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Swiss Academy
of Pharmaceutical
Sciences
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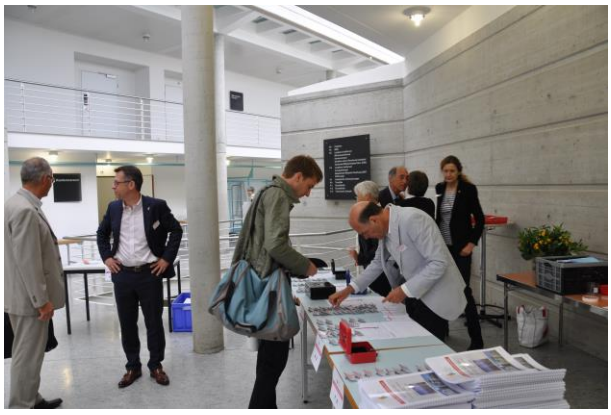
8th Swiss Pharma Science Day 2015

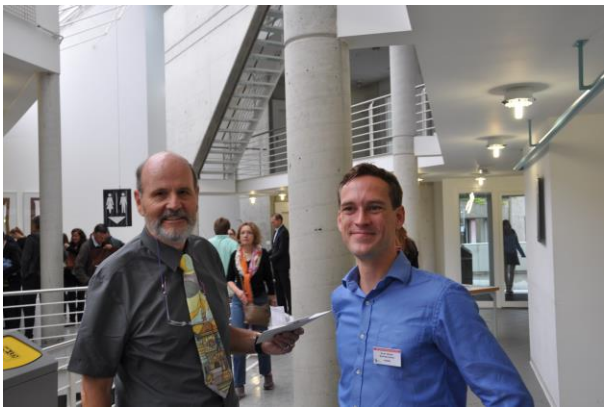
CONFERENCE REPORT and ABSTRACTS

Prof. Dr. Rudolf Brenneisen, Secretary General SAPhS

At its inception eight years ago, the Swiss Pharma Science Day (SPhSD) was intended as a gathering of Swiss pharmaceutical scientists working in academia and industry, with a special focus on the support of young scientists. This idea has been met with enthusiasm by pharmaceutical scientists and the support by the pharmaceutical industry and regulatory authorities. The organizers were thus highly motivated to make this year's event again a success for the participants, and pharmaceutical sciences in Switzerland as such.
Here is our report on the 8th SWISS PHARMA SCIENCE DAY of August 19, 2015 held again at the Pathology Langhans Auditorium of the University of Bern and the House of University of Bern. The SPhSD organizers Prof. Dr. Gerrit Borchard, president SAPhS, and Prof. Rudolf Brenneisen, secretary general SAPhS, welcomed about 130 (2014: 145) participants to the 8th SPhSD. Among the attendees were 39, 28, 26, 25, and 3 scientists from the University of Basel (including FHNW), University of Geneva-EPGL, ETH Zurich (including University of Zurich and ZHAW), industry, and other countries, respectively.



First participants appearing at the front desk



Prof. Rudolf Brenneisen, general secretary of SAPhS and SPhSD organizer, together with Prof. Oliver Germershaus, FHNW, organizer of pharmaLunch

Dr. Felix Wüst, SWISS PHARMA, together with Dr. Christine Moll and Francesca Vollenweider

Prof. Stefan Mühlebach, vice-president SAPhS and Frédéric Zwahlen, new president GSIA

The rows of the Langhans Auditorium slowly filling

Attendees studying the program

Morning Session, Lectures 1-3

Proff. Gerrit Borchard and Rudolf Brenneisen, welcoming audience and opening the SPhSD 2015

Prof. Stefan Mühlebach, moderator

The morning session was chaired by Prof. Stefan Mühlebach, vice-president SAPhS, introducing the first speaker:

Prof. Hartmut Derendorf, University of Florida at Gainesville (hartmut@cop.ufl.edu). Hartmut Derendorf is Distinguished Professor, V. Ravi Chandran Professor of Pharmaceutical Sciences and Chairman of the Department of Pharmaceutics at the University of Florida College of Pharmacy in Gainesville. Hartmut Derendorf has published over 440 scientific publications and 9 textbooks in English and German. He is editor or associate editor of 5 journals, such as the Journal of Clinical Pharmacology. Hartmut Derendorf has served as President of the American College of Clinical Pharmacology (ACCP) and President of the International Society of Antiinfective Pharmacology (ISAP). He was awarded the Distinguished Research Award, the Mentorship Award and the Nathaniel T. Kwit Distinguished Service Award of ACCP, the Research Achievement Award in Clinical Science of the American Association of Pharmaceutical Sciences (AAPS), the Leadership Award of the International Society of Pharmacometrics (ISOP) and the Volwiler Award of the American Association of Colleges of Pharmacy (AACP).

Hartmut Derendorf started his keynote lecture entitled ***„Modeling and Simulation to Streamline Drug Development – Examples from Earth and Space"*** by stating that the cost of drug development has exploded in recent years and risen to a level that soon will no longer be affordable to society. The public expectation of drug safety and guaranteed therapeutic success has become unrealistic. As a result, the number of new drug approvals can be expected to go down in the near future, a trend that is already noticeable in drug classes with low market potential due to short term therapeutic use, e.g. antibiotics. One reason for the high cost of drug development are many unnecessary studies where the results could have been predicted with reasonable certainty. PK/PD modeling is a tool that can be used to collect and integrate all the available information about a drug candidate and its class in order to make rational decisions on studies that will decrease the uncertainty of the compound. It is based on quantitative data on drug exposure and response and particularly well suited to address the question of dose finding and optimization. In the drug development process, it bridges the complete cycle from discovery to clinical use. The advantage of this approach is to define objective go/no-go decision criteria for the development process rather than relying on subjective empirical decisions. There is no way that today all developing questions can be answered by experimental evidence, and modeling and simulation is a powerful alternative approach. This modeling and simulation approach is of particular need in the field of new anti-infective agents where the rise of resistance has become an international threat to society. However, very few drug companies are currently developing new antibiotics due to the poor perspective of return on investment. However, the cost of anti-infective drug development can be dramatically lowered by applying pharmacometric concepts and selection of some key experiments based on pharmacokinetic/ pharmacodynamic (PK/PD) concepts. Using microdialysis, it is today possible to measure the local exposure at the infection site in both animals and humans. This PK information is much more useful than traditional serum pharmacokinetics. Furthermore, pharmacodynamic activities can be much better captured by analyzing time-kill curves rather than simple minimum inhibitory concentrations (MICs). Examples from various classes of antibiotic drugs will be presented where these concepts are applied and illustrated. Application of these concepts will help to develop new anti-infective treatments at low cost to combat resistance development with optimum efficacy and safety. These concepts are not only of interest on earth but also have applications in Space Exploration. As NASA is currently preparing for a mission to Mars there is great concern of medical need during such a mission. How can one make sure that the recommended dose for an astronaut in zero-gravity far away from home will be appropriate? Examples will be shown from ground-based pharmacokinetic and phamacodynamic studies to simulate zero-gravity as well as recent plans for the first complete PK/PD study on the International Space Station.

Prof. Hartmut Derendorf, keynote speaker…

…receiving after his speech a typical Swiss present

The second lecture on a topic in Biotechnology lecture was given by ***Prof. Annette Draeger, Institute of Anatomy, University of Bern*** (hartmut@cop.ufl.edu). Annette Draeger is Head of the Department of Cell Biology at the Institute of Anatomy, University of Bern. She studied medicine in Aachen, Hamburg, Lausanne and Melbourne. Her MD was followed by postdoctoral work at the MRC Laboratory of Molecular Biology in Cambridge, at the Institute of Pathology, University of Munich, and at the Institute of Molecular Biology of the Austrian Academy of Sciences in Salzburg. She is interested in mechanisms of plasma membrane repair and cellular damage control.

Her talk was on ***"Engineered Liposomes Sequester Bacterial Exotoxins and Protect from Severe Invasive Infections in Mice".*** Gram-positive bacterial pathogens that secrete cytotoxic pore-forming toxins, such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, cause a substantial burden of disease. Inspired by the principles that govern natural toxin-host interactions, her group has engineered artificial liposomes that are tailored to effectively compete with host cells for toxin binding. Liposome-bound toxins are unable to lyse mammalian cells *in vitro*. They use these artificial liposomes as decoy targets to sequester bacterial toxins that are produced during active infection *in vivo*. Administration of artificial liposomes within 10 h after infection rescues mice from septicemia caused by *S. aureus* and *S. pneumoniae*, whereas untreated mice die within 24-33 h. Furthermore, liposomes protect mice against invasive pneumococcal pneumonia. Composed exclusively of naturally occurring lipids, tailored liposomes are not bactericidal and could be used therapeutically either alone or in conjunction with antibiotics to combat bacterial infections and to minimize toxin-induced tissue damage that occurs during bacterial clearance.

Prof. Annette Draeger

Dominique Jordan, former president pharmaSuisse

The third lecture on a topic in Pharmaceutical Biology was given by ***Prof. em. Lars Bohlin of the Uppsala University in Sweden*** (lars.bohlin@fkog.uu.se). Lars Bohlin has been acting as head of the Department of Pharmacy and after that same position at the Department of Medicinal Chemistry during 2002-2005 and Vice-Dean of the Faculty of Pharmacy 2005-2008. He was elected as Inspektor by the students of Faculty of Pharmacy 1998-2003. Lars Bohlin recieved his Master of Science in Pharmacy degree at the Royal Institute of Pharmacy in Stockholm 1972 and Doctor of Pharmacy degree at Uppsala University 1978. He got his postdoctoral training with Professor Carl Djerassi at Stanford University and Professor Paul J. Scheuer at University of Hawaii 1978-1981. He was then appointed to the Faculty of Pharmacy at his present institution as Assistant Professor of Pharmacognosy 1981. He was promoted to Associate Professor 1986 and Professor of Pharmacognosy 1991. After his post-doctoral training he developed marine pharmacognosy in Sweden with the aim to identify structure-activity relationships with potential in drug discovery. His interests have focused on both ethnopharmacology with emphasis on anti-inflammatory natural products and also exploring host-defence in plant and marine organisms for molecules with anti-tumour activity. Furthermore, recently he has started a collaborative project with the objective to evaluate micro-fungi of natural origin as a source for molecules with inhibiting effect on antibiotic multi resistent bacteria. He has been a member of Cellbiology and Chemistry Drafting Committee within the Swedish Research Council, FORMAS. Furthermore, he has been involved as expert in international committees for evaluation of research or appointment of research positions in e.g. Germany, Austria, Switzerland, USA, Denmark, Norway, Canada, and South Africa. In 2001 he was involved as an international expert for evaluation of pharmacy education in Estonia and Finland. From 1992-1999 he was member of the Committee of the Swedish Pharmacopoeia and a member of the expert group no. 13 within the Euopean Pharmacopoeia Commission, Strasbourg. He has further been involved in several international comissions in relation to developing countries together with UNIDO and Swedish SIDA/SAREC. Frequently he is involved as PhD examiner and also guest lecturer in many countries. From 2011 he is acting as Chairman of the board at Folkuniversitetet, Uppsala. He has been subject editor for Phytochemistry Letters and member of advisory board of Planta Medica and Phytomedicine and present member of the Editorial Advisory Board of Journal of Natural Products. He has been supervisor for 15 PhD students and 6 licentiate exams and been an advisor to a number of post-docs and visiting scholars from many different countries. He has co-authored more than 150 research articles, reviews, and book chapters. In 2010 he is co-author of the 6th edition of the Pharmacognosy text book ”Drugs of Natural Origin - A Treatise of Pharmacognosy”. In 2013 he is co-author of a new text book ”Läkemedel från Naturen – en integrerad del av medicinen”. Furthermore, he has been involved in several patent applications and commercial development of bioactive natural products and present chairman of the board of Viogard AB with focus on non toxic growth inhibiting paints.

Lars Bohlin started his lecture on ***“Bioassays in Natural Product Research - 40 Years of Experience”*** by mentioning that the subject Pharmacognosy at Uppsala University, with a long history and connected to the famous Uppsala scientist Carl Linneaus. Today, teaching and research is focussed on bioactive novel molecules with medicinal potential from natural sources. The subject combines chemistry with biology in a multidisciplinary way. This type of approach is necessary in order to discover, describe, and communicate the richness of nature to overcome threats against biodiversity and the future sustainable use of natural resources. The ultimate purpose being to secure molecules with natural origin as potential leads in drug development and also to reveal potential new targets. Discovery of novel structure-activity relationships in nature has also an increased importance for inspiration of synthesis of natural product like compounds resulting in greater diversity but with less complexity and increased understanding of biological processes. Identifying bioactive molecules from complex biomasses requires careful selection and execution of relevant bioassays in the various stages of the discovery process of potential leads and targets. The aim of this lecture is to share his long-term experience in

bioassay-guided isolation, and mechanistic studies, of bioactive compounds from different organisms in nature. A long-term research has provided experience of selection and combination of bioassay models, which has led to an increased understanding of ethnopharmacological and ecological observations, together with in depth knowledge of mode of action of isolated compounds.

Prof. Lars Bohlin, the pharmacognosist from Sweden

Lifely discussion after Bohlin's lecture

Lunch Break and Poster Session

After the lunch break with an excellent Italian buffet, served by Gastronomy Inselspital, the participants were moving to the poster session exchanging scientific knowledge and experience with young academics. The break was also ideal for socializing and professionally networking. The abstracts of 54 posters can be found at the end of this report. As in previous years, 6 authors were evaluated by the reviewing board to receive awards for outstanding poster presentations. These awards were sponsored by the AKB Foundation, GSIA Foundation, Pharmaceutical Society of Zurich, Glatt Group, Zeller Söhne AG, and Vifor Pharma.

