070 Research unit called « Pharmacology of antimicrobial agents and antibioresistance», a multidisciplinary group working on antibiotics PK-PD with translational approaches, of which she took the leadership in January 2022. She has published over 80 peer-reviewed articles and her main research interest has been focusing for many years on antibiotics tissue distribution using microdialysis both in animals and human. She is now conducting research on the disposition of antimicrobial agents alone or in combination after systemic administration or nebulization to improve the treatment of severe lung infections and limit resistances and currently performing PK-PD *in vivo* studies in different infectious models. Sandrine Marchand participated in a JPIAMR project called Co-Action (2016-2019). She is currently workpackage leader in a European IMI project AB-Direct and is a partner in a second IMI project (GNA-NOW). She is also since 2021 involved in the Seq2Diag project in link to the French priority research plan “Antibioresistance” and she is associated with the U1070 unit in the ANR-BMTF CO-Protect project with the S. Wicha’s team. Sandrine Marchand is member of the ISAP (International Society of Anti-Infective Pharmacology) and EPASG (ESCMID PKPD of anti-infectives study group) groups.

Antimicrobials distribution in infected humans and animals

Sandrine Marchand

Inserm U1070, University of Poitiers

After a brief presentation of kinetic considerations of drug distribution in general, the presentation will focus on evaluating the impact of infection on antibiotic tissue distribution through an exhaustive review of the literature of microdialysis studies mainly performed in patients. The presentation will review for different tissues: subcutaneous tissue, lung, brain... microdialysis studies in patients allowing to understand or to apprehend the impact of infection on antibiotic tissue distribution. For subcutaneous tissue, the presentation will come back on studies in septic patients or in patients with a diabetic foot. In the lung, through different examples of *ex vivo* and *in vivo* microdialysis studies, the presentation will revisit the technical constraints of assessing the impact of infection on drug distribution despite well-designed and quality studies, with perhaps the need to return to more meaningful preclinical work. The same observation will be made for the study of antibiotics brain distribution in patients. This presentation will highlight the difficulty of assessing the impact of infection on the tissue distribution of antibiotics in clinical studies.

Session 3: Application of microdialysis in highly perfused organs

Keynote: Margareta Hammerlund-Udenaes

Margareta Hammarlund-Udenaes, PhD, is a Professor of Pharmacokinetics and Pharmacodynamics at Uppsala University, Sweden, since 1999, Professor Emerita since 2020. She has supervised 20 Ph.D. students and published more than 130 original articles. She is an Editor of the book Drug Delivery to the Brain – Principles, Methodology and Applications (Springer, 2014; 2nd Ed. 2022). Her h-index is 53 (Google Scholar). She was the Dean of the Faculty of Pharmacy at Uppsala University 2017 – 2020.

Dr**.** Hammarlund-Udenaes became a Fellow of the American Association of Pharmaceutical Scientists in 2005, an International Fellow of the Academy of Pharmaceutical Science and Technology in Japan in 2015, and an Honorary Member of the Swedish Academy of Pharmaceutical Sciences in 2016. She also received the Arïens Award in Pharmacology in the Netherlands in 2021 for her scientific achievements. She is a former Associate Editor of Pharmaceutical Research, former EAB member of the Journal of Pharmaceutical Sciences and presently an EAB member of Fluids and Barrier of the CNS. Among others, she was the Chair of the Gordon Conference on Barriers of the CNS in 2014 and of the 8th International Symposium on Microdialysis in 2016, and she is co-organizing the 14th Cerebrovascular Biology meeting to be held in Uppsala 2023.

The main scientific interest of Dr**.** Hammarlund-Udenaes is drug delivery to the brain, based on pharmacokinetic-pharmacodynamic principles with a translational perspective. This is a critical area for pharmaceutical research, because of the severe limitations imposed on the development of drugs for the CNS by the blood-brain barrier (BBB). The work to date has resulted in new innovative methods to measure brain drug delivery in discovery and development settings. It has revealed basic relationships of BBB function and brain physiology regarding the transport of exogenous compounds and has provided a new understanding of how drugs are handled by the BBB and the brain. Disease influence and the role of nanodelivery on BBB transport of drugs and peptides are also investigated.

Microdialysis of the brain for drug delivery studies

Margareta Hammarlund-Udenaes

Department of Pharmacy, Uppsala University, Sweden, mhu@farmaci.uu.se

Microdialysis is a crucial technique for understanding pharmacologically relevant drug delivery to the brain. By measuring the unbound concentrations with microdialysis, the transport of drugs across the blood-brain barrier (BBB) can be quantified. Brain unbound concentrations can also be related to receptor occupancy and pharmacological effects. Further, by knowing these relationships, relevant plasma concentrations and thereby dose can be estimated. The lecture will cover the state-of-art principles that were developed based on microdialysis results, to study CNS drug delivery and distribution, including the Kp,uu concept, both regarding the BBB as well as the partitioning of drugs into cells. Recent results regarding possible improvement of delivery by nano formulations and peptides will be covered. The main focus will be on small compounds.

Session 3: Application of microdialysis in highly perfused organs

Christina Scharf

Since 2022:	Graduate admitted for habilitation
Since 2022:	Head of the group „dialysis- and adsorption-procedures in critically ill patients“
Since 2014:	Member of the group „therapeutic drug monitoring“ (PD Zoller), first as doctoral candiate, than as postdoc
Since 2018:	Resident in anesthesiology
2022:	Master of business administration
2018:	Dissertation „Dr. med“
2017:	Degree in human medicine

Ex vivo microdialysis of meropenem in patients´ lung undergoing lung transplantation

Christina Scharf

Ludwig-Maximilians-University of Munich, Germany

Beta-lactam dosing is challenging in critically ill patients due to a huge pharmacokinetic variability. Therapeutic drug monitoring (TDM) is regularly performed in patients´ blood, but it does often not reflect the site of the infection. Pneumonia is a common infection in those patients, which can be treated with meropenem. It is unclear whether sufficient concentrations are reached at the target site „lung“ when therapeutic concentrations are measured in the blood.

As microdialysis cannot regularly be performed in the lung of intensive care unit (ICU) patients, the prospective ALF-trial (clinicaltrials.gov NCT03985605) investigated the suitability of meropenem measurement in explanted human lung tissue from patients undergoing lung transplantation via microdialysis. Furthermore, the measured meropenem concentrations with microdialysis were compared with the measurements in the blood, epithelial lining fluid and homogenized lung tissue.

In the presentation, you will receive details on the study design, the results obtained and the relevance for clinical practice.

Session 4: Quantifying target site-concentrations in peripheral tissues

Michael Boschmann

Education

1979-1985	Studies in Medicine, Otto-von-Guericke University, Magdeburg
1985	Diploma in Medicine (Dipl.-Med.)
1985	Boards: License to practice medicine
1985-1990	Training in Medical Biochemistry at the Institute of Biochemistry, Otto-von-Guericke University, Magdeburg
1989	Boards: Specialist in Medical Biochemistry
1990	Thesis (Dr. med.)
2004	Certificate: Basics in Clinical Trials
2007	Boards: Specialist in Clinical Pharmacology
2009	Certificate: Principal Investigator for Clinical Trials (according to Arzneimittelgesetz, AMG – German Pharmaceuticals Law)
2011	Certificate: Training Course for Investigators in Clinical Trials, Deutsche Gesellschaft für Pharmazeutische Medizin (DGPharMed)

Positions

1990-1991	Postdoc, Central Institute for Nutrition, Dept. of Energy Metabolism, Potsdam-Rehbrücke
1990-1991	Guest Scientist at the Institute of Biochemistry, Free University Berlin
1992-1995	Postdoc, German Institute of Human Nutrition, Dept. of Biochemistry & Physiology of Nutrition, Potsdam-Rehbrücke
1995-1997	Research Associate / Clinical Scholar, Lab Human Behavior and Metabolism (Hirsch/Leibel Lab), Rockefeller University, New York
1997-2002	Research Associate, German Institute of Human Nutrition, Dept. of Biochemistry & Physiology of Nutrition, Potsdam-Rehbrücke
2002-2007	Research Associate, Franz-Volhard Center for Clinical Research, Charité Universitätsmedizin Berlin, Campus Berlin-Buch
2008-	Project Leader and Coordinator, Franz-Volhard Center for Clinical Research at the Experimental & Clinical Research Center (ECRC), Charité Universitätsmedizin Berlin, Campus Berlin-Buch

Clinical microdialysis for monitoring interactions of hemodynamic, metabolic and neuroendocrine responses in peripheral tissues on healthy volunteers and patients

Michael Boschmann, MD

Experimental & Clinical Research Center – a joint cooperation between Charité Universitätsmedizin Berlin and Max-Delbrück Center for Molecular Medicine, Berlin, Germany

Clinical microdialysis is an elegant tool for monitoring hemodynamic and metabolic response to different physiological and pathophysiological challenges in easy accessible organs such as dermis, adipose tissue and skeletal muscle. Furthermore, microdialysis is a suitable method to study the interstitial space of tissue as one of the most important regulatory compartments.

In a first study, we evaluated the effect of standardized bicycle exercise at 50% VO_2max on tissue perfusion and metabolism in abdominal (aSAT) and femoral subcutaneous adipose tissue (fSAT) and skeletal muscle in eleven women and nine men. We found that lipids stored in muscle are rather used than lipids stored in adipose tissue for fueling the energy metabolism of muscle during exercise. During exercise, lipid mobilization is much greater in women than in men.

In a second study, we wanted to determine the hemodynamic and metabolic response to physiologically relevant atrial natriuretic-peptide (ANP) concentrations in adipose tissue (AT) and skeletal muscle (SM). ANP briskly stimulated AT lipid mobilization and systemic oxidation at plasma concentrations that are encountered in conditions such as heart failure. Therefore, ANP induced lipid mobilization might contribute to cardiac cachexia. Drugs that interfere with the natriuretic peptide system should be evaluated for potential metabolic side effects. Therefore, in a following study, we wanted to determine the metabolic and cardiovascular interaction of β-adrenergic receptors and ANP. We found that selected cardiovascular ANP effects are at least partly mediated by β-adrenergic receptor stimulation. ANP-induced changes in lipid mobilization and glycolysis are mediated by another mechanism, presumably stimulation of natriuretic peptide receptors, whereas substrate oxidation might be modulated through adrenergic mechanisms. After that, we hypothesized that ANP increases postprandial free fatty acid (FFA) availability and energy expenditure while decreasing arterial blood pressure. As a result, our data identified the ANP system as a novel pathway regulating postprandial lipid oxidation, energy expenditure, and concomitantly arterial blood pressure. The findings could have therapeutic implications. Finally, we tested the hypothesis that increased plasma ANP levels are associated with an increased catabolic (lipolytic) state of white AT in patients with chronic heart failure (CHF). Indeed, there was a direct correlation between plasma ANP levels and increased AT catabolic (lipolytic) state in CHF patients. This might contribute to AT wasting and the development of cardiac cachexia in patients with CHF.

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Session 4: Quantifying target site-concentrations in peripheral tissues

Nana Shi

Nana Shi came to Germany in 2019. She is a doctoral student at Institute of Allergology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin. Her research topics are Use of hollow microneedle patches for the detection of inflammation markers in the skin intersitial fluid (MISIT) and the use of microneedling in clinical research.

