SWISS PHARMA SCIENCE DAY 2022

SAPhS
Swiss Academy of Pharmaceutical Sciences

Friday 19 August 2022
Von Roll Campus University of Bern

«IMMUNOLOGY»

Intention

The SWISS PHARMA SCIENCE DAY (SPhSD) is an annual event of the Swiss Academy of Pharmaceutical Sciences (SAPhS, www.saphw.ch). The 1st SPhSD was held on 9 October 2008, at the University of Bern. For congress reports 2008-2021 including all lecture and poster abstracts see www.saphw.ch

The 15th SPhSD offers again a platform to present, in form of a poster session, the latest research results of Master and PhD students, as well as Post-Docs of the Swiss Academic Institutions for Pharmaceutical Sciences, i.e. ETH Zürich, University of Geneva, University of Basel, University of Bern, and University of Applied Sciences FHNW-School of Life Sciences Muttenz.

The poster session is embedded in a series of lectures given by invited distinguished scientists representing the broad field of pharmaceutical sciences, such as Pharmaceutical Biology, Biotechnology, Technology, Chemistry, Analytics, Engineering, Pharmacology, and Molecular Biology.

One of the primary goals of the SPhSD is to further stimulate professional and social contacts between the students still undergoing training, and Alumni having already a position in industry, hospital, public health administration or public pharmacy. Thus, cooperation and networking between the different institutions in academia and industry and the different fields of pharmaceutical sciences is being promoted.

Last but not least, the SPhSD represents an ideal platform to meet young engineers and scientists, who may be recruited for a position in the academia, hospital, industry, public health administration or public pharmacy.

Organizing Committee:

Prof. Rudolf Brenneisen, PhD, SAPhS
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Prof. Matthias Hamburger, PhD, SAPhS
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Program

10:00 – 10:15 **Welcome Addresses**

- Ursula von Mandach, Prof., PhD
  Co-President SAPhS
- Christian Leumann, Prof., PhD
  Rector University of Bern
- Rudolf Brenneisen, Prof., PhD
  Secretary General SAPhS

10:15 – 11:30 **Morning Session**

Chair: Verena Schröder, Prof., PhD,
Co-President SAPhS, University of Bern

10:15 – 10:45 **Keynote Lecture: Pfizer Research Prize 2022**

Stephanie Ganal-Vonarburg, Prof., PhD
Department for BioMedical Research DBMR
University of Bern:

«Gut Bacteria Can Program Antibodies»

10:45 – 11:15 **Lecture 2:**

Daniel Ricklin, Prof., PhD
Department of Pharmaceutical Sciences,
University of Basel:

«Mission Possible: Therapeutic Immune Modulation by Targeting the Human Complement System»

11:15 – 11:30 h **Discussion Lectures 1 and 2**

11:45 – 14:00 **Lunch Break and Poster Session**

Program (cont.)

14:00 – 16:15 **Afternoon Session**
Chair: Klaus Eyer, Prof., Dr. sci., ETHZ

14:00 – 15:00 **Short Oral Presentations – Selected Abstracts**

14:00 – 14:15 Nina L. Wittwer, Univ. of Basel, Univ. Hospital Basel
«Utilization of pharmacogenetic drugs in Switzerland» (P-IV-3)

14:15 – 14:30 Kevin Portmann, ETH Zurich
«Unravelling pro-inflammatory dynamics of individual cytokine-secreting cells through deep phenotypic single-cell analysis» (P-VI-2)

14:30 – 14:45 George Ramzy, Univ. of Geneva
«Patient-derived platform to leverage personalized treatment for colorectal carcinoma» (P-V-9)

14:45 – 15:00 Simone Aleandri, Univ. of Bern
«Lipidic based-gel with tunable release properties as a platform for local delivery of biotherapeutics» (P-II-2)

15:00 – 15:30 **Lecture 3: Theodor-Kocher Prize 2021**

Alexander Eggel, Prof., PhD
University Hospital Inselspital Bern and Department for BioMedical Research DBMR, University of Bern:

«Development and Implementation of a New Approach to Diagnose and Monitor Allergic Diseases»

15:30 – 16:00 **Lecture 4:**

Werner J. Pichler, Prof. em., MD
University of Bern and ADR-AC GmbH:

«Pharmacology Meets Immunology: the p-i Concept»

16:00 – 16:15 h **Discussion Lectures 3 and 4**

Program (cont.)

16:15 – 16:30	**Coffee break**
16:30 – 17:00	**Award Ceremony**
	SAPhS Fellow(s) 2022
	Poster Prizes
	Winner swissYPG Flash Talk (Pre-SPhSD event on 18 August 2022 at the House of the University of Bern)
17:00 – 17:15	**Closing Remarks**
17:15 – ca. 18:30	**Apéro**

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Lectures

L-1

Keynote Lecture: Pfizer Research Prize 2022

Stephanie Ganal-Vonarburg, Prof., PhD

Department for BioMedical Research DBMR, University of Bern

Biosketch:

Stephanie Ganal-Vonarburg studied molecular medicine at the University of Freiburg, Germany, and at the University of British Columbia in Vancouver, Canada. She subsequently completed her doctorate in molecular medicine and immunology in the laboratory of Prof. Andreas Diefenbach at the University of Freiburg and received her doctorate with honours (Dr. rer. nat.) in 2013. Stephanie came to Switzerland as a postdoctoral fellow in 2013 with the help of a Marie Curie scholarship from the European Union and an EMBO scholarship. From 2013-2019 she worked in Professor Andrew Macpherson's laboratory at the Department for BioMedical Research at the University of Bern, investigating the role of maternal microbiota in the development of the child's immune system and the impact of commensal colonization on B cell responses. The results of these studies were published in the journals „Science" and „Nature" in 2016 and 2020, respectively. In 2019, she was awarded with a Peter Hans Hofschneider Endowed Professorship. Since 2020, she is assistant professor at the DBMR of the University of Bern and at the Department for Visceral Surgery and Medicine (UVCM) of the Inselspital and heads her independent research group focusing on early-life microbial and environmental influences on host immune development.

Lecture Abstract:

«Gut Bacteria Can Program Antibodies»

Microbiota colonization causes profound B cell stimulation and immunoglobulin induction, yet mammals colonized with many taxa have highly complex individualized immunoglobulin repertoires 1,2. To deconstruct how the microbiota shapes the B cell pool and its functional responsiveness we have used a simplified model of defined transient microbial exposures by different taxa in germ-free mice 3. B cell immunoglobulin repertoire development was followed by deep sequencing and in single cells. Intestinal mucosal exposure generated oligoclonal responses which differed from germ-free controls or from the diverse repertoire generated after intravenous systemic exposure. The IgA repertoire, predominantly to cell-surface antigens, did not expand following dose escalation, whereas increased systemic exposure broadened the IgG repertoire to both microbial cytoplasmic and cell-surface antigens. These microbial exposures induced characteristic immunoglobulin heavy chain B cell repertoires mainly at memory and plasma cell stages. Whereas sequential systemic exposure to different taxa diversified the IgG repertoire and facilitated alternate specific responses, sequential mucosal exposure produced limited overlapping repertoires and attrition of initial IgA binding specificities. This shows a contrast between a flexible response to systemic exposure with the need to avoid fatal sepsis, and a restricted response to mucosal exposure reflecting the generic nature of mucosal microbial mutualism.

L-2

Lecture 2:

Daniel Ricklin, Prof., PhD

Molecular Pharmacy group, Department of Pharmaceutical Sciences, University of Basel

Biosketch:

Dr. Daniel Ricklin is Professor of Molecular Pharmacy at the University of Basel in Switzerland. Experimental drug discovery, ranging from small molecules to biomolecular drugs, is the central element of both his teaching and research activities. A pharmaceutical scientist by training, graduating from ETH Zurich, Dr. Ricklin gained experience in drug design, assay development, peptide chemistry and immunology during early academic career steps at the University of Rostock (Germany), the University of Basel (Switzerland) and the University of Pennsylvania (USA).
Since assuming the faculty position in Basel in 2017, his research program is focused on developing new therapeutic modalities for aberrant activation of host defense pathways in various clinical conditions, including immune, inflammatory, and hemolytic diseases and adverse transplant events. Dr. Ricklin is an expert on complement-targeted therapeutics and has contributed numerous articles, reviews, books, and invited lectures on this topic. He is co-inventor of several complement inhibitors, some of which are in clinical development.

Lecture Abstract:

«Mission Possible: Therapeutic Immune Modulation by Targeting the Human Complement System»

Therapeutic immune modulation as novel treatment strategy in oncotherapy has garnered considerable interest in recent years, and the term is therefore often associated with approaches that affect adaptive immune responses to cancer cells. In the shadow of these developments, however, therapeutic strategies that modulate the innate immune branch of host defense, and in particular the complement system, have seen a remarkable transformation from a “mission impossible” to a versatile treatment platform with several drugs on the market and even more clinical candidates with potential applications in indication areas ranging from autoimmune and inflammatory diseases to age-related and thromboinflammatory conditions and transplantation medicine.
The reason for the broad applicability of therapeutic complement modulators in a diverse set of disorders is rooted in the unique role and organization of complement as early-response cascade system with numerous connections to cross- and downstream defense pathways. While providing a valuable weapon against microbial intruders, particularly during early stages of life, improper activation of complement on biomaterials and host tissue can have severe adverse consequences and contribute to acute and chronic clinical conditions. Although the potential of therapeutic complement inhibition has long been recognized, concerns about the safety of the approach and its initial assessment in ill-suited indications have long delayed its clinical application. Developments during the past two decades have shown that complement inhibitors can be used safely and with both clinical and commercial success, thereby raising confidence in the approach and fueling development efforts.
Despite the growing arsenal of clinical complement inhibitors, there are still many open questions about the best-suited complement modulation strategies in individual indications. Moreover, development efforts for complex, multifactorial disorders such as sepsis or transplant rejection remain scarce, which opens important opportunities for academic drug discovery and development.

The fact that the complement system is comprised of some 50 therapeutic target proteins, including serine proteases and GPCRs, is accessible as extracellular, intravascular cascade system and is modulated by host- and pathogen-derived modulators that can be employed as templates renders academic development even more feasible and attractive.
This presentation provides an insight into the developments that finally turned a long-neglected field of therapeutic immune modulation into a “mission possible” with a promising future and highlights current breakthroughs and challenges. It also illustrates the great potential of complement-targeted modulator design as a treasure chest for academic drug discovery using examples from current research projects of the Molecular Pharmacy group at the University of Basel. These include protective biomaterial coatings, parasite-derived protease inhibitors and peptidic and glycomimetic complement activation modulators, which are developed and evaluated in close collaboration with academic, clinical and industrial partners. Finally, these projects are ideally suited to educate future pharmaceutical scientists about the growing and fascinating possibilities in experimental drug design, ranging from small molecules to bio- and nanopharmaceuticals.

L-3

Lecture 3: Theodor-Kocher Prize 2021

Alexander Eggel, Prof., PhD

University Hospital Inselspital Bern and Department for BioMedical Research DBMR, University of Bern

Biosketch:

Date of birth: 05.10.1982
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Phone: +41 31 632 22 87 DBMR
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The scientific journey of Dr. Eggel began at the University of Fribourg with basic studies in biology and later continued at the University of Bern where he received his PhD in experimental immunology. After a postdoctoral stay at Stanford University, he established an independent research group, which he is heading since 2013. In 2017, he received the “venia docendi” in experimental immunology from the Medical Faculty of the University of Bern where he also became an associated professor in translational immunology in 2021. Dr. Eggel is an expert in allergy, immunology and aging research with a broad knowledge in both therapeutic and diagnostic areas. His laboratory primarily focuses on the investigation of biologic mechanisms underlying both beneficial as well as pathologic type-2 immune responses. On the one hand, Dr. Eggel is seeking to get a better understanding about the development of allergies to come up with alternative diagnostic and therapeutic approaches. In his studies, he focuses on the generation and engineering of next generation antibodies or multifunctional alternative binding scaffolds. On the other hand, he is interested in age-related immunological changes and the possibility to manipulate regulatory functions of the immune system to systemically rejuvenate aged mammalian organisms. In both fields, he integrates molecular, cellular as well as systemic approaches to get a holistic view of the biological mechanisms.

Honors:
2021 Vontobel Award for Research on Age(ing), Vontobel Foundation, Zürich
2021 Theodor-Kocher-Prize 2021 from the University of Bern, Switzerland
2020 Swiss Immunology Early Career Award 2020 (SIECA 2020), SGAI
2015 Allergopharma Award, European Academy of Allergy and Clinical Immunology, Barcelona, Spain
2014 Pfizer Research Prize in the Category «Infectiology, Rheumatology and Immunology», Zürich, Switzerland
2013 Dr. Lutz Zwillenberg Prize from the University of Bern, Bern, Switzerland
2013 Young investigator travel grant, Swiss Society of Allergy and Immunology
2013 Best Abstract Prize, EAACI-WAO World Allergy & Asthma Congress, Milano, Italy
2013 Fondation ACTERIA Doctoral Thesis Prize in Allergology, Zürich, Switzerland
2013 Award for best poster in Translational and Clinical Allergology, Swiss Society of Allergy and Immunology, Bern, Switzerland
2009 Travel Scholarship for the European Congress of Immunology, European Federation of Immunological Societies, Berlin, Germany

Lecture Abstract:

«Development and Implementation of a New Approach to Diagnose and Monitor Allergic Diseases»

Authors: Noemi Zbären[1,2], Daniel Brigger[1,2], Daniel Bachmann[3], Arthur Helbling[2], Lukas Jörg[2], Michael P. Horn[4], Johannes M. Schmid[5], Hans-Jürgen Hoffmann[5], Jean-Pierre Kinet[6], Thomas Kaufmann[3*], and Alexander Eggel[1,2*]

[1] Department of BioMedical Research, University of Bern, Bern, Switzerland
[2] Department of Rheumatology, Immunology and Allergology, Inselspital, University Hospital Bern, Bern, Switzerland
[3] Institute of Pharmacology, University of Bern, Bern, Switzerland
[4] Department of Clinical Chemistry, Inselspital University Hospital, Bern, Switzerland.
[5] Department of Respiratory Diseases and Allergy, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[6] Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA.
*These authors contributed equally to the work.

Aim: Clinical management of allergic diseases is hampered by the lack of simple and safe tests to reliably identify culprit allergens and to closely monitor disease activity. Since allergy diagnosis is a complex multistep procedure, there is urgent need for standardized functional ex vivo assays allowing objective diagnosis, substantiating treatment choices and quantifying therapeutic success. Here, we present a novel functional cell-based assay that relies on passive sensitization of allergic effector cells with patient serum circumventing many current limitations in allergy diagnosis.
Methods: We genetically engineered conditional Hoxb8-immortalized myeloid progenitors from bone-marrow of transgenic mice expressing the human high-affinity IgE receptor (FcεRIα). Within 5 days, these cells can be differentiated into mature mast cells ("Hoxb8 MCs") in virtually unlimited numbers. Hoxb8 MCs were passively sensitized with patient sera, activated with various allergens and cellular responses were assessed by flow cytometry.
Results: We demonstrate that the established Hoxb8 MC assay can be used to accurately measure total IgE levels, identify culprit allergens, longitudinally monitor allergen-specific immunotherapy (AIT) and determine the timepoint of tolerance induction upon AIT in patients.
Conclusion: Our results indicate that this novel diagnostic test represents a valuable tool to support clinicians in the identification of IgE-mediated allergies and in the quantification of treatment efficacy as well as duration of therapeutic response.

L-4

Lecture 4:

Werner J. Pichler, Prof. em., MD

University of Bern and ADR-AC GmbH

Biosketch:
PICHLER Werner Joseph, MD, * 1949; Swiss
ADR-AC GmbH (www.adr-ac.ch); e-mail. Werner.pichler@adr-ac.ch
Specialist (FMH) internal medicine & clinical Immunology/Allergology, Switzerland
Laboratory specialist (FAMH) clinical Immunology/Hematology, Switzerland
1984 – 2014 Head of Division of Allergology and Prof. for clinical Immunology, Inselspital, University of Bern, Switzerland
2006: foundation of ADR-AC, a company devoted to diagnosis and research in drug hypersensitivity; head of research at ADR-AC till now
> 450 original contributions and reviews in international, peer reviewed journals;
main topics: drug hypersensitivity, clinical allergy/immunology; H-factor: 83
Main achievements: creation and development of “p-i concept” (1997 - 2022), a new model describing drug interactions with the immune system and some forms of drug hypersensitivity

Lecture Abstract:

«Pharmacology Meets Immunology: the p-i Concept»

Drugs (<1000D) are considered to be too small to interact as antigen with the immune system. Only if they bind covalently to proteins and thus form a new antigen, they are able to elicit immune reactions. However, research on the mechanism of drug hypersensitivity (DH) in patients has shown that a substantial part of immune-mediated DHR is due to a typical off-target activity of drugs on the highly variable immune receptors like human leukocyte antigens (HLA) and T cell receptor for antigen (TCR). This mechanism was termed pharmacological interaction with immune receptors, p-i concept (https://en.wikipedia.org/wiki/P-i_mechanism). One can differentiate between drugs which bind to certain HLA-proteins (p-i HLA) and drugs which bind to TCR (p-i TCR). The clinical manifestations of this non-covalent, labile interactions are DH, which are always due to T cell reaction, even if the drug binding occurred to HLA on antigen presenting cells. Indeed, the p-i concept is more than a simple off target activity of a drug, as such bindings can elicit a cascade of immune mechanism in general, some appearing after weeks to months in absence of incriminated drug.
p-i TCR: Using sulfamethoxazole (SMX) reactive T cell clones and TCR transfected hybridoma cells, the immune reactivity of T cells of a patient with reactivity to SMX and 11 related sulfanilamides (sulfapyrin, sulfadoxin etc) was analyzed functionally, by modelling and by molecular dynamics: SMX binds to TCR; the cross-reactivity to the related compounds could be explained by the fitting of the stimulatory compound into the binding site on TCRV□; an allosteric effect could be shown on a specific TCRV□20.1.
p-i HLA was investigated with e.g. abacavir: Abacavir binds exclusively to a peptide binding groove in HLA-B*57:01, which explains, why only individuals with this allele develop severe DH to abacavir; Binding of abacavir makes HLA-B*57:01 to look like HLA-B*58:01, which would be an allo-allele and differs from B*57:01 by only 4 amino acids. The finding that drug binding can transform self into “allo” explains some severe forms of DH (Stevens Johnson Syndrome, SJS) as SJS occur almost exclusively in DH and graft versus host disease.

The p-i concept links pharmacology with specific immunology. It explains some forms of DHR, and is important for management of DH and risk assessments of drugs. Moreover, it has a great impact for drug therapy in general, as it makes the specific immune receptors to potential targets for drug therapy.

References:

- Pichler WJ. Immune pathomechanism and classification of drug hypersensitivity. Allergy. 2019; Aug;74(8): 1457-1471.
- Pichler WJ, Watkins S, Yerly D. Risk Assessment in Drug Hypersensitivity: Detecting Small Molecules Which Outsmart the Immune System. Front Allergy. 2022; Feb 22;3: 827893.
- Pichler WJ. The important role of non-covalent drug-protein interactions in drug hypersensitivity reactions. Allergy. 2022; Feb;77(2): 404-415.

Posters

I. PHARMACEUTICAL BIOLOGY / PHYTOPHARMACOLOGY

P - I - 1

Aryltetralin lignans from *Hyptis brachiata* inhibiting human T-cell proliferation

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[3] *Centro de Investigaciones Farmacognosticas de la Flora Panamena (CIFLORPAN), Facultad de Farmacia, Universidad de Panama, 0824 Panama City, Panama*

Introduction: Enhanced proliferation and activation of T-lymphocytes is known to play a crucial role in the pathogenesis of autoimmune diseases and chronic inflammation. Current treatment options with immunosuppressant drugs often do not provide long-lasting relief of symptoms and show a gradual loss of efficacy over time, or they are accompanied by unwanted side effects [1].
Aims: Discovery of new immunosuppressive agents with the ability to decrease T-cell proliferation.
Methods: An in-house library consisting of 600 extracts from Panamanian plants was screened for inhibition of human T-cell proliferation. As one of the hits, a methanolic extract from the aerial parts of *Hyptis brachiata* (Lamiaceae) exhibited strong inhibitory effects. An HPLC-based activity profiling approach resulted in the targeted isolation of 7 aryltetralin lignans, 5 arylnaphthalene lignans, 2 flavonoids, 3 triterpenes, and cinnamyl cinnamate. The extract and the isolated substances were evaluated for their ability to inhibit T-cell proliferation in a CFSE-based system. Furthermore, the lignans were tested in a cell cycle arrest assay by measuring cellular DNA to determine the cell distribution in different cell cycle phases.
Results: The extract and the aryltetralin lignans inhibited T-cell proliferation in a concentration-dependent manner with an IC_{50} of 0.5 µg/ mL and IC_{50}s of 0.1 - 3 µM, respectively, without showing any signs of cytotoxicity at these concentrations. No relevant inhibition was observed for the arylnaphthalene lignans, flavonoids, and triterpenes. Further investigation using a cell cycle arrest assay revealed that the aryltetralin lignans potently inhibited mitosis similarly to podophyllotoxin.
Conclusions: Aryltetralin lignans isolated from *H. brachiata* were identified as potent T-cell proliferation inhibitors. Further investigation revealed that the isolated aryltetralin lignans possess inhibitory effects on cell division leading to cell accumulation in G2 phase. This suggests an interaction of these lignans with microtubule formation through inhibition of tubulin polymerization.

Keywords: T-cell proliferation, immunosuppression, aryltetralin lignans, *Hyptis brachiata*

Reference:
[1] Rosenblum MD, Remedios KA, Abbas AK. Mechanisms of human autoimmunity. J Clin Invest 2015; 125: 2228–2233.