Two SAPhS Fellows : Dr. Christine Moll and Prof. Kurt Hersberger

Science is appetite-stimulating

Prof. Jörg Huwyler, SAPhS board member, meeting Daniel Preisig, a colleague from University of Basel

Dr. Catherine Zahner, Zeller Söhne AG, and Prof. Stefan Mühlebach, Vifor Pharma

Dr. Maxim Puchkov, University of Basel

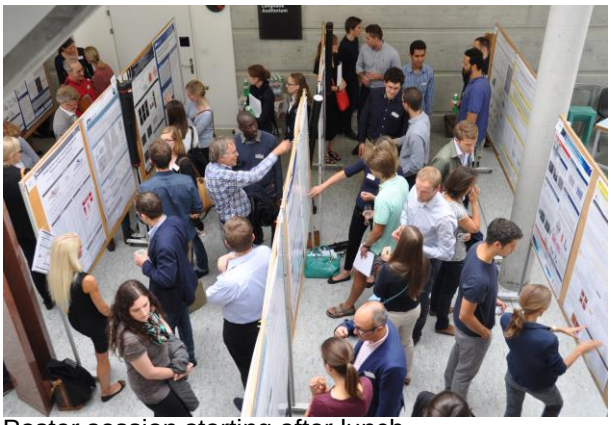
Academia collegues from Geneva and ETHZ: Proff. Borchard, Veuthey, and Gander

SAPhS board members: Philippe Tschopp, Prof. Ursula von Mandach, Dr. Astrid Czock

Two presidents: Michele Bordoni AKB, Prof. Gerrit Borchard SAPhS

Fabian Vaucher, new president of pharmaSuisse, and Dr. Andreas Stöckli, Mundipharma Medical

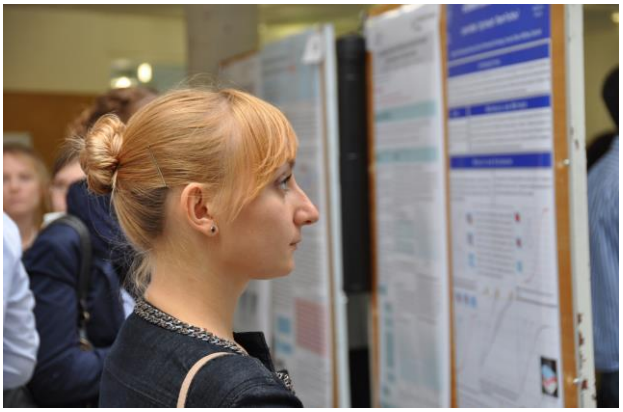
3 colleagues from ETHZ: PD Stefanie Krämer, Proff. Roger Schibli and Jean-Christophe Leroux



Poster session starting after lunch

Stella-Saphira Ehrenberger, explaining a colleague her poster

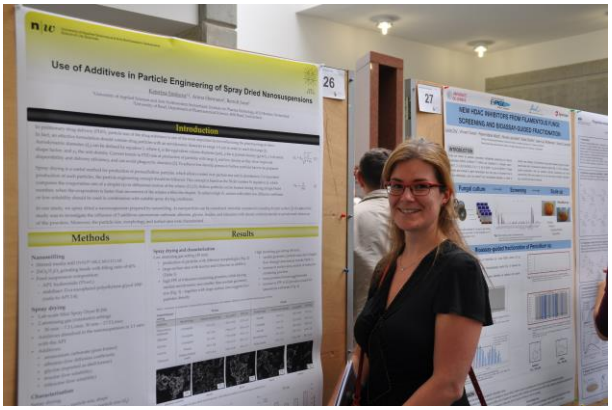
Prof. Bruno Gander, member of the poster reviewing board



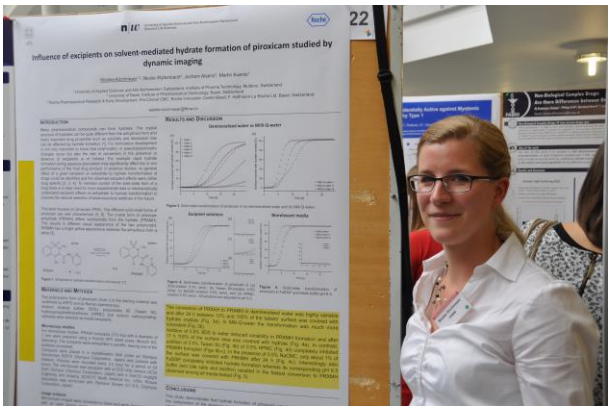
Unknown student

Dr. Steven Taub, CHUV, discussing the poster of Pierre Maudens, EPGL

Prof. Jörg Huwyler, poster reviewer board



Katerina Simkova, FHNW



Wiebke Kirchmeyer, FHNW

Dr. Maxim Puchkov, interviewing a poster presenter

Poster reviewing board evaluating the prize winners

Afternoon Session, Lectures 4-5

Dr. Christine Moll, vice-president SAPhS, chairing the afternoon session, introducing…

…Prof. Beat Ernst, talking about lectins

Prof. Dr. Beat Ernst, Dep. of Pharmaceutical Sciences, University of Basel (beat.ernst@unibas.ch), obtained his PhD at ETH Zurich. In 1979, he joined the group of Prof. Robert E. Ireland / California Institute of Technology in Pasadena as a postdoctoral fellow where he completed the total synthesis of tirandamycic acid. In 1981, he joined Ciba-Geigy Ltd. in Basel, became group leader in 1983, and head of the section Carbohydrate Chemistry and Biology in 1992. After the merger of Ciba and Sandoz in 1996, he moved to the therapeutic area Transplantation of Novartis. In 1998, he was appointed as Professor of Molecular Pharmacy at the University of Basel. His research interests are the chemical and enzymatic synthesis of therapeutically promising oligosaccharides and mimetics thereof, and the investigation of carbohydrate/protein interactions by NMR, surface plasmon resonance, microcalorimetry, X-ray crystallography, and molecular modeling. In 1991 he received the Werner-Price of the Swiss Chemical Society: "In Würdigung seiner bedeutenden Beiträge zur Naturstoff-Synthese und zur Entwicklung neuer Synthesemethoden". In 1993 he became Ciba Fellow "In Anerkennung der hervorragenden wissenschaftlichen Beitäge zur Chemie der biologisch relevanten kleinen Ringe, Naturstoffe und Kohlenhydrate". In 2003 he received the LearnTechNet Prize of the University of Basel and in 2013 the Phoenix Pharmazie Wissenschaftspreis.

The lecture of Beat Ernst in Molecular Pharmacy was entitled ***"Druggability of Lectins - Mission Possible****?".* In general, the druggability of lectins (the likelihood of being able to modulate a lectin with a small-molecule drug) is considered to be low. Thus, lectins are still carelessly neglected with regards to their therapeutic potential. Using the specific example of a bacterial lectin and a C-type lectin, we challenged the various pharmacodynamic and pharmacokinetic drawbacks traditionally associated with carbohydrate-lectin interactions. Urinary tract infections (UTI) are primarily caused by uropathogenic *Escherichia coli*. The infections are initiated by adhesion of the lectin FimH, located at the tip of bacterial type 1 pili, to the glycoprotein uroplakin 1A on bladder endothelial cells. In a detailed analysis, the binding of various FimH antagonists was analyzed by X-ray, ITC and NMR. In addition, their pharmacokinetic properties were optimized according to the requirements for oral administration. Finally, the most promising candidate was validated in an *in vivo* disease model, demonstrating its high therapeutic potential for the prevention and therapy of UTI. As a member of the family of C-type lectins, selectins play a key role in the body's defense mechanism against inflammation. They are involved in the initial steps of the inflammatory response: selectins mediate the tethering and rolling of leukocytes on the endothelial surface of blood vessels, which is a prerequisite for the subsequent firm adhesion and extravasation of leukocytes to the site of the inflammatory stimulus. However, excessive infiltration of leukocytes into the adjacent tissue can lead to acute and chronic reactions, as observed in reperfusion injuries, stroke, or rheumatoid arthritis. There-

fore, the antagonism of selectins is regarded as a valuable pharmaceutical option. The physiological selectin ligands are glycoproteins generally displaying micromolar affinities, with a carbohydrate epitope sLe^x that is essential for binding. In a detailed analysis by X-ray, ITC and SPR, the interactions of various glycomimetics of sLe^x with E-selectin were analyzed, leading to an in-depth understanding of the binding process. Based on this knowledge, two different fragment-based approaches led to the identification of high-affinity pan-selectin antagonists. One candidate is currently undergoing clinical evaluation.

The last lecture was given by ***PD Dr. Cristina Müller, Center for Radiopharmaceutical Sciences ETH-PSI-USZ, Paul Scherrer Institute Villigen*** (cristina.mueller@psi.ch). Cristina Müller is currently research group leader of the RadioTheragnostics group, Center for Radiopharma-ceutical Sciences, Paul Scherrer Institute, Switzerland. From 2009-2013 she was working at the Center for Radiopharmaceutical Sciences, Paul Scherrer Institute, Switzerland (SNF Ambizione Grant/Fellowship); 2008-2014/2015: Habilitation/Venia Legendi at the ETH Zurich, Dept. of Chemistry and Applied Biosciences; 2002-2005: PhD in Pharmaceutical Sciences, ETH Zurich, Dept. of Chemistry and Applied Biosciences and Paul Scherrer Institute, Center for Radiopharmaceutical Sciences. 2000: Federal Examination for Pharmacists, ETH Zurich, Dept. of Chemistry and Applied Biosciences; 1998-2000: Master of Science in Pharmaceutical Sciences, ETH Zurich, Dept. of Chemistry and Applied Biosciences and Paul Scherrer Institute, Center for Radiopharmaceutical Sciences; 1998: Federal Examination as Pharmaceutical Assistant, University of Bern; 1995-1997: Bachelor of Science, University of Bern. In 2001 Cristina Müller got the ETH Zurich Medal for Excellent Diploma Thesis in the Field of Pharmaceutical Sciences; in 2001 the Willi Studer Prize Award for Best Swiss Federal Examination; in 2007 the ETH Zurich Medal for Excellent Doctoral Thesis in the Field of Pharmaceutical Sciences; in 2007 the Young Investigator Award, First Place, Society of Nuclear Medicine, 54th Annual Meeting, Washington D.C., USA. In 2014 Cristina Müller was awarded the Ružička prize* 2014, ETH Zurich. She published over 50 peer-reviewed publications (>20 first authorships).

*The Ružička prize is since 1957 every year awarded to young scientist in or outside Switzerland for an outstanding paper in the field of general chemistry. 1969, the awardee was Richard R. Ernst (speaker at SPhSD 2013), who received 1991 the Nobel prize. The prize is dedicated to the ETH professor and Nobel prize winner Leopold Ružička.

The title of Cristina Müller's lecture was ***„Theragnostic Applications in Cancer Research: "Image & Treat" with Radioactive Folates"***. The goal of her research at the Center for Radiopharmaceutical Sciences has been to develop and evaluate tumor-targeted radioconjugates for nuclear imaging and radionuclide therapy of cancers. The study awarded by the Ružička prize and published in the Journal of Nuclear Medicine in 2014, reports on the application of a folate conjugate which was applied with the "matched pair" of scandium radionuclides for imaging and therapy. Scandium radionuclides have attracted the attention of researchers and nuclear physicians, due to their favorable decay characteristics for positron emission tomography (PET) imaging (^{44}Sc: $T_{1/2}$ = 3.97 h, $E\beta^+$ = 632 keV) and for therapeutic applications (^{47}Sc: $T_{1/2}$ = 3.35 d, $E\beta^-$ = 162 keV, $E\gamma$ = 159 keV). Recently, a novel DOTA-folate conjugate has been developed in Müller's laboratory, which revealed excellent *in vivo* tumor-targeting properties and low off-target accumulation. The aim of the study was to investigate this folate conjugate, labeled with scandium nuclides, for PET imaging and for radionuclide therapy in a preclinical setting with tumor-bearing mice. ^{44}Sc was produced at a cyclotron at the PSI by irradiating enriched ^{44}Ca targets with protons. ^{47}Sc was obtained from a high-flux reactor at the Institut Laue-Langevin (ILL) in Grenoble (France) by the irradiation of ^{46}Ca targets to produce ^{47}Ca which, in turn, decays to the desired ^{47}Sc. Separation of the Sc nuclides from Ca targets was carried out by chromatographic methods, yielding the desired nuclides with an excellent radionuclidic purity within a period of only ~20 min. Labeling of the DOTA-folate conjugate was performed under standard conditions at low pH (4.5) and elevated temperature (~95°C). ^{44}Sc/^{47}Sc-folates were prepared with a radiochemical yield of >96% at a specific activity of up to 10 MBq/nmol (^{44}Sc-

folate) and 13 MBq/nmol (^{47}Sc-folate), respectively. *In vitro*, the ^{44}Sc/^{47}Sc-folates showed FR-specific binding and uptake into KB tumor cells. PET imaging was performed with KB tumor-bearing mice 4 h after injection of ^{44}Sc-folate (2 MBq/mouse). The radioactivity accumulated to a high extent in the FR-positive tumor xenografts which were readily visualized using a dedicated small-animal PET scanner (Genisys^{8}, Sofie Biosciences). There was also relatively high and FR-specific uptake seen in the kidneys, which are known to express the FR. However, in the intestinal tract, as well as in the liver, radiofolate accumulation was not detec-table. Radionuclide therapy was conducted using ^{47}Sc-folate (10 MBq/mouse) in KB tumor-bearing mice. In this experiment, a significantly delayed tumor growth was observed in treated mice compared to untreated controls, which resulted in a >50% increase in survival time. With this study could demonstrate the suitability of using ^{44}Sc/^{47}Sc for radiotheragnostic purposes in a preclinical setting. This work indicates the potential of using ^{44}Sc/^{47}Sc in a clinical setting in the near future, allowing PET imaging and radionuclide therapy with chemically identical radiopharmaceuticals.

PD Dr. Cristina Müller, reporting her research in radiopharmaceuticals

Lively discussion after Cristina Müller's lecture

Recognitions and Awards – Fellows of SAPhS and Poster Prizes

The SAPhS nominates every year scientists who are distinguished by their outstanding research and professional contributions in the field of Pharmaceutical Sciences in Switzerland. The following three distinguished scientists were awarded **"Fellows of the SAPhS 2015"**:

Dr. Astrid Czock, pharmaSuisse, for her merits as head of „Science, Education and Quality"

Prof. Jörg Huwyler, University of Basel, for his merits in Science and Education of Pharmaceutical Technology.

Prof. Serge Rudaz, University of Geneva, for his merits in Pharmaceutical Analytics and achievements in the curriculum reform.

The following six **Poster Prizes** were awarded:

1st prize (CHF 1‘500), sponsored by the AKB Foundation and presented by its president Michele Bordoni to
Pierre Maudens, University of Geneva (group Prof. Allémann), poster P-19:
“Nanocrystals-Polymer Particles for Efficient Osteoarthritis Treatment“

2nd prize (1'000), sponsored by the Foundation of the Swiss Society of Industrial Pharmacists (GSIA), presented by the GSIA president Frédéric Zwahlen to
Alen Bozicevic, University of Basel (group Prof. Hamburger), poster P-1:
„Pollen Induced Asthma - Could Small Molecules in Pollen Exacerbate the Protein-Mediated Allergic Response?"

3rd prize (500), sponsored by the Pharmaceutical Society of Zurich (PharmGZ), presented by Vroni Jakob-Alther to
Sandhy Ananta, ETH Zurich (group Dr. Madduri), poster P-46:
„Dual Funtional Nerve Conduits Promote Directional Axonal Outgrowth"

Prize for the best poster in Pharmaceutical Technology (1'000), sponsored by Glatt Group, presented by Philippe Tschopp to
Wiebke Kirchmeyer, University of Applied Sciences and Arts Northwestern Switzerland (group Prof. Kuentz), poster P-22:
„Influence of Excipients on Solvent-Mediated Hydrate Formation of Piroxicam Studied by Dynamic Imaging"

Prize for the best poster in Pharmaceutical Biology (1'000), sponsored by Zeller Söhne AG, presented by Dr. Catherine Zahner to
Mark Issa, University of Geneva (group Prof. Cuendet), poster P-17:
„Bruceantin Controls the Proliferation of Multiple Myeloma Cancer stem Cells *In Vitro"*

Special Prize (500), sponsored by Vifor Pharma, presented by Prof. Stefan Mühlebach to
Matije Lucic, ETH Zurich (group Prof. Hall), poster P-42:
"Quantification of Antimirs in RISC by Chemical-Ligation RT-qPCR"

Acknowledgements and invitation to the 9th Swiss Pharma Science Day 2016

The organizers would like to thank all speakers for their excellent presentations, the reviewer board (Proff. Cuendet, Gander, Germershaus, Huwyler and Mühlebach) for evaluating the poster prizes, the photographer Philippe Tschopp, and the numerous people who helped to realize the SPhSD 2015.
Everybody is welcome to participate in the **9th SPhSD**, which will take place on **Wednesday August 31 2016**, again in Bern, at Pathology Langhans Auditorium.