Application of hollow microneedles for skin ISF harvesting in models of allergy and chronic urticaria

Nana Shi[1,2], Mikael Hillmering[3], Pelle Rangsten[3], Anna Lena Klein[4], Carolina Vera-Ayala[1,2], Sherezade Moñino-Romero[1,2], Marcus Maurer[1,2], Markus Renlund[3], Jörg Scheffel[1,2]

1 Dermatological Allergology, Allergie-Centrum-Charité, Department of Dermatology and Allergy, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
2 Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Allergology and Immunology, Berlin, Germany
3 Ascilion AB, Kista, Sweden
4 Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Dermatology Venereology and Allergology, Center of Experimental and Applied Cutaneous Physiology, Charité Universitätsmedizin Berlin, Germany

The pathomechanisms of chronic inflammatory skin diseases such as urticaria, or atopic dermatitis are complex and challenging to investigate. Histomorphometric analyses of skin biopsies combined with the analysis of blood, serum and skin microdialysis (SMD) samples are gold-standard tests in skin research. However, these tests are either invasive, samples are difficult to obtain with the risk of infection, or locally produced biomarkers in the tissue are often diluted below detection thresholds. To facilitate biomarker discovery in dermatology we have developed a hollow microneedle (hMN) chips-based dermal interstitial fluid (ISF) extraction method.

MN chips, comprising of 37, 420 µm long hollow microneedles were manufactured from monocrystalline silicon wafers and applied to human abdominal ex vivo skin for ISF extraction. On average, a volume of 12.6ul could be extracted with hMNs by application of -70kpa subpressure. The recovery of biomolecules was significantly enhanced in ISF extracted with hMN compared to SMD and independent of their size. In addition, we applied this method in ex vivo skin-serum transfer models for allergy and inducible urticaria i.e., peanut allergy, cold urticaria (coldU) and symptomatic dermographism (SD). MC degranulation, as indicated by an increase of histamine in the ISF, could be detected in response to injection of serum of a peanut allergic patient followed by injection of the antigen. Similarly, injection of SD or coldU serum followed by application of the respective trigger i.e., cooling and rewarming or scratching of the skin induced a detectable increase of histamine in the ISF.

In summary, we have developed a novel, accessible, efficient, and minimally invasive tool to sample ISF from the skin and used it successfully in ex vivo models of allergy and CindU to detect mast cell degranulation. Thus, analysis of ISF extracted by hollow microneedles is a promising tool for researching and diagnosing

Session 5: State of the art applications of microdialysis

Keynote: Nathalie Heuzé-Vourc'h

Nathalie Heuzé-Vourc'h, Research Director at INSERM, the National Institute of Biomedical Research in France. She leads a multidisciplinary team in the Research Centre for Respiratory Diseases (CEPR, INSERM U1100) in Tours, dedicated to 'Aerosol therapy and Biotherapeutics for Respiratory Diseases'. After graduating with her Ph.D. in oncology in France, she focused her research on lung diseases and obtained a postdoctoral position in the division of pulmonary and critical care medicine (Dr. Steven M. Dubinett) at UCLA, California. She gained interest in biotherapeutics working in a start-up (Agensys Inc., Santa Monica, California) developing anticancer monoclonal antibodies and was recruited in 2005 by INSERM as a young research scientist to continue working on this topic. She is currently supervising several projects on the multifaceted aspects of the delivery of biotherapeutics by inhalation to treat respiratory diseases, from formulation to preclinical safety, with both academic and private partners. She has published more than 70 peer-reviewed papers and book chapters. She is one of the leaders of the Laboratory of Excellence 'MabImprove', since 2011, from the French program 'Investments for the Future.' She is the co-founder of Cynbiose Respiratory, a CRO specialized in experimental models in the respiratory field and serves regularly as a consultant to the pharma industry on aerosol therapy.

Exploring the fate of inhaled therapeutic proteins in the lungs by microdialysis

Nathalie Heuzé-Vourc'h

Institut National de la Santé et de la Recherche Médicale (Inserm), France

Lung pharmacokinetic (PK) studies are required to characterize the kinetic of tissue/fluid deposition, transformation and clearance of inhaled drug developed for pulmonary disease. Classically, PK parameters are estimated by monitoring drug concentrations in the systemic circulation, then computed in mathematical compartmental models to predict the behavior of both local and systemically acting drugs. For biotherapeutics, like high-molecular weight proteins, that do not diffuse passively into organ/tissue compartments, indirect estimation of lung concentrations by modeling from plasma drug profiles is limited and sometimes biased. We developed a new method to quantify the time-course exposure of inhaled protein therapeutics by direct sampling in the lung parenchyma, of Non-human primates, by microdialysis. In this keynote, I will present the results of two protein therapeutics that behaved differently: a monoclonal antibody (mAb) and an immunomodulatory protein (recombinant flagellin). Overall, we successfully established the conditions for lung microdialysis of inhaled mAb targeting soluble-antigens, but this technique remains challenging. In contrast, we failed, for reasons that will be detailed, to implement lung microdialysis of flagellin*.

* The results presented come from a project, which received funding from Sanofi-Genzyme and by a public grant overseen by the French National Research Agency (ANR) as part of the "Investissements d'Avenir" program (reference: ANR-10-LABX−53-01) and already published (DOI: 10.1080/19420862.2018.1556081). They also result from the FAIR project, which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 847786.

Florie Le Prieult

1. Where and in what year did you receive your Ph.D.? Post-doc?
   - 2016: PhD in Neurobiology at University Medicine of the Johannes Gutenberg-University Mainz, Germany
   - 2016-2017: Post-doc in Neurobiology of Traumatic Brain Injuries at University Medicine of the Johannes Gutenberg-University Mainz, Germany
   - 2017-2018: Post-doc in *In vivo* Pharmacokinetics & Neuroscience Pharmacokinetics at AbbVie, Germany

2. What is your science background (what did you initially start studying) and how did that help you to transition to what you are doing now?

I studied Physiology and Bioengineering/Biotechnology throughout my university curriculum with a final specialization in neurobiology during my PhD. I progressively developed a strong interest in pathologies and got the opportunity to work in different areas, going from Cystic Fibrosis to cancer and finally neurodegenerative diseases. It directed me to therapies and how we can influence the efficacy of compounds by studying their kinetics in pre-clinical settings.

3. Do you have any recent publications that you would like to highlight? If so, please include the title of the publication and the journal.

One of my most recent paper is from 2021 and gather the data I will present at the conference, comparing and showing the potentials in drug development of both conventional microdialysis and cOFM, especially for large molecules purposes (Le Prieult, Florie et al. “Collecting antibodies and large molecule biomarkers in mouse interstitial brain fluid: a comparison of microdialysis and cerebral open flow microperfusion.” mAbs vol. 13,1 (2021)). We also published this year another paper focusing on microdialysis and Tau in the brain interstitial fluid (Barini, Erica et al., Neurobiology of aging vol. 109 (2022))

4. What is your biggest professional accomplishment?

Getting from PhD student to team leader in the timespan of 2 years.

5. Why do you do what you do?

Because I think that our research will make a difference in patients’ life and will get us to viable treatments for neurodegenerative diseases.

More details: CV on Linkedin

A window to the brain: on-line measurement of biologics concentrations and target engagement using microdialysis and cerebral open-flow microperfusion

Florie Le Prieult [a], Erica Barini [b/c], Loic Laplanche [a], Kerstin Schlegel [b], and Mario Mezler [a]

[a] Drug Metabolism and Pharmacokinetics, AbbVie Deutschland GmbH & Co. KG, Knollstrasse, Ludwigshafen, Germany
[b] Neuroscience Discovery, AbbVie Deutschland GmbH & Co. KG, Knollstrasse, Ludwigshafen, Germany
[c] now affiliated with Roche Holding AG, Basel, Switzerland

The determination of concentrations of large therapeutic molecules, like monoclonal antibodies (mAbs), in the interstitial brain fluid (ISF) is one of the cornerstones for the translation from preclinical species to humans of treatments for neurodegenerative diseases. Microdialysis (MD) and cerebral open flow microperfusion (cOFM) are the only currently available methods for extracting ISF, and their use and characterization for the collection of large molecules in rodents have barely started. For the first time, we compared both methods at a technical and performance level for measuring ISF concentrations of a non-target-binding mAb, trastuzumab, in awake and freely moving mice. Without correction of the data for recovery, concentrations of samples are over 10-fold higher through cOFM compared to MD. In vivo recovery (zero-flow rate method) revealed an increased extraction of trastuzumab at low flow rates. Technical optimizations have significantly increased the performance of both systems, resulting in the possibility of sampling up to 12 mice simultaneously as well as sampling from the same group of mice over two different time periods. Moreover, strict aseptic conditions have played an important role in improving data quality. The standardization of these complex methods makes the unravelling of ISF concentrations attainable for various diseases and modalities, starting in this study with mAbs, but extending further in the future to RNA therapeutics, antibody-drug conjugates, and even cell therapies. The possibility to perform PK/PD measurements in the same ISF samples is also now achievable and enable to further investigate target engagement in the relevant brain compartment which the interstitial space represents.

Disclosures: Florie Le Prieult, Loic Laplanche, Kerstin Schlegel, and Mario Mezler are employees of AbbVie and may own AbbVie stock. AbbVie sponsored and funded the study; contributed to the design; participated in the collection, analysis, and interpretation of data, and in writing, reviewing, and approval of the final publication. Erica Barini was an AbbVie employee and is now affiliated with Roche Holding AG.

Philipp Simon

Prof. Dr. Philipp Simon studied human medicine at the University of Leipzig, where he received his doctorate in 2010 and his habilitation in 2021. In 2018, he was recognized as a specialist in anesthesiology. After 2009, he took on various professional positions at the Department of Anesthesiology and Intensive Care Medicine, University of Leipzig Medical Centre most recently as senior physician and head of study coordination for clinical research. On April 1, 2022, Simon was appointed to the professorship of Anesthesiology and Operative Intensive Care Medicine with a focus on clinical research at the Faculty of Medicine of the University of Augsburg. He also took over as head of the Section of Operative Intensive Care Medicine at the Department of Anesthesiology and Operative Intensive Care at Augsburg University Hospital. In addition to numerous research projects on the visualization and individualization of mechanical ventilation using electrical impedance tomography, he is involved in rational antibiotic therapy in intensive care medicine and, in particular, in factors influencing pharmacokinetics using microdialysis technology.

Microdialysis in the obese: Leipzig study

Philipp Simon

Augsburg University, Germany

In addition to the choice of antibiotic and the duration of therapy, adequate dosage is decisive for the success of anti-infective therapy and the prevention of further development of resistance. This requires not only a sufficient level in the plasma, but also at the site of action in the peripheral tissue. In addition, obesity can influence pharmacokinetics, which can increase the risk of underdosing. In this research project, we are using microdialysis to investigate different antibiotic concentrations in the target tissue directly on the patient. The aim is to investigate factors such as body weight, but also disease, on the pharmacokinetics of antibiotics. In a larger clinical project, concentrations of various antibiotics were determined perioperatively in plasma and subcutaneous fat tissue of obese and normal-weight patients.

Session 6: Microdialysis in a clinical setting

Maximilian Edlinger Stanger

Maximilian Edlinger-Stanger is an anesthesia and critical care attending at the department of cardiac, thoracic and vascular anesthesia and intensive care medicine. His research interests are antimicrobial pharmacokinetics in cardiothoracic surgery and experimental models of ventilation-perfusion mismatch.