P - I - 2

Biosynthesis of dihydroxytropolone in *Streptomyces sp.*

L. Höing, R. Teufel

Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, 4056 Basel

Introduction: Tropolones are a group of natural products with potent metal-chelating properties that exhibit antibacterial, antiviral and antitumoral activity [1]. These compounds are hydroxylated derivatives of tropone that consists of a seven-membered, non-benzenoid, aromatic carbon-ring with an additional keto-group. In bacteria, the precursor for these compounds surprisingly originates from primary metabolism, i.e. the CoA-dependent catabolism of phenylacetic acid (paa). However, depending on the producing strain, different sets of enzymes are used to modify this precursor. For the Gram-positive *Streptomyces sp.*, the gene cluster encoding the dihydroxytropolone biosynthetic machinery was identified by gene-knockout studies [2].
Aims: In this study we investigated dihydroxytropolone formation in *Streptomyces sp.* by *in vitro* reconstitution of the biosynthetic pathway and elucidated the role of involved enzymes.
Methods: Heterologously produced enzymes were used to gain insight into the individual biosynthetic steps and reaction mechanisms of the partaking enzymes. Conducted assays were analyzed via HPLC and GC-MS, accumulating products were compared to chemically synthesized standards.
Results: Accordingly, the CoA-ester bond from the precursor molecule originating from phenylacetic acid catabolism gets cleaved off. In an unanticipated series of reactions comprising hydroxylation, decarboxylation and ring oxidation tropolone gets formed. This compound undergoes two consecutive ring-hydroxylations and is finally transformed to dihydroxytropolone. However, not all enzymes in the gene cluster contribute to the formation of the final product. Future investigations will focus on elucidating their overall role in the context of dihydroxytropolone formation.
Conclusions: The biosynthesis pathway for dihydroxytropolone could be reconstituted successfully *in vitro*. Taken together, the discovered enzyme functionalities substantially differ from the previously proposed roles that were based on gene knockout studies. Currently, the structure of the key enzymes is studied by X-ray crystallography in order to gain further insights into the unusual reaction mechanisms.

Keywords: tropolone, secondary metabolites, antibiotics, *Streptomyces sp.*

References:
[1] Duan Y et al. Chem BioChem 2020; 21 : 2384-2407
[2] Chen X et al. Appl Environ Microbiol 2018; 84

P - I - 3

Antiprotozoal activity of compounds isolated from *Psychotria leiocarpa*

O. Sevik Kilicaslan[1,2], M. Kaiser[3,4], P. Mäser[3,4], L. Carlos Klein-Júnior[5,6], and M. Cuendet[1,2]

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[5] *School of Health Sciences, Universidade do Vale do Itajaí, Itajaí*
[6] *Laboratory of Pharmacognosy and Quality Control of Phytomedicines, Faculty of Pharmacy, Universidade Federal do Rio Grande do Sul, Porto Alegre*

Introduction: With an estimated number of 241 million cases of malaria and 627,000 deaths from it in 2021, malaria remains one of the most important infectious parasitic diseases worldwide [1]. Due to increasing resistance against the drugs currently used to treat this illness, there is an urgent need to find new and more effective antimalarial therapies [2]. Over the years, medicinal plants have been an inspiring source for antimalarial compounds, such as quinine and artemisinin. The genus *Psychotria* is one of the most important within the Rubiaceae family and includes about 1'200 species. This genus is reported as a source of alkaloids and iridoids, which exhibited psychotropic, anti-inflammatory, antioxidant, antimutagenic, antimicrobial and antiprotozoal activities [3].
Aims: The present study focused on the discovery of new antiprotozoal agents from South American plants, specifically on the MeOH extract of the leaves of *P. leiocarpa*.
Methods: Air-dried and powdered leaves of *P. leiocarpa* (180 g) were macerated in MeOH at room temperature (3 x 24 h) to obtain 16.2 g of crude extract. Fractionation was performed using a flash chromatography system with various MeOH and H_2O mixtures. Then, 13 compounds were isolated on a reversed phase semi-preparative HPLC-UV system with H_2O/MeOH gradients. Their structures were elucidated by 1D, 2D NMR experiments and HRESIMS data. The extract and 12 of the isolated compounds were evaluated for their antiprotozoal activity towards *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, and *Plasmodium falciparum,* as well as for their cytotoxicity against rat skeletal myoblast L6 cells.
Results: The extract demonstrated an activity against the NF54 strain of *P. falciparum* (IC_{50} value of 8.8 µg/mL). Fractionation and isolation combined to LC-HRMS/MS-based dereplication provided 12 compounds including alkaloids, iridoids and phenolic acids.
Conclusions: One compound has not yet been described in the literature. The isolated compounds were tested for their antiprotozoal activity and cytotoxicity to determine their selectivity index and potential for future studies.

Keywords: Brazilian plant, natural products, parasitic diseases, malaria, *Psychotria leiocarpa*

References:

[1] World Health Organization. World malaria report 2021. 2021
[2] Dhorda M, Amaratunga C, Dondorp AM. Artemisinin and multidrug-resistant *Plasmodium falciparum* - a threat for malaria control and elimination. Curr Opin Infect Dis 2021; 34: 432-439
[3] Yang H, Zhang H, Yang C, Chen Y. Chemical constituents of plants from the genus *Psychotria*. Chem Biodivers 2016; 13: 807-820

II. PHARMACEUTICAL TECHNOLOGY

P - II - 1

Correlating functionality and physico-chemical properties of polysorbates for biopharmaceutical preparations

Y. Grether, D. Tobler, O. Germershaus

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Introduction and aim: The non-ionic surfactants Polysorbate (PS) 20 and 80 are amongst the most frequently used stabilizers in biopharmaceutical products. Therapeutic proteins are sensitive to adsorption at interfaces, frequently followed by unfolding and/or interface-induced aggregation. Surfactants such as PS reduce protein surface adsorption and thus improve stability. PS is normally used at concentrations above the critical micelle concentration (CMC). Apart from effective polysorbate concentration in the formulation and polysorbate degradation during shelf life, little is known regarding potential functionality-related characteristics of surfactants and its relation to product quality. With the overall aim of investigating a correlation between surfactant functionality and physico-chemical properties, we established several methods allowing straightforward characterization of Polysorbates with regards to potential functionality-related characteristics.
Methods: PS20 was purchased from, or donated by different excipient suppliers. The first number of the identifiers used in this study designates the supplier, the second letter the product code and the third symbol the batch. The phase inversion temperature (PIT) is the temperature at which the conductivity of an oil- water emulsion falls rapidly to 0. By raising the amount of surfactant in the emulsion the specific PIT for any surfactant ratio can be determined and the PIT deviation (PITd), which is related to the HLB (hydrophilic-lipophilic balance), can be calculated. Stress tests appraising functionality of surfactants were conducted by repeated freeze-thaw cycles of a monoclonal antibody formulation, and aggregation was assessed using turbidimetry, size exclusion chromatography (SEC), and flow imaging microscopy (Flow Cam).
Results: PITd data were all determined in triplicates, and significant differences could be detected (Fig. 1). Aggregation results revealed no significant differences in the turbidity and SEC analysis, but significant differences for the particle count (Fig. 2 and 3). Surfactant 1Aα was donated as a pharma-grade surfactant, whereas 2Aα was labelled as synthesis surfactant. Those results clearly indicate differences between the various suppliers and grade-classifications resulting in different funcitonality in biopharmaceutical formulations.

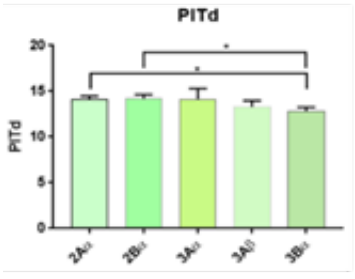

Figure 1: PITd of different PS20

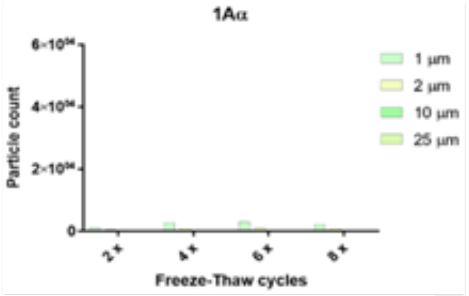

Figure 2: Particle count of PS20 (1Aα)

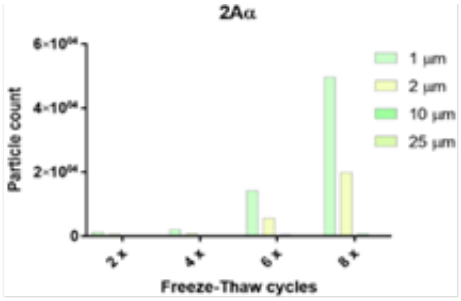

Figure 3: Particle count of PS20 (2Aα)

P - II - 2

Lipidic based-gel with tunable release properties as a platform for local delivery of biotherapeutics

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Introduction: Lipid mesophases are able to incorporate and release a plethora of molecules, spanning from hydrophobic drugs to small hydrophilic proteins, and therefore they have been widely used as drug delivery systems. However, their water channels of 3–5 nm do not allow the release of large hydrophilic molecules, such as monoclonal antibodies and therapeutic proteins [1].
Aim: To overcome this major geometrical constraint, we designed a gel by mixing monoacyl-glycerol lipids, generally recognized as safe for humans and/or animal use by the FDA, and phospholipids, to obtain a material with swollen water channels suitable to host and further release macromolecules [2].
Method: 1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DOPG), 1,2-dioleoyl-sn-glycero-3-phospho-l-serine (DOPS) and 1, 2-dioleoyl-3-trimethyl-ammonium propane (DOTAP) were added together with cholesterol (Chol) to a monoolein/water system to form a stable ultra-swollen cubic phase able to host and release a big macromolecule without disrupting the 3D structure of the gel. Small angle X-ray scattering (SAXS) and release experiments were used to elucidate how the size of the water channel, the phase identity of the gel and the electrostatic interaction between lipids and protein can be varied to achieve a tunable cargo release. Apoferritin (ApoF), a nanocage protein with a diameter of 12 nm, has been selected here as hydrophilic model protein to be embedded in our gel.
Results: When immersed completely in the release media, mesophases with a swollen water channel of 22 nm, composed of monoolein and doped with 5 mol% of DOPS and 10 mol% of Chol allowed us to achieve a protein release of 60 %, which is 120 times higher with respect to that obtained by employing non-swollen-LMPs composed only by monoolein. Swollen lipidic mesophases allow to administer locally biomacromolecules in those diseases easily reachable by a local application, such as rectal or vaginal cancer, thus reducing the drawbacks associated with a parenteral administration [2].
Conclusion: Using phospholipids to enlarge the water channels of bicontinuous cubic phases and Chol which keeps stable the fluid bilayer, we created swollen gels able to host and release the embedded ApoF. This safe and easy-to-manufacture release system could serve as a versatile platform to encapsulate biomacromolecules, and it paves the way for the mucosal application of ApoF and other biomacromolecules including HFt, mAb and antibody-drug conjugates in diseases such as rectal and vaginal tumors, that are easily reachable by local administration, reducing the systemic drawbacks associated with a parenteral administration.

Keywords: lipid mesophases, swollen water channels, apoferritin, protein delivery system, topical delivery

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P - II - 3

Vaginal administration of repurposed drugs for endometriosis treatment

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Introduction: Endometriosis is a gynaecological disease which involves fibrosis of pelvic organs such as the vagina and bowel [1]. Pirfenidone is an antifibrotic drug approved for the treatment of pulmonary fibrosis marketed as an oral tablet. Pirfenidone has the potential to be repurposed for endometriosis treatment; however, the side effects of orally administered pirfenidone were too severe for it to be recommended [2]. Here, we propose vaginal delivery of pirfenidone for endometriosis treatment to reduce side effects and improve efficacy.
Aims: Our aim was to formulate pirfenidone into vaginal dosage forms using standard pharmaceutical methods and additive manufacturing (3D printing).
Methods: We designed a semisoft ovule containing liposomal pirfenidone (Fig. 1, developed previously in our group [3]) manufactured using semisolid extrusion 3D printing. We also developed pirfenidone vaginal ovules using standard pharmaceutical excipients. Dosage forms were characterized according to pharmacopeial quality tests, bespoke *in vitro* release and *ex vivo* mucoadhesion assays.
Results: 3D printed ovules had a homogeneous pirfenidone distribution within the dosage form. They were superior to standard ovules in terms of dissolution behavior and *in vitro* release, dissolving slowly and releasing up to 94% of pirfenidone over a period of 8 h. They also showed advantageous *ex vivo* mucoadhesion properties.
Conclusion: This work demonstrates that pirfenidone can be manufactured into standard and 3D printed vaginal dosage forms, both as a free drug and in liposomal form, paving the way for clinical studies into vaginally administered pirfenidone for endometriosis treatment.

Keywords: vaginal drug delivery, drug repurposing, endometriosis, additive manufacturing

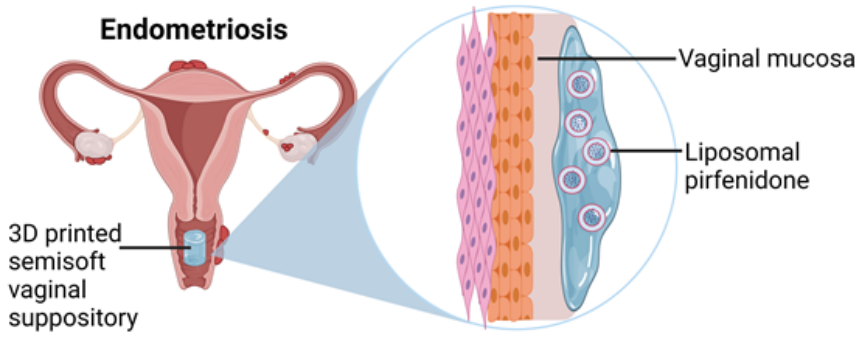

Figure 1: Schematic diagram of a semisolid dosage form for vaginal delivery of the repurposed drug pirfenidone. Figure created in BioRender.

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Composite pirfenidone gel for the local treatment of fibrosis in multiple organs

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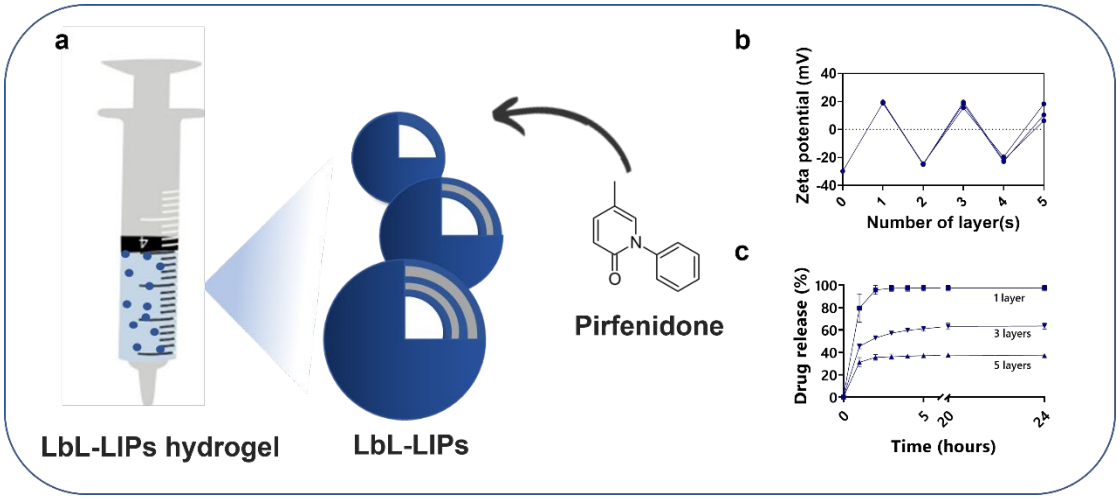
Introduction: Few therapeutic approaches are designed to effectively treat fibrotic diseases. Pirfenidone (PFD) is a small hydrophilic drug approved for the treatment of idiopathic pulmonary fibrosis and showing anti-fibrotic and anti-inflammatory properties in a variety of animals and *in vitro* models in different organs. Numerous studies using PFD are being conducted to treat local fibrosis-related conditions such as intestinal fibrosis, diabetic foot, and endometriosis. However, orally administered PFD – currently the only one approved dosage form – may be associated with significant side effects including gastrointestinal and neurological complications. Therefore, the therapeutic value of PFD can be improved by encapsulating it in drug delivery systems which allow its local and sustained release directly at the fibrotic site.
Aims: To improve PFD' safety profile, we designed a local drug delivery system consisting of layer-by-layer PFD-loaded liposomes (LbL-LIPs) embedded into a zinc alginate hydrogel.
Methods: PFD was encapsulated in liposomes using a passive loading method and characterized in terms of size and ζ-potential. The drug loading was quantified by HPLC and HPLC-CAD. Polymer deposition was monitored with ζ-potential and Cryo-TEM. LbL-LIPs hydrogel was prepared by ionotropic gelation, and its rheological properties assessed with an oscillation rheometer. For *in vitro* release studies, PFD release was monitored at both pH 4.5 and 7.4 using vertical diffusion cells.
Results: The encapsulation of PFD was optimized to obtain a drug loading of 85%. Further, the 150-nm liposomes were coated with up to 5 biopolymers – alginate and chitosan layers (Fig. 1b) without any purification step needed. LbL-LIPs were integrated into a zinc alginate hydrogel to provide an instantly crosslinked drug delivery platform. To mimic the vaginal and intestinal environment, *in vitro* release studies were performed at pH 4.5 and 7.4 (Fig. 1c). The results of the study demonstrate that, by adjusting the LbL thickness, a local and tunable release of PFD can be obtained.
Conclusions: Our composite PFD gel is the first tunable platform offering a mucosal delivery of an approved antifibrotic drug, and it paves the way to potential treatments directly to the fibrotic site.

Keywords: pirfenidone, intestinal fibrosis, endometriosis

Figure 1. a) Schematic representation of the composite hydrogel; b) Changes in ζ-potential at each biopolymer deposition; c) *in vitro* release studies of LbL-LIPs hydrogels at pH 7.4



P - II - 5

Hydroxyapatite microcapsules as multifunctional drug delivery devices

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Introduction: Modern drug delivery needs to use novel multifunctional materials for developing medicinal products and new therapies. Our previous work has shown that porous functionalized calcium carbonate particles are a prototype material with several functions, i.e., they can be used as filler, disintegrant, tablet hardness enhancer, and encapsulation carrier at the same time. Besides the multifunctionality, it is a biocompatible, biodegradable, and non-toxic compound. On the other hand, we have identified some downsides, such as a limited encapsulation efficiency. The latter results in drug depositions on the carrier's surface, which leads to a loss of material multifunctionality as the physicochemical properties change.
Aims: In this study we aimed to improve the former approach and design a new inorganic carrier material with a fundamentally different geometry: The template-inverted particles (TIP).
Methods: We developed a scalable process to manufacture TIP particles. The internal structure of TIP particles was elucidated using focused ion beam scanning electron microscopy (FIB-SEM) and SEM analysis of TIP cross-sections. Crystalline structures of TIP were determined from their x-ray powder diffraction (XRPD) spectra. The particle functionality, particularly the potential for drug encapsulation resulting from the unique core geometry, were evaluated by measuring water absorption capacity and mercury intrusion volume.
Results: TIP is a mono-material consisting of pure hydroxyapatite. Visual analysis confirmed the manifold cavity in the center of the particle that lend TIP its name. The intrusion volume data correlates with the water uptake capacity and reveal that TIP provides more than twice the loading space compared to the non-hollow precursor particle (TIP S1). TIP reveals a narrow size distribution with an average particle size of 20 µm. The high porosity of TIP ensures a rapid water uptake and tablet disintegration. Upon contact with water, TIP tablets immediately disintegrate into primary particles.
Conclusions: TIP is a novel class of multifunctional micro-delivery devices with a unique hollow cavity surrounded by a porous hydroxyapatite shell, enabling fast disintegration. TIP's unique feature is a hollow core that offers plenty of space for loading. The presented particles provide a solution for the problem of poor encapsulation efficiencies in commercially available carriers. TIP is a platform technology for formulation development of orally disintegrating tablets (ODT).

Keywords: oral drug delivery, template inverted particles (TIP), microcapsules, orally dispersible tablets (ODT)

P - II - 6

Stability studies of commercial corticosteroid suspensions

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Introduction: Particulate corticosteroid suspensions are widely used for epidural, transforaminal and perineural injection to treat lumbar pain [1]. The administration of these suspensions was associated with rare post-procedural complications that include neurovascular embolism, ischemia, and scarring of the nerves with the risk of paralysis and death [2]. We hypothesize that adverse events may be impacted by particle size of corticosteroid suspensions, as particles larger than erythrocytes (7-8 μm) are theoretically capable of blocking capillaries (diameter approx. 9 μm). Size may also be influenced by acidic pH (inflammation), plasma proteins such as albumin, and clinically co-administered solutions such as local anesthetics and iodinated contrast agents.
Aim: The aim of this study was to measure particle size of corticosteroid suspensions in biorelevant simulating fluids at different pH values and in presence of albumin, local anesthetics, and iodinated contrast agents.
Methods: Five commercial corticosteroid suspensions of triamcinolone (Triamcort 10, 20, 40 mg/mL, Kenacort A 10, 40 mg/mL), methyl prednisolone (DEPO-Medrol 40, 80, 200 mg/mL), and betamethasone (Diprophos 7 mg/mL, syringe and vial) were analyzed. The suspensions were mixed equivolumetrically with buffers at pH 6.0 and pH 7.4, albumin solution at 50 g/L, local anesthetics lidocaine 20 mg/mL and ropivacaine 7.5 mg/mL, and iodinated contrast agents iopamidol 370 mg/mL and ioxaglate 320 mg/mL for 24 h at 37 °C. Size measurements were performed by laser diffraction (Coulter LS 230 Particle Size Analyzer, Beckman Coulter, USA) with saline as dispersion medium and presented as diameter at the 50th (median diameter, D_50) and 90th percentile (D_90) of the volume distribution.
Results: The size of the corticosteroid suspensions varied from 9.4 to 11.7 μm (D_50) and 17.0 to 22.6 μm (D_90) for triamcinolone, 10.7 to 12.5 μm (D_50) and 17.8 to 20.7 μm (D_90) for methyl prednisolone, and 15.3 to 21.4 μm (D_50) and 26.2 to 35.5 μm (D_90) for betamethasone. Changes in particle size were small at pH 6.0 and in the presence of albumin, lidocaine, ropivacaine, iopamidol, and ioxaglate except for the methyl prednisolone suspension whose median diameter fell from 11.1 ± 0.3 μm to 6.2 ± 0.9 μm in the presence of albumin.
Conclusions: The diameters of the investigated commercial corticosteroid suspensions were between 9 and 20 μm and differed between suspensions. This particle size exceeds erythrocyte size such that the particles are theoretically capable of blocking capillaries. The size of the particles was similar at low pH and in presence of albumin, local anesthetics, and contrast agents except for one suspension. Investigations in biological fluids (e.g., serum) and of other particle properties (e.g., surface charge, morphology) are warranted to improve our understanding of stability-impacting parameters of injectable corticosteroid suspensions and maximize drug safety.