Sponsors SPhSD 2015

- AKB-Stiftung zur Förderung des Pharmazeutischen Nachwuchses
  Platin sponsor, sponsoring First Poster Prize and keynote lecture of Prof. Derendorf.
- Stiftung der Gesellschaft Schweizer Industrieapotheker (GSIA)
  Gold Sponsor, sponsoring Second Poster Prize and lecture of Prof. Bohlin.
- Mundipharma Medical Comp.
  Gold sponsor
- CSL Behring AG
  Gold sponsor
- Actelion Pharma Schweiz AG
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- GlaxoSmithKline Schweiz AG
  Silver sponsor
- Galexis AG
- Pharmazeutische Gesellschaft Zürich (PharmGZ)
- Glatt Group
  Sponsoring prize for best poster in Pharmaceutical Technology
- Max Zeller Söhne AG
  Sponsoring prize for best poster in Pharmaceutical Biology
- Vifor Pharma AG
  Sponsoring special prize and lecture of Dr. Cristina Müller
- Streuli Pharma
- PharmaSuisse
- Swiss Academy of Pharmaceutical Sciences (SAPhS)

Closing the SPhSD 2015 at the House of the University of Bern

It is a tradition to finish the Swiss Pharma Science Day with an apéro at the historic House of the University of Bern. Participants enjoyed tasteful Swiss wines and snacks facilitating socializing and networking.






















P-1

Pollen Induced Asthma - Could Small Molecules in Pollen Exacerbate the Protein-Mediated Allergic Response?

A. Bozicevic, M. De Mieri, M. Hamburger

Div. of Pharmaceutical Biology, Dep. of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland

Introduction: Plant pollen are known to be strong airborne elicitors of asthma in humans. *In vitro* data and clinical studies corroborate the involvement of small surface proteins present on the pollen grain. They modulate the immune system through IgE cross-linkage causing airway inflammation, and obstruction due to constriction of airways. At the physiological level, relaxation and constriction of airways is regulated by mechanisms involving proteins such as the lipid kinase PIP5Kγ and the cation channel TRPA1.
Aim: While the role of proteins is well established, a possible contribution of small molecules present in pollen to the clinical outcome of asthma has not been explored up to now. Therefore, we analyzed and compared the phytochemical profiles of pollen originating from 30 plant species causing varying degrees of pollen allergenicity.
Methods: Profiling was performed with high performance liquid chromatography (HPLC) coupled with electrospray ionization mass spectrometry (ESIMS), photodiode array (PDA) and evaporative light scattering (ELSD) detectors, and supported by microprobe nuclear magnetic resonance (NMR) spectroscopy and spectrophotometric analysis.
Results: The presence of conjugated polyamines, such as N^1,N^5,N^{10}-tricoumaroylspermidine, N^1-caffeoyl-N^5,N^{10}-dicoumaroylspermidine and N^1,N^5,N^{10},N^{15}-tetracoumaroylspermine was a characteristic feature of pollen from Asteraceae (*Ambrosia* and *Artemisia* ssp.), and compounds with Michael acceptor properties were also mainly present in pollen of these species.
Conclusions: Polyamines such as spermine and spermidine are modulators of the lipid kinase PIP5Kγ [1], and sesquiterpene lactones activate cation channel TRPA1 [2]. Thus, the possible contribution of these small molecules in the exacerbation of airway constriction should be explored in more details.

Keywords: *Ambrosia artemisiifolia*, pollen, asthma, conjugated polyamines, Michael acceptors.

References:
[1] Erle DJ and Sheppard D. J Cell Biol 2014; 205: 621-31.
[2] Avonto C et al. Angew Chem Int Ed Engl 2011; 50: 467-71.

P-2

Development and Validation of LC-MS/MS Quantification Methods for the Establishment of a Reliable Human *In Vitro* Blood-Brain Barrier Model

D.E. Eigenmann, E.A. Jähne, M. Oufir, M. Hamburger

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Introduction: We recently established and optimized an immortalized human *in vitro* blood-brain barrier (BBB) model, whereby 4 human brain capillary endothelial cell lines (hCMEC/D3, hBMEC, BB19, and TY10) were compared regarding their ability to produce endothelial cell layers with sufficient barrier tightness [1]. The hBMEC cell line was found to be most suited for the purpose.
Aims: In the present work, we aimed at validating this BBB model with a representative series of drugs which are known to cross the BBB to a different extent.
Methods: For each compound, quantitative LC-MS/MS methods were developed and validated according to current regulatory guidelines [2]. All positive and negative controls were screened in the *in vitro* BBB model in parallel with the barrier integrity marker sodium fluorescein (Na-F) which does not cross the BBB to a significant amount. Permeability coefficients (P_e) for all compounds were calculated and compared to the P_e values for Na-F.
Results: Bioanalytical quantification methods for 8 compounds have successfully been validated in terms of selectivity, precision, accuracy, and reliability according to EMA/FDA guidances [2]. During LC-MS/MS method validation, various biological and analytical challenges were encountered, such as analyte instability in Ringer HEPES buffer after long-term and short-term storage, interferences of co-eluting compounds during LC-MS/MS analysis, and inaccurate results attributable to the selection of unsuitable internal standards. If not addressed properly, these problematic areas may culminate in an incorrect determination of analyte concentrations in the matrix. Hence, appropriate measures were taken to meet these various challenges.
All positive controls (antipyrine, caffeine, diazepam, and propranolol) showed significantly higher P_e values than Na-F, indicating high BBB permeability. The negative controls (atenolol, cimetidine, and vinblastine) showed P_e values in a similar range than Na-F (with the exception of quinidine), indicating low BBB permeability [3].
Conclusions: We have successfully validated our human *in vitro* BBB model based on the hBMEC cell line [3]. As a next step, natural product-derived leads with promising *in vitro* activity are currently being screened for their ability to cross the BBB.

Keywords: Blood-brain barrier (BBB), permeability coefficient, validation, LC-MS/MS.

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P-3

Pharmacokinetics and *In Vitro* Blood-Brain Barrier Screening of Tryptanthrin, a Dual COX-2/5-LOX Inhibitor from *Isatis tinctoria*

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Introduction: The indolo[2,1-b]quinazoline alkaloid tryptanthrin (Fig. 1) was previously identified as a potent anti-inflammatory compound with an unique pharmacological profile. It is a potent inhibitor of COX-2, of 5-LOX catalyzed leukotriene synthesis, and nitric oxide (NO) production catalyzed by the inducible nitric oxide synthase (iNOS).
Aims: To further evaluate the potential of tryptanthrin as a new chemical entity, we investigated its pharmacokinetics (PK) properties and its potential to permeate the blood-brain-barrier (BBB).
Methods: PK properties of tryptanthrin were evaluated in a pilot study in male Sprague-Dawley rats (2 mg/kg bw i.v.). The ability of tryptanthrin (5 µM) to overcome the BBB was assessed in human and animal *in vitro* BBB models. For this purpose, quantitative UPLC-MS/MS methods were developed and validated according to current international guidelines [1,2].
Results: A half-life of 40.6 ± 3.33 min and a clearance of 1.00 ± 0.181 L/kg/h were found in the *in vivo* pilot study. In the immortalized human mono-culture BBB model (hBMEC cell line) the apparent permeability coefficient of tryptanthrin from apical to basolateral ($P_{app\ A\rightarrow B}$) was 37.0 ± 0.434 x 10^{-6} cm/s. In the primary co-culture rat/bovine BBB model a similar $P_{app\ A\rightarrow B}$ of 36.2 ± 0.899 x 10^{-6} cm/s was found. The P_{app} values of tryptanthrin were more than 10-fold higher than those of the barrier integrity marker NaF (< 3.2 x 10^{-6} cm/s). In the primary triple-culture rat BBB model, the $P_{app\ A\rightarrow B}$ of tryptanthrin was 83.4 ± 4.02 x 10^{-6} cm/s, and thus was more than 70-fold higher than the P_{app} of NaF. For bidirectional assays an efflux ratio (ER) <2 was calculated.
Conclusion: The method developed for the quantification of tryptanthrin in rat plasma, and the PK data from the pilot study will serve for the design of a full PK study addressing oral bioavailability. *In vitro* data obtained with all BBB models showed good correlation, and a high BBB permeation potential of tryptanthrin when compared to the negative control NaF. An ER < 2 indicated that tryptanthrin is not subjected to active transport.

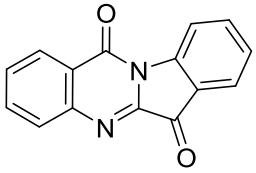

Fig. 1: Tryptanthrin

Keywords: *Isatis tinctoria*, tryptanthrin, pharmacokinetics (PK), blood-brain barrier (BBB), LC-MS/MS¨.

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P-4

Search for Alternatives to Copper in Organic Farming: Fungicidal Activity of a *Juncus Effusus* Medulla Extract and its Active Constituent, Dehydroeffusol, Against Downy Mildew and Apple Scab

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Introduction: Copper has been used since the 19th century for the control of plant diseases, and is still permitted in organic agriculture out of this tradition. In recent years, the utilization of copper has been criticized due to an unfavourable ecotoxicological profile [1]. Even though the amounts of copper have been significantly reduced, copper input is still above uptake by plants, and results in accumulation in the soil. Therefore, considerable efforts have been made in organic agriculture to identify ecologically safer substitutes.
Aims: The aim of this study is the search for copper substitutes of natural origin.
Materials and methods: An in-house library of plant and fungal extracts was screened *in vitro* for an inhibitory effect against several plant pathogens (fungi, oomycetes, bacteria) [2]. Hits were further assessed on grapevine and apple seedlings. The active constituent was identified by a procedure referred to as HPLC-based activity profiling which combines biological activity data with chemoanalytical information. Structure elucidation was performed by a combination of ESI-MS and NMR spectroscopy.
Results: As one of the hits, the ethyl acetate extract of *Juncus effusus* L. (Juncaceae) medulla showed strong inhibitory activity against *Venturia inaequalis* (apple scab) and *Plasmopara viticola* (grapevine downy mildew), with mean minimal inhibitory concentrations (MIC) (100%) of 35 µg/mL and 25 µg/mL, respectively. In a secondary assay on grapevine leaf discs inoculated with *P. viticola*, 94% inhibition was observed at a concentration of 0.5 mg/mL. When tested on grapevine and apple seedlings at a concentration of 0.5 mg/mL, the growth of these fungi was, on average, inhibited with 98% and 84% efficacy, respectively. Dehydroeffusol [3] was identified as the major active constituent, and showed mean MICs of 12 µg/mL against *V. inaequalis*, and 4.1 µg/mL against *P. viticola*, *in vitro*. Subsequent *in vivo* assessment of the pure compound revealed inhibition rates of 82% on grapevine seedlings, and 86% on apple seedlings at a concentration of 32 µg/mL.
Conclusion: The ethyl acetate extract of *J. effusus* showed potent activity *in vivo* against major fungi affecting food plants. Our results demonstrate that plants can provide promising opportunities for the replacement of copper in organic farming.

Keywords: Fungicides, copper, organic farming, *J. effusus*, dehydroeffusol.

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P-5

Production and Use of Human Platelet Lysate Formulations for GMP Cell Culture Systems

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Introduction: To date, fetal bovine serum (FBS or fetal calf serum, FCS) is commonly used as an additive in cell culture media. However the use of non-animal products for clinical production of cell therapies is preferred. Human platelet lysate (hPL) is a suitable alternative to FBS. Similar to FBS, hPL is rich in growth factors and can stimulate proliferation and expansion of many cell types.
Aim: To produce in-house and use hPL in clinical grade cell culture media.
Methods: Firstly, cellular growth with FBS and a commercial hPL® (hPL) was compared. Primary skin adult fibroblasts were seeded in triplicates in 6-wells plates (3'000 cells/cm^2) in DMEM with 10% FBS, 5% hPL or 10% hPL. Experimentation was repeated 4 times with various primary fibroblast cells. Next, we produced our own hPL. Platelet concentrates were frozen 3 times at −80°C and thawed at 37°C, and then centrifuged at ~5'300 g for 30 min to remove platelet membranes. Four batches of pooled human platelet lysate (phPL) were made: 3 with 10 donors each (phPL 1.1, 1.2 and 1.3) and 1 with 30 donors altogether (phPL 2). Fourty mL per donor were produced for each batch production. Each phPL was tested for aerobic and anaerobic sterility and cellular growth of the 4 phPLs was evaluated and compared with hPL. Primary skin adult fibroblasts (obtained from a human skin biopsy and cultured in DMEM with 5% hPL) were seeded in triplicates in 6-wells plates (3'000 cells/cm^2) in DMEM with 5% hPL, 5% phPL 1.1, 5% phPL 1.2, 5% phPL 1.3 or 5% phPL 2. Experimentation was repeated twice with primary fibroblast cells.
Results: Mean population doubling after 7 days of growth were 3.19 ± 0.34 with 10% FBS, 3.71 ± 0.23 with 5% hPL and 4.50 ± 0.43 with 10% hPL. Fourty-one platelet concentrates were manufactured in hPL with this process of preparation. All the phPLs produced were sterile and could be used for cell culture once passing quality assurance. Mean population doubling after 7 days of growth were 3.74 ± 0.62 with 5% hPL, 3.54 ± 0.08 with 5% phPL 1.1, 3.78 ± 0.26 with 5% phPL 1.2, 3.50 ± 0.24 with phPL 1.3 and 3.30 ± 0.12 with 5% phPL 2.
Conclusions: There is a higher population doubling with 5% and 10% hPL than with 10% FBS. Cellular growth is more rapid with hPL. Five % hPL is sufficient to support proliferation of primary fibroblasts. As well, new primary fibroblast cells can be obtained with hPL from human skin biopsies. hPL can easily be generated from platelet concentrate. The release of growth factors from platelet is effective due to cellular proliferation in all phPLs. The population doubling of phPLs are between 3.30 (phPL 2) and 3.78 (phPL 1.2) after 7 days of growth. The proliferation among phPLs and with hPL is homogenous over batch productions. All phPLs can be used as hPL for cell culture. hPL can efficiently replace FBS in primary fibroblast culture to provide non-xenogenic culture systems.

Keywords: Fetal bovine serum (FBS), human platelet lysate (hPL), pool of human platelet lysate (phPL), cell culture.

P-6

Quantification of Bufadienolides in *Bryophyllum pinnatum* Leaves and Manufactured Products by UHPLC-MS/MS

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Introduction: *Bryophyllum pinnatum* (Crassulaceae) is a succulent perennial plant native to Madagascar. It is used in anthroposophical medicine to treat psychiatric disorders, and as a tocolytic agent to prevent premature labour. Besides flavonoids, the plant is known to contain bufadienolides, which reportedly possess sedative and positive inotropic properties, as well as central nervous system related activities. Despite the possible toxicological relevance of bufadienolides, no reliable data are available on their content in plants and phytotherapeutic preparations.
Aims: The aim of the study was the quantification of the main 4 bufadienolides bersaldegenin-1-acetate, bryophyllin A, bersaldegenin-3-acetate, and bersaldegenin-1,3,5-orthoacetate in *B. pinnatum* leaves and manufactured products. The content of these compounds should be determined in various batches of leaves and press juices obtained from plants grown in Germany and Brazil.
Methods: Quantification was performed by UHPLC-MS/MS using MRM in positive electrospray ionization mode on an Agilent 6460 triple quadrupole mass spectrometer. Separations were performed on a Kinetex 1.7 μm XB-C18 column (100 x 2.1 mm i.d.) with a gradient of acetonitrile with 0.05% formic acid and water containing 10 mM ammonium formate and 0.05% formic acid. Bufalin was used as an internal standard. Reference compounds were previously isolated from the related species *B. daigremontianum*. Leaves were extracted with EtOH by pressurized liquid extraction (PLE). Press juices were centrifuged. The insoluble material was extracted with EtOH, while the supernatant was dried and re-dissolved in DMSO.
Results: Significant differences were observed between the analyzed samples, and even between leaves of individual plants. While the harvest time had no clear influence on the bufadienolide content, plants cultivated in Brazil were consistently found to contain significantly more bufadienolides than those grown in Germany. Interestingly, in all single plants investigated, the content of bufadienolides was markedly higher in young leaves. As to the press juice, which is the active ingredient in anthroposophic products, a significantly higher content of bufadienolides was also found in products obtained from plants cultivated in Brazil.
Conclusions: This study provides for the first time reliable data on the content of bufadienolides in *B. pinnatum* leaves and manufactured products.