Lung microdialysis during cardiothoracic surgery

Maximilian Edlinger-Stanger
Department of cardiac, thoracic and vascular anesthesia and intensive care medicine, Medical University Vienna, Austria

Pneumonia is one of the most common and serious complications after cardiothoracic surgery and is associated with increased mortality, length of stay, duration of ventilatory support, and health care costs. Patients undergoing cardiothoracic surgery present with multiple comorbidities, such as chronic obstructive pulmonary disease, interstitial lung disease, coronary artery disease, heart failure, diabetes mellitus, and peripheral artery disease. Optimal antibiotic treatment is of paramount importance in this critical patient cohort as target site concentrations have been shown to correlate with clinical outcomes in the treatment of pneumonia. However, pharmacokinetics of antimicrobial drugs in these patients differ significantly from those in healthy volunteers and exhibit large interindividual variability.

Perioperative factors may affect the pharmacokinetics of antimicrobial drugs: pathophysiologic sequelae of major cardiothoracic surgery, systemic inflammatory response syndrome after cardiopulmonary bypass, single-lung ventilation and atelectasis, ischemia-reperfusion syndrome, ventilation-perfusion mismatch, bleeding, fluid resuscitation and comorbidities. Employing standardized dosing regimens inferred from studies in healthy volunteers might cause inappropriate pharmacokinetics in patients undergoing cardiothoracic surgery, leading to treatment failure, toxicity, or development of antibiotic resistance. Therefore, pharmacokinetic data for antibiotics in lung tissue of these critically ill patients are of significant clinical interest.

Lung microdialysis allows the continuous measurement of free, unbound concentrations of antimicrobial drugs in the interstitium at an exact anatomical site – a distinguishing advantage compared to other techniques such as lung tissue homogenates or sampling of epithelial lining fluid. Microdialysis primarily measures drug concentrations in the extracellular compartment of the lung. Pulmonary pharmacokinetics of lipophilic drugs or intracellularly enriched drugs may not be studied adequately with microdialysis. During cardiothoracic surgery, microdialysis probes may be inserted into lung tissue under direct vision, which enables accurate placement and ensures patient safety. Recently published and ongoing studies employing lung microdialysis during cardiothoracic surgery will be discussed.

Session 7: Modelling and simulation of microdialysis data

Keynote: Elizabeth De Lange

Elizabeth de Lange, PhD, is Professor of Predictive Pharmacology at the Division of Systems Pharmacology and Pharmacy of the Leiden Academic Centre for Drug Research (LACDR) at Leiden University in the Netherlands. She combines advanced multi-level experiments (of which microdialysis is a key technique) and analytical techniques to produce smart data, and mathematical modelling, as a unique approach to build robust mathematical models for the prediction of drug effects in human, which is the ultimate aim of her research.

In the development of mathematical models that predict pharmacokinetic (PK)- pharmacodynamic (PD) relationships in human, in health and disease, she involves identification and characterization of key factors as well as their interdependencies. Particular emphasis lies on investigations on target tissues protected by special barriers, like the central nervous system (CNS).

This research has a comparative and integrative design to elucidate conditional influences of individual factors to the whole (Mastermind research Approach). This is a crucial basis for translation between species and conditions. A recent success is the development of a physiologically-based CNS PK model that is able to adequately predict drug distribution into and within multiple compartments of the rat and human CNS. Another highlight is the contribution to understanding the *in vivo* context of drug target residence time to predict the effects of drugs.

She has published over 145 papers, has provided >150 invited lectures, and has (co-)organized >85 scientific meetings. In 2013, she received the AAPS Fellow Award. In 2020, she received an Honorary doctorate from Uppsala University. In 2020 she received the Lewis Sheiner lecture award from the International Society of Pharmacometrics for her life time achievements in modelling and simulation.

Modelling and simulation of microdialysis data to understand PK and PD of the CNS in health and disease

Elizabeth de Lange

On behalf of the Predictive Pharmacology Research Group, Division of Systems Pharmacology and Pharmacy, Leiden Academic Centre for Drug Research (LACDR), Leiden University, The Netherlands

The development of successful central nervous system (CNS) drugs is still a challenge. It is hampered by inadequate consideration and integration of CNS pharmacokinetics (PK), pharmacodynamics (PD) and disease complexity (reductionist approach). Improvement will result from integrative approaches in data collection and modelling.

Membrane transport of and interaction with targets is driven by unbound drug exposure. To obtain time-course data on unbound drug concentrations, but also of (potential) biomarkers of effects and/or disease, in vivo microdialysis is a key technique. In the brain, this technique can be applied in experimental animals but, for ethical reasons, not (or at best highly limited) in human. Therefore, we have to rely on preclinical studies combined with translational physiologically based pharmacokinetic (PBPK) modeling to predict the human situation. This will improve the understanding of time- and condition dependent interrelationships between CNS PK, target binding kinetics, and PD, which is key to predictions in different conditions (diseases, species, etc).

In this presentation, we discuss in house state-of-the art multi-level animal experimental designs to generate smart data that can be condensed and stored in advanced mathematical models according to her Mastermind Research Approach. Specifically, we discuss the use of drug properties to predict CNS target site(s) PK using our time-course based CNS PBPK model. Then, for selected compounds, drug-target binding kinetics can be included to predict target occupancy (TO)-time profiles in humans. Furthermore, we suggest a pharmaco-omics approach to provide multilevel and paralleled data on systems processes. With this, we anticipate that clinical trials on CNS active compounds will be better informed, while using fewer animals, while also needing fewer individuals and samples per individual for proof of concept in humans.

Alexia Chauzy

Dr. Alexia Chauzy (female, born 1989, French) is an associate professor of Pharmacology at the faculty of Pharmacy of the University of Poitiers since 2019. She studied Pharmacy and obtained her Ph.D. in September 2018 at this same University. Alexia Chauzy is also member for 8 years of the INSERM U1070 Research unit called « Pharmacology of antimicrobial agents and antibioresistance», a multidisciplinary group working on antibiotics PK-PD with translational approaches. Her main research interests focus on population PK, PK-PD and PBPK modeling of antibiotics. She also conducts research on antibiotics tissue distribution using microdialysis in both animals and human and performs *in vitro* (time-kill curves, checkerboard) experiments to characterize efficacy of antibiotics alone or in combination. She recently developed an in-house hollow fiber infection model to study the *in vitro* efficacy of antibiotics by mimicking their pharmacokinetics in a patient. During her PhD, Alexia Chauzy participated in a European IMI project (Combacte-Care) and she is now involved in several projects of the unit. She is also currently co-director of two PhD students. Alexia Chauzy is member of the ISAP (International Society of Anti-Infective Pharmacology) and EPASG (ESCMID PKPD of anti-infectives study group) groups.

Minimal PBPK modeling of antibiotics CNS distribution

Alexia Chauzy[a]

[a] Université de Poitiers, Inserm U1070, Poitiers, France

Understanding antibiotic concentration-time profiles at the central nervous system (CNS) is crucial to treat severe life threatening CNS infections, such as nosocomial ventriculitis or meningitis. Yet CNS distribution is likely to be altered in patients with brain damage and infection/inflammation. Our objective was to develop a physiologically-based pharmacokinetic (PBPK) model to predict brain concentration-time profiles of antibiotics with different physicochemical properties and to simulate the impact of pathophysiological changes on CNS profiles. A minimal PBPK model consisting of three physiological brain compartments was developed from metronidazole and cefotaxime concentrations previously measured in plasma, brain extracellular fluid (ECF) and cerebrospinal fluid (CSF) of brain-injured patients (n=8 and 10, respectively). Volumes and blood flows were fixed to their physiological value obtained from literature. Diffusion clearances characterizing passive transport across the blood brain barrier (BBB) and blood–CSF barrier (BCSFB) were estimated from system- and drug-specific parameters. Active efflux transport across the BBB was derived from a Caco-2 model. The PBPK model described well unbound metronidazole and cefotaxime pharmacokinetic profiles in plasma, ECF and CSF. Simulations showed that with metronidazole, an antibiotic with extensive CNS distribution simply governed by passive diffusion, pathophysiological alterations of membrane permeability, brain ECF volume or cerebral blood flow would have no effect on ECF nor CSF pharmacokinetic profiles. However, with cefotaxime, an antibiotic with low permeability and substrate for efflux transporters, higher concentrations would be observed in ECF and CSF when membrane permeability increases and brain transporters are inhibited. This work will serve as a starting point for the development of a generic minimal PBPK model to describe the CNS distribution of a wide range of antibiotics and optimize their dosing regimens in patients infected or not in a multicenter clinical trial.

Session 7: Modelling and simulation of microdialysis data

David Busse

Winner of the Hartmut Derendorf Microdialysis Presentation Award at the Microdialysis Student's Day 2021

After completing the 2-year “Innovative Medicines” traineeship program at AstraZeneca, Sweden, David joined the Graduate Research Training Program “PharMetrX” in the working group of Prof. Dr. Charlotte Kloft at the Freie Universtät Berlin, where he assessed means of dose-individualisation in obese and morbidly obese patients with a strong focus on model-based integration of microdialysis data. Having received the Hartmut Derendorf Microdialysis Award in 2021, he has been invited to present at the 9th International Symposium on Microdialysis.

Model-based integration of target-site drug exposure is key for antibiotic dose-individualisation in obese patients

David Busse (1,2), Philipp Simon (3,4), Robin Michelet (1), Niklas Hartung (5), Lisa Schmitt (1,2), Hermann Wrigge (3,4,6), Wilhelm Huisinga (5), Charlotte Kloft (1)

Dept. of Clinical Pharmacy and Biochemistry, Institute of Pharmacy, Freie Universitaet Berlin, Germany (1) and Graduate Research Training program PharMetrX, Germany (2), Dept. of Anaesthesiology and Intensive Care, University of Leipzig Medical Center, Germany (3), Integrated Research and Treatment Center (IFB) Adiposity Diseases, University of Leipzig, Germany (4), Institute of Mathematics, University of Potsdam, Germany (5), Dept. of Anaesthesiology, Intensive Care and Emergency Medicine, Pain Therapy, Bergmannstrost Hospital Halle, Germany (6)

Background: Clinical pharmacokinetic/pharmacodynamic (PK/PD) targets represent a proxy for antibiotic exposure at the infection site and are typically derived from studies in plasma of nonobese patients. Due to lack of alternatives, they are often employed in probability of target-attainment (PTA) analysis in other populations, including the obese. Since drug penetration into the target-site might differ in obese patients, it remains unclear if these conventional targets are valid for this special patient population. This analysis aimed at leveraging microdialysis-derived target-site exposure data of four antibiotics to (i) characterise target-site exposure over a wide range of body mass and (ii) assess the validity of currently employed conventional PK/PD targets in obese patients.

Methods: Based on plasma and target-site data obtained via microdialysis from 30 obese (BMI_{mean}±SD=47±9 kg/m^2) and 30 nonobese patients (BMI_{mean}±SD=25±3 kg/m^2), four nonlinear mixed-effects PK models were developed. Half of them received a 30-min infusion of 1000 mg meropenem/600 mg linezolid and half 8000 mg fosfomycin/4500 mg piperacillin+tazobactam before abdominal surgery.