Keywords: corticosteroids, suspension, particle size, inflammation

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P - II - 7

Formulation and delivery strategies for sustained topical drug delivery to treat nail disorders

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Introduction: Limited permeation and poor drug diffusion are widely known factors responsible for the failure of topical treatments. The low permeability of skin and nail plate combined with frequent poor drug solubility leads to insufficient drug delivery to these tissues. Despite numerous studies trying to enhance topical drug delivery, current treatments still lack improvements.
Aims: The main goal of this thesis is to combine formulation and delivery strategies to create local drug depots for sustained topical drug delivery with applications to treat skin and nail disorders. Previous work has shown promising results using fractional laser ablation to enhance drug targeting and drug delivery in the nail by mechanically removing the nail plate's barrier. Using a sustained release formulation that could fill the pores created by the laser and deliver the drug for a more extended period would reduce the number of applications and the overall duration of treatment, thus potentially improving compliance and treatment outcome. The first project of this work has focused on developing a formulation for sustained delivery of terbinafine (TBF), an antifungal agent, to the nail.
Methods: A thermoresponsive poloxamer 407 (P407) gel was formulated. The rheological and thermo-gelation properties of the gels were investigated to evaluate their properties as potential drug carriers. P407 was selected because of its thermosensitive properties (liquid and gel depending on the temperature), as well as the considerable insight in human use gathered for years. A thermosensitive formulation could easily fill and gelify in the micropores at the temperature of the nail (32°C) to increase the residence time and decrease treatment frequency. In parallel, size reduction of antifungal drugs was performed by wet milling to obtain nanocrystals that will be incorporated in the gel. Previous work has shown that a high drug loading and an extended release could be achieved by using nanocrystals. The amount of drug in the pores created by laser ablation could be higher by using nanosized drug particles, and the active surface area in contact with the nail would be increased. Lastly, using a drug suspension avoids the issue of solubility.
Results: TBF nanocrystals were successfully obtained by wet milling with an average D.v (90) of 1.97 µm. Differential scanning calorimetry analysis showed that the melting point and enthalpy of TBF nanocrystals were similar to commercial and recrystallised products, meaning that TBF crystallinity was not altered after wet milling. Suspensions containing 10% (w/w) of TBF nanocrystals in 15 to 20% (w/w) of P407 were successfully formulated. Rheological studies demonstrated that 10% (w/w) of TBF nanocrystals in formulation slightly decreased the state transition of P407. Nevertheless, a state transition between 25°C and 32°C has been achieved with 16 and 17% (w/w) of P407.
Conclusions: TBF nanocrystal-loaded P407 gel containing 10% (w/w) of TBF nanocrystals with a state transition between 25°C and 32°C was successfully formulated. This project aims to continue towards *ex vivo* experiments using laser porated porcine hooves and then healthy and onychomycotic human nails in order to evaluate the *in vitro* ungual delivery of the formulation.

Keywords: topical drug delivery, sustained release formulation, fractional laser ablation, nail

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Screening and optimization of Tubacin-loaded liposomes using Plackett-Burmann design

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Introduction: Tubacin is a histone deacetylase inhibitor (HDACI). This compound has high lipophilicity (Log P≈7) and is insoluble in water. Consequently, tubacin has been rather used as a research tool than as a drug [1]. Liposomal formulations are suitable for the encapsulation of highly lipophilic drugs. Conventional formulation strategies rely on trial and error development. Quality by design approaches can reduce experimental efforts and costs. The use of design of experiments (DoEs) enable understanding of process variables and their impact on results. A Plackett-Burmann design (PBD) was used for the screening and optimization of critical parameters involved in the tubacin-loaded liposomes development.
Aim: This project aims for an intravenous administration of tubacin-loaded liposomes. Specific liposome sizes, as well as physical and encapsulation efficiency (EE%) and stability during storage are needed to obtain an optimal drug delivery system. DPPC, cholesterol and TPGS are used for liposome preparation by ethanol injection. Hence, several parameters such as lipid mass, temperature, tubacin concentration, loading time, DMSO volume and rotation speed were all screened by the PBD in order to understand the importance of these variables.
Method: Liposomes were prepared by ethanol injection following the PBD table. EE% was obtained using ultra high performance liquic chromatography (UPLC). Sizes were assessed by dynamic light scattering (DLS) in both batch and flow mode coupled with asymmetrical flow field flow fractionation (AF4). Images of the particles were taken by transmission electron microscopy (TEM).
Results: EE% of tubacin varied between 9% and 38%. Liposome sizes ranged between 122 nm and 235 nm while polydispersity indices (PDIs) were all below 0,17. Hence, the optimization was done to increase the EE%. Amounts of TPGS and DPPC as well as DMSO volume were all found to have a significant impact on the EE%, but only TPGS was positively correlated with the EE%. Hence, the amount of TPGS was increased to 4 and 5 mg, respectively. 5 mg of TPGS reached 80% of EE%, and size, PDIs and EE% were stable during 14 days of storage at 4°C.
Conclusion: Significant variables in the liposome preparation process were identified using a PBD formulation. This information resulted in the formulation of tubacin-loaded liposomes of acceptable sizes, PDIs and EE%. Stability of these properties at 4°C storage was also achieved.

Keywords: liposomes, tubacin, design of experiment, Plackett-Burmann

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P - II - 9

Cyclodextrin-based microneedle patch for nanoparticulate vaccine delivery

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Introduction: The *stratum corneum* is a difficult barrier to overcome, especially for poorly permeable molecules such as proteins. Microneedles have received increasing attention in transdermal drug delivery due to painless application, the possibility of self-administration and good stability. The submillimeter structures can pierce the skin and the drug load is deposited in the underlying dermal layer. Vaccines can benefit from a local delivery to the *dermis*, where antigen presenting cells reside and induce immune responses. Dose-sparing has been observed after intradermal vaccination [1], underlining the benefit and importance of microneedles in this context. We have recently developed a nanoparticulate vaccine that led to enhanced T-cell activation in mice after immunization with a model peptide [2]. To further increase its efficiency, we aim to deliver it *via* microneedles.
Aims: The objective of this work is to develop a dissolving microneedle patch with a suitable matrix and mild production parameters to deliver a nanoparticulate vaccine. The patch should be able to penetrate the skin and deposit the vaccine in the *dermis*. A fast dissolving separating back layer needs to be included to deposit the needles in the skin.
Methods: Microneedle patches were fabricated using a molding technique, by pouring matrix suspension of cyclodextrins with peptide-loaded amphiphilic cyclodextrin-based nanoparticles into silicon molds followed by several rounds of centrifugation and drying. An interlayer of dissolving polymer (e.g. PVA, Gelatin) was cast on top before the rigid back layer was applied. Microneedles were characterized by bright-field, fluorescence, and scanning electron microscopy (SEM). Uniformity of content was measured and mechanical testing was carried out with a texture analyzer. *Ex vivo* studies with porcine ear skin were used to assess penetration efficiency and incision depth. The vaccine release was investigated in Franz cells with porcine skin.
Results: A reproducible technique with mild conditions was developed to fabricate uniform microneedle patches and incorporate the nanoparticulate vaccine. Imaging by SEM revealed sharp tips with a diameter of around 10 µm and presence of nanoparticles in the tips. *Ex vivo* studies with porcine skin showed efficient needle penetration with incision depths of up to 600 µm in skin slices. After a few minutes of application, the back of the patch could be removed and separated from the microneedles.
Conclusions: The developed microneedle patch shows promising properties in the delivery of nanoparticulate vaccines. The dissolving interlayer allowed for deposition of the needles in the dermal layer of the skin. Further studies will focus on the efficiency of the vaccine after *in vivo* administration *via* microneedle patch.

Keywords: microneedles, vaccine, nanoparticles

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P - II - 10

Particles in biopharmaceutical formulations: mechanistic study developing tools for particle characterization in close container

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Introduction: The presence of sub-visible & visible particles in liquid protein formulations is a common finding. Although the clinical and quality impact of these particles is difficult to define, the pharmaceutical community agrees on the need to monitor them for potential immunogenicity risk [1]. Research on mechanistic formation and kinetics of inherent particles are conducted through stability studies to determine product specific profiles over the shelf life. The necessity to develop innovative technologies to get a fully comprehensive characterization of visible particles in closed containers has become more and more important.
Aims: The objective was to develop an innovative prototype to perform nondestructive visible particle characterization and chemical identification in a closed container (vial or syringe) filled with liquid drug product.
Methods: The developed prototype is composed of an innovative visual inspection setup based on light sheet microscopy to perform robust detection, accurate sizing, quantification and 3D localization of visible particles in a closed container. The integration of a Raman video probe to the visual inspection unit enables performing in-situ chemical identification of the detected particles.
Results: A comparative study evaluating the performance of the prototype with the three methods (manual visual inspection, fluidic imaging system & filtration method) currently used to characterize visible particles present in parenteral drug products was performed to highlights these new capabilities of measurement.
Conclusions: The application of this new tool provides the opportunity to improve formulation development by enabling a new approach to study the mechanistic formation and kinetics of particles. The analysis of particles in a closed container on regular time points will provide indications to understand better protein aggregation pathways and the possible factors that affect or control the protein aggregation process.

Keywords: particle characterization, protein aggregates, visual inspection, Raman spectroscopy

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P - II - 11

Development of a gastro-resistant formulation for a complex mixture of microorganisms

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Introduction: The intestinal microbiota is very diverse. It consists of several thousand species of microorganisms, including bacteria. An imbalance of the bacterial microbiota is called dysbiosis. This phenomenon can cause various diseases such as inflammatory bowel disease, obesity and diabetes, allergies, autoimmune diseases, cardiovascular diseases, and *Clostridioides difficile* infection (CDI) [1]. CDI is a major health problem and one of the most important nosocomial infection. Its treatment is challenging because of frequent relapses [2]. However, fecal microbiota transplantation (FMT) is a promising option for the treatment of CDI, but its current formulations need optimization.
Aims: The goal of the project is to develop a gastro-resistant formulation by a method of encapsulation of a model microorganism.
Methods: Bacterial culture of *Escherichia coli* was done in 2xYT medium. Then, encapsulation by ionotropic gelation using sodium alginate, trehalose and sodium pyruvate was carried out prior to coating and freeze-drying. Various coatings were tested: xanthan gum (XG), cellulose acetate phthalate (CAP) and Eudragit S100. Finally, the viability of the bacteria was tested using flow cytometry and counting on solid 2xYT culture medium: before and after coating, after freeze-drying and after dissolution test of the European Pharmacopeia 10 (5.17.1). The test consists of immersion of the particles in 0.10M HCl during 2 h followed by dissolution of those particles in PBS pH 6.8 and 7.4.
Results: The size of the particles was around 1 mm and the batches were homogenous. A formulation composed of 2.5% Eudragit and 0.5% TEC in a 0.07 M NaOH solution showed 63.4% viability in PBS solution at pH 6.8 and 63.6% viability in PBS solution at pH 7.4 after immersion in 0.10 M HCl for 2 h. The plate-counting results are 3.22×10^7 CFU/g of particles in the PBS solution at pH 6.8 and 1.25×10^7 CFU/g of particles in the PBS solution at pH 7.4.
Conclusions: By adding a cryoprotectant and a plasticizer to the coating formulation make it possible to preserve the physical properties of the microparticles after freeze-drying [3]. A very promising formulation with good bacterial viability by flow cytometry and counting on solid culture medium was obtained. The next steps would be the application of this formulation to a mixture of microorganisms and stool samples.

Keywords: fecal microbiota transplantation, bacteria formulation, alginate particles, gastro-resistant formulation

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Development of a novel microencapsulation formulation for fecal microbiota transplantation

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Introduction: *Clostridioides difficile* infection (CDI) is one of the most important nosocomial infection with more than 124,000 cases per year in Europe and a mortality rate of 15-17%. The standard of care (SoC) is an antibiotic treatment using fidaxomicin or vancomycin. Unfortunately, the relapse rate is high (~35%), and the SoC is significantly less effective against the recurrent infection (rCDI). CDI is associated with a gut dysbiosis, so treatments to modulate the microbiota have showed promise to treat rCDI. Fecal microbiota transplantation (FMT) is an adjuvant treatment against rCDI from the second recurrence episode and has an efficacy of 90%. The formulation of purified donor stool requires further optimization, because its administration routes are uncomfortable: naso-duodenal/jejunal tubes, colonoscopy, enema or 30 voluminous oral capsules [1,2].
Aims: This project aims to produce a novel formulation for FMT. This will allow to simplify FMT administration and to enhance the handling and storage of fecal samples. Moreover, a dry, stable, compact, gastro-resistant and efficient formulation of live microbiota can promote research related to microbiota manipulation therapies.
Methods: Model bacteria strains were first investigated. The chosen strains were: *Escherichia coli*, *Enterococcus faecalis* and *Lactobacillus paracasei.* Then, the method was applied to purified stool. Concentrated bacteria suspensions or stool was mixed with a solution containing sodium alginate, sodium pyruvate and trehalose. The obtained mixture was then extruded through a syringe in a calcium chloride solution under magnetic stirring to form particles by ionotropic gelation [3]. Then, particles were collected by filtration and rinsed. Samples were snap-frozen in a trehalose solution and freeze-dried overnight on automatic cycle. Characterization was carried by optical microscopy and the viability of the entrapped bacteria was assessed by plate-counting and flow cytometry using a LIVE/DEAD™ BacLight™ Bacterial Viability Kit.
Results: Spherical gel beads of a mean size of ~2 mm were obtained. Particles were reproducible between all batches. A high-loading of viable microorganisms was obtained for model strains and fecal samples. For plate-counting, values ranged from 10^{15} to 10^{17} CFU/g for single and mixed model strains and from 10^{6} to 10^{8} CFU/g for fecal samples**.** This corresponded to a viability of 30% to 60% for flow cytometry.
Conclusions: Robust batches were obtained in terms of particles' appearance and size. Highly-loaded alginate particles with encapsulated live bacteria were successfully produced for both model strains and fecal samples. This novel formulation is promising as the technology was applicable to both model strains and the gut microbiota. The next steps will be to develop a gastro-resistant formulation and to assess the stability and viability of the microbiota over time during storage.

Keywords: fecal microbiota transplantation, bacteria formulation, alginate particles

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P - II - 13

Tablet formulation of soy polyenylphosphatidylcholines (PPCs) for liver fibrosis therapy

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Introduction: Liver fibrosis is a pathological pathway of the intrinsic wound healing response, characterized by widespread collagen and extracellular matrix accumulation, and caused by the myofibroblastic activation of human hepatic stellate cells (HSCs) [1]. PPCs' antifibrotic effect on liver fibrosis was proven, among others [2], by our group thanks to an *in vitro* fibrosis model based on the human immortalized HSC line LX-2 [3].
Aims: The aim of this work was to formulate a high lipid content tablet with a novel soy phospholipid S 80 M developed from Lipoid GmbH (Ludwigshafen, Germany) that possesses advantageous technological properties.
Methods: To prepare soy PPCs S 80 M tablets, manual dry granulation was used to obtain granules that were successively compressed to tablets employing a single punch press. All relevant pharmacopoieal tests were performed both on granules (flowability, bulk and tapped density) and tablets (uniformity of mass, friability, hardness, and lipid recovery). Phospholipids were extracted from tablets and, to represent more likely physiological pathway after ingestion, were reconstituted both in a buffer or like protein-free chylomicrons (PFC)-like emulsions to test them on our established *in vitro* fibrosis model.
Results: Final concentration of up to 70% w/w S 80 M was formulated in tablets that complied with major pharmacopoeial requirements. Lipid extracts and emulsions had the same antifibrotic effect on the activated, fibrotic LX-2 cell model. The biological activity obtained was the same as with only pure S 80 M liposomes [3], showing an increased intracellular lipid droplets' storage and a decrease in collagenous fibres.
Conclusions: We successfully formulated a high concentration of bioactive soy PPCs in tablets that comply with major pharmacopeial requirements. The *in vitro* tests of this oral formulation showed that the typical antifibrotic effect observed with PPCs' liposomes was retained. Antifibrotic effects can be increased by co-formulation of soy PPCs with a hepatoprotectant such as silymarin or an experimental antifibrotic API.

Keywords: tablets, soy phospholipids, liver fibrosis, pharmacopoeia, *in vitro* model

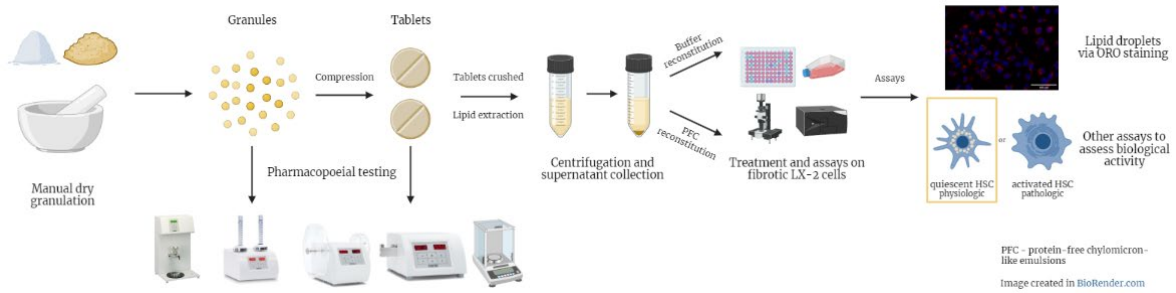

Figure 1. Tablet production and lipid extraction to test S 80 M

Disclosure statement:
S 80 M was kindly donated by Lipoid GmbH. No study sponsors had any involvement in study design, data collection, or interpretation of data presented in this communication. PL has consulted to Lipoid and Sanofi and received research grants from Lipoid, Sanofi-Aventis Deutschland and DSM Nutritional Products Ltd; she is currently Specialist for Pharmaceutical Quality in the Human Medicines Expert Committee (HMEC) at Swissmedic, the Swiss Agency for Therapeutic Products.

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P - II - 14

Application of deep learning to the analysis of time-resolved (4D) micro-computed tomography data

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Introduction: Deep learning applications in image analysis are a promising development that is slowly making its way into pharmaceutical research. Especially in areas that exceed the capabilities of classical approaches such as the analysis of medical image data, specifically tomography data, computer vision diagnostics based on convolutional neural networks (CNNs) find widespread application. The focus thus far has been mostly on the classification of static images. However, much more information can be obtained by considering time-resolved computed tomography (CT) data.
Aims: We aim to provide a methodological toolbox to facilitate the reconstruction and segmentation of time resolved x-ray based tomographic microscopy (µCT) data, thereby enabling quantitative analysis. We aim to demonstrate the immense value of this imaging technique, as well as the utility of our image analysis methodology by generating, reconstructing, and segmenting an extensive set of time-resolved µCT data capturing the disintegration of pharmaceutical tablets.
Methods: Our work makes use of a custom software pipeline to handle the reconstruction of the immense amount of raw data generated by time resolved microscopic tomography. The data was acquired at the TOMCAT tomographic microscopy beamline of the Swiss Light Source particle accelerator at Paul Scherrer Institute. The heterogeneous image quality inherent to the time resolved µCT imaging technique necessitated the application of a deep learning-based image segmentation approach. Thus, the application of a CNN enabled quantification of the dynamic process of tablet disintegration captured in the data.
Results: Our proposed image reconstruction and segmentation pipeline rapidly delivers consistent and accurate results, thereby enabling and facilitating quantitative analysis. The trained CNN given the task of image segmentation outperforms humans in precision as well as consistency. Preliminary analysis of the resulting data reveals unprecedented insights into tablet disintegration.
Conclusions: Our work demonstrates a way of harnessing the untapped potential of artificial intelligence coupled with time-resolved µCT imaging in pharmaceutical applications. The application of deep learning to our proposed image-processing pipeline makes it both powerful and easily adaptable.

Keywords: deep learning, time-resolved tomographic microscopy, tablet disintegration

P - II - 15

Evaluation of lipid nanoparticle formulations for efficient DNA delivery to the liver

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Introduction: Gene therapy is a promising strategy to treat inherited monogenetic diseases. Commonly used viral vectors for nucleic acid delivery are hampered by immunogenicity issues, safety concerns and high production costs. In contrast, non-immunogenic and cost-effective non-viral alternatives are of increasing demand. Especially lipid nanoparticles (LNPs) have gained considerable attention. LNP systems for gene delivery usually consist of four lipid components (i.e., ionizable or cationic lipid, helper lipids, cholesterol, and a PEG-lipid) and nucleic acid. The ionizable lipids have been optimized for nucleic acid encapsulation and intracellular delivery, facilitating the release from the endosomal compartment to the cytosol following cellular uptake. To date, LNPs are the most clinically advanced nano-sized delivery system for nucleic acids. The great effort put in the development of ionizable lipids with increased *in vivo* potency brought LNPs from the laboratory benches to the FDA approval of Patisiran (*Onpattro*, Alnylam Pharmaceuticals) in 2018 and mRNA-based vaccines against SARS-CoV-2 (*Spikevax*, Moderna; *Comirnaty*, BioNtech). However, there are still challenges including the composition of LNP, as well as biological hurdles that includes the delivery of the nucleic acid from the cell membrane to the nucleus.