Keywords: *Bryophyllum pinnatum*, bufadienolides, UHPLC-MS/MS, anthroposophic medicine.

P-7

Discovery of Natural Products Potentially Active Against Myotonic Dystrophy Type 1

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Introduction: Myotonic dystrophy type 1 (DM1) is a genetically inherited muscle disorder that is characterised by progressive muscle wasting and weakening, cataracts, and cardiac conduction defects. At present there is no cure or effective treatment for this disabling disease.
Aims: Identification of new small molecules of natural origin targeting DM1 via an HPLC-based activity profiling approach.
Methods: A collection of 70 pure compounds and more than 2'100 extracts from different plants and fungal strains were screened with a novel DM1-based biochemical assay for their ability to inhibit the formation of the pathogenic complex formed between (CUG)n-RNA and the splicing-factor muscleblind-like 1 (MBNL1). Active constituents were tracked using HPLC-based activity profiling, an approach which combines bioactivity data, structural information from online HPLC-UV-MS and offline microprobe NMR analyses, and database searches.
Results: Ten extracts from different plant species were found to be active (≥ 50% inhibition at 100 µg/mL). Methylenetanshinquinone and 1,2-dihydrotanshinquinone were found to be the most active compounds in *Salvia miltiorrhiza*. The β-carboline alkaloid harmine was responsible for the activity of *Peganum harmala*, and the iridoid glycoside auroside was identified as the active constituent in *Lamium album*. The HPLC profiles suggested the presence of tannins in the remaining 7 active extracts. Retesting of these extracts after tannin removal by filtration over polyamide confirmed the nonspecific interaction of the original extracts with the protein-based screen. In addition, the protoberberine alkaloid berberine was identified as a potent hit from the library of pure compounds.
Conclusions: Overall, this study identified several small molecules of natural origin which are promising hit compounds in (CUG)n-MBNL1 complex inhibition. In a secondary cellular assay some of the identified small molecules partially reversed the splicing defects associated with DM1. Detailed secondary *in vitro* and *in vivo* investigations on these compounds are ongoing.

Keywords: HPLC-based activity profiling, natural products, myotonic dystrophy type 1, tanshin-quinones, alkaloids.

P-8

Non Biological Complex Drugs: Are There Differences Between Originators and Similars?

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Introduction: Absolute iron-deficiency anemia (IDA) may be caused either by a significant decrease in iron absorption or by severe blood loss [1]. In order to treat iron deficiency, either oral administration of iron salts or iv administration of iron carbohydrate drugs are applied. The latter belong to a new class of drugs, called Non-Biological Complex Drugs (NBCDs) [2]. Iron sucrose (Venofer®) is a colloidal suspension composed of polynuclear iron(III)-hydroxide cores, which are surrounded by a sucrose shell. Over the last decades, different intended copies of Venofer® were introduced on various markets. However, clinical and non-clinical evidence suggests that the treatment with these similars (ISSs) may not lead to the same results as with the Originator drug [3].
Aims: The aim of this work is to present a full physicochemical characterization of both the Originator product and ISSs in order to lay the basis for an assessment of the safety and exchangeability of the different preparations.
Methods: Dynamic light scattering (DLS), transmission electron microscopy (TEM) and He-focused ion beam microscopy (He-FIB) were used to determine size and shape of the colloidal products. The molecular weight (m.wt.) distribution was investigated via GPC analysis, the charge of the colloidal suspension determined by zeta potential (ZP) measurements. The colorimetric method using chromazurol B was used to quantify the amount of labile iron, and *in vitro* dissolution kinetics assays were performed.
Results: DLS analysis revealed that Venofer® is composed of particles with a size below 10 nm, with a monomodal distribution in Number. By contrast, two ISSs showed a bimodal distribution in Number. The shape of the different complexes was furthermore investigated by TEM and He-FIB. The images obtained confirmed the size and spherical shape of all iron sucrose complexes. The molecular weight distribution was investigated via GPC analysis, obtaining an average m.wt. between 34 and 60 kDa for Originator and ISSs, in agreement with the USP monograph for iron sucrose injection. The net charge was shown to be approximately −50 mV for all complexes investigated. The amount of labile iron was determined via a colorimetric method with chromazurol B. Significant differences between Originator and ISSs were evaluated. Finally, the *in vitro* dissolution kinetics of both Venofer® and ISSs were examined, obtaining significant differences in T_{75} values for the Originator and the ISSs.
Conclusions: The extensive physicochemical characterization of Venofer® and ISSs reported in this work represents a first approach that may help to explain the differences in the clinical efficacy of these drugs. Data obtained for DLS and ZP were confirmed by 2 independent laboratories. Clear differences between Originator and Similars were assessed with the different techniques. However, the use of a single assay was not considered sufficient to disclaim the quality of such colloidal preparation. Further investigations with human samples will be carried out in order to clarify the interactions of the nanoparticles with the immune system.

Keywords: Non-biological complex drugs (NBCDs), iron sucrose (IS), iron sucrose similars (ISSs).

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P-9

Prescribing Patterns of Proton Pump Inhibitors in Patients with Use of NSAIDs – A Comparison Between Switzerland and the UK in Primary Care

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Introduction: Proton pump inhibitors (PPIs) have proven to be effective in reducing the risk of gastro-duodenal bleeding caused by non-steroidal anti-inflammatory drugs (NSAIDs). Despite of having only little adverse drug reactions they became more common prescribed during the last years. We are not aware of any studies describing the PPI prescribing pattern in the Swiss ambulatory setting.
Aims: To examine the frequency of concurrent prescribing of PPIs and NSAIDs (on the same day and within ± 90 days of a NSAID prescription) in Switzerland and the UK. We further aimed at assessing co-prescribing of PPIs in individuals with co-medication of either NSAIDs with systemic steroids, or NSAIDs with anticoagulants.
Methods: We conducted a descriptive study on the health resource utilization (HRU) by using claims dataset provided by the large Swiss health insurance Helsana and data from the Clinical Practice Research Datalink (CPRD). We included patients of all ages who received at least one prescription of an NSAID between January 1, 2010 and December 31, 2013. They must not have received another NSAID prescription in the previous 180 days. We exclusively focussed on the first-time NSAIDs prescription during the study period.
Results: In Switzerland, 17.5% of all 645'158 patients with a first-time NSAID prescription were identified to have a concurrent prescription of a PPI on the same day (31.8% within ± 90 days). The concurrent prescribing of PPIs in patients with NSAIDs and systemic steroids on the same day was lower (16.4%), but higher in patients with NSAIDs and anticoagulants (33.0%) (45.5% vs. 52.6% within ± 90 days). In the UK, 18.9% of 981'938 patients with a first-time NSAID prescription were identified to have a concurrent PPI prescription on the same day (30.1% within ± 90 days). Significantly higher prescribing rates of PPIs were observed in patients with a co-medication of NSAIDs and systemic steroids (35.5%) or anticoagulants (46.4%) on the same day (52.2% vs. 60.7% within ± 90 days). In Switzerland, the most frequently concurrent prescribed NSAID/PPI was diclofenac (38.9%) and pantoprazole (64.6%), followed by ibuprofen (30.6%) and esomeprazole (16.1%). In the UK, the most frequently concurrent prescribed NSAID/PPI was naproxen (47.6%) and omeprazole (71.8%), followed by ibuprofen (23.2%), and lansoprazole (26.3%).
Conclusions: The frequency of co-prescribing of PPIs in patients with co-medication of NSAIDs and systemic steroids or anticoagulants showed to be significantly higher in the UK. The general concurrent prescribing pattern of PPIs in patients with NSAID use only did not show any discrepancies.

Keywords: PPIs, NSAIDs, prescribing patterns**,** claims data**,** health resource utilization.

P-10

Standardised Documentation of Interventions: Pharmacist's Perceptions

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Introduction: In daily practice, pharmacists are not used to routinely document their interventions in a standardized way. A classification could help to record interventions and data generated provide a pool for epidemiological studies. To increase patient safety while transferring between care settings and to ease seamless care, the structure of the classification system should be similar across the settings but provide different levels of details, and should be integrable into patient file. To suit the community pharmacy setting, we adapted an existing classification system for pharmaceutical interventions, which stemmed from the GSASA system implemented in several Swiss hospitals [1]. Both systems were previously validated and reached a good inter-rater reliability. To further develop and implement it successfully, we invited pharmacists to participate in a focus group.
Aims: To obtain the experiences and perceptions of practicing pharmacists, to further develop an existing classification system for pharmaceutical interventions for community pharmacy.
Methods: We conducted a focus group interview with practicing and experienced pharmacists, working in different institutions. As preparation, the participants documented a standard case using the classification system to become acquainted with. The first question "Why is it important to document what we are doing?" allowed the panellists to express 2 to 3 reasons on paper sheets, which were gathered and discussed. The core content, structure, and order of the system were discussed to evaluate the level of agreement. The panellists had the possibility to accept, reject or revise each item, and made suggestions. Agreement > 50% indicates the acceptance of the item. The interview was recorded on audio tape and anonymously transcribed. The transcript was reread and analysed using thematic analysis.
Results: Out of 11 pharmacists invited, 9 joined the focus group. The panellists consisted of 6 community pharmacists (3 of them developed or previously used a documentation system), and 3 clinical pharmacists who routinely work with a classification system in their hospital. The panellists recognized the importance of documenting their interventions and believed that this may allow traceability, facilitate communication within the team and other health care professionals, and increase patient safety and quality of care. The interview also aimed to refine and validate the classification system to create a new version. Of 51 subcategories, 47 were accepted (92.2%, mean agreement 91%), 4 needed revision (7.8%), none were rejected. The major change, based on the participant's suggestions, was the addition of a new category ("communication: individuals involved"), which resulted in the suppression of a subcategory ("report to pharmacovigilance center") and the reword of 2 subcategories ("information to physician", "clarification in patient notes").
Conclusions: As stated by the panellists, documentation of pharmacist's interventions should enhance traceability and information flow through communication within team and with other health care professionals. This should lead to an improvement of patient safety and visibility of pharmacist's activities. Documentation can also have a teaching and learning function to increase quality and performance. The panellist's suggestions allowed to refine the classification system, resulting in a final version which will be tested in a cross-sectional study with community pharmacists.
Keywords: Pharmaceutical Care, classification system, interview.

Reference:
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P-11

No Association Between Type II Diabetes Mellitus and Incident Osteoarthritis of the Hands - A Population-Based Case-Control Study

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Introduction: The association between diabetes and all osteoarthritis has been studied with conflicting results. However, there is emerging evidence that metabolic syndrome is associated with incident osteoarthritis of the hand specifically (HOA).
Aims: We analyzed the association between type II diabetes mellitus (T2DM) and HOA specifically in a large observational study.
Methods: We conducted a BMI-matched (1:1) case-control study using the UK-based Clinical Practice Research Datalink of cases aged 30 to 90 years with an incident diagnosis of HOA from 1995 to 2013. In multivariate conditional logistic regression analyses, we calculated ORs for incident HOA in patients with T2DM, stratified by T2DM severity (HbA_{1C}), duration, and pharmacological treatment. We further evaluated effect-modification by presence of other metabolic diseases (hypertension, hyperlipidaemia, obesity).
Results: Among 13'500 cases and 13'500 controls, we observed no statistically significant association between T2DM and HOA risk (OR 0.94, 95% CI 0.86-1.03), regardless of T2DM severity, duration, or pharmacological treatment. Having hypertension did not change the OR, whereas we observed a trend towards increased ORs in overweight T2DM patients with co-occurring hyperlipidaemia with or without coexisting hypertension (OR 1.31 95% CI 0.91-1.89, without hypertension; OR 1.22, 95% CI 0.97-1.55, with hypertension).
Conclusions: Our results provide evidence that T2DM is not an independent risk factor for HOA. Previously observed increased risks for HOA in patients with the metabolic syndrome may be driven by an underlying diagnosis of hyperlipidaemia especially in overweight patients.

Keywords: Osteoarthritis of the hand, diabetes, hyperlipidaemia, CPRD.

P-12

Simulations of Drug Release Profiles as a Function of Different Geometrical Designs

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Introduction: By changing the release pattern of existing drug substances, therapy can be optimized. However, there are no standard methods available for modeling of drug release profiles as a function of geometrical arrangements.
Aims: Explore simulated release profiles from complex geometries using discrete element calculation method based on 3-dimensional cellular automata.
Methods: The simulations were done with 3-dimensional cellular automata (F-CAD 2.0). For validation intrinsic dissolution tablets were produced with Medel'Pharm Styl'One compaction simulator.
Results: The simulations were made for 4 example tablet geometries. Cylindrical surface features variable release rate that decreases linearly. A double-core tablet yielded in a complex release pattern. A lense-shaped core provided a constantly accelerating release rate due to the lense-shaped core was accessible for the medium at 6 different points. After reaching a maximum at 70% drug release, the release rate decreased. The triple-layer tablet was used to show the influence of the layer position on the release rate. With this model, the method for selecting a controllable barring material was proposed.
Conclusions: It was demonstrated that simulations of drug release profiles as a function of different geometrical designs was possible. Simulated release rate pattern revealed a complex behavior with respect to different tablet geometries. It is a time effective and cost saving way to find the tablet-geometry with the desired optimized release profile.

Keywords: Drug release simulation, F-CAD**,** geometrical designs, tablet.

P-13

Enzyme-Loaded Liposomes for Enhanced Alcohol Detoxification by Peritoneal Dialysis

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Introduction: Alcohol abuse represents a growing burden for society, especially among young people susceptible to binge-drinking. Severely intoxicated patients are subjected to haemodialysis (HD), due to its higher clearance capacity over the fairly simple and more convenient peritoneal dialysis (PD).
Aims: Capitalizing on recent results discussing the enhanced extraction efficiency of PD supplemented with transmembrane pH-gradient liposomes [1], the current project aims to improve the efficiency of PD, by enhancing alcohol clearance through the use of liposome-anchored enzymes, namely alcohol oxidase (AO) and catalase (CAT). Here we describe the acylation of AO and CAT and their physicochemical characterization to facilitate liposome loading.
Methods: N-(Succinimidyloxy-glutaryl)-L-α-phosphatidylethanolamine, dioleoyl was conjugated to AO and CAT at increasing phospholipid:protein molar ratios (50:1 – 200:1), and the residual activity was measured. The degree of modification was assessed by quantifying the free lysine residues normalized to the native enzymes. Conjugates were further characterized by reverse phase (RP) and size exclusion chromatography (SEC).
Results: Hydrophobic modification decreased the free amine residues and conjugation was further confirmed by RP analysis while the residual activity was well preserved (>90%). A conjugation ratio- dependent loss in monomeric protein was observed by SEC, strongly suggesting that aggregates are formed as a result of successful hydrophobization.
Conclusions: The currently employed conjugation ratio is higher than in previously published reports. We therefore believe the here demonstrated conjugation degree should be sufficient for subsequent liposome loading by detergent dialysis.