To describe target-site PK, a physiologically-motivated approach based on lean body weight (LBW [1]) and a drug- and structural PK parameter-specific estimated fraction of fat mass (FM scaling factor) has been employed ("LBW/FM" method) to scale flows and central V with LBW, and peripheral V with LBW and FM [2,3,4].

To assess the validity of currently employed conventional PK/PD targets in obese patients, Monte-Carlo simulations of drug exposure in plasma and at target-site were performed for obese and nonobese patients (n=1000, NONMEM version 7.4.3) and the "fraction of dosing interval for which unbound concentrations exceed MIC" (%$fT_{>MIC}$, meropenem/piperacillin) and "unbound AUC/MIC" (linezolid/fosfomycin) were calculated for selected MIC values in plasma and at target site.

Results: For the LBW/FM method, precision of the FM scaling factor for all drugs was acceptable (relative standard error (RSE)≤45.1%) and 12.8%—34.0% of the peripheral volume were scaled by FM and 66.0%—87.2% by LBW. When *only* plasma data were integrated in model development, precision of the FM scaling parameter was inadequate (RSE=46.0%-172%) and the impact of FM on the peripheral volume was underestimated (0.200%—17.1% scaled by FM).

In obese versus nonobese patients, no difference in target-site penetration was found for piperacillin/tazobactam, fosfomycin and linezolid. Consequently, conventional PK/PD targets, developed in mostly nonobese patients, were deemed suitable for the assessment of antibiotic PK/PD through PTA analysis. Yet, these targets did not apply to morbidly obese patients receiving meropenem due to lower target-site exposure. New PK/PD targets corresponding to target-site exposure were derived by matching percentiles of %$fT_{>MIC}$ of virtual nonobese patients in plasma to target-site. 100%$fT_{>MIC}$ related to multiples of MIC were required as plasma-based PK/PD targets in morbidly obese patients.

Conclusions: An effect of FM on the peripheral V using the LBW/FM method represented a physiologically plausible approach. This suggested that dosing of antibiotics should be governed by the physiologically-motivated LBW/FM method, applicable to a wide body composition range. It was demonstrated that only the availability of ISF data in adipose tissue resulted in precise estimates of the impact of FM on PK parameters. The discrepancy between PK/PD targets in obese versus nonobese patients for meropenem indicated altered penetration into target site and therefore inadequacy of conventional PK/PD targets for meropenem leading to possible overestimations of PTA. Thus, the derived new PK/PD targets for this special population should asessed in clinical studies.

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Session 8: Regulatory acceptability and future direction of microdialysis

Mariette Heins

Mariette completed her pharmacist's degree at the university of Groningen. While working at a hospital pharmacy she realized she found research more appealing than optimizing patient-doctor-treatment interactions. Researching therapeutic protein engineering and production of cytokines the next couple of years, though highly interesting, lacked the direct link to therapeutic application and treatment. In 2011 she started working as a study director at Brains On-Line, focusing on drug development research using microdialysis as a tool. Since 2017 the company is part of Charles River Laboratories. At this time, Mariette is director at the Groningen site of CRL, responsible for science and operations.

Microdialysis in translational exposure and distribution

Mariette Heins
Charles River, the Netherlands

Microdialysis is well known for being a useful tool in research allowing elucidation of freely available neurotransmitters / analytes in the interstitial fluid of the brain. Advancements in probe design and membrane use have allowed us to sample from many different soft tissues and optimize the set up for use of various analytes of interest. The use of these optimized probes in combination with surgical developments and refinements allow us to collect samples from several compartments, e.g. brain ISF, CSF and blood, within one animal. Combined data has been shown to be useful in PK/PD calculations and predictions and allows more comprehensive translation of exposure and distribution from rodents to higher species and humans. Here, I will provide some examples of our work in rodents and NHPs using both a classical small molecule and antibodies. Demonstrating the strength of combining microdialysis with serial sample collection from other compartments in preclinical research.

Markus Zeitlinger

Markus Zeitlinger studied medicine at the Medical University of Vienna and graduated in 2000. He completed his training as specialist in Internal Medicine and Clinical Pharmacology and advanced to his main current position as Head of the Department of Clinical Pharmacology. In 2007 he received his post-graduate diploma in Clinical Research. Beside clinical trial design his scientific interests cover antimicrobial agents with focus on early phases of clinical research and pharmacokinetics/pharmacodynamics (PK/PD). He has published over 250 peer reviewed publications and book chapters in particular in the areas of antimicrobial agents, vaccines and imaging and is ad hoc reviewer of over 30 journals. As scientific expert to the European Medicines Agency (EMA) he was actively involved in more than 350 scientific advice procedures given by the agency, including 1/3 of all rapid scientific advice procedures during the Covid-19 pandemic. Furthermore he holds key positions in several national and international scientific societies as well as in a number of national and six European research consortia. These international functions include chairing of the PK/PD working group of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) as well as being member of the executive committee of the Federation of European Pharmacological Societies (EPHAR).

Acceptability of microdialysis data for registration agencies

Markus Zeitlinger [a]

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Modern drug development is time consuming and cost intensive. At several steps in preclinical and clinical phases, assessment of tissue pharmacokinetics is mandatory or at least recommended. Among other techniques microdialysis is specifically recognized by regulatory guidelines like EMA and FDA. The technique might be also helpful is obtained intensive PK profiles in the pediatric population or for extension of indications, e.g. of antimicrobials.

In addition to traditional drug development, registration of follow up drugs called genetic or biosimilar drugs has got more and more attention in recent decades. These drugs bridge their claim of efficacy and safety based on comparability to the originator drug. Microdialysis might be particular powerful in the assessment of bioequivalence for topically applied dermatological drugs. For biosimilars the handicap of the large molecular size of these drugs has to be overcome.

In summary microdialysis is a powerful tool in modern drug development acknowledged by regulatory agencies. Validation of the technique is the key factor for regulatory purposes.

Oral talks selected from abstracts:

Session 1: Characterisation and optimisation of microdialysis techniques

Laura van Smeden

Laura van Smeden (MSc) studied Biomedical Engineering at Eindhoven University of Technology and graduated in the group of Molecular Biosensing (MBx, https://www.tue.nl/en/research/research-groups/molecular-biosensing/) under the supervision of Professor Menno Prins (founder of Helia Biomonitoring: https://www.heliabiomonitoring.com/).
Laura started working as a PhD student in 2019 in the same group, focussing on continuous monitoring of metabolites and inflammation markers. The sensing approach is based on Biosensing by Particle Mobility, where she worked on the detection of creatinine, cortisol, IL-6, and TNFα. Currently, her research aims to combine microdialysis with continuous biosensing for patient monitoring applications to improve personalized healthcare.

Continuous cortisol monitoring by combining microdialysis with Biosensing by Particle Mobility

Laura van Smeden[a,c], Maud Linssen[a,c], Khulan Sergelen[a,c], Arthur de Jong[b,c], Junhong Yan[d], Menno Prins[a,b,c,d]

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[d] Helia Biomonitoring, Eindhoven, the Netherlands.

Objectives:

The ability to continuously measure concentrations of small molecules is important for biological studies, drug research, and patient monitoring. An example is cortisol, a steroid hormone involved in a wide range of medical conditions, which has widely fluctuating levels that cannot be continuously monitored with laboratory-based assays. We demonstrate a cortisol sensor that allows continuous monitoring of cortisol in blood plasma with microdialysis sampling [1].

Methods:

We have developed a continuous real-time sensing technology based on reversible single-molecule interactions, called Biosensing by Particle Mobility (BPM) [2-4]. The sensor consists of particles functionalized with anti-cortisol antibodies and a sensing surface functionalized with cortisol-analogues. Due to single-molecule interactions between antibody and analogue, the particles transiently bind to the sensing surface, which is detected optically via the motion of the particles. Increasing cortisol concentrations reduce the number of binding events, as the cortisol occupies binding sites on the antibodies. The sensor is reversible and gives a continuous sensor signal that depends on the cortisol concentration. We performed measurements in buffer, in filtered blood plasma, and in microdialysate sampled from human blood plasma at 37°C that was spiked with cortisol.

Results:

The sensor detects cortisol in the high nanomolar to low micromolar range and can monitor varying cortisol concentrations over multiple hours, showing response to increases as well as decreases in cortisol concentration. Results are demonstrated in buffer, in filtered blood plasma, and in human blood plasma sampled using a microdialysis catheter.

Conclusion:

We developed a sensor for continuous cortisol monitoring in combination with microdialysis sampling. We expect that the combination of BPM sensing and microdialysis sampling will lead to flexible bioanalytical systems for diverse applications in fundamental biological research and patient monitoring.

References:

[1] Van Smeden et al, in preparation.
[2] Visser, Yan, et al., Nat. Commun. 9: 2514 (2018)
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[4] Lin, et al., ACS Sens. 6: 1980 (2021)

Eline Hermans

Eline Hermans graduated as a medical doctor in 2018 from the Ghent University and began her specialty training in Pediatrics. In 2019 she started her PhD with funding from the Research Foundation Flanders (FWO), investigating the influence of sepsis and critical illness on the tissue distribution of antibiotics in children. She performs clinical and preclinical microdialysis studies, using the pig as a model for a sick child. Eline lives in Ghent and like a true Ghentian**,** her most treasured possessions are her bicycle and her inflatable kayak for exploring this beautiful city.

A piglet model of pediatric sepsis to investigate tissue penetration of antibiotics in children: a microdialysis study on piperacillin-tazobactam.

Eline Hermans[a,b], Evelyn Dhont[a,c], Peter De Paepe[a,d], Johan Vande Walle[e], Mathias Devreese[b], Pieter De Cock[a,f]

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[f] Hospital Pharmacy, Ghent University Hospital, Belgium

Objectives: Antibiotics are the cornerstone in the treatment of sepsis. Microdialysis (MD) data in adults suggest an impaired antibiotic tissue penetration in the case of sepsis. Tissue pharmacokinetics (PK) remain largely understudied in children. Juvenile pig models have proven to provide an accurate prediction of PK behavior in pediatric patients. This study aimed to investigate the influence of sepsis on the tissue penetration of piperacillin (PIP) - tazobactam (TAZ) in a piglet model.

Methods: In 16 piglets, PIP-TAZ was administered over 4 days (75 mg/kg IV over 30 minutes, 6h dosing interval). Blood and MD samples (muscle) were collected in first-dose and steady-state (>24h) conditions. On day 3 and 4, in 10 piglets a continuous lipopolysaccharide (LPS) infusion was administered to induce a septic state. In the 6 control animals (no LPS) time effects during the study period were evaluated. Non-compartmental PK analysis was used to quantify the tissue penetration (Area Under the concentration-time Curve (AUC) ratio tissue/plasma). The AUC ratios were pairwise compared between the healthy and septic states in each piglet, data are reported as mean ± SD.

Results: For PIP, the AUC ratio in first-dose conditions was significantly lower in the septic state (0.84 ± 0.22) compared to the healthy baseline measurement (1.06 ± 0.46) (P = 0.042). In steady-state conditions, comparable results were found with an AUC ratio of 0.80 ± 0.22 in the septic state and 1.09 ± 0.27 during baseline (p=0.009). Comparable results for TAZ were observed. There were no time effects observed in the control group.