Aims and Methods: In our current study, different LNP formulations were designed using ionizable lipids of the clinically approved LNP formulations (i.e., DLIN-MC3-DMA, SM-102, ALC-0315) to identify an efficient and safe vector for delivery of DNA to the liver. LNP formulations were generated by microfluidic mixing and characterized for their physico-chemical particle properties (i.e., size, ζ-potential, encapsulation efficiency, shape). Different biological key processes were determined including cellular and nuclear uptake as well as transgene expression in human hepatoma cells (i.e., HuH-7) and murine primary hepatocytes. Systemic circulation properties of LNP formulations *in vivo* were estimated using a zebrafish model. Ultimately, LNP biodistribution was assessed in a pilot experiment with the lead formulation by intravenous injection in wildtype mice.

Results: All LNP formulations efficiently encapsulated DNA and resulted in small spherical particles with sized ranging from 80 to 120 nm. Particle surface charge was determined to be negative for all formulations. SM-102-based LNP formulation displayed the highest rate of cellular and nuclear uptake. Accordingly, SM-102-based LNP formulation resulted in highest transgene expression in HuH-7 cells as well as murine primary hepatocytes. Systemic circulation was comparable for all LNP formulations. Interestingly, only MC3-based LNPs showed significantly decreased circulation after 24 hours. Besides, particle extravasation following administration into systemic circulation was highest for MC3-based formulation and lowest for ALC-0315-based formulation. A pilot experiment assessing biodistribution with SM-102-based LNP formulation showed main delivery of DiD-labelled lipids to the lung, liver, and spleen.

Conclusions: Although physico-chemical particle characteristics of the assessed LNP formulations are similar, significant differences of their effects *in vitro* and *in vivo* were observed. The SM102-based LNP formulation displayed most efficient uptake into cells and nuclei resulting in high transgene expression *in vitro*. In addition, SM-102-based LNPs were shown to circulate well without excessive extravasation. We conclude that SM-102-based formulation offers a promising candidate as an efficient and safe vector for delivery of DNA to the liver. Future work will focus on targeting strategies to promote more efficient and specific delivery following systemic administration.

Keywords: gene delivery, lipid nanoparticles, liver; zebrafish, biodistribution

III. PHARMACOEPIDEMIOLOGY

P - III - 1

Recording of chronic diseases and adverse obstetric outcomes during hospitalizations for a delivery in the National Swiss Hospital Medical Statistics Dataset between 2012 and 2018: An observational cross-sectional study

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Introduction: The prevalence of chronic diseases during pregnancy and adverse maternal obstetric outcomes in Switzerland has been insufficiently studied. Data sources which reliably capture these events, are scarce.
Methods: We conducted a nationwide observational cross-sectional study (2012–2018) using data from the Swiss Hospital Medical Statistics (MS) dataset. To quantify the recording of chronic diseases and adverse maternal obstetric outcomes during delivery in hospitals or birthing centers (delivery hospitalization), we identified women who delivered a singleton live-born infant. We quantified the prevalence of 23 maternal chronic diseases (ICD-10-GM) and compared results to a nationwide Danish registry study. We further quantified the prevalence of adverse maternal obstetric outcomes (ICD-10-GM/CHOP) during the delivery hospitalization and compared the results to existing literature from Western Europe.
Results: We identified 577,220 delivery hospitalizations, of which 4.99% had a record for ≥1 diagnosis of a chronic disease (versus 15.49% in Denmark). Moreover, 13 of 23 chronic diseases seemed to be substantially under-recorded (8 of those were >10-fold more frequent in the Danish study). The prevalence of 3 of the chronic diseases was similar in the 2 studies. The prevalence of adverse maternal obstetric outcomes was comparable to other European countries.
Conclusions: Our results suggest that chronic diseases are under-recorded during delivery hospitalizations in the MS dataset, which may be due to specific coding guidelines and aspects regarding whether a disease generates billable effort for a hospital. Adverse maternal obstetric outcomes seemed to be more completely captured.

Keywords: pregnancy, chronic diseases, obstetric outcomes, observational research, Hospital Medical Statistics

P - III - 2

The use of metamizole and other non-opioid analgesic and characterization of its users in Switzerland, a descriptive study

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Introduction: Metamizol has been withdrawn or withheld from the market in many countries (e.g. France, USA, UK) due the risk of agranulocytosis. In Switzerland, metamizol is among the most frequently prescribed non-opioid analgesic drugs (NOADs).
Aims: We aim to investigate the use of metamizol and other NOADs in Switzerland over time, and to characterize users of different NOADs with regard to age and co-medications.
Methods: We conducted a retrospective descriptive study using the claims database of the largest Swiss health insurance company (Helsana). We identified all claims (ATC-codes) of metamizol, ibuprofen, diclofenac, and paracetamol in patients aged 18 years or older. We evaluated the number of dispensed defined daily doses (DDD) of each NOAD from 2014 to 2019. To characterize users of different NOADs, we identified the first claim of each NOAD of interest (metamizol, ibuprofen, diclofenac and paracetamol) per patient in 2019. Among four resulting cohorts, we excluded patients with <180 days of enrollment prior to this first claim. We captured patients' age and whether they had claimed (yes/no) oral anticoagulants (OA), platelet inhibitors (PI), antihypertensives (AH) or glucose lowering drugs (GL) within 180 days prior to this first NOAD claim.
Results: Paracetamol was the most frequently claimed NOAD (792'553 DDD/100'000 patients in 2014), without relevant change until 2019 (+4%). We quantified 13'945 claimed DDD of metamizol per 100'000 patients in 2014, with the largest increase of 80% to 20'136/100'000 in 2019 (4th most frequently claimed NOAD in 2019). The number of claimed DDD (/100'000) of ibuprofen steadily increased by 23% between 2014 (375'075) and 2019 (457'982), whereas those of diclofenac decreased by 21% from 421'266 in 2014 to 335'492 in 2019. Metamizol users had the highest median age of 57 years (y), interquartile range (IQR) 41y-73y, followed by diclofenac users (55y, 41y-68y), paracetamol users (54y, 37y-71y), and ibuprofen users (46y, 33y-60y). Metamizol users more frequently claimed OA (13%) compared to users of other NOADs (5% ibuprofen, 6% diclofenac, 10% paracetamol), PI (18%) (8% ibuprofen, 13% diclofenac, 16% paracetamol), AH (40%) (22% ibuprofen, 32% diclofenac, 36% paracetamol) and GL (11%) (6% ibuprofen, 8% diclofenac, 10% paracetamol) in the 180 days before the first NOAD claim in 2019.
Conclusion: Metamizol is a popular and increasingly used NOAD in Switzerland, which seems to be preferentially prescribed to elderly patients with cardiovascular/metabolic comorbidities and/or undergoing anticoagulant pharmacotherapy. Given its incompletely understood safety profile, safety studies for metamizol are needed.

Keywords: descriptive study, metamizole, usage, claims, user characterization

P - III - 3

What are the factors associated with the willingness to have medications deprescribed in older patients with multiple chronic conditions and polypharmacy?

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Introduction: As the global population is ageing, more people suffer from multiple chronic conditions (multimorbidity), which is often accompanied with the use of several medications (polypharmacy). Polypharmacy is associated with the use of inappropriate medications. Deprescribing – the stopping or reducing of inappropriate medications – is a promising way to reduce inappropriate medication use. However, patients face several barriers related to their willingness to have medications deprescribed. Further, there currently is mixed evidence on the patient sociodemographic and clinical characteristics associated with the willingness to have medications deprescribed in older patients with multimorbidity and polypharmacy.
Aims: The aims of this sub-study were to describe the willingness to have medications deprescribed of older adults with polypharmacy and multimorbidity and to assess which sociodemographic and clinical patient factors are associated with the willingness to have medication deprescribed.
Methods: Baseline data from the «Optimising PharmacoTherapy In the multimorbid elderly in primary CAre» (OPTICA) study was used [1]. Descriptive statistics was used to describe participating patients' demographic and clinical factors and selected questions from the «revised Patients' Attitudes Towards Deprescribing» (rPATD) questionnaire describing patients' hypothetical willingness to have medications deprescribed [2]. Ordinal logistic regression analyses were used in a three-step approach to investigate the association between sociodemographic and clinical factors associated with patients' willingness to have medications deprescribed.
Results: Data from 298 trial participants who replied to the patient version of the rPATD questionnaire were analysed. The median age was 77 years and 45% of the participants were female. The median numbers of medications for chronic use and chronic conditions were both seven. Most participants reported being satisfied with their current medications (>90%), as well as having a good understanding of the reasons why their medications were prescribed (>90%). However, 88% were also willing to deprescribe one or more of their medications if their doctor said it was possible. In the main model with sociodemographic and selected clinical characteristics, a higher number of prescribing omissions (aOR 1.47; 95% CI 1.07 to 2.03; p-value 0.019) was associated with a higher likeliness to be willing to have medications deprescribed. After adding information on the types of chronic conditions and medications, having a cardiological condition (aOR 0.71; 95% CI 0.54 to 0.93; p-value 0.014) was associated with a reduced likeliness to be willing to have medications deprescribed, while having a selective beta blocking agent (aOR 1.41; 95% CI 1.02 to 1.96; p-value 0.037) and/or an angiotensin II antagonist (aOR 1.59; 95% CI 1.00 to 2.52; p-value 0.049) was associated with a higher likeliness to be willing to have medications deprescribed.
Conclusion: Although most older adults with multimorbidity and polypharmacy were satisfied with their current medications, they were willing to deprescribe one or more of their medications if their doctor said it was possible. The different types of chronic medications and chronic conditions that were found to be associated with the participants' willingness to have medications deprescribed should be further investigated to determine if they could be used in future interventions designed to optimize medication use in older adults with multimorbidity and polypharmacy.

Keywords: older adults, polypharmacy, multimorbidity, deprescribing, patient attitudes

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IV. CLINICAL PHARMACY / CLINICAL PHARMACOLOGY

P - IV - 1

Diabetes management and deprescribing in specialized palliative care

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Introduction: Symptom control to optimize quality of life is essential in patients receiving specialized palliative care. Diabetes is a common comorbidity in palliative care patients [1]. Polypharmacy is highly prevalent and associated with a decline in quality of life [2]. However, there are only very few thematically meaningful guidelines on diabetes management at end of life. Recommendations on deprescribing of antidiabetic drugs and glucose monitoring remain underreported [1,3]. Existing recommendations are generally based on clinical experiences rather than evidence. To provide guidance, insights in current practices are needed.
Aims: We aimed to provide an overview of diabetes management, with emphasis on drug therapy and deprescribing, and glucose monitoring, in palliative care.
Methods: We conducted a scoping review (PubMed, Embase) on diabetes management and deprescribing in palliative care patients with a life expectancy of one year or less. Based on this information, we performed an online survey on diabetes management and deprescribing recommendations from health care professionals working in various palliative care settings.
Results: In the scoping review, 50 publications published between 1993 and 2022 were included. Most articles were reviews or observational studies with recommendations on therapy management in type I (17/50, 34%) and type II (28/50, 56%) diabetics. No intervention studies were identified. Half of the articles addressing maximum blood glucose level (14/28, 50%) recommend a value of 15 mmol/L. Of the articles addressing minimal blood glucose level, 56% (15/27) recommend values between ≥5mmol/L and <7mmol/L. Interventions at levels of ≥20 mmol/L were recommended in 67% (6/9) of the articles. Urine glucose and HbA1c monitoring were found to be of little clinical relevance (3/50, 6%, respectively 12/50, 24%), with 67% (8/12) of the articles rating HbA1c monitoring clinically irrelevant, or even recommending against it. Of the articles addressing drug therapy in type I diabetics, 63% (10/16) recommended insulin dose reduction, and 38% (6/16) recommended discontinuation of insulin therapy. For type II diabetics, recommendations for therapy adaptions encompassed discontinuation of oral antidiabetics (20/33, 61%) and considering discontinuation of insulin therapy (13/33, 40%). Experts with (8 palliative care and 5 hospice physicians, 2 palliative care consultants, and 3 clinical pharmacists) and without working experience (3 endocrinologists) completed the survey (21/48, 43.8%), of whom only 2 reported knowing and using guidelines for diabetes management in palliative care. 76.2% (16/21) agreed with the targeted blood glucose level of 5-15 mmol/L identified in the literature. All except for 2 participants (19/21, 90.5%) even considered a maximum blood glucose level of 20 mmol/L to be tolerable under certain circumstances. 80.9% (17/21) agreed with the statement that the HbA1c value is no longer of relevance in palliative care. None of the participants (20/20, 100%) apply urine glucose monitoring. A majority (12/21, 57.1%) stated reasons to discontinue drug therapy in type I diabetics, but 42.9% (9/21) recommended continuing insulin therapy at the lowest possible dose until death to avoid discomforting effects of ketoacidosis. In type II diabetics, 95.2% (20/21) stated reasons to discontinue drug therapy. Several participants emphasized that life expectancy is not the sole criterion for deprescribing antidiabetic therapies. Patient's symptoms and a risk-benefit analysis must also be considered.

Conclusions: Diabetes management and deprescribing are of great clinical relevance, as diabetes is a frequent comorbidity in palliative care patients. Although trends towards desirable targeted blood glucose levels were identified, it was not possible to provide explicit diabetes management and deprescribing recommendations for palliative care. Diabetes management remains highly patient-individual. No consensus was reached between the literature and the survey on the optimal timing for discontinuation of oral antidiabetic therapy, dose reduction of insulin, and discontinuation of blood glucose monitoring. However, some recommendations for diabetes management and deprescribing identified in the literature pertaining to minimal and maximal blood glucose levels were approved by the experts and should be pursued.

Keywords: diabetes management, deprescribing, palliative care

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P - IV - 2

Prescription trends and deprescribing in outpatient specialized palliative care

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Introduction: In palliative care, complex medication regimens with a high prevalence of polypharmacy (on average 7.1-7.8 drugs daily) [1,2] may increase the occurrence of drug-related problems, encompassing medication errors and adverse drug reactions. Deprescribing is particularly relevant in palliative care where therapeutic goals change drastically with the decision in favor of symptom management and quality of life. However, the need for discontinuation of medication can vary greatly over time and needs regular consideration, especially towards perceived medication appropriateness. Guidance on deprescribing in specialized palliative care is limited and urgently needed. To emphasize the clinical relevance of deprescribing in specialized palliative care, it is essential to investigate prescription trends and to identify potentially inappropriate medications (PIMs).
Aims: We aimed to investigate prescription trends with a focus on polypharmacy and deprescribing, and to identify indicators for PIMs in outpatient specialized palliative care.
Methods: We performed a descriptive medication analysis in patients receiving care by a mobile palliative care team (MPCT). Regular and as-needed medication was assessed at three time points (t_0 first assessment, t_1 first changes to medication, t_2 death or transfer to an inpatient setting). In parallel, we conducted a scoping literature review on indicators for PIMs and deprescribing in outpatient palliative care. Prescription trends identified in the medication analysis were linked to the findings of the scoping review.
Results: In the scoping review, 20 publications (5/20, 25%) and one guideline were included. Of the 15 studies applying criteria to identify PIMs provided by one of these five guidelines, «Beers Criteria», «Medication Appropriateness Index» or their own developed criteria, analgesics were reported to be prescribed most frequently (8/15, 53%), followed by psychoactive (7/15, 47%) and gastroprotective drugs (7/15, 47%). The 3 most frequently detected PIMs were blood pressure-lowering (7/15, 47%) and cholesterol-lowering drugs (7/15, 47%), followed by gastroprotective drugs (5/15, 33%), antithrombotic drugs (4/15, 27%), and vitamins/minerals (4/15, 27%). For the medication analysis, 75 patients (49/75, 65% male; MPCT involved 27 days on average [1-157 d]) met the inclusion criteria. In patients with changes to their medication (70/75, 93%), a total of 3257 drugs were prescribed (regular 1426/3257, as-needed 1831/3257). Regular medication increased (+13%) between t_0 (n=479) and t_1 (n=539) and decreased (-24%) between t_1 and t_2 (n=408). As-needed medication increased (+87%) between t_0 (n=378) and t_1 (n=707), and (+6%) between t_1 and t_2 (n=746). The overall number of prescriptions increased (+45%) between t_0 (n=857) and t_1 (n=1246) and decreased (-7%) between t_1 and t_2 (n=1154). In patients with changes to their medication, polypharmacy was present in 66% (46/70) at t_0, 76% (53/70) at t_1 and 54% (38/70) at t_2. Overall, polypharmacy was present in 31% (23/75) and absent in only 1 of the patients at all 3 time points. Most prescriptions per patient on average were identified within the as-needed medication at t_2 (11 ± 4 drugs).
Conclusions Linking prescription trends of MPCT patients with indicators for PIMs and deprescribing identified in the literature helped to emphasize the clinical relevance of deprescribing in outpatient specialized palliative care. The findings could help to develop new pharmacy services and to provide guidance towards a safe and effective medication regimen in highly vulnerable specialized palliative care outpatients.

Keywords: palliative care, mobile palliative care teams, potentially inappropriate medications, deprescribing

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P - IV - 3

Utilization of pharmacogenetic drugs in Switzerland

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Introduction: In Switzerland pharmacogenetic testing is not routinely implemented into primary care, although 167 substances (PGx drugs) on the Swiss market contain information about pharmacogenetics in their drug label, of which 93 are deemed to be actionable [1].
Aims: The aim of this study was to assess the prevalence of PGx drug prescriptions in the Swiss population and to identify the most commonly used PGx drugs and thereby the most relevant PGx genes.
Methods: We identified 90 drugs with dosing recommendations from the Pharmacogenetic Knowledgebase involving 24 genes. We assessed the utilization of those drugs between 2016 and 2020, using claims data from a large Swiss insurance company (Helsana). Helsana covers approximately 1.2 million persons with basic health insurance per year. We calculated absolute and relative numbers of PGx drug exposure, and ranked the substances and associated genes based on the number of exposed persons.
Results: We identified 1 626 058 persons who were registered at Helsana for at least one year during the study period, with 885 866 (54.5%) of them being registered during the whole 5-year period (2016-2020). Persons who were registered for the whole 5-year period had a mean age of 43.4 (±10.9) years and 52.4% were women. In the 5-year period, 55.9% of persons had at least one drug claim and 91.5% of them were exposed to at least one PGx drug. The top 3 PGx drugs were ibuprofen (31.2%), pantoprazole (27.7%) and tramadol (12.3%). The total number of users varied from 1 for imipramine to 276 392 for ibuprofen. At least 9 out of 10 PGx drug users were exposed to drugs of the musculo-skeletal system (38.7%), the alimentary tract and metabolism (37.8%), or the nervous system (31.8%). *CYP2C19* (65.7%), *CYP2C9* (64.6%), and *CYP2D6* (48.5%) were the genes with the highest numbers of persons exposed to at least one associated PGx drug during the whole 5-year period. Seven genes (*CYP2C19*, *CYP2C9*, *CYP2D6*, *SLCO1B1*, *HLA-B*, *MT-RNR1*, and *VKORC1*) were accountable for at least 94% of all potential drug-gene interactions throughout all time-periods.
Conclusions: The prevalence of PGx drug prescriptions is high in the Swiss population, suggesting that a substantial amount of the Swiss population could benefit from preemptive pharmacogenetic testing. As we identified a number of different relevant genes, we think a preemptive testing panel would be more favorable than single-gene testing.

Keywords: pharmacogenetics, drug utilization, Switzerland, claims data, Helsana

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The risk of acute infection in association with newly diagnosed depression: a cohort study

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Introduction: Two cohort studies suggested an up to 60% increased risk of infections in patients with depression compared to patients without depression. However, confounding and surveillance bias may have played a role.
Aims: To assess the risk of acute infections in patients with diagnosed depression compared to patients without diagnosed depression accounting for surveillance bias and confounding.
Methods: We conducted a cohort study using the UK primary care Clinical Practice Research Datalink (CPRD, 2000-2019). We identified patients with an incident diagnosis of depression (exposed cohort). For each exposed patient, we identified one comparison patient of the same age and sex without a diagnosis of depression prior to the cohort entry date (CED) of the corresponding exposed patient. Comparison patients had to have ≥1GP visit within 14 days prior to the CED. We followed patients from day 1 after the CED for a maximum of 2 years or until censoring. The primary outcome was a composite of diagnosed acute infection (respiratory, gastrointestinal, urogenital, or septicemia). We used fine stratification based on a propensity score (PS) to control for 32 baseline covariables including lifestyle factors, comorbidities, comedications, and health-care utilization. We estimated incidence rates (IR) and IR ratios (IRR) of acute infections comparing diagnosed depression to no depression using negative binomial regression.
Results: After PS-fine stratification, we included 285 922 patients with newly diagnosed depression and 285 921 comparison patients. We quantified a weighted IR of 97.32 infections/1000 person-years (py, 32 965 infections in 338 725.2 py) in patients with diagnosed depression, and IR 83.67 /1000 py (40 840 infections in 488 121.5 py) in patients without depression. After PS-matching, the risk of diagnosed acute infection in patients with depression (vs no depression) was slightly increased (IRR, 1.18; 95% CI, 1.16-1.20). Excluding patients with any of the recorded baseline comorbidities or comedications reduced the PS-weighted IRR to 1.07 (95% CI, 1.04-1.09). Not requiring ≥1GP visit within 14 days before CED for comparison patients resulted in a PS-weighted IRR of 1.54 (95% CI, 1.52-1.55).
Conclusions: After controlling for surveillance bias and confounding, we observed a less increased incidence of diagnosed acute infections in patients with diagnosed depression (vs no depression) than previously suggested. The slight relative risk increase further diminished after excluding patients with underlying comorbidities, suggesting residual confounding by disease severity.