Keywords: Alcohol, detoxification, peritoneal dialysis, liposomes, enzymes.

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P-14

Development of Mucoadhesive Drug Delivery Systems by Precipitation of Chitosan on Drug-Loaded Microparticles

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Introduction: Multiparticulate dosage forms consist of small sub-units containing the drug and having the advantage of better distribution and less local irritation in the gastrointestinal tract compared to single-unit dosage forms. Mucoadhesive formulations potentially prolong and normalize the gastrointestinal residence time, and increase bioavailability due to their adhesive interactions with the mucosal membrane [1]. Recently, our group has described a simple solvent-evaporation method to load various model drugs into porous calcium carbonate microcarriers (Omyapharm) [2].
Aims: To develop mucoadhesive microparticles by coating drug-loaded calcium carbonate microcarriers with the mucoadhesive polymer chitosan using a precipitation method. Furthermore, we aimed to develop a particle-retention assay for measuring mucoadhesivity under dynamic flow conditions simulating the shear stress in the gastrointestinal tract.
Methods: The model drug metronidazole benzoate (MBZ) was loaded into Omyapharm microparticles with a drug load of 40% (w/w) by solvent evaporation [2]. Precipitation of chitosan on MBZ-loaded microparticles was done by slowly increasing the pH of the chitosan solution to pH 7. After separation from aqueous phase, the dried chitosan-coated particles were assayed for drug, chitosan and calcium content. The mucoadhesivity was evaluated by measuring particle retention on porcine colonic mucosa using a custom-built flow channel. In the same experiment, drug release was analyzed by HPLC and compared with the USP IV flow-through method.
Results: Drug load and chitosan content were close to expected values, indicating a good control of the precipitation process. Microparticles with highest chitosan content (33.3%, w/w) showed best mucoadhesive performance with a retained fraction of 81.6 ± 6.2% after 30 min. Drug release in the flow-channel was comparable as measured with the USP IV method, indicating more complete drug release for particles with higher chitosan content.
Conclusions: The presented chitosan-precipitation method is well suited for preparation of mucoadhesive microparticles and was recently published [3]. The good mucoadhesivity makes such chitosan-coated microparticles a promising formulation strategy for drug delivery to the intestinal mucus layer. This can be useful for improvement of the bioavailability of poorly water-soluble drugs, or for local treatment of colonic diseases with less systemic adverse drug effects.

Keywords: Mucoadhesive microparticles, chitosan, poorly water-soluble drugs, colonic delivery.

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P-15

Bioanalytical Validation Issues of an UHPLC-MS/MS Method for Quantitation of DOPAC in Sprague Dawley Rat Plasma, and Application to Pharmacokinetic Studies

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Introduction: 3,4-Dihydroxyphenylacetic acid (DOPAC) is a major metabolite of the dietary flavonoid quercetin, which is formed by the intestinal microflora. It has been shown that DOPAC exerts an anxiolytic effect after intraperitoneal application in mice [1]. However, the fate of DOPAC, and its pharmacological activity in the CNS are largely unknown.
Aim: Determination of DOPAC plasma levels in Sprague Dawley rats and investigation of its pharmaco-kinetic properties.
Methods: A sensitive UHPLC-MS/MS method for measurement of DOPAC levels in lithium heparin Sprague Dawley (SD) rat plasma was developed using $^{2}H_5$-DOPAC as an internal standard (IS), and validated according to international regulatory guidelines [2, 3]. An Agilent UHPLC Infinity 1290 coupled to an Agilent QQQ6460 triple quadrupole mass spectrometer (Agilent Technologies, USA) was used. The method was used in a pharmacokinetic study in rats, whereby DOPAC was administered intra-venously (i.v) at doses of 1, 2 and 4 mg/kg body weight. Blood samples taken from 0 to 12 h were analyzed by WinNonlin software (version 5.2.1, Pharsight, St. Louis, MO, USA).
Results: Major bioanalytical issues during development of the DOPAC LC-MS/MS method were encountered, and solved to reach acceptance criteria of the main regulatory guidelines [2, 3]. Given that DOPAC is also an endogenous metabolite of dopamine, the use of a “surrogate” matrix was required. Bovine serum albumin (BSA; 60 g/L) was used in this case. Protein precipitation with acetonitrile was selected as the best extraction method. Non-specific adsorption of DOPAC to plastic tubes was avoided by coating with 0.2% Tween 20. Stability of DOPAC in BSA solution did not meet the regulatory require-ments at room temperature (RT) after 4 h, and after 3 freeze and thaw (F/T) cycles at -80°C. The stability of DOPAC was confirmed at RT after 2 h, and after 1 F/T cycle at -80°C. Calibration curve was quadratic ($R^2 \geq 0.998$) with 1/X weighting factor, in the range of 30-3000 ng/mL. After i.v. administration, pharmacokinetics of DOPAC followed the first-order two-compartment body model with a high clearance of 1.5 L/h/kg and a short half-life of 20 min.
Conclusions: The UHPLC-MS/MS method is specific, selective, precise, accurate, and capable to produce reliable results. The high clearance and the short half-life of DOPAC suggest that the compound is either cleared rapidly via metabolism or excretion. At present, it is unclear how CNS-related pharmacological effects reported for DOPAC are to be explained.

Keywords: DOPAC, LC-MS/MS, validation, “surrogate” matrix, pharmacokinetics.

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P-16

Evaluation of New Stationary Phase Chemistries and Analytical Conditions for the Analysis of Basic Compounds in SFC

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Introduction: Supercritical fluid chromatography (SFC) has made a remarkable comeback in the last few years. A number of technical improvements brought to SFC instrumentation make this approach compatible with the most recent stationary-phase technologies, including columns packed with fully porous sub-2 µm particles, generating kinetic performance similar to those observed in UHPLC. In the same way as LC in its time, SFC has a challenge to cope with: the analysis of basic compounds. A lot of compounds with pK_a values above 8 tend to exhibit strong peak distortion (tailing, shouldering, split peaks) which could strongly compromise resolution and sensitivity.
Aims: Characterize new stationary phase chemistries in order to identify the best kit for UHPSFC method development. Try to find analytical conditions that would allow using a pure CO_2/methanol mobile phase.
Methods: In this study, we have evaluated 3 new SFC stationary phases (2-picolylamine, diethylamine and 1-aminoanthracene) by injecting a set of 39 basic pharmaceutical compounds. For each column, we have also tested different analytical conditions: first with no additive at all, secondly using an additive (ammonium formate) in the injection solvent, and last with the same additive in the mobile phase. Finally, the results were compared with existing stationary phase chemistries (diol, 2-ethylpyridine and hybrid silica).
Results: In terms of retention, the behavior of the 3 columns was very different, the 1-aminoanthracene being very retentive when the diethylamine had the lowest retention. All the columns that have been tested do not give acceptable results in the absence of additive with a lot of tailing and distorted peaks. The use of additive in the injection solvent only had a real positive impact with the 2-picolylamine and diethylamine columns. This can be a good tip to get acceptable results with a mobile phase composed of only CO_2 and methanol. However, the best results were still obtained with the additive in the mobile phase for each column. Finally, the 6 columns showed good complementarity with different selectivity and retention characteristics. The best kit for the analysis of basic compounds that seemed to arise from this study is composed of hybrid silica, 2-picolylamine and diethylamine.
Conclusions: The best kit for basic drugs method development would be constituted of 2-picolylamine, hybrid silica and possibly diol or diethylamine columns.

Keywords: UHPSFC, basic compounds, stationary phase, additive.

P-17

Bruceantin Controls the Proliferation of Multiple Myeloma Cancer Stem Cells *In Vitro*

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Introduction: Multiple myeloma (MM) remains an incurable malignancy in spite of the development of novel therapies. The cancer stem cells (CSCs) hypothesis offers an explanation to the reason the majority of MM patients relapse, develop resistance and eventually die from the disease. CSCs are characterized with self-renewal, differentiation and resistance. Thus, compounds targeting MM-CSCs may significantly improve the prognosis of MM patients. Here we report that bruceantin shows promising activity against MM-CSCs.
Aims: The main goal of this project was to investigate the anticancer effects of bruceantin on MM-CSCs.
Methods: Highly tumorigenic MM-CSCs (CD44+, CD133+, SSEA 3/4+ and OCT4+) were isolated from a newly diagnosed patient with MM. The effects of bruceantin on cell proliferation, cell migration and angiogenesis were assessed using esterase activity assay, scratch assay and HUVECs angiogenesis assay, respectively. In order to identify a possible mechanism of action, the expression of stem cell-specific genes were measured by qPCR. An inhibitor of the Notch signaling pathway was employed in order to examine the possible involvement of Notch in mediating the effects of bruceantin on MM-CSCs proliferation.
Results: Bruceantin potently inhibited MM-CSCs proliferation and migration at 24 h and in a dose-dependent manner. Bruceantin also inhibited angiogenesis. Quantitative real time PCR analysis of gene products implicated in stem cell maintenance and differentiation revealed that bruceantin caused an alteration in the expression of Notch1, RUNX1, PBX1, HES1, NFKBIA, CHUK and IRF1. The use of a Notch inhibitor appeared to block the antiproliferative effects of bruceantin in MM-CSCs.
Conclusions: Bruceantin inhibited the proliferation of MM-CSCs, likely through Notch. The ability of bruceantin to inhibit MM-CSCs migration and angiogenesis *in vitro* warrants further investigation in other MM-CSCs models and potentially in *in vivo* models.

Keywords: Multiple myeloma, cancer stem cells.

P-18

Evaluation of the Intestinal Absorption Mechanism of Casearin X in Caco-2 Cells with Modified Carboxylesterase Activity

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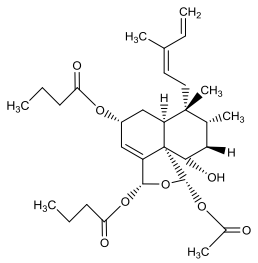
Introduction: The clerodane diterpene casearin X (CAS X, Fig. 1), isolated from the leaves of *Casearia sylvestris*, is a potential new drug candidate due to its potent *in vitro* antitumor activity at low concentrations [1]. The prediction of the intestinal absorption is an important step in the drug development process. Due to the high lipophilicity (Clog P = 5.7) and the ester-containing drug character of CAS X, problems in the permeability across Caco-2 cells can therefore be expected.
Aims: The intestinal absorption mechanism of CAS X should be evaluated using Caco-2 cell monolayers with or without active carboxylesterase (CE) hydrolysis. Furthermore, a mass balance study under both experimental conditions comparing the transport from A → B and B → A should be performed.
Methods: Cells were cultured as described previously [2]. The inhibition of CE-mediated hydrolysis was achieved by pretreatment of the monolayers with bis-*p*-nitrophenyl phosphate (BNPP) [3]. An HPLC-MS method was developed and validated for the quantification of CAS X.
Results: A significant amount of CAS X disappeared during both, absorptive (A → B; 89.2 ± 2.5%) and secretory (B → A; 73.3 ± 2.9%), permeability experiments in Caco-2 cell monolayers without CES inhibition. Recoveries significantly increased over 80% in Caco-2 cell monolayers in the presence of the inhibitor BNPP. Under CE-inhibited conditions, CAS X was rapidly transported in the absorptive direction, with a P_{app} value of 66.87 ± 2.33 x 10^{-6} cm/s. No significant efflux of CAS X was observed in secretory experiments (P_{app} = 7.54 ± 0.62 x 10^{-6} cm/s). Besides, the efflux ratio (P_{ratio}) was 0.11, suggesting that the uptake mechanism by transporters are involved. The mass balance study revealed that a large amount of CAS X remains inside the cells (62.2 ± 3.4%) which can be explained by the lipophilic character of the compound.
Conclusions: The estimation of P_{app} was only possible under CE-inhibited conditions, in which CAS X is able to cross the Caco-2 cells monolayer, probably by active transport, with no significant efflux, but with a high retention of the compound inside the cells. These findings suggest that CAS X behaves like a prodrug in an *in vitro* cell culture model which can be hydrolyzed by CE, in particular by hCE-1, which is specific for Caco-2 cells.

Fig. 1 Casearin X



Keywords: Casearin X; Caco-2 cells; absorption; inhibition; carboxylesterase.

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P-19

Nanocrystals-Polymer Particles for Efficient Osteoarthritis Treatment

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Introduction: The development of a new drug delivery system for intra-articular (IA) injection is essential for the treatment of osteoarthritis (OA), the most common type of arthritis affecting 60 % of the world's population under 65 years [1].
Aims: The aim of this study was to develop Nanocrystals-Polymer Particles (NPPs) as highly loaded particles with extended release properties for a local and efficient osteoarthritis treatment.
Methods: PH-797804 a selective ATP-competitive p38 mitogen-activated protein kinase (MAPK) inhibitor, currently in phase II clinical trial (NCT01102660), was chosen as active pharmaceutical ingredient (API). Subsequently, the steps of the study were, (i) to formulate API nanocrystals and protect them against a crystalline regrowth (Wet-milling, followed by SEM, X-ray diffraction, DSC and DLS characterization), (ii) to synthesize a fluorescent polymer useful for the intravital tracking of particles, (iii) to encapsulate a high payload of API (from 3 to 35 % w/w) in 10 to 25 µm-particles (obtained with a Spray dryer 4M8TriX and characterized by LD, SEM, UHPLC), (iv) to assess the *in vitro* drug release, (v) to evaluate the human synoviocytes viability (MTT test) and (vi) to investigate the *in vivo* activity of the particles in an antigen-induced arthritis (AIA) mice model.
Results: PH-797804 nanocrystals were produced by a recrystallization/wet-milling process and optimally stabilized by vitamin E TPGS [2]. Nanocrystals had a monomodal size distribution. NPPs obtained by spray-drying had up to 35 % drug loading, with 14.2-µm mean size diameter. They released their content over months. NNPs were non-toxic to human synoviocytes at $100\times IC_{50}$. Finally, *in vivo* experiments on AIA murine model showed a good retention of NPPs in the joint during 4 weeks, a significant reduction of the inflammation and inhibition of several cytokines (e.g. IL-1β and IL-6).
Conclusion: New formulations were successfully developed to offer a controlled, sustained release of API from biocompatible polymeric particles containing nanocrystals. They were successfully tested in an arthritis mouse model. NPPs, as a new pharmaceutical technology, could be a safe drug delivery system to treat locally a disease during a long time with a high drug dose available.

Keywords: Nanocrystals, microparticles, osteoarthritis, p38 MAPK inhibitor.

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P-20

Designing Lipid Microdomains on an Inorganic Carrier by Hot-Melt Extrusion

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Introduction: More than 60% of the newly developed active compounds are poorly water-soluble. Among the different formulation strategies for such compounds, solid dispersions (SDs) represent one of the most efficient ways to overcome solubility and bioavailability issues [1]. Over the last two decades, hot-melt extrusion (HME) has become a commonly used process for manufacturing of SDs. The active ingredient is commonly embedded in a carrier that is composed of a polymer and other excipients such as plasticizers or fillers [2]. Other amorphous SDs could be obtained by HME while adsorbing a compound on an inorganic carrier. It was shown that interactions between an acidic compound moiety and functional groups of an adsorbent were leading to amorphization of the active compound [3]. The goal of SDs is to disperse a compound in an amorphous or molecular state. Nevertheless, a major issue is the compound thermodynamic stability that can recrystallize or can be expulsed from a crystalline carrier.