Conclusion: In this juvenile pig model, experimental endotoxemia impaired the PIP-TAZ tissue penetration. The results of this study warrant further research into the tissue PK of septic children to optimize antibiotic dosing in this population. Correlating the results of this study with future clinical data could endorse the applicability of the piglet model for drug research in septic children.

Chisomo Zimphango

Chisomo is a PhD candidate at the department of Clinical Neurosciences, University of Cambridge. His work focuses on integration of microdialysis with spectrometric sensors for continuous online detection of relevant cerebral metabolites in traumatic brain injury patients.
Aside postgraduate commitments, Chisomo is an enthusiast for innovation and entrepreneurship, current co-president of Building Bridges in Medical Sciences (BBMS), University of Cambridge.

Continuous real time monitoring of cerebral metabolites using microdialysisspectroscopic sensor in traumatic brain injury (TBI)

Chisomo Zimphango[a], Farah C. Alimagham[a], Monica J. Killen[a], Agnieszka Zakrzewska[a], Adam Young[a], Tanya Huttera,[b], Keri L.H Carpenter[a], Peter J. Hutchinson[a]

[a] Division of Neurosurgery, Department of Clinical Neurosciences, University of Cambridge, UK

[b] Walker Department of Mechanical Engineering, The University of Texas at Austin, USA

Objectives: Using cerebral microdialysis (CMD) to interrogate the local brain metabolism in traumatic brain injury (TBI) is a well-regarded technique to reveal the brain's energy state[1]. Though the sampling of molecules using this technique is continuous, assessment of relevant metabolites is through offline bedside analysers and thus achieved hourly. Here, the purpose was to measure relevant clinical metabolites continuously in real-time using cerebral microdialysis integrated with a spectroscopic sensor.

Methods: For in vitro studies, CNS perfusion fluid was pumped at 0.3 microL/min, using an M Dialysis 107 pump, through a CMD catheter (M Dialysis 71, 100 kDa cutoff) sited in an external solution composed of CNS perfusion fluid plus human serum albumin (5 mg/mL) and sodium azide (0.05%), to which pure glucose, lactate, and pyruvate (cited as the three most relevant metabolites in TBI [2]) were added. The microdialysates from this in-vitro brain model were continuously delivered to the mid-IR detector for online analysis. For clinical studies, adult patients with severe TBI (severity evaluated using CT) were assessed for eligibility. Two TBI patients were monitored online using CMD coupled to the mid-IR sensor, for various periods.

Results: For in vitro studies, the microdialysis-spectroscopic system detected reductions in concentrations of evidenced by examining the fingerprint region (ranging from ~900 to 1200 cm^{-1} in our case). Strong peaks characteristic of glucose, lactate, and pyruvate attributed to stretching vibrations of C–O and C–C were identified at wavenumbers 1036, 1124 and 1176 cm^{-1} for glucose, lactate, and pyruvate respectively. For clinical studies, preliminary results have showed our system's ability to continuously monitor brain microdialysates. Sophisticated analyses like partial least square regression (PLSR) to translate the mid-IR absorbance data into the actual concentrations of glucose, lactate and pyruvate are in progress [3].

Conclusion: To date, the continuous real time monitoring of brain metabolites using microdialysis-integrated spectroscopic sensor are promising and when examined along with other physiological aspects of TBI patients have potential to help improve patient outcomes [4].

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Session 4: Quantifying target site-concentrations in peripheral tissues

Davide Bindellini

Davide Bindellini is a PhD student at the Department of Clinical Pharmacy and Biochemistry, Institute of Pharmacy, at Freie Universitaet Berlin. His work focuses primarily on drug therapy optimisation for special populations. In particular, he conducts research on antibiotic therapy optimisation in obese patients and cortisol replacement therapy in paediatric patients.

Semi-mechanistic model-based analysis of plasma and target-site cefazolin pharmacokinetics and protein binding in obese and nonobese patients to evaluate current dosing regimens adequacy

Davide Bindellini[a,b], David Busse[a,b], Philipp Simon[c,d], Robin Michelet[a], David Petroff[e], Linda Aulin[a], Christoph Dorn[f], Markus Zeitlinger[g], Hermann Wrigge[h], Wilhelm Huisinga[i], Charlotte Kloft[a]

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[i] Institute of Mathematics, University of Potsdam, Germany

Objectives: Cefazolin (CEZ) is frequently used for the treatment of skin and soft tissue infections (SSTIs). Obesity is a risk factor for surgical site infections [1] and drugs pharmacokinetics (PK) can be altered in obese patients [2]. However, data for CEZ PK in the interstitial space fluid of subcutaneous adipose tissue (SSTIs target site) in obese patients are scarce. Target-site PK data can be obtained using microdialysis, as recommended by EMA [3]. The aims of this mechanistic model-based analysis were (i) to identify covariates explaining PK differences between obese and nonobese patients, and to quantify (ii) CEZ protein binding kinetics and (iii) penetration index (PI) in obese versus nonobese patients.

Methods: Data from 15 obese and 15 nonobese patients, receiving a single dose of 2 g CEZ (30-min i.v. infusion) [4], were analysed. PK data were available over 8 h in plasma (n_{total}=240, $n_{unbound}$=120) and at target site (n=591) obtained using two microdialysis catheters (one per upper arm). Retrodialysis was used as calibration method for microdialysis measurements. Nonlinear mixed-effects (NLME) PK modelling was applied. Theory-based allometric scaling and a mechanistic approach based on lean body weight (LBW), fat mass (FM) and a drug-related scaling parameter R (LBW/FM approach) [5] were evaluated. In the latter, central volume was scaled with LBW and peripheral volume was scaled partly with LBW (R) and FM (1-R). Linear and saturable binding models were evaluated. The model was leveraged to evaluate PI (unbound CEZ AUC_{0-t} in target-site:plasma) between patient groups.

Results: A two-compartment NLME PK model best described the data. Allometric scaling of distribution parameters using the LBW/FM approach was chosen based on model performance and mechanistic interpretability [5]: A larger impact of LBW than FM on CEZ distribution was found (R=76.4%, 95%CI=64.9%-86.0%). No covariates were included on CEZ clearance. CEZ plasma protein binding was best characterised via nonlinear saturable binding. PI was evaluated at 4 and 8 hours: At both times, no statistically significant differences were observed between patient groups (p=0.683 and p=0.345, respectively).

Conclusion: LBW/FM approach [5] described well the differences in CEZ PK between patient groups. Even though covariates on CEZ clearance were expected, the addition led to unstable models or unidentifiable parameters. CEZ plasma protein binding results were in line with previous studies [6]. Lastly, no impact of obesity was found on CEZ PI both at 4 and 8 hours. The final model will be leveraged to assess current CEZ dosing regimens adequacy for obese and nonobese patients.

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Axel Tingskull

Proteomic Study of Brain Microdialysis Samples from Subarachnoid Haemorrhage Patients with and without Cerebral Vasospasm

Axel Tingskull[a], Bijar Ghafouri[b], Peter Zsigmond[a], Fredrik Ginstman[a]

[a] Department of Neurosurgery, Linköping University Hospital, Sweden
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Objectives: To investigate the protein profile in brain microdialysate and their correlation with development of cerebral vasospasm after a subarachnoid haemorrhage.

Methods: The study is designed as a retrospective explorative case-control study. Microdialysis samples were collected from the biobank of the neurosurgery department at Linköping University Hospital. Eligibility criteria were previous subarachnoid haemorrhage with (cases) or without (controls) subsequent cerebral vasospasm. Cerebral vasospasm was defined as increased velocity of blood flow in *arteria cerebri media* measured with transcranial doppler and clinical signs of delayed cerebral ischaemia. Included controls were matched by age, sex, and type of perfusate fluid used. Brain microdialysate were sampled every two hours and were pooled into six-hour groups before analysis. During periods when patients showed clinical signs of vasospasm, analysis were made on original two-hour sampled vials, to improve resolution of data. Controls were pooled into six-hour groups over three consecutive days corresponding to vasospasm periods in the cases. The proteome was analysed using nano liquid chromatography in combination with tandem mass spectrometry (nLC-MS/MS). Multivariate data analysis followed by bioinformatics was used to find potential biomarkers for vasospasm.

Results: Eight patients (median age: 62,5, 4 male and 4 female) who suffered a subarachnoid haemorrhage with severe cerebral vasospasm and eight patients (median age: 61,5, 4 male and 4 female) who suffered subarachnoid haemorrhage without subsequent cerebral vasospasm were selected from a database. All samples were collected in the clinical setting from 2014 thru 2020. Every patient had two microdialysis catheters placed, one in each hemisphere. The proteomic identified 78 proteins in brain dialysate samples from two patients. Multivariate data analysis showed significant discrimination between the time points.

Conclusion: This study shows that proteomic in combination with cerebral microdialysis is a valuable tool to investigate protein changes in brain that might indicate vasospasm in patient with subarachnoid haemorrhage.

Wisse van Os

Wisse van Os is a PhD student at the Clinical Pharmacology department of the Medical University of Vienna. His research interests lie primarily in pharmacokinetics and pharmacodynamics of antibiotics. In his thesis work he focuses on predicting antibiotic activity based on target site concentrations, using *in vitro* infection models and pharmacometric approaches.

Lefamulin exposure in soft tissues: population pharmacokinetics and pharmacokinetic/pharmacodynamic target attainment

Wisse van Os[a], Markus Zeitlinger[a]

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Objectives: Lefamulin is a novel antibiotic, currently approved for community-acquired pneumonia. Clinical development for acute bacterial skin and skin structure infections (ABSSSIs) is considered. We aimed to evaluate whether the currently approved dosing strategy may be appropriate to treat ABSSSIs.

Methods: The pharmacokinetic (PK) data was obtained in a previously published trial in which 12 healthy males received one dose of 150mg lefamulin as iv infusion [1]. Unbound concentrations in interstitial space fluid (ISF) of subcutaneous adipose and skeletal muscle tissue were measured using microdialysis (µD) over 24h. Plasma concentrations were also obtained. A population PK analysis was performed using nonlinear mixed-effects modelling and included 250 µD and 144 plasma samples. Observations below the quantification limit (2.2% of total) were excluded. A published saturable protein binding model was used to convert total to unbound plasma concentrations [2]**.** µD data was modelled using an integrated dialysate-based approach [3]. To describe tissue distribution we evaluated models in which ISF concentrations are scaled to compartments of the plasma model and models in which separate tissue compartments are estimated. An unbound drug area under the curve (*f*AUC) to minimum inhibitory concentration (MIC) ratio of 14 was used as exposure target [4] in the PTA analyses. By weighting PTA over MIC distributions [5], the cumulative fraction of response (CFR) was calculated for *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Results: In accordance with previous analyses [2,6], plasma PK was best described with a three-compartment model. Tissue concentrations were best described with one extra compartment each. To stabilise model predictions, the distribution volumes of these compartments were fixed to physiological values [7]. A power function on drug concentrations in the central compartment was needed to capture nonlinear drug distribution into tissues. For the current dosing regimen of 150mg q12h, and using 90% PTA as cut-off, simulations suggested a susceptibility breakpoint for ABSSSIs of 0.06mg/L, regardless of whether unbound concentrations in plasma or ISF were used as exposure measure. This breakpoint splits the MIC distribution of *S. aureus* and *S. pneumonia*. CFR values for *S. aureus* were 86%, 61%, and 62% when using free drug in plasma, subcutis and muscle as exposure measure, respectively. For *S. pneumoniae* the CFR was lower still: 71%, 45% and 46% for plasma, subcutis and muscle, respectively. Simulating 300mg q12h increased the CFR to ≥94% and ≥80% for *S. aureus* and *S. pneumoniae*, but this dose is insufficiently supported by safety data.