Keywords: depression, acute infections, cohort study

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Association of the Swissmedic safety alert regarding skin cancer and the use of hydrochlorothiazide and other antihypertensives in Switzerland

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Introduction: Over the past 5 years, studies consistently revealed an increased risk of non-melanoma skin cancer in association with hydrochlorothiazide (HCT). Swissmedic issued a direct healthcare professional communication (DHPC) on November 18th 2018.
Aims: We aimed to evaluate the association between the DHPC and the use of HCT and other first-line antihypertensives in Switzerland.
Methods: We performed an interrupted time-series analysis using claims data from the Swiss health insurance Helsana between January 1st 2014 and February 28th 2020. Within monthly intervals (30 days), we quantified the total dispensed defined daily doses (DDD) per 10'000 insured individuals of preparations containing 1) HCT, 2) ACE-inhibitors and angiotensin-receptor blockers (ACEI / ARB), 3) calcium-channel blockers (dihydropyridine-type, CCB) and 4) thiazide-like diuretics (bendroflumethiazide not on Swiss market). We conducted segmented linear regression (adjusted for seasonality) to quantify the average number and the trend of monthly dispensed DDD per 10'000 individuals prior to the DHPC, as well as the immediate change (four months lag period) and the slope change over time after the DHPC.
Results: We identified a yearly average of 1'121'043 insured individuals during the study period. We observed an average number of dispensed DDD of HCT of 11'713 / 10'000 prior to the DHPC, which was already declining (trend = - 26.6 DDD/10'000 per month, p<0.0001). The DHPC was associated with an immediate 5.9% decline in the number of dispensed DDD of HCT (- 692.5 DDD/10'000, p=0.007) and a slope change of - 79.8 DDD/10'000 per month (p=0.0046). The average number of dispensed DDD (60'822/10'000) of ACEI / ARB increased prior to the DHPC (104.8 DDD/10'000 per month, p<0.0001), but the DHPC was not associated with a significant immediate effect (-2'324, p=0.0962) or slope change (-101.8, p=0.5019). The same was the case for CCB (average = 18'366 DDD/10'000 per month before DHPC; trend = 61.4, p<0.0001; immediate effect = -193, p=0.7187; slope change = -2.3, p=0.9681). Thiazide-like diuretics were not frequently used (1'546 DDD/10'000 per month before DHPC; trend = 6.7, p<0.0001; immediate effect = -63.3 DDD/10'000, p=0.1839; slope change = 12.5 DDD/10'000, p=0.0173).
Conclusions: Between 2014 and end of February 2020, the use of HCT (in DDD) decreased by 31%. The DHPC was associated with a moderate immediate and prolonged accelerated decline in the use of HCT in Switzerland. Due to the lack of bendroflumethiazide, this decrease appears to be mainly compensated by a 6.6% increase in ACEI / ARB, the most frequently used antihypertensives. Use of CCB increased by 22%, although at a third of the use volume of ACEI / ARB. The DHPC per se was not associated with a change in trend of the use of ACEI / ARB and CCB.

Keywords: hydrochlorothiazide, skin cancer, pharmacoepidemiology

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Diabetes mellitus, antidiabetic use and the risk of treated depression in the UK

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Introduction: Diabetes mellitus (DM) and depression are highly prevalent chronic diseases, both affecting quality of life and reducing life expectancy. Two previous studies from Denmark on this topic yielded conflicting results.
Aims: To assess the association of DM and antidiabetic treatment with the risk of incident treated depression in primary care in the UK.
Methods: In the UK-based Clinical Practice Research Datalink (CPRD) database, we identified patients with incident, treated depression (date of first recording named 'index date', [ID]) and matched them (1:1 on age, sex, calendar time and GP practice) to controls free of recorded depression. In a second analysis, we only included cases of depression among a subgroup of patients with diabetes mellitus type 2 (T2DM) and matched them (1:4 on age, sex, calendar time, and diabetes duration) to diabetic controls. Patients were 18 to 90 years at the index date with >3 years of history in the database and without a history of alcoholism, cancer or HIV. We conducted conditional logistic regression and adjusted the final model for BMI, smoking and alcohol status, several comorbidities, use of antipsychotic or hypnotic drugs at any time before the ID, and number of GP visits in the year before the ID.
Results: We identified 254'559 patients with incident treated depression between 1995 and 2020. Patients with T1DM (adj. OR 1.34, 95% CI 1.22-1.48) as well as patients with T2DM (adj. OR 1.72, 95% CI 1.63-1.81) were more prevalent among cases with depression. We observed the highest relative risk for treated depression in men with T2DM aged 18 to 49 years (adj. OR 2.17, 95% CI 1.82-2.59) whereas women with T2DM of the same age had a lower risk (adj. OR 1.47, 95% CI 1.26-1.72).
The analysis nested in patients with T2DM encompassed 3'827 cases with depression and 14'481 controls. Patients had a mean duration of DM of 3.7 years (SD ± 3.2 years) at the date of the depression diagnosis. Patients who were treated with metformin alone had an increasing risk of depression according to the number of Rx for metformin (1-4 Rx: adj. OR 1.15, 95% CI 0.97-1.37; 5-19 Rx: adj. OR 1.42, 95% CI 1.23-1.64; ≥20 Rx: adj. OR 2.14, 95% CI 1.82-2.52) compared to non-users. Users of metformin plus one or more other antidiabetic drug (sequential use was possible) had a two to threefold increased risk of treated depression compared to non-users.
Conclusions: Compared to the general population, patients with DM were at an increased risk of treated depression. Users of metformin (monotherapy or combined therapy) had an increased risk of depression compared to non-users of antidiabetic drugs.

Keywords: observational study, diabetes mellitus, depression

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Prolonged isolated soluble dietary fibre supplementation in overweight and obese patients: A systematic review with meta-analysis of randomised controlled trials

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Introduction: The prevalence of overweight and obesity is rising rapidly, currently affecting 1.9 billion adults worldwide. Prebiotic dietary fibre supplementation could be a promising approach to improve weight loss and reduce metabolic complications in overweight and obese patients due to modifications of the microbiota composition and function. Previous systematic reviews and meta-analyses addressing similar questions revealed discordant evidence and/or are outdated.
Aims: The aim of this study was to synthesize current clinical evidence for soluble dietary fibre supplementation in overweight and obese patients and its effect on body weight and metabolic parameters.
Methods: We searched MEDLINE, Embase, Google Scholar, and forward and backward citations for randomised controlled trials (RCTs) with isolated soluble dietary fibre supplementation for at least 12 weeks in overweight and obese patients measuring body weight, published through April 2022. We expressed the results as mean differences (MDs) using the random effects model of the metafor package in R and assessed risk of bias using the Cochrane RoB2 tool. We conducted the study according to the PRISMA guidelines and registered the protocol on PROSPERO (CRD42022295246).
Results: In total, we identified 22 RCTs with an intervention duration ranging from 12 to 52 weeks with supplemented dietary fibre doses from 2.6 g to 29 g per day. The participants with dietary fibre supplementation showed a significantly higher reduction in body weight (MD -1.25 kg, 95% CI -2.24, -0.25; 27 RCTs; 1'428 participants) accompanied by a significant decrease in BMI, waist circumference, fasting blood insulin, and HOMA IR compared to the control group. There was no significant effect on the percentage of body fat, fasting blood glucose, HbA1c, and C-reactive protein.
Conclusions: Certainty of evidence was high, paving the way for the implementation of isolated soluble dietary fibre supplementation into clinical practice.

Keywords: body weight, dietary fibre, microbiome, overweight and obesity, systematic review and meta-analysis

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Dietary fiber intake and its association with ultra-processed food consumption in the general population of Switzerland

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Introduction: Adequate dietary fiber intake is important for health and well-being. Ideally, dietary fiber requirements are covered with minimally or unprocessed foods rather than with ultra-processed foods (UPF). UPF usually have a high energy density, added consumption promoting substances, such as salt or sugar, and low satiating capacity. Recent studies have shown that UPF can promote obesity despite their high fiber content.
Aims: We aimed to investigate the distribution of dietary fiber intake in the general population of Switzerland. Of particular interest was how the recommendations for dietary fiber intake are followed by subgroups of the population and the role of UPF regarding coverage of the dietary fiber requirements.
Methods: Data were obtained from the cross-sectional Swiss National Nutrition Survey *menuCH* [1]. Dietary intake was assessed with 24-h dietary recalls and foods were classified according to their processing degree using the *NOVA* food classification system [2]. Sociodemographic and lifestyle factors were obtained through a self-administered questionnaire. We summarized demographic, anthropometric, and dietary parameters for the whole population and subgroups according to absolute and relative fiber intake. We analyzed the association between fiber intake and quartiles of UPF consumption (in % food weight and % energy provided) by fitting multinomial logistic regression models. Data were weighted to achieve representation of the Swiss population by using the *menuCH* weighting strategy [3].
Results: Data obtained from 2'057 individuals were included in the analysis, of which 87% had a dietary fiber intake below 30 g/day. Median (interquartile range) absolute and relative fiber intake was 19 (15-25) g/day and 9 (7-12) g/1000 kcal/day, respectively. Participants with high UPF consumption had lower odds of being in the medium and high dietary fiber intake group (15-30 and ≥30 g/day, 10-14 and ≥14 g/1000 kcal/day) compared to participants with low UPF intake (<15 g/day, <10 g/1000 kcal/day). The odds of being in the medium or high dietary fiber intake groups decreased linearly across quartiles of UPF consumption (*p* for trend <.001).
Conclusions: Dietary fiber intake is insufficient in all population groups in Switzerland. UPF consumption is inversely and dose-dependently associated with dietary fiber intake. To increase dietary fiber intake in the population, public health measures should be implemented aimed at discouraging UPF consumption and increasing dietary fiber intake via minimally or unprocessed foods.
Keywords: dietary fiber, ultra-processed foods, recommendations, 24-h dietary recall, recommended dietary allowance

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Characteristics of patients with incident delirium during inpatient rehabilitation: a descriptive study

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Introduction: Delirium is an etiologically non-specific organic brain syndrome that leads to poor outcomes in rehabilitation. Characterizing patients with delirium during rehabilitation is important to identify factors that are potentially associated with delirium occurrence.
Aims: To characterize patients with incident delirium episodes during rehabilitation.
Methods: We conducted a retrospective descriptive study using data from the electronic health records (EHR) of ZURZACH Care, a Swiss group of rehabilitation centers. EHR records comprise medical notes suggestive of delirium occurrence (validated in a previous study [1]), patient and rehabilitation characteristics such as age, sex, length of stay and rehabilitation disciplines, as well as routinely recorded clinical data such as diagnoses, administered drugs, degree of disability and disease burden. The study base comprised all rehabilitation stays of all patients who were admitted to inpatient rehabilitation at ZURZACH Care, Rehaklinik Bad Zurzach, Switzerland, between 1 January 2015 and 31 December 2018. Incident delirium episodes were defined as those rehabilitation stays during which incident delirium took place. We calculated the cumulative incidence of delirium during rehabilitation. Within the study base and separately within incident delirium episodes, we summarized continuous variables as means with standard deviations, and categorical variables as absolute and relative frequencies.
Results: Within the study base comprising 10'503 rehabilitation stays, we identified 125 validated incident delirium episodes (cumulative incidence: 1.2%). Patients with incident delirium episodes were more often male (56.0% *vs.* 44.6%), on average older (mean age: 77.2 *vs.* 66.8 years), had a longer mean rehabilitation stay (33.1 *vs.* 25.3 days), a higher disease burden (Cumulative Illness Rating Scale [CIRS]: 18.8 *vs.* 14.7), and a higher average number of administered drugs (9.0 *vs.* 6.9) than those of the study base. The average degree of disability of patients with incident delirium episodes was higher at admission (Functional Independence Measure [FIM] 45.6 *vs.* 75.9) and improved less during rehabilitation (+16.1% *vs.* +21.0%) than in the study base. Rehabilitation most frequently took place in neurology discipline (32.9%) and also most incident delirium episodes (71.2%) occurred in this discipline.
Conclusions: Our findings identified key characteristics of patients with incident delirium during inpatient rehabilitation. Further observational studies are needed to identify risk factors of developing delirium during rehabilitation.

Keywords: neurocognitive disorders, delirium, rehabilitation, descriptive study

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Pharmacist-led pharmacogenetic testing and counselling: A study design addressing depression therapy in psychiatric practice

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Introduction: Up to 50% of patients suffering from major depressive disorder do not respond sufficiently to the prescribed first-line antidepressant therapy, resulting in an immense burden for the patient. Finding the right drug and dosage for the patient remains challenging in many cases and interindividual differences in response are frequently observed. One reason for interindividual differences in drug response is based on genetic predisposition. The accumulating evidence on drug-gene interactions is already highlighted in numerous drug labels and recommendations for pharmacogenetic (PGx)-guided drug selection and dosing are available for more and more drugs, including antidepressants. Still, PGx-guided antidepressant therapy is not yet routinely applied in psychiatric practice. We hypothesize that PGx information can be beneficially incorporated alongside other factors like drug-drug interactions and disease modalities to guide drug selection and dosing in the treatment of major depression.

Aim and Methods: With the aim to investigate the effect of pre-emptive PGx testing in combination with a clinical medication review in psychiatric practice, we designed a prospective, randomized, parallel arm, open label clinical trial. Adult patients suffering from unipolar moderate to severe depressive episodes are recruited in the inpatient setting. Patients requiring a change in antidepressant therapy will be randomly assigned to either the intervention or control arm. The intervention is a consultation of the psychiatrist with a clinical pharmacist concerning antidepressant selection and dosing. This consultation includes genotyping and thereof evidence-based genotype interpretation in addition to a clinical medication review considering the individual patient history with previous therapy failures or adverse drug events, medical and laboratory data (incl. serum drug levels where available) as well as current co-medication. Patients in the control arm receive antidepressant treatment without prior PGx assessment and without a pharmacist consultation (standard care). The primary study outcome is treatment response based on the Hamilton Depression Rating Scale (HAM-D17) after four weeks of treatment.

Results: Patient recruitment is ongoing in two psychiatric clinics in German-speaking Switzerland. Based on a sample size calculation (power = 80 %, α = 5 %), we plan to enroll 85 patients each in the intervention and control arm (n = 190).

Conclusion: The herein described study design was developed and implemented in close collaboration of clinical pharmacists with psychiatrists. We expect valuable and relevant insight into pre-emptive PGx testing and counselling as a clinical pharmacy service in psychiatric practice and its potential benefits for major depression patients.

Keywords: pharmacogenomics, pharmaceutical care, clinical pharmacy, personalized medicine, major depression

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Development of a pocket card to guide medication counseling at hospital discharge

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Introduction: Medication related problems are frequent, especially during care transitions such as hospital discharge [1]. They can jeopardize patient safety, and therefore, hospitals need to establish measures in order to prevent them and thus increase medication safety. Counseling patients about their medications at hospital discharge has the potential to reduce medication related problems [2].
Aims: The first aim of this study was to analyze the process of medication counseling during hospital discharge at the University Hospital of Bern. Based on these findings, the second aim was to propose a structured framework for medication counseling for the study hospital.
Methods: This quality improvement project was conducted in the Department of General Internal Medicine at the University Hospital of Bern. In a first part, we conducted semi-structured face-to-face interviews with health care professionals involved during the discharge process and with patients discharged home via follow-up phone call. In addition, a structured literature review in PubMed, Embase, and CINAHL was conducted to identify important medication counseling topics. All the results served as a basis for the development of a pocket card to systematically guide the residents through medication counseling during the discharge process. The pocket card was reviewed by 14 physicians and 3 pharmacists.
Results: We included 40 studies in our literature review and found that medication counseling can have a positive impact on patient safety. The interviews identified that a structured discharge medication counseling on oral anticoagulants in the studied department is conducted. Otherwise, medication counseling is done, but in an unsystematic manner. The findings of the interviews and the literature review identified 4 important part for our pocket card: (1) preparation for the medication counseling: clarifying the need for participation of relatives or an interpreter, preparing written documentation, and conducting medication reconciliation. (2) Users are asked to state the counseling purpose. (3) The main section guides the explanation of medication changes, indications, dosages including as needed medications, and time until action onset. The need to point out interaction risks is noted. For special medications or patients at risk for medication related problems it is described that patients should be counseled on the following: procedure if a medication is forgotten to be taken, specific, important adverse drug reactions and their management, special use instruction (e.g. devices) and storage, and self-monitoring. (4) Patients are advised to organize home medications and arrange a family doctor appointment. Adherence importance and possible aids, such as medication dispensers, are emphasized and contact details are provided. The user should ask for clarifications and check patient understanding by asking them to describe in their own words what they know (teach-back-method). The back of the card includes explanations, e.g. what medications might be important when it comes to missed doses.
Conclusions: We successfully developed a pocket card to guide the medication counseling process. We strive to implement the pocket card to improve the discharge process and follow-up on the impact and satisfaction of healthcare professionals and patients, which could ultimately have a positive impact on our patients' medication safety.

Keywords: medication counseling, medication safety, hospital discharge, clinical pharmacy

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Identification of risk factors for drug-related readmissions – a scoping literature review

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Introduction: Hospital readmissions due to medication-related problems and adverse drug reactions occur regularly and are an emotional burden for patients and caregivers as well as an economic challenge for health care systems. Some hospitals have implemented clinical pharmacy services at or after discharge that may have the potential to reduce medication-related readmissions. However, in many European countries including Switzerland, pharmacists' resources are insufficient to provide such discharge services to all inpatients, requiring prioritization of patients most likely to benefit.
Aims: The aim of this scoping literature review was to identify risk factors for drug-related 30-day readmissions to inform the first round of a Delphi study.
Methods: We conduct a scoping literature review in the Medline, Embase and CINAHL electronic databases to identify publications assessing risk factors for 30-day drug-related readmissions. The reference lists of included publications will be searched for additional relevant publications. Two reviewers independently screen titles and abstracts for inclusion. Relevant full-texts will be reviewed by one reviewer and confirmed by another. Risk factors mentioned in more than one publication are used to inform the first round of a following Delphi Study.
Results: After deduplication, 1'159 titles and abstracts were screened. This process is almost complete and first results will be ready for the conference. Preliminary results indicate that increased age, polypharmacy, number of drug changes at last hospital stay and number of hospitalizations before readmissions increase the risk for drug-related readmissions. Additionally, the following drug-related problems associated with 30-day readmission have been found to date: (1) underprescribing, among others in patients with heart failure and unstable angina, (2) adherence related problems including use deviating from the prescription and difficulty using dosage forms, (3) insufficient ambulatory monitoring, for example of insulin, antithrombotic and diuretic therapy, (4) overprescribing, (5) overdose, (6) underdose, (7) drug-drug interactions, (8) suboptimal drug selection and (9) transition errors. Additionally, 12 therapeutic classes are associated with an increased risk for readmissions due to adverse drug reactions including (1) antithrombotic agents, (2) antibiotics, (3) diuretics, (4) calcium channel blockers, (5) antineoplastic agents, (6) immunosuppressant agents, (7) opioids, (8) antiepileptics, (9) benzodiazepines, (10) antidepressants and (11) drugs used in nicotine dependence.
Conclusions: The scoping literature review will be useful in identifying risk factors for drug-related readmissions to inform the first round of a Delphi Study. In a next step, we will conduct the Delphi study to identify relevant risk factors to be incorporated into an electronic algorithm to flag patients at risk for medication-related readmissions to a general internal medicine department. We are looking for preventable readmissions in order to prioritize these patients for clinical pharmacy services at hospital discharge.

Keywords: drug-related hospital readmission, medication safety, risk assessment, clinical pharmacy

P - IV - 13

Heart rate variability features in patients with postbariatric hypoglycaemia after Roux-en-Y gastric bypass measured by a wearable device

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Introduction: Post-bariatric hypoglycemia (PBH) is a late complication of bariatric surgery, particularly with Roux-en Y gastric bypass (RYGB). Patients affected by PHB demonstrate distinct postprandial glucose patterns, with very rapid rises in glucose shortly after food intake, followed by a rapid decline resulting in hypoglycemia 1-2 h later. Since hypoglycemia stimulates the sympathetic nervous system, measuring heart rate variability (HRV), which reflects the balance between the sympathetic and parasympathetic nervous system, could be useful for real-time early detection of hypoglycemia.

Aims: To investigate HRV patterns during the postprandial glucose trajectory in PBH patients using a wearable device during real life conditions.

Methods: Patients with confirmed PBH after RYGB recorded meal intake and wore a blinded CGM sensor (Dexcom G6) and smartwatch (Garmin Vivoactive 4S, modified to log photoplethysmography data continuously) over 50 days. Filtering and HRV feature engineering were performed using the python package FLIRT [1]. According to postprandial glucose trajectory following a meal declaration, we defined five distinct time points: baseline, turning point 1, peak, turning point 2, and nadir. Five HRV features (RMSSD, SDNN, pNN50, LF, HF) were analyzed at these points. Postprandial events affected by sensor artefacts, missing data or repeated meal intakes were excluded. Events were stratified into 3 subgroups based on nadir glucose levels: euglycaemia (≥3.9 mmol/L), Level 1 hypoglycemia (<3.9 - 3.0 mmol/L) and Level 2 hypoglycemia (<3.0 mmol/L). We used repeated measure linear mixed effects models to assess differences in HRV features between the different time points and glycemic levels.

Results: We included 9 patients with post-RYGB PBH (8 female, median (IQR) age 48 (10) years, BMI 30.2 (12.4) kg/m^2, HbA_1c 5.5 (0.5) %, 8 (6) years since RYGB). Out of 1'444 recorded meals, 367 (25%) were included in the analysis. Thereof 182 (50%) ended in euglycemia, 137 (37%) in Level 1 hypoglycemia, and 49 (13%) in Level 2 hypoglycemia. Overall, all assessed HRV features significantly decreased from baseline to glucose peak. After the peak, an increase in HRV was observed. Median (IQR) nadir glucose was 4.6 mmol/L (0.8) for euglycemia, 3.5 mmol/L (0.4) for Level 1 hypoglycemia, and 2.5 mmol/L (0.6) for Level 2 hypoglycemia. Mean duration of Level 1 and Level 2 hypoglycemia was 30.0 (20.0) min and 25.0 (10) min, respectively. HRV features did not significantly differ according to level of nadir glucose.