Aim: The aim of this study was to molecularly design novel structures for SD. Thus, lipid microdomains were designed on an inorganic adsorbent in a polymeric carrier by HME. Unlike the traditional melt adsorption method, where the compound is stabilized through interactions with the adsorbent, a totally new approach was followed. By creating interactions between an adsorbent and an acidic lipid plasticizer, it could be possible to inhibit the lipid recrystallization. The obtained microdomains of non-crystalline lipid could then eventually accommodate a poorly water-soluble compound.

Methods: Hydroxypropylcellulose (HPC; Klucel EF), stearic acid (SA), and Neusilin US2 were used as polymeric carrier, acidic plasticizer, and adsorbent, respectively. Premixes were prepared by mixing 10% (w/w) SA with different ratios of HPC and Neusilin. HME was performed at 160 °C. In a second step, β-carotene (BC, 3 % (w/w)) was employed as poorly-water soluble model compound. SA and BC physical state was assessed by XRPD and AFM. Interactions between SA and Neusilin were studied by ATR-FTIR and temperature variable ATR-FTIR.

Results: XRPD and AFM analyses demonstrated a decrease in SA crystallinity with increasing amount of Neusilin. In the sample containing 70/10/20 % (w/w) HPC/SA/Neusilin, no SA crystallite could be detected with the two methods showing its complete amorphization. ATR-FTIR spectra exhibited clear vanishing and shifts in different SA vibrations bands with an increase in Neusilin concentration. The same observations were made in the spectra of molten SA. This indicated that SA did not recrystallize following HME and was essentially present as an oily phase. Amorphization of SA was most likely due to H-bonds and interactions with Al^{3+} and Mg^{2+} ions present on Neusilin surface. This was proven by bands shifts and appearance of a COO^- band in presence of the adsorbent. Finally, the extrudate containing 67/10/20/3 % (w/w) HPC/SA/Neusilin/BC was the only one that did not show any BC crystalline XRPD peak.

Conclusion: This study suggested that it was possible to inhibit SA recrystallization by creating ion/dipole interactions and H-bonds with Neusilin. Amorphous lipid microdomains were hence created by the incorporation of an appropriate Neusilin concentration. These microdomains enabled the amorphous dispersion of BC in the extrudate and thereby confirmed feasibility of the new approach to design amorphous SD.

Keywords: Hot-melt extrusion, adsorbent, lipid microdomains.

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P-21

Hydrogels in Three-Dimensional Cell Culture as a Scaffold to Mimic Human Subcutaneous Tissue

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Introduction: In 2014 the FDA approved 41 drugs - the highest number of approvals since 1996 - among which more than ¼ were biologics [1]. Therapeutic proteins are often injected subcutaneously, offering many advantages such as self-administration. However, this route of administration can lead to immunogenic injection site reactions even after weeks or months of well-tolerated treatment. This adverse effect is partly due to aggregation of the injected protein in the subcutaneous space.
Aims: In order to study the protein aggregation and immune response in the subcutaneous space, we aim to develop a 3D cell culture model incorporating immune cells such as dendritic cells. Importantly, hydrogels mimicking 3D extracellular matrix must exhibit viscoelastic properties comparable to subcutaneous tissue.
Methods: For this purpose, we first used as a cell model human lung carcinoma cell line A549 and cell proliferation in hydrogels was followed using the WST-1 assay at day 0, 1, 3 and 7. Hydrogels were selected based on their ability to form a viscoelastic gel under optimal cell culture conditions (37 °C) and their availability as a sterile and endotoxin-free product. Hydrogel cytocompatibility was assessed on agarose (Sigma-Aldrich®) at different concentrations (0.5, 0.35, 0.25 %), basement membrane extract containing laminin, collagen IV and proteoglycans (Geltrex® Matrix, Gibco® by Life Technologies™), and crosslinked hyaluronic acid (Fortelis® Extra, Anteis S.A.). Elastic Young's modulus in compression was determined on theses hydrogels using a TA-XT Texture Analyzer (Stables Micro Systems Ltd).
Results: All the tested gels revealed to have a good cytocompatibility. Geltrex® Matrix, Fortelis® Extra and agarose 0.5 % allowed only a slight proliferation (1.5 to 2.3-fold increase at day 3). In contrast, low-concentration agarose gels (0.35 and 0.25 %) allowed for increased proliferation – up to 4-fold at day 3. The decrease of mechanical constraint due to lower polymer concentration was shown for agarose, with the cell proliferation rate increasing inversely related to the agarose concentration. All 3 extracellular matrix equivalents provided adequate cytocompatibility and cell viability. Young's modulus measurements showed values comprised between 14 and 73 kPa. These values are close to or within the range previously reported [2] for human subcutaneous fatty tissue.
Conclusions: Today, 3D cell cultures are extensively developed in various research fields such as oncology because of their higher biological relevance compared to standard 2D cell culture. Screening with A549 cells gave us a good overview of the cytocompatibility of the selected hydrogels. Further work is now required on a more appropriate cell line [3] to design a 3D culture model closer to the *in vivo* reality and study the immunogenicity of therapeutic proteins.

Keywords: 3D cell culture, hydrogels, cell viability, Young's modulus.

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P-22

Influence of Excipients on Solvent-Mediated Hydrate Formation of Piroxicam Studied by Dynamic Imaging

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Introduction: Many pharmaceutical compounds are known to form hydrates during manufacturing or dissolution. Since the crystal structure of a hydrate can be quite different from the anhydrous form, there are several important solid-state characteristics affected such as for example solubility and dissolution rate [1]. Therefore, it is important to identify hydrate formation or polymorphism of drug candidates early on as part of a pharmaceutical salt- and polymorph screening. This work focuses on the compound piroxicam (PRX) that has been studied previously regarding different solid state forms [2]. Piroxicam anhydrate (PRXAH) has a substantially different crystal form compared to that of the hydrate which results in different colours of the two polymorphs.
Aims: The current work aims to clarify excipient effects as well as to elucidate the kinetics of hydrate conversion in simulated intestinal medium. A particular aim of this study was to use dynamic surface imaging and quantitative image analysis for improving a mechanistic understanding.
Methods: For microscopy studies, we prepared compacts with a diameter of 7 mm by compacting 110 mg of PRXAH. The compact was embedded in paraffin but one of the surfaces was left uncovered. These compacts were placed in a crystallization dish under a microscope and were covered with media. Pictures were recorded every 0.5 h for a period of 24 h. The obtained images were converted to black-and-white (binary) images.
Results: The conversation of PRXAH to PRXMH in water was exhibiting substantial variability. In the presence of the excipients Tween 80 and HPMC, the formation of PRXMH was completely inhibited. Probably this inhibition was based on excipient absorption on the surface of the compact. NaCMC decreased also the transformation but a small amount of hydrate (1 % of the surface) was still formed. The transformation of PRXAH in phosphate buffer was increased compared to pure water. Fasted state simulated intestinal fluid (FaSSIF) inhibited nucleation and solid-state transformation.
Conclusions: In this study it was shown that certain excipients are able to inhibit pseudopolymorphic transformation of PRX during a time period of 24 h. It was further shown that the start of nucleation and the transformation rate in pure water were rather variable, which was probably due to amplified noise factors such as for example variations in pH, impurities or ionic strength in water. With the analysis of dynamic imaging we found a reliable and fast tool to determine conversion kinetics.

Keywords: Piroxicam, hydrate formation, excipient effects, image analysis.

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P-23

Anthraquinones with Potent Quinone Reductase Inducing Activity from the Roots of *Morinda lucida* Benth

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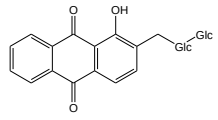
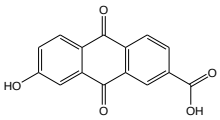
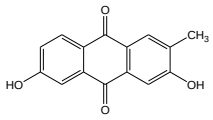
Introduction: *Morinda lucida* Benth (Rubiaceae) is a tree endemic to West Africa. The plant is used by the local population in the diet and as a traditional medicine. *Morinda sp.* are known to contain various anthraquinones, which were previously correlated to different biological activities such as anticancer [1]. Quinone reductase (QR) is a phase II enzyme involved in cancer chemoprevention. Its induction may prevent carcinogenesis by reducing electrophilic compounds [2]. Moreover, an elevation of other phase II enzymes such as glutathione S-transferase (GST) is closely correlated to QR induction making this enzyme a good biomarker [2].
Aims: Identification of anthraquinones with potent QR inducing activity from a methanolic extract of the roots of *Morinda lucida* Benth using bioassay-guided fractionation strategies.
Methods: Successive chromatographic methods such as RP C_{18} medium pressure liquid chromato-graphy (MPLC) were performed to isolate compounds from the methanolic extract of the roots of *Morinda lucida* Benth. Spectroscopic and spectrometric techniques were carried out to determine the structure of the compounds. Finally, their ability to induce QR was tested on 2 murine hepatoma cell lines; the wild-type Hepa 1c1c7 and c35, a mutant cell line defective in a functional Aryl hydrocarbon receptor (AhR).
Results: Activity-guided fractionation led to 4 anthraquinones already isolated in other species of *Morinda* and one anthraquinone never described before as natural compound (**2**). The QR inducing activity of compounds **1**, **2** and **5** in Hepa 1c1c7 cells (expressed as the concentration to double the activity (CD)) was in the micromolar range, while compounds **3** and **4** were active in nanomolar.



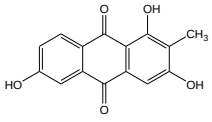
1
CD = 10.2 µM



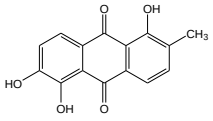
2
CD = 7.0 µM



3
CD = 0.29 µM



4
CD = 0.25 µM



5
CD = 21.6 µM

Conclusions: Anthraquinones can be regarded as good candidates for cancer chemoprevention due to their ability to induce QR activity in the nanomolar range and for their low toxicity. Moreover, *Morinda sp.* and other plants from the Rubiaceae family are rich in anthraquinones and part of the diet.

Keywords: *Morinda lucida*, quinone reductase, cancer chemoprevention, bioassay-guided fractionation, anthraquinones.

References:
[1] Brown AC. Phytother Res 2012; 26: 1427-40.
[2] Cuendet M et al. J Nat Prod 2006; 69: 460-3.

P-24

Compatibility and Stability of Levetiracetam in Commercial Parenteral Nutrition Admixtures

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Introduction: Antiepileptic drugs are frequently used in critically ill patients for seizure control or prevention, e.g. in brain trauma, in cancer or in neonates. Such patients often also require parenteral nutrition (PN). The admixing of levetiracetam, a frequently indicated antiepileptic drug to the PN is then often requested for convenience due to the lack of available i.v. lines. Because of the complex composition and the numerous potential interactions with the components of a PN admixture, such an admixture is not appropriate without physicochemical assessment and documentation [1]. The assessment of compatibility and stability of levetiracetam in this PN admixture in current practice conditions would provide a convenient and safe PN and drug treatment.
Aim: The aim of this study was to assess the physicochemical stability and compatibility of the frequently used levetiracetam in (commercialized) ready to use PN admixtures.
Methods: Levetiracetam Sandoz® concentrate for infusion (500 mg/5 mL, Sandoz Pharmaceuticals AG, Rotkreuz, Switzerland) was directly injected to a ready-to-use commercial PN admixture (Nutriflex® Lipid Special and Nutriflex® Omega Special, B Braun, Medical AG, Sempach, Switzerland). Stability tests were performed over seven days at +4 °C, room temperature +22°C and at +37°C (using a water bath) with an usual levetiracetam dose of about 20 mg/kg b.wt. The stability of lipid emulsion was observed by light microscope (BX51 Olympus, 15 visual fields/ sample were analysed with 1000x magnification and oil immersion) and by visual inspection (colour changes, creaming, precipitates). Moreover, the pH was determined and the concentrations of levetiracetam were checked by LC-MS/MS (Dionex Ultimate 3000 Thermo Scientific, AB Sciex QTRAP® 4500, determination of recovery in serum 82% and limit of determination 0.001 mg/mL) at: 0.4 mg/mL, 1.6 mg/mL and 4.8 mg/mL.
Results: Levetiracetam i.v. was shown compatible and stable over seven days in Nutriflex® lipid and omega special at 3 different temperatures. No changes in pH occurred over 7 days and no microscopic OOS or visual changes were observed. Except for the samples stored at +37°C, which showed yellowish discolorations. The mean value of the largest lipid droplet in each visual field (MLT_{max}) over 7 days was 2.4 ± 0.08 µm not different from the emulsion without the drug. No trend for an increase in the mean droplet size was seen when adding 10 mM of magnesium sulfate. The concentrations of levetiracetam at different temperatures were stable over 7 days and ranging 100 ± 15% of the theoretical value.
Conclusions: The present study investigated the compatibility and stability of 2 commercially available PN admixtures and levetiracetam i.v. up to 7 days in these PN admixtures and at usual handling/storage temperature. These results show a valuable approach how to determine stability/compatibility of drugs with PN in daily practice to support complex therapeutic schemes in the clinical setting. The demon-strated example of levetiracetam admixed to PN is important in patient therapy.

Keywords: Antiepileptic drug, parenteral nutrition, physicochemical stability and compatibility.

Reference:
[1] Thomson FC, Naysmith MR, Lindsay A. Hosp Pharm 2000; 7: 155-64.

P-25

Electromembrane Extraction : a New Technical Development

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Introduction: Electromembrane extraction (EME) is a relatively recent electro-assisted extraction method. EME consists in the migration of charged compounds under an electrical field, through a supported liquid membrane (SLM) impregnated on a microporous hollow-fiber. This method has already shown high potential for the extraction of low molecular weight basic compounds of medium polarity.
Aims: In this study, a new set-up of EME device was developed. The main attribute of this original device, directly inspired from microdialysis, relies in the continuous refreshment of the acceptor compartment, enabling higher preconcentration factors compared to the conventional static set-up, and good recoveries.
Methods: Standards solutions (50 ng/mL) of 15 neuropeptides were extracted with the new EME set-up. For the first time to our knowledge, a polypropylene hollow-fiber with a 50-µm wall thickness was used. Pressure and voltage were applied thanks to an Agilent Electrophoresis 7100 CE system. Several parameters such as the SLM composition (nature of the organic solvent and carrier), the voltage, the extraction time, and the flow rate of the acceptor phase were evaluated towards preconcentration factor and recovery. Extracts were analyzed by RP-UHPLC-MS/MS with SRM using an Agilent QqQ 6490 MS system. Preconcentration factors and recoveries were estimated by comparison with a standard solution injected in the same conditions.
Results: Although alcohols have been described the most suitable solvents for peptide extraction, the addition of decanone to a nonanol SLM (1:1, v/v) stabilized the extraction process and offered better results for a few peptides. The addition of DEHP as a carrier to the SLM was found to enhance the selectivity for polar peptides. The high electrical field (about 200/cm) significantly influenced the extraction process. High extraction times (up to 45 min) had no significant influence on the preconcen-tration factor, but greatly improved recovery (up to 70%). Decreasing the flow rate of the acceptor phase enabled a high preconcentration factor (up to 50-fold).
Conclusions: A polypropylene hollow-fiber with 50 µm wall thickness was used for the first time for electomembrane extraction. The original design of the set-up allowed for the renewal of the acceptor compartment and high preconcentration factors were obtained.

Keywords: Electromembrane extraction, peptide, UHPLC-MS/MS.