Conclusion: Lefamulin tissue exposure following the currently approved dosing regimen may be insufficient to treat ABSSSIs.

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List of posters:

P01: Amrei-Pauline Konrad, *The successful application of in vivo microdialysis starts in vitro: a workflow for posaconazole*

P02: Laura van Smeden, *Continuous cortisol monitoring by combining microdialysis with Biosensing by Particle Mobility*

P03: Sara R. Thomas, *Perfusate considerations for coupling in vivo microdialysis sampling with microchip electrophoresis*

P04: Eline Hermans, *The translation from in vitro experiments to in vivo microdialysis trials: a case study of piperacillin-tazobactam*

P05: Eline Hermans, *A piglet model of pediatric sepsis to investigate tissue penetration of antibiotics in children: a microdialysis study on piperacillin-tazobactam*

P06: Miriam Moser, *Impact of Nimodipine on cerebral metabolism after subarachnoid hemorrhage as a function of its pharmacodynamics and –kinetic characteristics*

P07: Florian Slimano, *Plasma and tissue pharmacokinetics of a new Phosphodiesterase-4 inhibitor in rats*

P08: Chisomo Zimphango, *Continuous real time monitoring of cerebral metabolites using microdialysis-spectroscopic sensor in traumatic brain injury (TBI)*

P09: Maria Sanz Codina, *Biomarkers as model covariates for predicting moxifloxacin tissue penetration in septic patients*

P10: Lovisa Tobieson, *Perihemorrhagic tissue metabolic crisis increases with delay to surgical evacuation of intracerebral hemorrhage: a microdialysis study*

P11: Wisse van Os, *Lefamulin exposure in soft tissues: population pharmacokinetics and pharmacokinetic/pharmacodynamic target attainment*

P12: Felix Leon Mueller, *Optimal design of future clinical microdialysis trials based on non-clinical investigations: On the example of linezolid*

P13: Frida Bällgren, *Oxycodone active uptake across the blood brain barrier and the blood-cerebrospinal fluid barrier in male and female rats*

P 01

The successful application of *in vivo* microdialysis starts *in vitro*: a workflow for posaconazole

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Objectives: Posaconazole (POS) is an azole antifungal agent, indicated for prophylaxis and treatment of invasive fungal infections [1]. To guide optimal dosing, knowledge about infection-site exposure is essential and can be assessed via microdialysis (µD) [2]. Although POS is known to exhibit variable pharmacokinetics (PK) in plasma [1], the target-site variability is currently unknown. Therefore, this project aims to develop an *in vitro* workflow including methodological steps needed for the development of an µD assay, paving the way for successful *in vivo* application to reliably quantify infection-site PK of POS in patients.

Methods: A workflow was developed based on extensive literature research, using data-bases such as PubMed and Google Scholar. To the best of our knowledge, no µD method for POS was yet published. Since POS is a highly lipophilic drug with a log K_{ow} of 4.15 [3], the focus lay on upcoming challenges, searching for literature particularly addressing lipophilic compounds. The experience of our working group with µD of voriconazole, also an azole antifungal, was beneficial for the workflow development [4]. Possible challenges and solutions were identified for the two main parts of relevance to develop a reliable µD assay: a highly sensitive, validated bioanalytical method and demonstration of compatibility of POS and µD equipment *in vitro*.

Results: The developed workflow consisted of three parts: (i) a validated bioanalytical method using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for quantification of POS in microdialysate and plasma, (ii) investigation of the feasibility of a µD assay *in vitro*, and (iii) characterisation of unspecific binding kinetics of POS to µD material [2] and approaches to reduce unspecific binding if warranted. As a first step, the bioanalytical method was successfully developed using LC-MS/MS for quantification POS in small microdialysate samples requiring 2 µL injection volume. Reduced sensitivity due to matrix effects caused by physiologic NaCl could be mitigated by addition of ammonium formate to the mobile phase. Feasibility studies for the µD assay are performed subsequently using a static *in vitro* µD system with CMA 63 µD catheters and physiologic NaCl as perfusate. Different media mimicking the probe-surrounding matrix, e.g. interstitial space fluid and plasma, are investigated to better link *in vitro* and *in vivo* µD application. Catheters are calibrated using the method of retrodialysis whereby the influence of experimental factors, e.g. flow-rate and membrane length, on the relative recovery are determined.

Conclusion: The developed workflow outlines the methodological steps needed for the development of an *in vitro* µD assay which are crucial to ensure successful *in vivo* application of µD in first in human clinical trials, allowing reliable quantification of infection-site PK of POS in patients. Such quantification, amalgamated with knowledge about pharmacodynamic effects, could aid in characterising the interpatient variability and identifying factors influencing PK, which constitutes a step towards optimising POS therapy.

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P 02

Continuous cortisol monitoring by combining microdialysis with Biosensing by Particle Mobility

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Objectives:

The ability to continuously measure concentrations of small molecules is important for biological studies, drug research, and patient monitoring. An example is cortisol, a steroid hormone involved in a wide range of medical conditions, which has widely fluctuating levels that cannot be continuously monitored with laboratory-based assays. We demonstrate a cortisol sensor that allows continuous monitoring of cortisol in blood plasma with microdialysis sampling [1].

Methods:

We have developed a continuous real-time sensing technology based on reversible single-molecule interactions, called Biosensing by Particle Mobility (BPM) [2-4]. The sensor consists of particles functionalized with anti-cortisol antibodies and a sensing surface functionalized with cortisol-analogues. Due to single-molecule interactions between antibody and analogue, the particles transiently bind to the sensing surface, which is detected optically via the motion of the particles. Increasing cortisol concentrations reduce the number of binding events, as the cortisol occupies binding sites on the antibodies. The sensor is reversible and gives a continuous sensor signal that depends on the cortisol concentration. We performed measurements in buffer, in filtered blood plasma, and in microdialysate sampled from human blood plasma at 37℃ that was spiked with cortisol.

Results:

The sensor detects cortisol in the high nanomolar to low micromolar range and can monitor varying cortisol concentrations over multiple hours, showing response to increases as well as decreases in cortisol concentration. Results are demonstrated in buffer, in filtered blood plasma, and in human blood plasma sampled using a microdialysis catheter.

Conclusion:

We developed a sensor for continuous cortisol monitoring in combination with microdialysis sampling. We expect that the combination of BPM sensing and microdialysis sampling will lead to flexible bioanalytical systems for diverse applications in fundamental biological research and patient monitoring.

References:

[1] Van Smeden et al, in preparation.
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P 03

Perfusate considerations for coupling *in vivo* microdialysis sampling with microchip electrophoresis

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Objectives: Electrophoretic separations can suffer from poor compatibility with high ionic strength samples including microdialysis (MD) perfusates. Therefore, the use of lower ionic strength alternative perfusates has been investigated for on-line studies with microchip electrophoresis even though these could lead to changes in brain chemistry [1]. In these studies, the effect of the sodium chloride (NaCl) concentration in the perfusates on the *in vivo* recovery of endogenous ions and catecholamines was investigated. Recoveries were also determined as a function of time following a perfusate switch from artificial cerebral spinal fluid (aCSF) to a low-NaCl aCSF perfusate.

Methods: Our group has developed a microdialysis microchip electrophoresis with electrochemical detection (MD-ME-EC) method that utilizes a background electrolyte compatible with aCSF-like sample matrices. The microchip was fabricated with the pyrolyzed photoresist film working electrode on a quartz substrate bonded to a PDMS substrate containing a separation channel and the microdialysis interface as previously described [1]. Sample flow into the microchip was maintained at 1 µL/min for all experiments. Sample injection was carried out by using gated injection by floating the separation potential for 1 s thus allowing the syringe pump to push a sample plug into the electrophoresis channel. Detection of analytes was accomplished at 0.8 V vs. silver/silver chloride reference electrode with a platinum counter electrode connected to a potentiostat. Liquid chromatography with electrochemical detection was performed for the detection of catecholamines as previously described [2]. The sodium (Na^+) content of rat brain microdialysis samples obtained *in vivo* using perfusates containing 10-40% of the physiological Na^+ concentration was determined using capillary electrophoresis with capacitively coupled contactless conductivity detection. *In vivo* experiments utilized Sprague-Dawley rats with a microdialysis cannula/probe implanted into the striatum.

Results: In these experiments it was determined that at the lower NaCl concentrations of 10 and 20%, the sodium content in the perfusate increased to 15 and 26% respectively, however for 40% NaCl there was no significant change in the concentration of Na^+ in the perfusate . It was also shown that the lower NaCl perfusate (10% to 40%) content generated a reduction of the catecholamine recoveries from the tissues by 40–65%.

Conclusion: It was demonstrated that a 20% NaCl aCSF perfusate is compatible with MD-ME-EC and still provides adequate analyte recovery *in vivo*.

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P 04

The translation from *in vitro* experiments to *in vivo* microdialysis trials: a case study of piperacillin-tazobactam.

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Objectives: Calibration is an essential aspect of microdialysis research. In drug trials, retrodialysis by drug (RD) and retrodialysis by internal calibrator (RC) are the most frequently used calibration techniques. In this study, we aimed (1) to evaluate these calibration techniques for piperacillin (PIP) - tazobactam (TAZ) by comparing the recovery rates (RRs) during RD and RC, both *in vitro* and *in vivo*, and (2) to assess the reliability of *in vitro* to *in vivo* extrapolation of microdialysis methodology and RR for PIP-TAZ.

Methods: Microdialysis catheters with a membrane length of 10 mm were used. The flow rate was set to 2 µL/min. During RD both PIP and TAZ were added to the perfusion solution (NaCl 0,9%). Benzylpenicillin was chosen as internal calibrator for PIP and TAZ. This methodology was first tested *in vitro* and was subsequently applied in a preclinical study in sixteen piglets and a clinical study in five pediatric intensive care (PICU) patients. Both calibration techniques were performed in each animal and study patient. Microdialysis catheters were placed in the paraspinal and thigh muscle, respectively. Data are reported as mean ± standard deviation.

Results: Following the *in vitro* experiments, both RD and RC were deemed successful for PIP and TAZ, with mean RRs of 47 ± 6% (PIP) and 59 ± 5% (TAZ) for RD and 52 ± 5% for RC. In the piglet study, in general, RC recovery (29 ± 10%) rendered higher RRs and resulted in more consistent and reliable tissue concentration-time curves than RD (22 ± 10% PIP; 27 ± 11% TAZ). In the first two PICU study patients both calibration techniques performed poorly with very low RRs: 0.2 ± 0.4% (PIP) and 1.5 ± 0.7% (TAZ) for RD and 7 ± 6% for RC. As these discrepancies between the preclinical and clinical *in vivo* RRs were unexpected, methodological changes were made in the subsequent study patients. Reducing the flow rate from 2 to 1 µL/min led to an important increase of the RR for RC (24 ± 9%), but satisfactory RR for RD could not be reached. Additionally omitting TAZ from the perfusion solution during RD significantly improved the performance of RD for PIP (23 ± 8 %).