Conclusions: A decrease in HRV, indicating sympathetic activity, was consistently observed with postprandial glucose excursions. However, no such patterns were observed at the initiation of hypoglycemia and HRV features did not discriminate postprandial periods ending in eu- vs. hypoglycemia. Further research is needed to determine whether the lack of hypoglycemia-induced changes in HRV in PBH patients is indicative of dysautonomia and whether wearable devices can guide the development of PBH-specific hypoglycemia prediction algorithms.

Keywords: heart rate variability, post-bariatric hypoglycemia, Roux-en-Y gastric bypass, continuous glucose monitoring, photoplethysmography

Reference:

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V. MOLECULAR PHARMACOLOGY / MOLECULAR MEDICINE

P - V - 1

New tools to study HDAC6 activity in multiple myeloma

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Introduction: Multiple myeloma (MM) is a plasma cell malignancy with poor prognosis and therapeutic options, especially in relapse and refractory patients. Histone deacetylase 6 (HDAC6) is overexpressed in MM patients and plays a central role in the acquisition of resistance to conventional anti-proteasome treatments. HDAC6 is thought to stimulate the aggresome clearance pathway and manage misfolded protein degradation.
Aims: To use CRISPR-Cas9 to generate engineered RPMI 8226 MM cells: HDAC6 knockdown (KD), HDAC6 knockout (KO), as well as specific knockout cell lines of CD1 (CD1-KO), CD2 (CD2-KO) and ZnF-UBP (ZnF-UBP-KO) domains. These cell lines will serve to get insight into the role of HDAC6 and its various domains in MM.
Methods: MM cell lines were generated through CRISPR-Cas9 editing and clonal selection. The expression levels of HDAC6 and its catalytic activity were measured by western blot, as well as the expression levels of its main partner proteins. The evaluation of the catalytic activity of HDAC1 in cells was assessed using a UHPLC-MS method by calculating the ratio between the deacetylated and acetylated substrate. As a control, the WT cell line was treated with panobinostat, a pan-HDAC inhibitor, at its IC_{50} concentration [1].
Results and Discussion: Western blot analyses confirmed the decreased expression of HDAC6 in the HDAC6-KD RPMI 8226 and the lack of expression in the HDAC6-KO RPMI 8226 cell lines, compared to non-edited cells (RPMI 8226). Cytoplasmic tubulin is one of the main substrates deacetylated by HDAC6. Therefore, in agreement with a down-regulation of HDAC6 protein expression, acetylated tubulin was up-regulated in both cell lines, indicating a decrease in HDAC6 activity. At the same time, Hsp90 protein expression was not affected by changes in HDAC6 expression, probably due to its ubiquitous nature inside the cells. However, HSF1 protein expression slightly increased with HDAC6 decrease. HDAC1 is the most abundant HDAC isotype in the cells. HDAC1 enzymatic activity increased with the reduction and loss of HDAC6 expression. Therefore, there may be a compensatory mechanism that should be further investigated. The characterization of RPMI 8226 HDAC6-CD1-KO, HDAC6-CD2-KO and HDAC6-ZnF-UBP-KO is currently being carried out.
Conclusions: These preliminary results indicate that the alteration of HDAC6 levels could lead to important changes in various signaling pathways. Therefore, the generation of cell lines defective in HDAC6 and its different domains could provide good tools to study the impact of HDAC6 activity in cells and evaluate its pathological role in MM in order to propose new therapeutic approaches.

Keywords: Multiple myeloma, HDAC6, aggresome, CRISPR-Cas9

Reference:

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P - V - 2

Right on time to defeat cancer: investigating the connection between the circadian rhythm and lung tumorigenesis

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Introduction: Lung cancer is one of the most prevalent reasons for cancer-related death worldwide. The circadian system is a mechanism allowing to anticipate changes in geophysical time. Emerging studies provide evidence in favor of an interconnection between cell cycle and circadian clock in mammalian cells. Upon malignant transformation, both the cell cycle and the circadian clock undergo significant changes, although the causality stays unclear in most cases.
Aims: We aimed to identify molecular mechanisms underlying the connection between circadian clock disruption and lung tumorigenesis in humans.
Methods: A549, A427, H1264 and H727 cell lines corresponding to different subtypes of non-small cell lung cancer (NSCLC), as well as primary lung cancer and adjacent non-tumorous cells were analyzed for molecular clockwork. Cells were transduced with *Bmal1-luciferase* (*luc*) and *Per2-luc* lentivectors, synchronized *in vitro* with dexamethasone, and circadian bioluminescence was recorded at population and single cell levels. In parallel, confluence was monitored in an IncuCyte®. The impact of *siClock*-mediated clock perturbation on cancer cells was evaluated by circadian bioluminescence recording and IncuCyte® live cell analysis.
Results: NSCLC cells bore circadian rhythmicity that varied among different cell lines. A549 cells showed regular rhythm until they reached stationary phase leading to an abrupt drop in the expression of *Bmal1-luc* and *Per2-luc* followed by dysregulation of the rhythm. Knockdown of *CLOCK* gene in A549, A427 and H727 cell lines decreased their proliferation.
Conclusions: The identified alterations in rhythmicity among various NSCLC cell lines underline the heterogeneity of lung cancer and demonstrate the need for a tailored approach to each lung cancer subtype and potential application of core clock alterations for diagnostics of lung cancer. Our data showed suppressed cancer cell growth following clock disruption and identified core-clock components as novel promising therapeutic targets. Further mechanistic studies in cell lines, as well as the characterization of core-clock component changes in primary lung cancer cells established from patient biopsies will have direct implications for current efforts on identifying novel therapeutic targets and on improving the diagnosis of these malignancies.

Keywords: circadian rhythm, *CLOCK*, lung cancer, primary cells

P - V - 3

HDAC6 ZnF-UBP domain inhibitors: a strategy to overcome multiple myeloma resistance?

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Introduction: Multiple myeloma (MM) is a plasma cell malignancy with poor prognosis, and therapeutic options with low efficacy in relapse and refractory patients. Histone deacetylase 6 (HDAC6) is overexpressed in MM patients and plays a central role in the acquisition of resistance to conventional anti-proteasome treatments. Besides displaying its deacetylase activity through its catalytic domains CD1 and CD2, HDAC6 is also thought to stimulate the aggresome pathway as a possible resistance mechanism through its ZnF-UBP C-terminal domain. This domain is capable of recognizing ubiquitinated motifs of misfolded proteins and send them to the aggresome through the microtubule network [1].
Aims: To test the ubiquitin-displacing potential of ZnF-UBP inhibitor candidates through protein-protein interaction (PPI) assays. The identification and characterization of ZnF-UBP inhibitors will be useful to explore their impact in MM cell lines, alone or in combination with other anti-MM agents.
Methods: A library of 60 ZnF-UBP inhibitor candidates was produced aiming at chemically blocking the aggresome pathway. HDAC6-ubiquitin interaction was measured in presence of these compounds using a fluorescence polarization assay. Besides, the total HDAC catalytic activity in RPMI 8226 cells was assessed using a UHPLC-MS method by calculating the ratio between the deacetylated and acetylated substrate. As a positive control, cells were also treated with panobinostat, a pan-HDAC inhibitor [2].
Results and Discussion: Seven compounds showed an inhibition of HDAC6-ubiquitin interaction with an IC_{50} value of 2-5 µM. A bioluminescence resonance energy transfer assay is currently being set up to assess HDAC6-ubiquitin interaction in a cell-based model. Besides, deacetylase catalytic activity and cell proliferation were not affected by these compounds.
Conclusions: The results suggest that these compounds may specifically inhibit the HDAC6 ZnF-UBP domain and, consequently, the aggresome formation. Combinatory treatments with other anti-MM agents will be performed in order to find possible synergistic effects in various sensitive and resistant MM cell lines.

Keywords: multiple myeloma, HDAC6, ZnF-UBP domain, aggresome

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P - V - 4

Pan-HDAC/PI hybrids: a new challenge for resistance in multiple myeloma

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Introduction: Even if novel therapies improved the treatment landscape, multiple myeloma (MM) still remains an incurable disease due to high rates of relapse and resistance. Combined and targeted therapies proved to be more beneficial compared to monotherapy approaches in reestablishing responsiveness to drug-resistant MM, leading to an improvement in patient median overall survival. Resistance to proteasome inhibitors (PI), one of the first line therapy, is often a major obstacle to the successful treatment of MM. After development of resistance, MM cells bypass the proteasome pathway by using the alternative aggresome-autophagy pathway for protein degradation in which histone deacetylase 6 (HDAC6) is a key player. Therefore, targeting the proteasome and HDACs simultaneously has been emerging as a promising strategy to address resistance in MM.
Aims: To evaluate the antiproliferative activity and the mechanism of action of pan-HDAC/PI hybrids in MM.
Methods: Eleven hybrid compounds, bearing both HDAC and proteasome inhibitors were synthesized. All hybrids were composed of the pharmacophore of entinostat (pan-HDAC inhibitor) and the one of bortezomib (PI), and their cytotoxic activity was evaluated in MM cells. The antiproliferative activity of the compounds was measured in RPMI 8226 cells after 72-h treatment using the XTT assay, and those that showed an IC_{50} value < 500 nM were tested in RPMI 8226 cells resistant to proteasome inhibitors. The HDAC inhibitory activity was evaluated in RPMI 8226 cells treated for 8 h using a UHPLC-MS-based method and the proteasome inhibitory activity was monitored using a fluorogenic peptide substrate. Furthermore, the cytotoxicity of the most active compounds of the library was tested in a 3D spheroid model made of RPMI 8226 cells, mesenchymal stem cells and endothelial progenitor cells in a ratio 2:1:1. The antiproliferative activity in 3D was measured after 48-h treatment using the CTG assay.
Results: The compound with the strongest antiproliferative activity had an IC_{50} value of 9.5 nM in RPMI 8226 cells, 18.9 nM in cells resistant to proteasome inhibitors, and 90 nM in a 3D spheroid model. Moreover, the compound inhibited 28% of HDAC enzymatic activity at 10 µM in RPMI 8226 cells and it displayed an IC_{50} value of 23.6 nM for inhibitory activity of the proteasome.
Conclusions: The most active compounds showed antiproliferative activity in the low nM range in various MM models. The substitution of entinostat with a selective HDAC6 inhibitor is currently being pursued with the idea of increasing specificity and decreasing side effects.

Keywords: multiple myeloma, proteasome inhibitors, HDAC inhibitors, resistance, hybrids

P - V - 5

Insight into the interaction profiles and functional modulation of complement-related integrin receptors using an integrated screening platform

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Introduction: While the complement system serves as a first line of defense against pathogens and endogenous threats, inappropriate complement activation is involved in several clinical conditions. Cell adhesion and inflammatory signaling via complement receptors (CR) of the β_2 integrin family can serve as critical disease contributor, but the poor druggability of integrin targets and the shortage of validated assay systems render CR a scarcely explored therapeutic option.
Aims: In this project, we therefore compiled a library of the major ligand-binding domains (αI) of the β_2 integrins to enable direct binding and functional assay, with an emphasis on the complement receptors CR3 and CR4. Furthermore, we employ this platform to develop and assess tool compounds able to disentangle the promiscuous ligand binding profile of CR3 and its implication in pathologies [1].
Methods: Recombinant forms of the αI domain of all four β_2 integrin family members (i.e., CR3, CR4, LFA-1, and CD11d/CD18) were expressed in *E. coli* and purified by affinity chromatography. Whereas surface plasmon resonance (SPR) was used to determine affinity and kinetic profiles, functional assays are used to determine the functional and selectivity spectra of the proteins.
Results: By optimizing the expression parameters and selecting appropriate purification tags, we expressed the αI domains of all four β_2 integrins with high yield and purity. SPR studies confirmed activity and selectivity of the recombinant CR3 and CR4 αI domains for complement opsonins C3b, iC3b and C3d with distinct kinetic profiles. Bead- and cell-based adhesion assays were employed to confirm and expand upon the molecular characterization. Moreover, the effect of cofactors and peptidic and small molecule modulators are currently investigated with this platform.
Conclusion: It is anticipated to use the recombinant αI domains of β_2 integrins in functional studies and for CR3/CR4-targeted drug development, with a potential expansion to the other β_2 family members at a later stage. Tool compounds developed within this project are expected to provide valuable insight into the (patho-)physiology of CR3 and may help to identify potential therapeutic approaches for autoimmune, inflammatory, and age-related diseases.

Keywords: innate host defense, integrins, complement

Reference:

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P - V - 6

Molecular insight into the target interaction profile of the factor H-binding peptide 5C6

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Introduction: Upon contact with blood, non-self surfaces such as biomaterials or transplants can trigger the complement system and generate inflammatory mediators. These undesirable defence reactions are enabled by the absence of complement regulators on such surfaces. One option to mitigate adverse complement activation is the recruitment of the abundant regulator factor H (FH) to the material surface using FH-binding coatings. Our groups previously described a cyclic peptide, termed 5C6, with potent FH-recruiting capacity and assigned the broad core section of FH, comprising complement control protein (CCP) domains 5-18, as general contact region [1,2]. Using a truncation library of FH segments produced in HEK cells, we identified a five-domain segment of FH as minimum binding site for 5C6.
Aims: To further characterize the binding mode and selectivity of 5C6 for proteins of the FH family, we combined recombinant protein expression with direct binding and functional studies.
Methods: Recombinant factor H fragment containing the short consensus repeats CCP10-14 (rFH10-14) was cloned in pET15b vector, expressed in *E. coli* and purified by affinity chromatography. The binding of carboxyfluorescein labelled 5C6 to rFH10-14 was evaluated by microscale thermophoresis assay (MST). Flow cytometry method was used to assess the ability to recruit fluorescently labelled rFH10-14 and full-length plasma purified FH on the surfaces of streptavidin-coated magnetic beads that were pre-coated with N-terminally biotinylated 5C6 peptide. Similar bead-based test system was used for assessing the ability of 5C6 coated surface to diminish complement derived C3b/iC3b deposition after incubation in normal human serum. Considering the homology of FH10-14 with FH-related protein 5 (FHR5), we explored potential cross-reactivities of 5C6 with FHR5 using Western blotting, ELISA and MST analysis.
Results: A recombinant FH fragment rFH10-14 was expressed in *E. coli* and assessed for its interaction with 5C6 by MST. rFH10-14 produced in bacteria showed potent binding to 5C6, with a comparable profile to FH10-14 and FH8-15 produced in eukaryotic cells or plasma-purified FH. We also explored potential cross-reactivities of 5C6 with FHR5 using a number of methods but did not detect any relevant interactions. The FH-recruiting and complement-modulating capacities of 5C6 coatings were confirmed by a bead-based assay. 5C6, but not a scrambled derivative, was able to recruit rFH10-14 and full-length FH. The active 5C6 coating also prevented C3b/iC3b deposition on beads when exposed to serum.
Conclusions: Despite the absence of structural data, the combination of segment libraries and recombinant FH segments enabled us to map the 5C6-capturing site to a defined area in the core section of FH. The availability of a functionally active rFH10-14 fragment that can be produced in bacteria will largely facilitate structural studies.

Keywords: complement, complement regulators, factor H, factor H related protein 5, factor H-binding peptides

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P - V - 7

Structure-activity assessment of the leech-derived complement inhibitor BD001

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Introduction: BD001, a leech derived protein, inhibits two serine proteases C1s (classical, CP) and MASP2 (lectin, LP) needed for complement activation [1]. However, the structural determinants for activity and target selectivity have only partially been described, due to its complex structure, also hampering the assessment of the protein as therapeutic option [2]. We aimed at improving the production of BD001 in *E.coli*.
Aim: This study aimed at developing BD001 derivatives with enhanced target activity and distinct pathway selectivity profiles, based on in silico predictions and protein engineering.
Material and Methods: We used computational tools to perform a mutational scan of BD001 and predict changes in C1s affinity. We selected several mutants for experimental testing. The activity of recombinant BD001 and derivatives was tested in functional complement inhibition assays. Direct binding studies with C1s and other host defense proteases were performed with SPR. Finally, we developed complement activation ELISAs in animal sera of translational interest to determine the species specificity of BD001.
Results: Despite its disulfide-rich nature, we managed to express BD001 and its mutants in *E. coli*. The panel of BD001 mutants for our SAR study showed distinct activity profiles in the functional assays, the experimental activity changes did not always correlate with *in silico* predictions. Intriguingly, some mutants differentially affected the overall efficacy or selectivity profiles, thereby suggesting a path for rational optimization of BD001. In addition to its reported activity for human complement, BD001 showed potent CP and LP inhibition in monkey and mouse serum.
Conclusion: Our initial SAR study provided insight into the molecular determinant of target activity and selectivity of BD001, thereby paving the way for more precise protein engineering. As the use of off-the-shelf tools for mutant screening is of limited predictive value, we are establishing enhanced computational methods for future optimization steps. Overall, our findings establish BD001 as an interesting lead for further development towards treatment options for complement-related diseases, with the species specificity studies indicating the feasibility for preclinical evaluation of the inhibitor in disease models.

Keywords: complement inhibitor, gigastasin, serin proteases, leech protein, complement system

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P - V - 8

An exchange with implications: Towards new derivatives of the complement inhibitor compstatin with improved species specificity profiles

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Introduction: Compstatin, discovered at the University of Pennsylvania in 1996, is a cyclic peptide that inhibits the activation of C3, the central component of the complement system. This innate immune pathway has been implicated in several clinical conditions, thereby sparking an interest to develop complement-targeted therapeutics. Through years of research, compstatin was continuously optimized and a PEGylated derivative, pegcetacoplan, was approved by the FDA in 2021. While facilitating clinical development, compstatin's narrow species specificity for human/primate C3 prevented its evaluation in many preclinical disease models.
Aim: Our groups therefore aim at identifying and developing compstatin derivatives capable of inhibiting complement activation in mouse and rat models.
Methods: We use structural insight from a clinical candidate of the compstatin class and homology modelling to describe molecular determinant of species specificity, and *in silico* methods to predict compstatin derivatives with activity for mouse or rat C3. Promising candidates are produced using solid phase peptide synthesis, cyclized via a disulphide bridge, and tested for binding affinity for mouse/rat C3b (using surface plasmon resonance) and complement-inhibitory activity (using ELISA).
Results and Conclusion: Structural analysis unexpectedly revealed that the narrow species specificity of compstatin is determined by a reduced number of high-quality drug-target contacts in the binding pocket rather than by steric hindrance. By selectively substituting amino acids in sequence of an early compstatin analog (Cp01; Acl[CVWQDWGAHRC]T), we discovered initial lead peptides that show notable binding to mouse C3b. These will serve as templates to increase the affinity for mouse C3 by stepwise modification of the peptide, thereby modulating the species specificity profile of this important drug class.

Keywords: complement system, compstatin, peptide

P - V - 9

Patient-derived platform to leverage personalized treatment for colorectal carcinoma

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Introduction: Although cancer treatment has improved over the past decades, effective systemic treatment options are still lacking for colorectal cancer (CRC) patients, mainly due to standardized treatment protocols that fail to identify patients with poor prognosis.
Aim: Establish a platform combining patient-derived organoids and mathematical modeling to identify personalized treatment for colorectal carcinoma patients.
Methods: Using our phenotypically driven method, The Therapeutically Guided Multidrug Optimization (TGMO), we were able to identify 4 synergistic drug combination (ODC) with minimal experimental effort, in different CRC 3D complex cell models, both naïve and chronically treated with FOLFOXIRI (first line chemotherapy treatment).
Results: We validated the activity of our ODC on freshly isolated patient-derived samples from primary colorectal cell carcinoma, cultured in an optimized manner to obtain a single patient-derived organoid (PDO) at a clinically relevant size of 300-400 µm. Our ODC inhibited up to 90% of PDO viability in 3 different patients PDO, outperforming the activity of the corresponding monotherapies as well as FOLFOXIRI at clinically used dose. Furthermore, we performed a similar TGMO-based screen with 50 drug combinations directly on PDO isolated from CRC patients. Our 2nd order linear regression analysis of the modelled data showcased patient specific synergistic ODC that inhibited viability with up to 80%, while maintaining higher activity than the FOLFOXIRI.
Conclusion: The TGMO combined to PDO technology consists of a robust platform to leverage personalized drug-mixtures in a clinically relevant time frame of 2 weeks post-tumor resection, through rapid experimental effort and mathematical data modelling, by interrogating a small sample of the patient derived tissue.

Keywords: primary colorectal cancer, patient-derived, organoids, drug combinations, artificial intelligence

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The development of collectin-11 antagonists to limit ischemia-reperfusion injury

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Introduction: Collectin-11 (CL-11, CL-K1) is an initiatory protein of the complement innate immune pathway. It is highly relevant in the context of ischemia-reperfusion injury in renal transplantation, having recently been demonstrated to play an important role in both acute kidney injury and late-stage/chronic renal inflammation. In a murine renal transplantation model, preventing CL-11 binding to glycans on the renal cell surface reduced complement-mediated tissue damage and neutrophil infiltration while improving overall renal function [1]. CL-11 is known to bind glycans, which are a class of biomolecule that decorate the extracellular surfaces of cells, thereby acting as a first point of contact with neighbouring cells and molecules. Glycans play a critical role in numerous biological processes, including inflammation, cell adhesion, and host-pathogen recognition, and as a result glycan-binding proteins have been rapidly gaining interest as targets in drug development programs [2]. Although glycan structures have extensive structural diversity, they suffer from weak binding affinities and inherently poor pharmacokinetic properties. In order to improve their drug-like properties, «glycomimetic» compounds can be designed which mimic the structure and function of the native glycan, yet show improved affinity, specificity, and bioavailability [3].
Aims: We therefore explored the option to design «glycomimetic» antagonists of CL-11 with enhanced affinities and improved pharmacokinetic properties.
Methods: Molecular dynamics simulations and docking studies were performed to better characterize the ligand-binding mode of CL-11 and identify potential extended binding site motifs. Based on these results, different glycomimetic libraries were constructed and evaluated *in vitro* using a thermal denaturation-based nano-differential scanning fluorimetry assay, and a polymer-based competitive binding assay.
Results: Over 100 glycan-based structures have been synthesized and evaluated with the best inhibitors showing improvements of 50-fold in affinity, advancing our efforts at developing a therapeutic entity against ischemia-reperfusion injuries and further evaluating the physiological role of CL-11.
Conclusions: The development of high affinity CL-11 inhibitors may provide a novel therapeutic for ischemia-reperfusion injury.