P-26

Use of Additives in Particle Engineering of Spray-dried Nanosuspensions

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Introduction: Particle engineering via spray drying is a very useful tool for tuning particles' size, distribution, shape, density, and cohesiveness. Following this concept, dry powders for inhalation comprising large porous particles can be prepared. This is especially useful for pulmonary delivery, where particle size and density of a formulation play major role in delivery efficacy [1]. However, to achieve large geometric size and low density, suitable spray drying additives have to be selected. Spray drying can be also employed to formulate composite nanoparticles-containing microparticles. Nano-particles themselves offer improved bioavailability and dose uniformity, and allow formulation of poorly water-soluble substances.
Aim: The aim of this study was to investigate the influence of 5 additives (ammonium carbonate, albumin, glycine, leucine, and trileucine) with density-control potential on aerodynamic behaviour of spray dried nanosuspension. Moreover, the particle size, morphology, and surface area were characterised.
Methods: Budesonide nanosuspension was prepared by wet media milling. Subsequently one of the selected additives was added to the feed and the whole mixture was spray dried to form composite microparticles. Aerodynamic behaviour was asses using the next generation impactor (NGI). NGI mimics the lungs and allows estimating the amount of powder deposited in the lungs by calculating the fine particle fraction (FPF).
Results: In our work, we prepared composite microparticles by spray drying a drug nanosuspension at 2 atomising gas settings. At lower atomising gas setting, the FPF values for all excipients except for tri-leucine ranged between 18.9 and 28.5%. Trileucine composite particles reached FPF of 53.0 ± 2.7%. Since higher atomising gas setting causes decrease of geometric particle size, even better results were reached this way. All powders except with ammonium carbonate had FPF above 55%, with trileucine having the highest FPF of 68.7 ± 2.0%. The highest surface area (~ 25 m^2/g) was formed when trileucine was co-spray dried at high atomising gas setting.
Conclusion: We investigated the influence of 5 additives, namely ammonium carbonate, albumin, glycine, leucine, and trileucine, on properties of spray dried nanosuspension. Under the same spray drying conditions, the particle size, surface area, morphology, as well as fine particle fraction of the powders varied with the additive used. Despite expectations, ammonium carbonate did not form porous particles and aerodynamically performed worst even at high spray gas setting. Irrespective of the spray gas setting, trileucine seemed to be the best additive in terms of FPF and surface area. However, if one would take into account also price of the substances, use of other additives, such as albumin or leucine, together with optimisation of spray drying conditions would seem as better choice.

Keywords: Spray drying, particle engineering, trileucine, pulmonary delivery, fine particle fraction.

Reference:
[1] Chow AHL et al. Pharm Res 2007; 24: 411-437.

P-27

New HDAC Inhibitors from Filamentous Fungi: Screening and Bioassay-Guided Fractionation

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Introduction: Fungi are known to produce secondary metabolites presenting a wide range of biological activities, one of them being the inhibition of histone deacetylases (HDACs). These enzymes are classified in 4 classes: classes I (HDAC1, 2, 3, 8), II (HDAC4, 5, 6, 7, 9, 10), III (SIRT1-7), and IV (HDAC11). They are involved in gene transcription and may play an important role in various pathologies, such as cancer and neurological diseases. Various HDAC inhibitors (HDACi) have been isolated from microorganisms (eg. trichostatin A, rhomidepsin). More specifically, several filamentous fungi have been shown to produce HDACi: cyclic tetrapeptide FR235222 (*Acremonium* sp.) [1], epicocconigrone A and epicoccolide B (*Epicoccum nigrum*) [2].
Aims: Identification of new HDAC inhibitors from filamentous fungi and comparison of 2 detection methods in an enzyme-based assay.
Methods: In order to isolate and identify new HDAC inhibitors, a screening of 30 crude ethylacetate (EtOAc) extracts of filamentous fungi was performed using an enzyme-based assay with a fluorescence detection method. The most active extract was fractionated on a preparative flash chromatography. The HDAC inhibitory activity of fractions collected was tested using the assay with 2 detection methods: fluorescence and mass spectrometry (MS). The most active fraction was purified on a preparative chromatography system. Natural products were finally characterized by NMR analysis.
Results: The most active candidate selected during the screening was a species of *Penicillium*, with an HDAC inhibitory activity of 86% at 100 µg x mL^{-1}. After fractionation, 5 fractions presented an HDAC inhibition above 80% at 100 µg x mL^{-1} by using a fluorescence detection method, whereas only 2 fractions presented an HDAC inhibition above 65% at 100 µg x mL^{-1} when a MS detection method was used. The different percentages oberved between the 2 detection methods may be explained by the presence of intrinsic fluorescence and/or trypsin inhibitors in fractions. The compounds isolated from the most active fraction were identified as salirepol (IC_{50} = 50.3 µM) and patulin (IC_{50} > 100 µM).
Conclusion: The fractionation of *Penicillium* sp. crude AcOEt extract allowed the isolation of a bioactive compound, salirepol. Moreover**,** the comparison of both detection methods to measure HDAC inhibition showed that the MS detection method is particularly appropriate for screening natural products since quenching due to fluorescent molecules and/or trypsine inhibitors could be avoided.

Keywords: Screening, bioassay-guided fractionation, filamentous fungi strains, HDAC inhibition.

References :
[1] Mori H et al. J Ant 2003; 56: 72-79.
[2] Amrami EL et al. J Nat Prod 2014; 71: 49-56.

P-28

Polyamine-Conjugation to RNA Looptomirs for Enhanced Target Binding Affinity

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Introduction: Therapeutic oligonucleotides have a tremendous potential in medicine. However, their development as drug molecules faces major technical obstacles such as poor cell penetration, sensitivity to nucleases and low affinity for their targets. Shortmers (antisense oligonucleotides <15 nt) are a promising approach towards oligonucleotides with more drug-like properties, requiring however chemical modification to maintain target binding affinity, and knowledge of ideal binding sites.
Aims: We have previously shown that 2'-OMe 13mer and 14mer looptomirs targeting pre-let-7a-2 restore synthesis of mature let-7, a tumor suppressing miRNA in many human cancers, by selectively blocking oncogenic Lin28 binding [1]. Here, we present the design of optimized shorter looptomirs carrying polyamine conjugates. Polyamines are known to preferably interact with RNA and stabilize duplex structures. Due to their positive charge at physiologic pH, conjugation to oligonucleotides creates zwitterionic molecules with favorable properties in terms of cellular penetration and hybridization affinity to their target RNA.
Methods: Polyamine-oligonucleotide conjugates were prepared in both a pre- and a post-synthetic chemical approach using copper-catalyzed Huisgen cycloaddition (CuAAC), conferring considerable versatility with respect to the ribose moiety (DNA, RNA, 2'-OMe or 2'-MOE) and compatibility with insertion of phosphorothioates or functional labels such as fluorescent dyes. Melting temperature to complementary RNA and hybridization thermodynamics were assessed using UV absorbance; overall duplex conformation was investigated using circular dichroism (CD). Binding affinities (K_D) and kinetics (k_a, k_d) of short looptomirs to a miRNA hairpin were determined by surface plasmon resonance (SPR).
Results: 7mer, 9mer, 10mer and 13mer polyamine-conjugated looptomirs targeting pre-let-7a-2 were synthesized. High increases in thermal stability to complementary RNA were observed upon polyamine insertion, while CD studies confirmed that overall duplex geometry was not altered. In a 10mer looptomir, single modifications were found to enhance binding affinity to the target pre-miRNA hairpin about 10-fold, bis-modification about 100-fold, owing to both accelerated association and decelerated dissociation rates. Ongoing studies focus on investigating the performance of polyamine-modified short looptomirs in dedicated cellular assays.
Conclusions: Polyamine-conjugation to looptomirs is a promising approach towards high activity shortmers with improved pharmacokinetic and pharmacodynamic properties.

Keywords: Antisense, looptomir, shortmer, let-7, SPR.

Reference:
[1] Roos M et al. Nucleic Acids Res 2015; 43: e9

P-29

How to Predict Cytochrome Metabolism by QM Atomic Charge-Based Descriptors

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Introduction: Drug metabolism is a complex process that involves diverse enzyme catalyzed chemical transformations. Drug discovery requires experimental characterization of both the metabolites and the metabolic processes involved, which is impractical at large scale. Computational tools offer an inexpensive complementary strategy to predict the sites in a molecular structure that are liable to metabolic transformation. These models assist medicinal chemists already in the early stages of the drug development process [1]. We present a strategy for predicting the sites of metabolism (SoM) of cytochrome-catalyzed reactions. The approach is based on atomic partial charges calculated with quantum chemistry (QC) methods. Partial charges are used as building blocks for atom descriptors that encode the molecular charge distribution. With help of these descriptors, machine-learning models are trained on a metabolic reaction database.
Aims: Provide proof-of-concept for QC derived molecular representations for SoM prediction.
Methods: We employed QC software packages for calculating atomic partial charges. The workflow and descriptor creation are implemented in the Python programming language. Support Vector Machines and Random Forest models were trained on a cytochrome metabolism database taken from the literature that comprises 680 molecules.
Results: Certain partial charge schemes yield partial charges that are nearly conformation-independent, which allows for a one-conformer approach. We obtained machine-learning models with good predictive performance based on these partial charge schemes. Combination with other established descriptors led to robust and accurate models.
Conclusions: Atomic partial charges represent the molecular charge distribution and thus offer valuable information for modeling reactivity problems, such as cytochrome-mediated metabolism.

Keywords: Metabolism, molecular modeling, machine learning, quantum chemistry.

Reference:
[1] Kirchmair M et al. Nat Rev Drug Discov 2015; 14: 387-404.

P-30

Plant Extract Screening to Discover New Compounds against *Chlamydiales*

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Introduction: *Chlamydiales* are strict intracellular bacterial pathogens responsible for many infections in humans and animals. As (i) the incidence of *Chlamydia* infection in the world's population is still increasing and (ii) antibiotic resistance rises rapidly, it is essential to identify new drugs that target the chlamydial developmental (intracellular replication) cycle.
Aims: To achieve this goal we developed and optimized a screening method to identify new molecules, mainly produced by plants, that are active against transcriptional factors (TFs) from the chlamydial pathogen *Waddlia chondrophila*, thought to be involved in the developmental cycle.
Methods: For this purpose, we developed and optimized a screening method using the *Chlamydial* specific TF EUO to set up a screening method based on a transcriptional interference assay in the heterologous host *Escherichia coli*. Therefore, the *euo* gene was cloned in two different inducible plasmids and its expression was verified by immunoblotting. *LacZ*, bioluminescence (LuxAB) and the kanamycin resistance protein NptII were evaluated as transcriptional reporters for EUO activity. The most suitable for the final screening in a 96 well-plate was transformed together with the plasmid showing the best expression of EUO in *E. coli*.
Results: From the 3 reporter plasmids tested, the *lacZ* reporter system was selected, due to its ease in kinetic studies and quantification. The screening method with a good Z-factor was chosen for the measurements and the standard Miller assay protocol was adapted to 96 well plates. About 180 plant extracts from the Swiss Alps were tested for their potential inhibitory effects. Results showed that the method developed here is suitable for high-throughput screening.
Conclusions: Further molecules such as pure compounds will be tested. Screening methods for the 9 other selected TFs from *Waddlia chondrophila*, which are conserved in other species of the *Chlamydiae* phylum and likely involved in their development, will be established. Overall, the approach presented here shows a new method to study genetically intractable microbes.

Keywords: *Chlamydia, Waddlia chondrophila,* transcription factors, reporter system.

P-31

Targeting Furin: Selective Liposomal Drug Delivery to Rhabdomyosarcoma

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Introduction: Childhood sarcomas account for about 15% of pediatric cancers with rhabdomyosarcoma (RMS) being the most common soft tissue sarcoma in children. Although current treatments (*i.e.* surgery, chemotherapy) can achieve good responses, late side effects represent a heavy burden for cancer survivors and are a major complication in pediatric oncology. To this end, a cyclic peptide (RMS-P3) with strong affinity for RMS *in vitro* and *in vivo* has been identified to selectively deliver drugs to the tumor [1].
Aims: There is a need to find more effective and less toxic therapies for pediatric sarcomas. In the present work, we aim to improve the efficacy of existing chemotherapies against RMS and to minimize long-lasting side effects by increasing drug localization at the tumor site with a long-circulating anticancer liposomal formulation, decorated with the RMS-specific peptide. Such vesicles will selectively recognize furin, a proprotein convertase, overexpressed on the surface of RMS.
Methods: The original sequence of the RMS-specific peptide was modified in order to improve its water solubility. The binding of RMS-P3 to furin was validated by surface plasmon resonance (SPR), and the effect of the targeting peptides on the activity of the enzyme was investigated by incubating human recombinant furin with the fluorogenic substrate peptide Boc-RVRR-AMC (7-amino-4-methylcoumarin) [2]. To generate the targeted vesicles, RMS-P3 and a NHS ester of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[carboxyPEG-2000] (DSPE-PEG-NHS) were dissolved in anhydrous DMSO or phosphate buffered saline at different molar ratios and stirred for 24 h at room temperature. The coupling reaction was verified by matrix-assisted laser desorption/ionization and quantified by high-performance liquid chromatography. Vincristine (VCR), one of the drugs for first line RMS treatment, was chosen in this study as therapeutic agent and was encapsulated into 130-nm liposomes following a trans-membrane pH-gradient procedure.
Results: Both, the original and the modified RMS-specific peptides successfully inhibited the cleavage of the fluorogenic substrate by furin. SPR confirmed the binding of RMS-P3 to furin with an estimated K_d of 1 µM. The best coupling conditions were found to take place in DMSO with a 5 times excess of DSPE-PEG-NHS *vs*. peptide. The VCR was encapsulated with an entrapment efficiency of 90% for both targeted and non-targeted liposomes and a release of 20% of the drug from the peptide-decorated vesicles in the presence of 100% serum within 24 h showed a slow leakage of VCR from the liposomes.
Conclusions: These liposomal vesicles are a promising step toward development of safer and more efficient therapies against RMS. The selective binding of the RMS-specific liposomes will then be tested *in vitro* on RMS cell lines overexpressing furin and later on *in vivo* in RMS-bearing mice.

Keywords: Rhabdomyosarcoma, tumor cell targeting, PEG-coated liposome, furin, vincristine.

References:
[1] Hajdin K. PLoS One 2010; 5: e10445.
[2] Anderson ED. EMBO J 1997; 16: 1508–1518.