Conclusion: We documented that an *in vitro* optimization of experimental design does not always translate into satisfactory RRs *in vivo* and thus a successful measurement of tissue concentrations. Also, extrapolation of RRs between different *in vivo* settings should be done with caution, as was shown by the marked differences in RRs between the preclinical piglet study and initial PICU patients. The finding in the clinical study that simultaneous RD for PIP and TAZ seemed to reduce the loss rate of both compounds, requires further study.

P 05

A piglet model of pediatric sepsis to investigate tissue penetration of antibiotics in children: a microdialysis study on piperacillin-tazobactam.

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Objectives: Antibiotics are the cornerstone in the treatment of sepsis. Microdialysis (MD) data in adults suggest an impaired antibiotic tissue penetration in the case of sepsis. Tissue pharmacokinetics (PK) remain largely understudied in children. Juvenile pig models have proven to provide an accurate prediction of PK behavior in pediatric patients. This study aimed to investigate the influence of sepsis on the tissue penetration of piperacillin (PIP) - tazobactam (TAZ) in a piglet model.

Methods: In 16 piglets, PIP-TAZ was administered over 4 days (75 mg/kg IV over 30 minutes, 6h dosing interval). Blood and MD samples (muscle) were collected in first-dose and steady-state (>24h) conditions. On day 3 and 4, in 10 piglets a continuous lipopolysaccharide (LPS) infusion was administered to induce a septic state. In the 6 control animals (no LPS) time effects during the study period were evaluated. Non-compartmental PK analysis was used to quantify the tissue penetration (Area Under the concentration-time Curve (AUC) ratio tissue/plasma). The AUC ratios were pairwise compared between the healthy and septic states in each piglet, data are reported as mean ± SD.

Results: For PIP, the AUC ratio in first-dose conditions was significantly lower in the septic state (0.84 ± 0.22) compared to the healthy baseline measurement (1.06 ± 0.46) (P = 0.042). In steady-state conditions, comparable results were found with an AUC ratio of 0.80 ± 0.22 in the septic state and 1.09 ± 0.27 during baseline (p=0.009). Comparable results for TAZ were observed. There were no time effects observed in the control group.

Conclusion: In this juvenile pig model, experimental endotoxemia impaired the PIP-TAZ tissue penetration. The results of this study warrant further research into the tissue PK of septic children to optimize antibiotic dosing in this population. Correlating the results of this study with future clinical data could endorse the applicability of the piglet model for drug research in septic children.

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P 06

Impact of Nimodipine on cerebral metabolism after subarachnoid hemorrhage as a function of its pharmacodynamic and -kinetic characteristics

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Objectives:

In aneurysmal subarachnoid hemorrhage (SAH) patients nimodipine is approved for prevention and treatment of ischemic neurological deficits. As a calcium channel blocker nimodipine is a potent vasodilator, which might counteract the delayed-onset cerebral vasospasm following SAH. Additionally, a neuroprotective effect of nimodipine is assumed, as it reduces intracellular calcium, which plays an essential role in cellular apoptosis. However, due to its systemic vasodilative effects nimodipine can induce marked systemic hypotension, which might otherwise impair cerebral perfusion and brain metabolism. To this end, we investigated the impact of orally administered nimodipine on systemic blood pressure, cerebral perfusion pressure (CPP), brain tissue oxygen tension (pbtO2), and brain metabolism, as measured by cerebral microdialysis.

Methods:

In this retrospective study 27 consecutive patients with poor grade aneurysmal SAH undergoing multimodal neuromonitoring and administration of oral nimodipine were included. ICP, pbtO2, CPP and blood pressure were recorded continously, microdialysis samples were taken every 1-2 hours. Nimodipine 60mg was given orally every 4 hours. To investigate the influence of nimodipine as a function of its pharmacodynamic characteristics on cerebral metabolism before and 2 hours after its administration, mixed linear models were conducted.

Results:

Following nimodipine administration mean arterial blood pressure decreased significantly from 105.22 mmHg 15 min before administration to a minimum value of 102.83 mmHg ($p<0.001$; CI -2.619; -2.167) after 30 min, which was paralleled by a significant drop of CPP from 85.91 mmHg to a minimum of 83.49 mmHg ($p<0.001$, CI -2.658; -2.189). Consequently, after nimodipine application pbtO2 significantly decreased after 60 min from 24.54 mmHg at baseline to 24.09 mmHg ($p<0.001$, CI -0.604; -0.308). Cerebral metabolism (glucose, pyruvate, glutamate, glycerol, L/P ratio) did not change significantly during the whole observation period of 4 hours following nimodipine administration, therefore the changes might not have been clinically relevant.

Conclusion:

Oral nimodipine can induce a certain decline of mean arterial blood pressure, which might result in reduction of cerebral perfusion and oxygenation. Nevertheless, cerebral metabolism was not impaired in our cohort as CPP and pbtO2 stayed within their physiological ranges. Therefore, at least orally administered nimodipine and its vasodilative effect does not significantly deteriorate patient's cerebral metabolism.

P 07

Plasma and Tissues pharmacokinetic of a new Phosphodiesterase-4 inhibitor in Rats

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Objectives:

Phosphodiesterase inhibitors have been developed for the treatment of inflammatory diseases as chronic obstructive pulmonary disease (COPD). The first PDE4 inhibitor (PDE4i) approved, roflumilast, could be responsible for psychiatric disorders because of a great distribution to central nervous system (CNS). The development of new PDE4i with low CNS distribution is ongoing. The aim of this study is to perform the plasma and tissue pharmacokinetic of 1056, a new PDE4i derived from zardaverine, in comparison with roflumilast.

Methods:

After a recovering period of surgical procedures, microdialysis probes were inserted in the caudate (CMA/11) and in the left lateral lobe of liver tissue (CMA/20) of Wistar Rats (n=24). Plasma samples and dialysates for the determination of 1056 and roflumilast were drawn between 0 and 300 min after intravenous administration of 5 or 1 mg/kg (1056) and 5 mg/kg (roflumilat). 1056 and roflumilast were measured by UPLC-UV. Non-parametric pharmacokinetic analysis was performed using PRISM (version 8.0, GraphPad software, USA). CNS and liver distribution of 1056 and roflumilast was estimated by the area under the curve (AUC) ratio.

Results:

Plasma pharmacokinetic between 1056 and roflumilast (5 mg/kg) showed a greater elimination half-life (124.6±51.4 vs. 63.6±16.4min), $AUC_{0\text{-}5h}$ (288,021±45,234ng.min/mL vs. 988,807±451,619ng.min/mL) and volume of distribution (656.8±312.0 vs. 173.7±54.3) for roflumilast vs. 1056, respectively. CNS C_{max} of 1056 (5mg/kg) was 16.5±11.7ng/mL (in comparison with C_{max} plasma 1452.1±993.1ng/mL) and the AUC was 3,476±2,648ng.min/mL with a CNS/plasma AUC ratio of 0.38±0.32%. Liver tissue pharmacokinetic parameters of 1056 were closed to plasma unbound 1056. Liver/plasma AUC ratio for 5mg/kg and 1mg/kg were 21.7% and 21.1%, respectively.

Conclusion:

The new PDE4i 1056 has many pharmacokinetic differences with roflumilast. First the lower volume of distribution and the greater AUC could be due to a less liver metabolism than for roflumilast. Secondly the CNS/plasma AUC ratio reflects a low distribution rate from plasma to CNS that could avoid central AE reported with roflumilast. The liver/plasma AUC ratio is consistent with a probable but no major liver metabolism, as supported by plasma pharmacokinetic parameters. Further preclinical studies should explore the toxicity of 1056 in order to confirm the CNS safety of 1056.

References:

P 08

Continuous real time monitoring of cerebral metabolites using microdialysis-spectroscopic sensor in traumatic brain injury (TBI)

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Objectives: Using cerebral microdialysis (CMD) to interrogate the local brain metabolism in traumatic brain injury (TBI) is a well-regarded technique to reveal the brain's energy state[1]. Though the sampling of molecules using this technique is continuous, assessment of relevant metabolites is through offline bedside analysers and thus achieved hourly. Here, the purpose was to measure relevant clinical metabolites continuously in real-time using cerebral microdialysis integrated with a spectroscopic sensor

Methods: For in vitro studies, CNS perfusion fluid was pumped at 0.3 microL/min, using an M Dialysis 107 pump, through a CMD catheter (M Dialysis 71, 100 kDa cutoff) sited in an external solution composed of CNS perfusion fluid plus human serum albumin (5 mg/mL) and sodium azide (0.05%), to which pure glucose, lactate, and pyruvate (cited as the three most relevant metabolites in TBI [2]) were added. The microdialysates from this in-vitro brain model were continuously delivered to the mid-IR detector for online analysis. For clinical studies, adult patients with severe TBI (severity evaluated using CT) were assessed for eligibility. Two TBI patients were monitored online using CMD coupled to the mid-IR sensor, for various periods.

Results: For in vitro studies, the microdialysis-spectroscopic system detected reductions in concentrations of the relevant molecular species when concentrations were changed in a step-down manner. This was evidenced by examining the fingerprint region (ranging from ~900 to 1200 cm^{-1} in our case). Strong peaks characteristic of glucose, lactate, and pyruvate attributed to stretching vibrations of C–O and C–C were identified at wavenumbers 1036, 1124 and 1176 cm^{-1} for glucose, lactate, and pyruvate respectively. For clinical studies, preliminary results have showed our system's ability to continuously monitor brain microdialysates. Sophisticated analyses like partial least square regression (PLSR) to translate the mid-IR absorbance data into the actual concentrations of glucose, lactate and pyruvate are in progress [3].

Conclusion: To date, the continuous real time monitoring of brain metabolites using microdialysis-integrated spectroscopic sensor are promising and when examined along with other physiological aspects of TBI patients have potential to help improve patient outcomes [4].

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P 09

Biomarkers as model covariates for predicting moxifloxacin tissue penetration in septic patients

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Objectives: Pathophysiological changes during sepsis lead to variability in pharmacokinetics (PK). However, determining the concentration at the infection site in individual patients is challenging. We aim to identify biomarkers that can predict antibiotic tissue penetration in septic patients. We also want to develop a population PK model to describe the tissue distribution of moxifloxacin in septic patients, and investigate whether including the selected biomarkers as covariates can explain part of the observed variability and help in predicting antibiotic tissue penetration.

Methods: We analysed previously published data of 10 patients with sepsis who had participated in a microdialysis study. [1] Tissue PK was assessed in skeletal muscle and subcutaneous adipose tissue (sc) using microdialysis on days 1, 3 and 5 of treatment with 400 mg of moxifloxacin once daily. Free concentrations in plasma were calculated using the mean unbound fraction of moxifloxacin at 1 and 10 h after administration. We selected biomarkers according to their pathophysiological relevance to tissue penetration [2] and performed Spearman rank order correlations between the selected biomarkers and tissue penetration. Currently we are working on a nonlinear mixed-effects model to be able to better describe and separate the inter-individual and inter-occasional variability in tissue penetration, and investigate the capacity of selected biomarkers to explain these levels of variability.