Keywords: drug development, complement, glycomimetic, ischemia-reperfusion injury

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Synergistic low-dose drug combination is effective against various cancer cell types

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Introduction: The current clinical treatments of colorectal carcinoma (CRC) and renal cell carcinoma (RCC) are both set back by the development of resistance to the current clinical treatments and important side effects [1]. Therefore, new approaches allow overcoming resistance and leading to fewer side effects are necessary. Synergistic low-dose drug combinations can address both of these issues as the different drugs can tackle several pathways simultaneously and the synergy between the compounds allow reducing the clinical doses of each treatment. We have discovered a promising low-dose synergistic drug combination active in CRC, RCC and melanoma subtypes [2]. This combination composed of the two tyrosine kinase inhibitors (erlotinib and dasatinib) and two histone deacetylase inhibitors (tacedinaline and tubacin), was shown to induce a significant decrease of cancer cell viability by inhibiting spindle pole clustering and thus inducing abnormal mitosis and cell death. Our drug combination represents therefore a promising starting point for a new therapeutic strategy.
Aims: This study aims to establish preliminary *in vitro* data on both treatment-naïve and chemotherapy-resistant cell lines. The cell line-dependent efficacy of our drug combination against CRC and RCC cell lines will be evaluated in 2D and 3D cell cultures.
Methods: Multiple human CRC cell lines and their chemotherapy-resistant clones were treated with our drug combination and corresponding monotherapies. The ATP levels after 72 h of incubation with the treatments was characterized using the CellTiter-Glo (Promega) reagent.
Results: The drug combination reduced cancer cell viability between 20% and 90% in a cell type dependent manner. The efficacy was lower in CRC than in RCC cells, which can be explained as the drug combination was optimized in renal cancer cells. All chemotherapy-resistant cells, apart from A498, showed similar responses to the drug combination compared to the treatment-naïve cells.
Conclusions: These preliminary *in vitro* results show that our drug combination varies in efficacy in different cell lines, but that the acquired resistance to 1st line clinical treatment (chemotherapy) generally does not influence the effect of the drugs on cell viability. We will next quantify the spindle multipolarity of these cell lines and test for correlations with the efficacy. Finally, we will test the combinations on mice xenografts of these cell lines to assess their in vivo efficacy and safety.

Keywords: drug combination, cancer treatment, drug resistance

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VI. ANALYTICS

P - VI - 1

High-sensitivity ICP-MS as tool for the *in vitro* and *in vivo* characterization of future radiometal-based theranostics

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Introduction: Recently several radiotheranostics have been proved by the FDA namely [^{177}Lu]Lu-PSMA-617 (PLUVICTO™, Novartis) and [^{177}Lu]Lu-DOTATATE (LUTATHERA®, Novartis) [1-2]. However, the development and preclinical characterization of new radiopharmaceuticals for diagnosis and therapy requires specialized and expensive A/BC-type laboratories and equipment. Due to the increasing interest in nuclear theranostics, it would be highly desirable to have a complementary technique in place that allowed the preclinical characterization of future radiometal-based theranostic agents in conventional research laboratories and without the need of handling radioactivity. Since the high sensitivity of inductively coupled plasma mass spectrometry (ICP-MS) allows the detection of metals in trace quantities, it could be a valuable alternative to pre-clinically characterize future radiotheranostics.
Aims: The aim of this project was to investigate whether ICP-MS can be used for the preclinical characterization of future radiometal-based theranostic candidates and if it delivers comparable results to the state-of-the-art radioactive assays with the same sensitivity and accuracy. For proof-of-concept, the PSMA targeting ligand PSMA-617 labeled with lutetium-175 and lutetium-177, respectively, was used as reference.
Methods: The affinity as well as the total uptake and internalization of [^{175}Lu]Lu-PSMA-617 was determined in *in vitro* cellular experiments using ICP-MS for the measurement of the metal lutetium-175 and the results were compared to those for [^{177}Lu]Lu-PSMA-617. Methods were established using microwave assisted nitric acid digestion, to analyze organ samples of a biodistribution study of [^{175}Lu]Lu-PSMA-617 in a mouse model bearing PSMA-positive tumor xenografts.
Results: Comparison of the uptake and internalization of the two ligands, [^{175}Lu]Lu-PSMA-617 and [^{177}Lu]Lu-PSMA-617, in PSMA-positive PC-3 PIP and LNCaP cells revealed, that the two methods, namely the ICP-MS and γ-counting, gave similar results with only 2-3% difference. The K_D value of [^{175}Lu]Lu-PSMA-617 was successfully determined by ICP-MS and was 13.4-25.6 nM (95% CI), whereas it was 11.4-18.4 nM (95% CI) for the [^{177}Lu]Lu-PSMA-617. The biodistribution profile of the ^{175}Lu- and ^{177}Lu-labeled PSMA-617 in PC-3 PIP tumor bearing mice 1 h p.i. and 4 h p.i. were comparable with significant uptake in the PC-3 PIP tumor and in the kidneys.
Conclusions: This study demonstrated that high-sensitivity ICP-MS is a valid technique to be used as an alternative to the state-of-the-art radioactive assays. It could serve for the screening of ligands with new scaffolds, linkers, and chelators regarding their *in vitro* serum stability, and for the preclinical *in vitro* and *in vivo* evaluation.

Keywords: inductively coupled plasma mass spectrometry (ICP-MS), nuclear theranostics

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Unravelling pro-inflammatory dynamics of individual cytokine-secreting cells through deep phenotypic single-cell analysis

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Introduction: Recent years have seen the introduction of new therapeutic concepts, such as chimeric antigen receptor T cells, bispecific antibodies and immune checkpoint inhibitors. Since these therapies are based on modulation of the immune system, risk assessment for adverse pro-inflammatory reactions has become a necessity in pre-clinical development. *Ex vivo* cytokine release assays (CRAs) have been the gold standard during the past 10 years. These tests incubate the drug of interest with healthy donor cells for stimulation and subsequent pro-inflammatory cytokine release is measured which allows for *in vivo* predictions. Today's tests are time consuming and based on endpoint measurements, completely ignoring the dynamics of a potential immune answer.
Aims: Here, we present an alternative assay to study cytokine release with unprecedent sensitivity and dynamic resolution.
Methods: To achieve this, single peripheral blood mononuclear cells (PBMCs) are encapsulated into 40 pL water in oil emulsions, immobilized and imaged over time. Encapsulation of functionalized nanoparticles enables the measurement of multiple fluorescence-based sandwich-immunoassays in every emulsion, together with cell viability and surface markers, allowing the simultaneous measurement of up to 3 different cytokines per cell over time.
Results: Proof of concept experiments have shown, that the incubation time of cells and drug can be drastically reduced and cytokine secretion can be detected as early as 1 h after incubation.
Conclusions: These new single-cell analysis approaches provide insights into detailed immunological and inflammatory processes, helping to understand cytokine secretion behavior during an immune response. In future, we aim to expand the usability of these assays to study cytokine secretion in patients suffering from various auto-inflammatory diseases and to use it in diagnostic analyses of life-threatening conditions, such as cytokine storms.

Keywords: single-cell analysis, cytokine release assay, cytokine storm, droplet-based microfluidics

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Terpenomics for authenticity control of natural products using mass spectrometry and chemometrics

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Introduction: Essential oils (EOs) are natural products, which find wide application as flavour and fragrance agents in the food and cosmetic industry as well as phytopharmaceuticals in health care [1]. An EO can consist of only a few or up to more than 100 individual compounds that can appear in varying concentrations with different stereochemistries. Main constituents of EOs are terpenes, which can be classified by their number of isoprene units and can have a similar mass and structure, which makes analysis challenging [2]. Due to production costs and high trading prices, EOs are subject to adulteration. Current conventional methods for the quality and authenticity control of EOs focus on the analysis of a few selected markers. This approach however does not appear sufficient to characterise an EO thoroughly and can jeopardize patient safety and misguide consumers. An extended method for the quality control of EOs is based on compound pattern analysis of e.g. terpenes which has been applied successfully for the geographic allocation of pine oil [3]. Terpenomics, a holistic study of terpenes in natural products, has the potential to improve current quality control methods and therefore provide better patient safety when using phytopharmaceuticals.
Aims: The present work focuses on identifying novel methodologies for the quality control of natural product that are fast, convenient and are based on a holistic approach.
Methods: Volatile components of natural products were analysed using dielectric barrier discharge ionization-mass spectrometry (DBDI-MS). DBDI is a soft ionization technique that takes place at atmospheric pressure. Samples can be placed without any pre-treatment directly in front of the ionization source. Volatile components are ionized and introduced into the MS surface. In this approach, samples were analysed using a triple quadrupole MS (TripleQuad 3500, AB Sciex) by producing a Q_3 scan with mass range 50-400 Da. This methodology produces a characteristic fragment pattern that can be subsequently analysed with chemometrics such as hierarchical cluster analysis (HCA). HCA is an unsupervised classification method that calculates the proximity of values to one another and separates them based on their (dis)similarities.
Results: The described methodology was able to provide in depth characterization of natural products. Fragment pattern analysis of volatile components in combination with chemometrics showed that natural products were distinguishable by their origin, composition, species and the plant organ. Further, the methodology provided indications of mislabelling and potential adulterations.
Conclusions: DBDI-MS was successfully applied to distinguish natural products in detail. The methodology does not require any sample pre-treatment, is fast and convenient due to the lack of chromatographic separation and the acquired data can be used for model training to classify natural products based on their origin, composition, species and plant organ.

Keywords: terpenomics, authenticity control, chemometric analysis, patient safety, DBDI

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P - VI - 4

Characterization of constrained peptides by CID-MS/MS fragment pattern analysis

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Introduction: Peptides represent an unique class of pharmaceutical compounds due to their exceptional position in the chemical space between traditional small molecules (molecular weight (MW) < 500 Da) and large therapeutic proteins (MW > 5000 Da). However, various chemical modifications are necessary to overcome pharmacokinetic weaknesses such as low oral bioavailability. A common strategy for optimization is to constrain alpha-helical peptides via side chain to side chain macrocyclization. This so-called peptide-stapling can provide several advantages such as enhanced protease stability, cell permeability or 3-dimensional structure conservation [1]. Hydrocarbon stapling is probably the most commonly used type of constrain, but numerous other chemistries for peptide stapling have been reported in recent years [2]. But along with these new chemical approaches in peptide drug development also the importance of quality control of these peptidomimetics increases. Traditionally, sequence confirmation of linear peptides can be achieved by MS/MS analysis. Depending on the activation type chosen for fragmentation, different fragment ions resulting from backbone fragmentation can be observed. Whereas mainly b/y fragments can be found upon collision induced dissociation (CID), electron transfer dissociation (ETD) for example results mainly in c/z fragments [3]. However, literature addressing the behaviour of constrained peptides in MS/MS analysis and resulting fragmentation is missing so far.
Aims: The goal of this study was to reveal differences in the fragment pattern of hydrocarbon stapled peptides differing in staple type and position, to provide a starting point for the development of future quality control and structure elucidation processes of constrained peptides and peptidomimetics by MS/MS analysis.
Methods: The peptides were synthesized by solid phase peptide synthesis on a rink amide resin. Hydrocarbon stapling was conducted via ring closing metathesis (Grubbs Catalyst 2nd generation). The peptides were analysed by MS/MS analysis performed on an ion trap mass spectrometer (LTQ XL, Thermo Fisher Scientific) with an ESI source in positive mode. Fragments were generated by CID with a normalized collision energy of 35%.
Results: Distinct fragmentation patterns for the hydrocarbon stapled peptides were obtained. Sequence coverage of the linear segments via b/y fragments was achieved as expected for CID fragmentation. Surprisingly, additional fragments, unusual for CID fragmentation (a-type), were observed at high relative intensities for the stapled peptides. The abundance of these fragments was specific for staple position as well as for staple type. Sequence coverage within the staple is missing.
Conclusions: It was shown that MS/MS fragmentation of different hydrocarbon stapled peptides resulted in patterns specific for staple type and position. There are indications that stapled peptides show a fragmentation pattern different from common linear peptides highlighting the importance of further investigation of novel peptidomimetics in a context of quality control. Furthermore, a possible influence of peptide conformation on the resulting fragmentation should be investigated further.

Keywords: stapled peptides, MS/MS, CID, peptide drug development, quality control

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VII. PHARMACOLOGY / BIOPHARMACY

P - VII - 1

Erlotinib distribution in *rSlco2b1*$^{-/-}$ vs *hSLCO2B1*$^{+/+}$ rats – preliminary data

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Introduction: Erlotinib (Tarceva) has been approved in Switzerland for the treatment of non- small-cell lung cancer (NSCLC) in 2005 and later for pancreatic cancer. *In vitro* data suggest that this small molecule is a specific substrate of the Organic Anion Transporting Polypeptide (OATP) 2B1 [1]. The OATP2B1 transporter that belongs to the SLC family of transporters is ubiquitously expressed with high abundance in the pharmacokinetically relevant organs: liver, kidney, and small intestine. Despite numerous reports on endo- and exogenous substrates, the understanding of the *in vivo* role of OATP2B1 in pharmacokinetics of its substrate drugs is still limited [1].
Aims: As a probe substrate could significantly contribute to the understanding of the role of hOATP2B1, the aim of this study was to further investigate the potential of erlotinib as an *in vivo* substrate of the hOATP2B1 (human transporter) applying the newly established rat models *rSlco2b1*$^{-/-}$ and *hSLCO2B1*$^{+/+}$.
Methods: In this study, we compared the distribution of erlotinib in the liver, kidney, small intestine and brain of *rSlco2b1-/-* and *hSLCO2B1*$^{+/+}$ rats, respectively. In detail, 1 h after the intravenous administration of 12.4 mg/kg into the tail vein, 200 µL of serum was collected, and tissues were harvested. Erlotinib and its major metabolite OSI-420 were quantified applying a newly developed and validated LC-MS/MS method. Furthermore, mRNA expression of genes involved in erlotinib metabolism (*cyp3a1*, *cyp3a2* as well as *abcb1a, abcb1b, abcg2 and ugt1a1*) was determined in liver and small intestine of untreated animals applying real-time PCR.
Results: The *in vivo* distribution experiment revealed in all tissues studied (liver, kidney, small intestine, brain, and muscle) a clear trend towards higher erlotinib concentration in *hSLCO2B1+/+* rats. This was also seen for its main metabolite OSI-420. Calculating the OSI-420 to erlotinib ratio indicated an increased metabolite formation in the liver of *hSLCO2B1+/+* animals suggesting increased Cyp3a1 enzyme activity. Upregulation of this key metabolizing enzyme (*Cyp3a1)* was confirmed by real-time PCR and western blotting in untreated animals.
Conclusions: We are reporting preliminary data from a distribution study. Our data indicate that erlotinib is metabolized faster in hSLCO2B1+/+ rats' suggesting elevated activity of the main metabolising enzyme – Cyp3a1. Further studies focusing on the mechanism underlying Cyp3a1 induction in hSLCO2B1+/+ rats are warranted.

Keywords: erlotinib, human OATP2B1, CYP3A1, SLC drug transporters, metabolism

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P - VII - 2

In vitro and *in vivo* testing of a tablet formulation with double control mechanism for colonic drug delivery

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Introduction: Efficient and selective colonic delivery has not yet been achieved. This is relevant for local treatment of bowel diseases. Both premature and delayed release, causing systemic side effects and therapy failure, respectively, should be prevented.
Aims: We aimed to develop enteric-coated matrix tablets with dual release control mechanism and demonstrate specific colonic delivery *in vivo*.
Methods: Controlled colonic release (CCR) tablets using the plant polysaccharide xyloglucan as matrix were enteric coated with Eudragit® FS30D. Tablets containing either caffeine or 5-aminosalicylic acid (5-ASA) were analyzed in *in vitro* dissolution experiments simulating gastric, upper and low small intestinal (SI), and colonic stage, the latter containing microbial xyloglucanase. Drug release and matrix hydrolysis based on reducing sugars were measured. Drug and metabolite plasma levels were measured over 96 h after tablet administration to landrace pigs. Data was analyzed by physiologically based pharmacokinetic modeling and *in vivo* drug release rates and absorbed amounts from SI and colon were estimated by model-dependent deconvolution using immediate release (IR) tablets and intravenous solutions as reference.
Results: Optimized CCR tablets released less than 13% drug during *in vitro* gastric and SI stages. Xyloglucanase (0.1 or 1 U/mL) added to the colonic stage accelerated and allowed for complete release within the expected colonic transit time. Xyloglucan hydrolysis in the presence of enzyme indicated erosion-controlled release of both drugs. CCR tablets showed a delayed t_{max} and a decreased c_{max} compared to IR tablets (Table 1), indicating prolonged drug release into the colon. Bioavailability values and calculated absorbed masses concurred. Release rates in the colon were larger than in the SI, demonstrating microbiome-triggered release. Absorption from the SI was much smaller than from the colon.
Conclusions: Colonic drug delivery was achieved by the developed CCR tablets employing two internally triggered control mechanisms. Comparability between pigs and humans implies clinical relevance of the results.

Keywords: colonic drug delivery, matrix tablet, landrace pig, pharmacokinetic deconvolution

Table 1 Deduced pharmacokinetic parameters from animal study (mean ± SE)

Formulation	Parameter	Caffeine	5-ASA
IR	Dose (µmol)	1'030	1'306
	t_{max} (h)	2.7±0.8 (n=8)	4.8±1.1* (n=8)
	c_{max} (µM)	33.4±2.2 (n=8)	7.5±2.0* (n=8)
	Absolute systemic bioavailability (%)	77.4±5.7 (n=8)	60.2±6.2** (n=8)
CCR	Dose (µmol)	1'030	1'306
	t_{max} (h)	27.5±2.5 (n=6)	13.2±1.8* (n=8)
	c_{max} (µM)	12.9±1.2 (n=6)	1.6±0.9* (n=8)
	Absolute systemic bioavailability (%)	55.8±5.6 (n=6)	38.5±8.5** (n=8)
	Drug release rate in the SI (µmol/h)	9.1±1.9 (n=6)	19.6±5.9 (n=8)
	Drug release rate in the colon (µmol/h)	27.9±5.7 (n=6)	90.3±33.7 (n=8)
	Drug absorbed from the SI (µmol)	25.4±5.2 (n=6)	52.3±15.7** (n=8)
	Drug absorbed from the colon (µmol)	548.6±61.7 (n=6)	506.8±132.4** (n=8)

* Based on the drug metabolite Ac-5-ASA, ** Based on the sum of parent drug 5-ASA and drug metabolite Ac-5-ASA

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St. John's wort has an unexpected impact on CYP3A metabolism in rats

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Introduction: Nuclear Receptors (NR) play a pivotal role in maintaining homeostasis in living beings by transcriptional regulation of their target genes upon ligand binding. One well-known NR is the pregnane X receptor (PXR) which accepts a variety of xenobiotics as activating ligands. Importantly, for PXR species specific ligands have been reported with rifampicin and pregnenolone-16α-carbonitrile as two well-known examples that solely activate the human or the rodent PXR, respectively. Another compound known to bind to the human PXR is the St. John's wort (SJW) constituent hyperforin. For this molecule selectivity for human PXR has also been reported in *in vitro* experiments [1]. However, there are *in vivo* studies in rats reporting decreased plasma concentrations of Cyp3a substrates after pretreatment with SJW while enzyme activity and/ or expression was shown to be increased [2, 3].
Aim: It is aim of this study to further investigate the impact of hyperforin on rat Cyp3a metabolism comparing two SJW formulations. One formulation was Hyperiplant® which contains a high amount of hyperforin (3-6 mg/ 100 mg extract) and the other was Rebalance® containing very low amounts of hyperforin (<0.2 mg/ 100 mg extract).
Methods: To test for rPxr mediated transactivation cell based reporter gene assays using the synthetic reporter construct CYP3A4-XREM-Luciferase were performed. Furthermore, impact of the formulations on Cyp3a1 mRNA expression was tested in rat hepatoma cells quantified by real-time PCR. The same experiments were conducted with the human PXR using human liver cells. Subsequently, an *in vivo* rat study was conducted in which rats were treated with Hyperiplant® (400mg/kg) or Rebalance® (400mg/kg) on 10 consecutive days before expression of hepatic Cyp3a1 on protein (Western blot analysis) and mRNA (real-time PCR) level was assessed. Activity of Cyp3a1 was determined detecting the formation of 6-β-OH-testosterone in microsomes isolated from rat liver using high-performance liquid chromatography.
Results: In presence of the rat Pxr, both formulations showed transactivation of the Luciferase reporter gene, and both formulations increased Cyp3a1 mRNA expression in the rat hepatoma cells. However, in both experiments hyperforin did not exert a transcriptional effect. Importantly, in the human system induction or transactivation was limited to Hyperiplant® and hyperforin. In rats, expression of hepatic Cyp3a1 was significantly induced after Hyperiplant® treatment. No such effect was observed for Rebalance®. Hepatic abundance of Cyp3a1 protein was significantly increased upon both treatments with a higher effect for Hyperiplant®. However, Hyperiplant®, but not Rebalance® increased hepatic Cyp3a-activity in rat liver microsomes.
Conclusions: Treatment of rats with the SJW extracts mimics the PXR-mediated changes in drug metabolism reported for humans. However, our *in vitro* data suggest that the impact on rat Cyp3a1 is independent of the hyperforin content and may involve another so far unknown mechanism.