Results: Concentration of uric acid (r= -0.557, p= 0.001) and total proteins (r= -0.478, p= 0.013) best correlated with moxifloxacin subcutaneous penetration (C_{max} sc/C_{max} plasma free). Uric acid might relate to severity of illness and is also elevated in patients with reduced urinary excretion. Decreased plasma protein binding leads to an increase in free plasma fraction which might cause an increase in volume of distribution and a shorter elimination half-life. GPT (r= -0.489, p= 0.006), a hepatic biomarker which can be elevated in case of muscular damage, best correlated with moxifloxacin muscle penetration (C_{max} muscle/C_{max} plasma free).

Conclusions: Various biomarkers have negatively correlated with tissue penetration in this study. Identifying at-risk patients for decreased target site antibiotic penetration by an easily available biomarker could benefit antibiotic treatment in sepsis by estimating antimicrobial tissue penetration at the bedside. To which extent variability in tissue penetration can be explained by biomarker levels is currently being investigated using nonlinear mixed-effects modelling.

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P 10

Perihemorrhagic tissue metabolic crisis increases with delay to surgical evacuation of intracerebral hemorrhage: a microdialysis study

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Objectives:

Intracerebral hemorrhage (ICH) is the most devastating and debilitating form of stroke and medical treatment options are limited. Surgical evacuation of the blood clot can be lifesaving in instances of raised intracranial pressure (ICP), but has failed to show improved outcome in previous studies. A recent meta-analysis of surgical clot evacuation, however, showed improved outcome with surgery, and this effect was stronger when surgery was performed early post ICH onset [1]. In this study we aim to investigate the metabolic profile in brain tissue using microdialysis in the perihemorrhagic zone (PHZ) and seemingly normal cortex (SNX) of ICH patients undergoing surgical clot evacuation as a function of time to surgery.

Methods:

In this observational study, 34 patients subjected to surgical evacuation of supratentorial ICH received two cerebral microdialysis catheters (CMA 71): one in the PHZ and one in the SNX in a region remote from the ICH. The microdialysate was analyzed for energy metabolites (glucose, lactate, pyruvate, glycerol, glutamate) every two hours bed-side in the Neurocritical Care Unit. Multivariate data analysis was performed including principal component analysis and subsequent regression analysis using orthogonal projection of latent structures (OPLS), using time to surgery as predictive variable.

Results:

Microdialysis data was retrieved for 4144 data points. Mean values for glucose were lower in PHZ compared to SNX whereas lactate, pyruvate, glycerol and glutamate were higher in PHZ compared to SNX.

After pruning outliers, 4123 data points were included in the model. OPLS showed a significant regression model ((CV-ANOVA, $p<0.001$, F 81.3, df4), R2x0.404, R2Y0.0736, Q2 0.0753) for altered metabolic pattern as a function of time from *ictus* to surgery. R2Y for OPLS regression model was larger for PHZ (0.163; CV-ANOVA, $p<0.001$, F98.97, df4) compared to SNX (0.0875, CV-ANOVA, $p<0.001$, F 47.13, df4) suggesting that the metabolic pattern in the PHZ is more influenced by time to surgery than in the SNX.

Conclusion:

Time delay from *ictus* to ICH surgery correlates with a pattern of metabolic crisis in brain tissue, particularly in the vulnerable PHZ. These results could be pathophysiological evidence of the clinical benefit of early surgery, particularly for the vulnerable brain tissue in the PHZ.

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P 11

Lefamulin exposure in soft tissues: population pharmacokinetics and pharmacokinetic/pharmacodynamic target attainment

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Objectives: Lefamulin is a novel antibiotic, currently approved for community-acquired pneumonia. Clinical development for acute bacterial skin and skin structure infections (ABSSSIs) is considered. We aimed to evaluate whether the currently approved dosing strategy may be appropriate to treat ABSSSIs.

Methods: The pharmacokinetic (PK) data was obtained in a previously published trial in which 12 healthy males received one dose of 150mg lefamulin as iv infusion [1]. Unbound concentrations in interstitial space fluid (ISF) of subcutaneous adipose and skeletal muscle tissue were measured using microdialysis (μD) over 24h. Plasma concentrations were also obtained. A population PK analysis was performed using nonlinear mixed-effects modelling and included 250 μD and 144 plasma samples. Observations below the quantification limit (2.2% of total) were excluded. A published saturable protein binding model was used to convert total to unbound plasma concentrations [2]**.** μD data was modelled using an integrated dialysate-based approach [3]. To describe tissue distribution we evaluated models in which ISF concentrations are scaled to compartments of the plasma model and models in which separate tissue compartments are estimated. An unbound drug area under the curve (*f*AUC) to minimum inhibitory concentration (MIC) ratio of 14 was used as exposure target [4] in the PTA analyses. By weighting PTA over MIC distributions [5], the cumulative fraction of response (CFR) was calculated for *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Results: In accordance with previous analyses [2,6], plasma PK was best described with a three-compartment model. Tissue concentrations were best described with one extra compartment each. To stabilise model predictions, the distribution volumes of these compartments were fixed to physiological values [7]. A power function on drug concentrations in the central compartment was needed to capture nonlinear drug distribution into tissues. For the current dosing regimen of 150mg q12h, and using 90% PTA as cut-off, simulations suggested a susceptibility breakpoint for ABSSSIs of 0.06mg/L, regardless of whether unbound concentrations in plasma or ISF were used as exposure measure. This breakpoint splits the MIC distribution of *S. aureus* and *S. pneumonia*. CFR values for *S. aureus* were 86%, 61%, and 62% when using free drug in plasma, subcutis and muscle as exposure measure, respectively. For *S. pneumoniae* the CFR was lower still: 71%, 45% and 46% for plasma, subcutis and muscle, respectively. Simulating 300mg q12h increased the CFR to ≥94% and ≥80% for *S. aureus* and *S. pneumoniae*, but this dose is insufficiently supported by safety data.

Conclusion: Lefamulin tissue exposure following the currently approved dosing regimen may be insufficient to treat ABSSSIs.

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P 12

Optimal design of future clinical microdialysis trials based on non-clinical investigations: On the example of linezolid

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Objectives: Pharmacokinetic/pharmacodynamic (PK/PD) targets for antiinfective therapies are typically derived from plasma PK and used to optimise dosing regimens of antiinvectives. Yet, often infections are not located in plasma but extracellularly in the interstitial space fluid (ISF) and plasma and ISF PK might differ. Thus, target-site related PK/PD targets are needed as suggested for linezolid (LIN) [1]. To establish such targets, quantification of the pharmacologically active unbound drug in ISF using microdialysis (µD) is required. Observed higher variability in *in vivo* µD studies has hindered the derivation of target-site related PK/PD indices and the source of this variability is not fully understood [2]. The aim of this investigation was to identify possible sources of variability to improve the study design for future clinical µD trials.

Methods: I*n vitro, ex vivo,* and *in silico* approaches were used to explore the target-site and method-related variability in µD. A static and a dynamic *in vitro* µD system (sIVMS/dIVMS) were used to assess intra- and inter-catheter variability for µD of LIN in artificial ISF (aISF) via µD. The inter- and intra-catheter *in vitro* variability was implemented and fixed in a previously developed population PK (popPK) model of LIN (based on clinical data) [3] and the remaining parameters were re-estimated, after which changes in PK parameter estimates were evaluated. In an *ex vivo* approach the impact of the localisation of µD catheters, in the deep and shallow subcutaneous (s.c.) adipose tissue were investigated in retrodialysis settings.

Results: Inter- and intra-catheter variability in *in vitro* studies using aISF was 4.10% coefficient of variation (CV) (95%CI=3.40%-4.80%) and 2.55% CV (2.12%-2.97%), respectively, compared to 26.1% CV (16.7%-33.8%) and 27.2% CV (21.8%-32.0%) for the *in vivo*-based LIN model. In the *ex vivo* setting, a retrodialysis variability, quantified using linear mixed-effect modelling, split into inter-catheter and residual variability (RV) (including intra-catheter variability), of 9.20% CV (RV=7.35%) in the shallow and 21.4% CV (RV=10.4%) in the deep s.c. adipose tissue was determined compared to 16.0% CV (RV=18.1%) *in vivo* [1]. Implementing the *in vitro* measured variability in the popPK model deteriorated the predictive performance of individual µD concentrations assessed in visual predictive checks. Yet, the central tendency remained adequately.

Conclusion: The retrodialysis variabilities in the deep adipose tissue were consistent with those obtained from the *in vivo* studies, which illustrated the impact and importance of catheter implementation and position during clinical trials. Furthermore, the results indicated that the contribution of the target site to the overall variability might be higher than the contribution of µD catheter-related parameters. This finding was corroborated by the decrease in the accuracy of individual predicted µD (target-site) concentrations upon fixing estimated to in vitro derived µD-associated variability in the popPK model. To further improve µD trials and derive improved PK/PD indices, tissue related parameters causing the variability need to be investigated and quantified.

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P 13

Oxycodone active uptake across the blood-brain barrier and the blood-cerebrospinal fluid barrier in male and female rats

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Objectives: Oxycodone transport across the blood-brain barrier (BBB) has previously been documented using cerebral microdialysis, with an unbound brain-to-plasma concentration ratio ($K_{p,uu,striatum}$) of 3, indicating significant active uptake [1]. This rare phenomenon has been associated with a not yet characterized proton-coupled organic cation antiporter [2]. Dissimilar drug transport properties across the blood-cerebrospinal fluid barrier (BCSFB) and the BBB are often recognized, and mainly attributed to differences in the expression profiles of transporters [3]. To understand the antiporter contribution to oxycodone CNS disposition in CSF compartments, we investigated the transport of unbound oxycodone across the BCSFB and its brain-CSF exchange using microdialysis.

Methods: Microdialysis probes were implanted into *vena jugularis* and two of the following areas: the lateral ventricle (LV) (reference site for BCSFB transport), *cisterna magna* (CM) and striatum (reference site for BBB transport). Individual *in vivo* recovery was assessed by the calibrator method [4] using oxycodone-D3. Sprague-Dawley rats were given a 60-min constant-rate infusion of 0.3 mg/kg oxycodone followed by dialysate collection in 10-min intervals up to 240 min. Samples were analysed using UPLC-MS/MS, with oxycodone-D6 as an internal standard. Phoenix WinNonLin 8.3 was used for non-compartmental analysis. The areas under the concentration-time curves were used to calculate $K_{p,uu}$-values, where values above unity indicate predominant active uptake. Data are presented as mean±SD.

Results: Findings clearly show that the phenomenon of active uptake is present not only at the BBB but also at the BCSFB, as $K_{p,uu,LV}$ was 3.41±0.74 (n=10). The uptake across the BBB was 1.3-fold higher than that across the BCSFB as $K_{p,uu,striatum}$ was 4.44±1.02 (n=17, p=0.036). The exposure in striatum was 1.6-fold higher than that in CM as $K_{p,uu,CM}$ was 2.68±1.01 (n=9, p=0.017). This suggests that CSF needs to be used as a surrogate for unbound oxycodone brain concentrations with precaution. The BBB transport (p=0.78), the BCSFB transport (p>0.99) and the exposure in CM (p>0.99) were not subjects to sex differences.

Conclusion: The findings clearly show that oxycodone active uptake is present at both the BBB and the BCSFB, without sex differences. The active uptake across the BCSFB suggests probable functional involvement of the antiporter also at the blood-CSF interface. As the exposure in striatum was higher than that in both LV and CM, CSF needs to be used as a surrogate for brain concentrations with precaution.

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