Keywords: PXR, CYP3A, species-specificity, hyperforin, St. John's wort

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P - VII - 4

Effects of St. John's wort formulations on P-glycoprotein expression in rats

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Introduction: P-glycoprotein (Pgp) is an efflux pump highly expressed in various pharmacokinetically relevant tissues including intestine, liver and kidney. In these organs, Pgp limits intestinal drug absorption, and enhances hepatobiliary and renal drug elimination. In the brain, Pgp is highly expressed in the apical membrane of endothelial cells limiting penetration across the blood-brain barrier. One of the transcriptional regulators of Pgp is the pregnane X receptor (PXR). This nuclear receptor is ligand-activated by various xenobiotics and increases Pgp expression and activity. St. John's wort (SJW) is an herbal medicine used to treat mild depression. Importantly, SJW contains several bioactive constituents of which hyperforin is known to activate human, but not the rat PXR [1]. Nevertheless, treatment with SJW was reported to increase intestinal Pgp expression in rats [2]. Hyperiplant® and Rebalance® are two SJW formulations containing high and low concentration of hyperforin, respectively.
Aims: The aim of this project was to compare the effect of a 10-day oral treatment with Hyperiplant® and Rebalance® on Pgp expression in rats to further investigate the impact of SJW formulations in this species.
Methods: Seven weeks old Wistar rats were subjected to a 10 days oral treatment with 400 mg/kg of Hyperiplant® or Rebalance® suspended in H_2O containing 0,5% methylcellulose and 0,1% Tween 80. As a control Wistar rats were treated for 10 days with the suspension mixture. Pgp mRNA and protein levels were analyzed in small intestine, liver and brain performing real-time quantitative PCR and Western blot analysis, respectively. Immunohistochemical staining applied to sections of small intestine, liver and brain was used to reveal the location of the transporter.
Results: No changes were detected in Pgp mRNA expression in small intestine, liver or brain of the treated rats. Although we were able to replicate the intestinal induction of Pgp protein in rats treated with Hyperiplant®, no such effect on Pgp protein expression was observed in other tissues or by Rebalance®. Immunohistochemical staining of Pgp applied to sections of small intestine, liver or brain revealed the expected location of the transporters with no influence of Hyperiplant® or Rebalance® on the subcellular localization.
Conclusions: Neither Hyperiplant® nor Rebalance® treatment had an impact on the Pgp mRNA expression in intestine, liver, kidney or brain. Accordingly, it is mostly likely that the observed induction of Pgp protein in the intestine of rats treated with Hyperiplant® is not linked to the transcriptional regulator PXR.

Keywords: Pgp, PXR, St. John's wort, Hyperiplant®, Rebalance®

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P - VII - 5

Investigation of hematopoietic efflux transport of coproporphyrins

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Introduction: Interaction with Organic Anion Transporting polypeptides (OATP)1B can lead to serious clinical drug-drug interactions (DDI). Therefore, thorough evaluation of the potential for an OATP1B interaction for new molecular entities (NME) in drug development is warranted. Recently, coproporphyrin (CP) I and CPIII have been proposed as biomarkers to predict OATP1B-mediated DDIs. CPs are by-products of heme biosynthesis and readily eliminated from the body with hepatic OATP1B-mediated uptake considered as the rate-limiting step in CP clearance. 80% of total CPs is produced in red blood cells (RBC), however, the mechanism of CPs elimination from RBCs has not been investigated so far. In mice the half transporter Abcb6 has previously been shown to be expressed on RBCs and to mediate the efflux transport of CPIII. Moreover, studies investigating the human RBC plasma membrane proteome revealed the expression of efflux transporters, namely MRP1, MRP4, MRP5, ABCB6, and BCRP. Considering that erythrocytes are easily accessible to any compound, modulation of efflux transporter activity on RBCs may impact serum CP levels irrespective of OATP1B function.
Aims: The objective of our current studies was to investigate efflux transporters that might be involved in the hematopoietic efflux transport of CPs.
Methods: Human leukemic K562 cells were treated with 300 nM imatinib to induce heme synthesis, or left untreated for three days. Upon termination of the treatment, heme production was assessed by means of intracellular hemoglobin (Hb) content applying benzidine staining. In parallel, CP concentrations in the supernatant were quantified with LC-MS/MS. We further investigated mRNA and protein expression of ABCC1/MRP1, ABCC4/MRP4, ABCC5/MRP5, ABCB6, and ABCG2/BCRP applying real-time qPCR and Western Blot analysis, respectively. Efflux transporter candidates were further tested for their transport activity towards CPs as a substrate. Here, we used HeLa cells transiently overexpressing a reported uptake transporter for CPI (OATP1B1) or CPIII (OATP2B1) alone or in combination with one of the efflux transporter candidates and monitored cellular accumulation of CPs.
Results: Treating K562 cells with imatinib for 3 days, we observed a significant accumulation of Hb and a concomitant increase in extracellular CP levels. In the presence of imatinib, we further detected all efflux transporter candidates but ABCC4 to be significantly upregulated on mRNA level. However, on protein level only BCRP was upregulated, while MRP4 presented with a molecular weight shift in Western blot analysis. Testing BCRP and MRP4 in transport experiments, we observed that both transporters mediated the efflux transport of both CPI and CPIII.
Conclusion: In our current studies, we provide first indication that active transport might be involved in the hematopoietic efflux of CPs in human RBCs, with BCRP and MRP4 being the most interesting candidates to be followed up in future studies.

Keywords: OATP1B, drug-drug interaction, coproporphyrins, biomarker, red blood cells

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Differences in hepatic disposition of atorvastatin in *SLCO2B1*-knockout and humanized rat model

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Introduction: Organic Anion Transporting Polypeptides (OATP) are responsible for the hepatic uptake of atorvastatin. One member of this transporter family is OATP2B1 (encoded by the *SLCO2B1* gene) which is ubiquitously expressed and well characterized for its interaction with atorvastatin [1,2].
Aims: We hypothesized that a knockout of the endogenous *Slco2b1* in rats should reduce disposition of atorvastatin into hepatocytes compared to *wildtype* animals while an OATP2B1-humanized rat model should facilitate cellular accumulation of atorvastatin.
Methods: Prior to animal studies, *in vitro* uptake experiments of atorvastatin in transiently transfected and vTF7-infected HeLa cells were performed in order to determine transport kinetics of the rodent Oatp2b1. Rat models were custom-made based on Wistar rats applying the CRISPR/Cas9 technology. In *Slco2b1*$^{-/-}$ rats the endogenous gene is deleted, while in the *SLCO2B1*$^{+/+}$ rats the coding sequence of the human orthologue was inserted into the *Slco2b1* gene locus. In the atorvastatin distribution study, male rats (n = 7 per genotype) received 2 mg atorvastatin per kg bodyweight intravenously. One hour after tail vein injection, blood and liver were collected. Obtained specimens were analyzed for atorvastatin content using a validated bioanalytical method (LC-MS/MS). Furthermore, expression and abundance of drug transporters known to be involved in hepatic atorvastatin handling were measured by RT-qPCR and Western blotting analysis, respectively.
Results: We validated atorvastatin as a substrate of the rodent Oatp2b1 with an affinity (Km) of 0.47 µM (95% CI: 0.12 to 6.12) and a maximal transport rate (Vmax) of 2.04 fmol µg^{-1} min^{-1} (95% CI 1.29 to 10.85). Rat serum quantified for atorvastatin revealed a reduction of atorvastatin levels by approximately 40% in *SLCO2B1*-humanized rats compared to *Slco2b1*-knockouts rats (mean ± SD: 70.13 ± 46.69 µg mL^{-1} *vs.* 42.44 ± 21.31 µg mL^{-1}; p = 0.1839, ordinary one-way ANOVA with Fisher's LSD test) while no difference between knockout and *wildtype* was observed. Surprisingly, liver levels in *Slco2b1*-knockout rats showed to be increased (mean ± SD: 406.0 ± 127.5 µg g^{-1}) compared to *wildtype* (mean ± SD: 283.8 ± 55.75 µg g^{-1}; p = 0.0209 one-way ANOVA with Fisher's LSD test) and reduced in *hSLCOB2B1*$^{+/+}$ rats (mean ± SD: 240.2 ± 42.88 µg g^{-1}; p = 0.0025, one-way ANOVA with Fishers LSD test compared to *Slco2b1*$^{-/-}$). The analysis of mRNA expression and protein abundance of other drug transporters involved in the uptake of atorvastatin revealed no major differences between *wildtype*, *Slco2b1*-knockout and *SLCO2B1*-humanized rats.
Conclusion: Although we expected to see reduced hepatic uptake of atorvastatin with higher serum levels in rats lacking the transporter, we now report higher serum and liver levels in Oatp2b1-deficient animals. Furthermore, we observed lower serum and liver levels in *SLCO2B1*-humanized animals. The obtained hepatic atorvastatin levels contradicted our hypothesis that indicates a difference in intracellular handling. Further studies investigating the *in vivo* pathway and metabolism of atorvastatin are warranted to elucidate the mechanism of the reduction in humanized rats.

Keywords: OATP2B1, atorvastatin, liver, *in vivo*, drug transport

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P - VII - 7

Lung-on-a-chip platform for inhalation drug screening

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Introduction: Chronic lung diseases are a global health and economic concern, leading to millions of deaths every year. However, there is a huge lack of prognostic and therapeutic strategies to combat these chronic illnesses. This is majorly due to the lack of complex distal lung models, which makes it challenging to find new targets for therapeutics. Animal models are considered as standard models when it comes to new target discovery and studying complex pathomechanisms. But animal differ considerably in their anatomy, physiology, cellular composition, and proportions compared to the human lungs. Organs-on-chip platforms on the other hand are able to resemble the true cellular composition of human alveolar barrier without the ethical and high expense concerns. Therefore, such an *in vitro* setup could be a valuable tool in drug discovery for studying chronic respiratory diseases.
Aim: The goal of this project is to validate drug activity of inhaled corticosteroids on a human derived triple culture composed of alveolar epithelial, differentiated THP-1 cells (macrophages) and lung microvasculature endothelial cells.
Methods: To mimic the air-blood barrier of the distal lung a triple-culture consistent of immortalized alveolar epithelial cells (^{AX}iAECs) [1], provided by Alveolix; human lung microvasculature endothelial cells (hLMVECs) and differentiated THP-1 macrophages were seeded on the AX12 Lung-on-Chip system [2] from Alveolix. By inducing air-liquid interface on apically seeded epithelial cells and macrophages together with cyclic stretch, an imitation of the human alveolar barrier was achieved. Measurement of the trans electrical barrier resistance (TER) was performed to analyse barrier integrity. Cells were initially exposed to an inflammation inducing nanoparticles and/or toxic compound and subsequently treated with progressive doses of inhaled corticosteroid (Fluticasone). Inhalation like -exposure was performed using the Cloud system from Vitrocell AG [3].
Results: Our results demonstrated that the nanoparticles and PHMG caused severe alveolar barrier damage leading to disrupted and inflamed alveolar barrier on-chip. Furthermore, with inhaled Fluticasone treatment, we could show decreased inflammation as measured from immunostainings using a cellular damage marker.
Conclusion: The findings of this project give an insight into a new innovative lung-on-chip platform that could be used to conduct preclinical drug efficacy and toxicity studies. Inclusion of relevant physiological conditions of cyclic stretch and air liquid interface were found to play an imminent role in uptake of aerosolized nanoparticles and corticosteroids. Hence, we anticipate that such *in vitro* models will be valuable for preclinical studies and will reduce animal experimentations.

Keywords: lung-on-chip, inhalation, air-blood barrier, COPD, preclinical studies

References:
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P - VII - 8

Evaluation of tau P301L mice for legumain PET radiotracer development in Alzheimer's disease

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Introduction: Current methods to monitor Alzheimer's disease (AD) onset and progression focus on the detection of beta-amyloid plaques (AB) and tau neurofibrillary tangles (NFT). While functional imaging of AB and NFT offers diagnostic value for patients suffering from AD, attempts to ascertain prognostic value have been unsatisfactory. The lysosomal asparagine endopeptidase legumain was found to be elevated in AD patients. Furthermore, legumain is causally linked to the processing of both amyloid precursor protein and tau to their pathological products [1,2]. In conjunction with neuroinflammation, legumain is hypothesized to precede the accumulation of AB and NFT, making it potentially amenable to serve as a prognostic marker in positron emission tomography (PET). The translatability of imaging probes rests on the establishment of appropriate mouse models for preclinical evaluation.
Aims: Our objective was to characterize a candidate mouse model (tau P301L) with regard to its application in legumain PET tracer development.
Methods: Tau P301L overexpressing B6.Dg-Tg(Thy1.2-TauP301L)183Nitsch mice abbreviated as tau P301L were selected based on single cell RNA sequencing analysis and confocal microscopy [3]. Subsequently, tau P301L mice were bred and sacrificed prior to onset of symptoms at age 5 months. Heterozygous tau P301L and homozygous non-transgenic littermates were compared. Brains were dissected post-euthanasia and processed directly. To investigate whole brain gene transcription, real-time quantitative PCR was performed. Flow cytometry was employed to identify and quantify immune cell populations. Whole brain distinction between active and prolegumain was achieved with western blot.
Results: RNA sequencing analysis of microglia yielded upregulation of neurodegeneration related genes (7 genes, increase ≥ 2-fold, p-value ≤ 0.05). Confocal microscopy was positive for the presence of legumain. Real-time quantitative PCR showed an increase in neuroinflammation marker IL-6 transcription (increase = 2.5-fold, p-value ≤ 0.05) but no change in total legumain transcription (increase = 1.2-fold, p-value ≥ 0.05). Flow cytometry revealed no significant difference in the number of lymphocytes ($CD45^+$ cells) between conditions (p-value > 0.05). Western blot indicated the expression of active legumain to a similar extent in transgenic and control mice.
Conclusions: Despite promising results in preliminary RNA sequencing analysis and confocal microscopy, no difference in legumain expression and activity was found between P301L mice compared to non-transgenic littermates. We conclude that this model of tau P301L overexpressing mice is not suitable for legumain PET tracer development. Using the established techniques, further mouse strains need to be evaluated.

Keywords: legumain, AEP, tau, amyloid beta, Alzheimer's disease, PET

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P - VII - 9

Boosting buccal absorption of biopharmaceuticals with a bioinspired suction device

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Introduction: Biopharmaceuticals have revolutionized the prevention and treatment of many diseases, such as infectious diseases, diabetes and cancer. However, they are typically administrated via the oral route due to their high molecular weight and relative instability. Although decades of research have been conducted in the quest for oral formulations, success has been scarce. In fact, only a few of those drugs have been marketed as oral formulations, while their bioavailability is typically less than 1%, with large inter- and intra-patient variations. As a result, novel approaches moved towards complex technologies such as microneedle injections in the gastrointestinal tract.
Aims: In contrast, we aim for a simple platform technology that can boost the diffusion of macromolecular drugs through the buccal mucosa. We hypothesized that a non-invasive stretching force could strongly enhance drug absorption. Based on the unique structure of octopus suckers, we designed and investigated a self-applicable suction patch device for the buccal mucosa.
Methods: The device was manufactured by 3D printing using a self-made biodegradable polymer (poly(β-thioester) and characterized thoroughly. Further, the diffusion properties and mechanistic investigation of the device were carried out *ex vivo* on fresh porcine buccal mucosa. The device was loaded with various fluorescent drug surrogates and applied under different conditions. A semi-quantitative image analysis method was established to measure the penetration depth, allowing a direct comparison of all conditions. Moreover, immunohistological stainings were utilized for a mechanistic investigation.
Results: It was found that the suction patch achieves up to two orders of magnitude higher adhesion than current state-of-the-art mucoadhesives and shows high resistance to shear forces. Most interestingly, we could boost the permeation of minimal permeable drug surrogates by synergism between mucosal stretching and PEs. Also, we identified critical permeation enhancing parameters that allowed drug surrogates with a size of 20 kDa to penetrate the buccal mucosa sufficiently. Finally, a possibly transient rearrangement or disassembly of the F-actin could be correlated to the permeation enhancing effect.
Conclusion: In conclusion, we present a novel and non-invasive route for administering biopharmaceuticals via a bioinspired device. The technology shows potential for clinical translation and provides new opportunities for drug delivery, permeation enhancer design and drug development.

Keywords: buccal drug delivery, bioinspired, 3D printing, biopharmaceuticals, non-invasive

This work has already been presented at the Annual Controlled Release Society Meeting in Montreal 2022.

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Zebrafish (*Danio rerio*) larva as an *in vivo* vertebrate model to study renal function

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Introduction: The study of renal function remains a challenge. *In vitro* cell based assays are approved to study e.g. ABC/SLC mediated drug transport but do not cover other renal functions as glomerular filtration and tubular reabsorption. For this purpose, *in vivo* studies are needed, which are time-consuming, expensive, and often rely on *in vivo* experimentation with higher vertebrates. However, they often need to be combined with *in vitro* or *ex vivo* transporter assays to provide mechanistic insights at a cellular level. In view of these limitations, there is a high unmet need for cost-effective and animal reducing *in vivo* test systems that include mechanistic studies at a cellular level and allow a translation to humans.
Aims: Since the zebrafish larva's (ZFL) pronephric kidney shares high similarity with the anatomy of nephrons in higher vertebrates, including mammals and does not count as an animal, we explored in the present study whether 3 to 4 days old ZFL have a fully functional pronephron. The aim is to use ZFL as an *in vivo* vertebrate model to study glomerular filtration, ABC/SLC mediated drug transport and folate receptor 1 mediated tubular reabsorption.
Methods: Polymers of interest were fluorescently labelled using click-chemistry (e.g. FITC-PEG) and characterized using Fluorescence Correlation Spectroscopy to determine purity and the hydrodynamic diameter. These polymers and fluorescent model substrates of specific drug transporters combined with their corresponding inhibitors were intravenously injected into 3-4 days old anaesthetized ZFL. Fluorescent test compounds were localized within the tubular volume or the central blood compartment using confocal fluorescence microscopy and recombinant marker zebrafish lines.
Results: Intravenous injection of fluorescent PEG and dextran derivatives of different molecular weights revealed a cut-off of 4.4 to 7.6 nm in hydrodynamic diameter for passive glomerular filtration, which agrees with corresponding values in rodents and humans. Distal tubular reabsorption of a FITC-folate conjugate, covalently modified with PEG_{2000}, was mediated by the folate receptor 1 (folr1). Transport experiments in the presence and absence of specific inhibitors confirmed functional expression in the proximal tubule of oat/slc22, mrp1/abcc1, mrp2/abcc2, mrp4/abcc4 and the ZFL p-glycoprotein analogue abcb4. These results were confirmed by corresponding *ex vivo* experiments in killifish (*Fundulus heteroclitus*) proximal kidney tubules.
Discussion & Conclusions: We conclude that ZFL has a fully functional pronephron at 4 days post fertilization and is, therefore, an attractive translational vertebrate screening model to bridge the gap between cell culture-based test systems and pharmacokinetic experiments in higher vertebrates.

Keywords: zebrafish, renal function, drug transporter, glomerular filtration, kidney tubule

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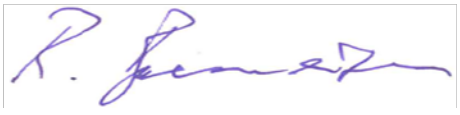

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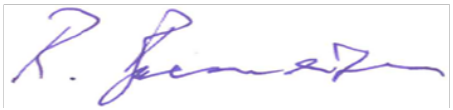

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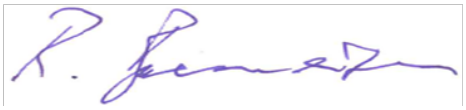

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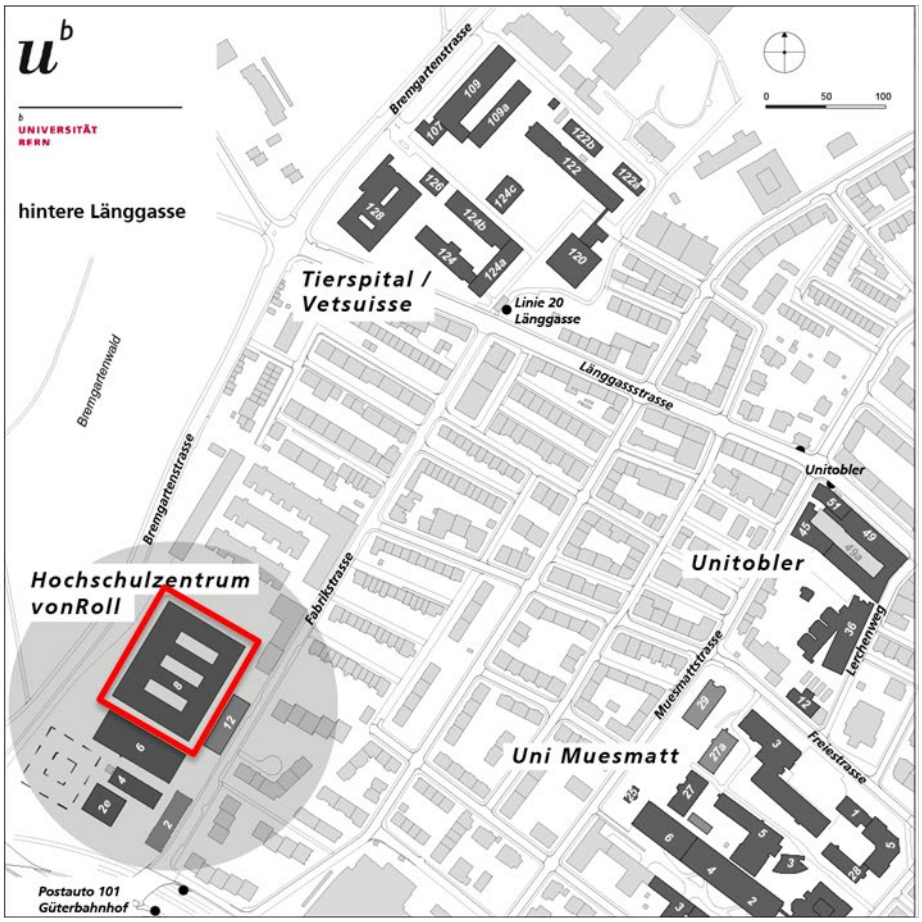

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