P-32

Single Particle Aerosol Mass Spectrometry (SPAMS 3.0)

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Introduction: To date, the Next Generation Impactor (NGI, Copley Scientific) is considered by regulatory bodies as the most suitable cascade impactor test for pressurized metered dose inhaler (pMDI) and dry powder inhaler (DPI) analysis. However, the NGI technique does not give any information regarding interactions among the active pharmaceutical ingredients (APIs) and excipients in the formulation on the level of the individual particles. The NGI cannot reveal if and what co-associations exist between an API and excipient or between multiple APIs [1]. Particle co-association is one postulated mechanism for a possible increased *in vitro* pharmaceutical performance of inhalation drugs [2]. Single particle aerosol mass spectrometry (SPAMS) is a novel analytical technique where the individual aerodynamic diameters as well as the chemical composition of individual aerosol particles can be determined in real-time [1].
Aims: Single Particle Aerosol Mass Spectrometry was used to determine individual aerodynamic diameters and chemical compositions of individual aerosol particles in real-time for inhalation drug products.
Methods: A Livermore Instruments Inc. SPAMS 3.0 was used to conduct the experiments. Aerosol particles are drawn into the SPAMS through an aerodynamic focus lens stack. Particles are pushed into a collimated beam which guides them via air stream through a scattering laser field and then directs them to a time-of-flight mass spectrometer, where individual particles after desorption and ionization are analyzed, both positive and negative ions simultaneously [3].
A size calibration curve was generated by analyzing Polystyrene and SiO_2 calibrant particles (both from Thermo Fisher Scientific Corporation, Fair Lawn, NJ) aerosolized using a disposable nebulizer. The aerosolized particles were dried using a diffusion dryer before being analyzed by the SPAMS 3.0. The Seretide® pMDIs were actuated through an adapter into a USP throat fitted to a 4-L reservoir, which was fitted to the SPAMS inlet. The actuation of the pMDIs was coordinated with a simultaneous 5-sec draw of air into the reservoir at a rate of 30 L/min through a side port. By comparison of mass spectrometric data, unique marker peaks were found for identifying each individual compound.
Results: The individually sized particles generated from the analysis of a Seretide® pMDI (contains fluticasone propionate and salmeterol xinafoate) were assembled into an aerodynamic particle size distribution (APSD) histogram of counts *vs.* size bins that mimic the size cutoffs of NGI stages. The APSD plots illustrate the ability of SPAMS to discern the APSD of actives, either individually or in combination, on a particle by particle basis.
Conclusions: The SPAMS 3.0 demonstrated its capability to analyze a wide range of pMDI formulations with accurate aerodynamic particle sizing, chemically specific for each API in real-time. SPAMS allows the determination of particle-particle co-association in addition to providing aerodynamic particle size information that can be generated with NGI.

Keywords: Inhalation, mass spectrometry, aerosol, single particle, cascade impactor.

References:
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[2] Haghi M et al. Respirology 2013; 18: 1197-1201.
[3] Fergenson DP et al. Resp Drug Deliv Eur 2013; 0: 3-6.

P-33

Normalization Strategies for Metabolomics Analysis of Urine Samples by UHPLC-QTOF-MS

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Introduction: Metabolomics attends to analyse the most important number of metabolites (mass < 1000 Da) in a biological system. This approach constitutes a potent method for assessing phenotype modifications caused by disease or environmental influences. Thanks to its non-invasive collection and its availability in large quantities, urine represents a major biological matrix. However, one important issue remains the variability of urine concentration, which implies data normalization.
Aims: The aim of this study was to evaluate various normalization strategies in the context of metabo-lomics analysis of urine samples by UHPLC-QTOF-MS.
Methods: Samples from 8 people (4 women, 4 men) at 3 time points were collected. Each sample and quality controls (QCs) were analyzed by UHPLC-QTOF-MS in negative and positive ESI mode. XCMS was used for data preprocessing and SIMCA 14 (Umetrics) for multivariate data analysis with Pareto scaling. Two data treatments, MS Total Useful Signal (MSTUS) and Probabilistic Quotient Normalization (PQN), were investigated as data normalization strategies. Creatinine concentration, osmolality and NMR measurements were used to estimate the sample concentration and calculate dilution factors.
Results: The major sources of variability observed in the data set were attributed from the analytical drift and the sample concentration. Various strategies were evaluated to circumvent both issues. MSTUS and PQN provided the best normalization strategies and reveal the intra- and inter-individual variability. The interpretation of normalized data by these pre-treatments offered complementary points of view. With data treatments, the analytical drift was corrected but not practically removed. Multiple injections of urine contaminate the mass spectrometer and decrease the intensity of the signal during the sequence. The analytical drift is related to both the number and the concentration of samples. To remove this analytical drift, creatinine concentration, osmolality and NMR measurements were used to estimate the sample concentration and calculate appropriate dilution factors before injections. Fixing a range of concentrations during the sample preparation removed the major part of the drift and improved the analytical condition and data acquisition before the normalization by MSTUS or PQN.
Conclusions: The best results were obtained by a sequential strategy, which consists to dilute all the samples to reach a common concentration before data normalization.

Keywords: Metabolomics, urine, normalization, analytical drift.

P-34

Effect of Silk Fibroin Purification Process on the Performance of Silk-Based Drug Delivery Systems

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Introduction: Silk is a natural biomaterial produced by silkworms (*Bombyx mori*), consisting of 2 proteins, fibroin and sericin, and has excellent properties for drug delivery. Sericin forms a glue-like envelope around the fibroin fibers. Silk fibroin (SF) itself consists of a heavy (ca. 350 kDa) and a light (ca. 25 kDa) chain which are connected by a disulfide bond [1]. During preparation of SF, silk is treated with alkali at elevated temperatures to remove sericin (degumming), potentially affecting SF integrity and molecular weight. We therefore hypothesize that the purification process conditions might influence performance of SF based drug delivery systems.
Aim: The aim of this study was to investigate the effects of different degumming conditions on drug delivery systems based on SF.
Methods: SF solutions were prepared as described previously [2]. Dried cocoons were boiled for different timeframes (5 min, 30 min, 1 h, and 2 h) in 0.02 M sodium carbonate. SF was washed several times with ultrapure water and dried overnight. After drying SF was dissolved either in 9 M lithium bromide or in Ajisawa's reagent (calcium chloride:ethanol:water in a molar ratio of 1:2:8) at 65°C. The samples were dialyzed against ultrapure water for 48 h. SDS PAGE was performed using a 12% gel according to the procedure of Laemmli [3]. SF films were produced by mixing 6.5% SF solution with the model drug solutions. The SF-drug solutions were dried overnight in 24-well-plates at room temperature. The films were left either untreated or treated with methanol (70%) or water vapor to induce β-sheet formation. For the drug release studies phosphate buffered saline was added and incubated at 37°C for one week.
Results: Degumming time had a pronounced effect on SF integrity. After 5 min degumming, 2 bands (25 kDa and 350 kDa) were observed, with slight degradation visible in the 350 kDa band. After 2 h of degumming, less protein with higher molecular mass remained and more proteins with smaller molecular mass were found. Analyzing the sericin release (amide bonds) during processing of the cocoons in boiling 0.02 M sodium carbonate for 2 h revealed that the majority of sericin is already removed within min. These changes of SF structure resulted in differences in the release of model drugs, underlining the importance of SF purification conditions.
Conclusions: SF is a promising natural biomaterial for controlled drug delivery. Prolonged degumming caused SF degradation resulting in faster drug release compared to less degraded SF chains. To sum it up, the drug release can be controlled by varying the processing methods of SF.

Keywords: Silk fibroin, drug delivery, biodegradable films, biomaterial.

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P-35

Medicinal Plants Traditionally Used in Senegal for Tuberculosis Treatment: Are They Active Against *Mycobacterium* Strains?

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Introduction: In West Africa, populations are used to take traditional medicine as a first aid against common health problems. In this aspect many plants are claimed to be used to treat Tuberculosis (TB) which remains one of the world's deadliest communicable diseases according to WHO [1].
Aims: The aim of my work is to study medicinal plants against TB and their possible interactions with manufactured drugs.
Methods: An ethnopharmacological survey conducted on 120 TB-patients and 30 healers was performed in Senegal from March to May 2014. This approach allowed the selection of 12 plants used in TB treatment. The selected plants were collected and extracted by a sequence of increasing polarity solvents and also by the traditional way (decoction). The extracts were screened for their antimycobacterial activity, using a validated host-pathogen assay model based on an antibiotic test against *Mycobacterium marinum* and an anti-infectious test on *Acanthamoeba castellani* infected by *M. marinum* [2].
Results: Twenty different extracts were screened with this model; two of them showing good activities (*Combretum aculeatum* and *Guiera senegalensis*) compared to the positive control drugs (rifampicin and ethionamide). The active extracts were analyzed by LC-PDA-MS and UPLC-HRMS for dereplication [3]. Microfractionation coupled to the assay enabled the identification of active fractions and the final purification of the active principles is currently under way. The approach combining bioguided isolation and UHPLC-TOF-MS analysis allows to the efficient dereplication of α- and β-anomers of Punicalagin present in *C. aculeatum*
Conclusions: In this study it was possible to demonstrate the *in vitro* and *in vivo* anti-*Mycobacterium* activity of the aqueous extracts of two medicinal plants used in Senegal against TB: *Combretum aculeatum* (Combretaceae) and *Guiera senegalensis* (Combretaceae). One active compound, punicalagin, was highlighted on *C. aculeatum*. It is an ellagitannin also found in *Terminalia chebula and Punica granatum.* This compound has been already described as active against many *Mycobacterium* strains. Bioguided isolation for the identification of other active compounds present in these extracts is under way. Since plants are sometimes used also with anti-TB drugs, possible interaction may occur. Studies on plants extracts ability to modulate the activity of different key cytochrome enzymes are planned.

Keywords: Tuberculosis, ethnopharmacology, Senegal, Combretaceae, host-pathogen assay.

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P-36

Delivery of Liposomes to Cells via Microinjection

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Introduction: Microinjection is a powerful cell manipulation technique, so far mostly used in biological sciences to study the function of macromolecules that are unable to cross the plasma membrane of living cells [1]. Compared to common *in vitro* incubation methods, microinjected materials can bypass the endosomal pathway, thereby avoiding final breakdown in the lysosomes. By employing microinjection in the field of drug delivery, it may become possible to modulate the characteristics of cells and use them as vehicles for drugs and enzymes.
Aims: In this study, liposomes were microinjected in HeLa cells, providing them with external self-contained vesicles, which may carry biologically active therapeutics. The main focus laid on investigating the stability and the toxicity of the injected lipid vesicles within the cell.
Methods: Four different liposome formulations were studied for their intracellular stability as well as their potential cytotoxicity. The formulations tested differed in terms of transition temperature of the main lipid and the presence of poly(ethylene glycol) (PEG) chains at the liposomal surface. The intracellular stability of the formulations was monitored over time after injection using a fluorescence dequenching assay with the dye/quencher pair pyranine/p-xylene-bis-pyridinium bromide. The integrity of the intra-cellular lipid vesicles containing the dye/quencher pair was verified by exposing the cells to Triton X-100, which causes the rupture of the liposomal membrane, and a corresponding increase in fluorescence intensity. The cytotoxicity of the injected formulations was evaluated employing a propidium iodide exclusion assay.
Results: The PEGylated formulation composed of lipids with a high transition temperature (41°C) was the most stable, remaining intact even after two days within the cell. Interestingly, all liposome formu-lations appeared to be well tolerated by the cells, since the mortality did not exceed that of the buffer injected cells.
Conclusion: The microinjection of liposomes is shown to be a promising, non-toxic, easy-to-perform method to provide cells with new organelle-like entities, which, depending on the formulation, may show sufficient stability to be used in drug delivery applications.

Keywords: Microinjection, liposome, toxicity, stability.

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P-37

Content of Free Anthraquinone Aglyca in Anthranoid-Containing Herbal Laxatives of the European Pharmacopoeia (Ph. Eur.)

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Introduction: The monographs of the European Pharmacopoeia for laxative herbal drugs undergo a modernization for the determination of the hydroxyanthracene glycosides to HPLC techniques whereby a determination/limitation of aglyca is actually discussed for Sennae folium and fructus, due to the postulated mutagenic and genotoxic effects of the aglyca, especially aloe-emodin [1, 2]. With regard to a possible limitation for aglyca, the determination of their content in the laxative herbal drugs would be reasonable. However, there are up to now no reliable and comparable data available yet for the content of free aglyca.
Aims: The aim of the this study was to provide reliable, comparable analytical data on the distribution and content of the aglyca aloe-emodin, chrysophanol, emodin, rhein, and physcion in different batches of the laxative herbal drugs *Aloe capensis*, Frangulae cortex, Rhei radix, Rhamni purshiani cortex and Sennae folium and fructus measured by UHPLC.
Methods: The drugs were extracted with acetonitrile/$NaHCO_3$ aq. and diluted with acidified water. An ACQUITY HSS T3 column was used as stationary phase. The mobile phase consists of acidified water and acetonitrile/methanol in a gradient. The detection was at 435 nm.
Results and Discussion: The Rhei radix sample demonstrates the presence of all reference aglyca. The separation is shown in Fig. 1. Results of several samples in regard to the variations and the quantitative content are presented in detail on the poster.

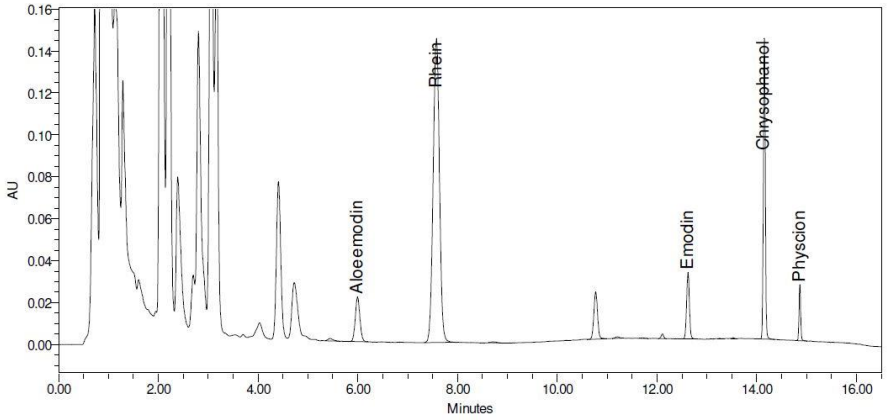

Fig. 1: UHPL chromatogram from a Rhei radix sample

With respect to a possible limitation of aloe-emodin, the herbal drugs of Sennae showed the lowest content of about 1% whereas Rhei radix contains a much higher amount of around 5%.
Conclusions: The presented method is simple, precise and a helpful tool to support the discussion, if the analyses of aglyca in anthranoid-containing laxatives will be important to regulate.

Keywords: Aloe-emodin, anthranoid aglyca, herbal laxatives, HPLC.

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P-38

Anti-Angiogenic Strategies for Chemoembolization of Liver Tumors

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Introduction: Angiogenesis inhibition is an evolving concept in the management of hypervascular solid tumors, such as hepatocellular carcinoma of the liver. Patients bearing this type of tumor can be treated by injection of hydrogel microbeads loaded with anti-angiogenic drugs – a treatment called Transarterial Chemoembolization (TACE). The injected embolic beads block the tumor vessels, starve the tumor and at the same time, inhibit tumor neoangiogenesis.
Aims: In this study, embolic beads loaded with the multi-tyrosine kinase inhibitor sunitinib [1] were evaluated in a rabbit tumor model with regard to local drug delivery and successive drug diffusion into the tissue.
Methods: Adult New Zealand white rabbits were implanted with VX2 tumors in the left liver lobe. Two different routes of administration of sunitinib were compared: 5 animals were injected sunitinib-eluting DC Beads® (1.5 mg sunitinib) into the artery feeding the tumor, and 7 animals received 6 mg of sunitinib p.o. daily over 14 days. Sunitinib concentrations in the tumor and in the healthy liver tissue were analyzed by LC-MS/MS [2] and drug fluorescence over 14 days. Sunitinib tissue biodistribution was visualized by sunitinib fluorescence in cryosections with an Axio Z1 Imager (Carl Zeiss, Feldbach, Switzerland), using a GFP filter for fluorescence, and quantified with ImageJ 1.49t software (NIH, Bethesda, Maryland, US).
Results: Both at early and late time points after the transarterial treatment, presence of sunitinib-eluting microbeads resulted in high intra-tumoral sunitinib levels, despite a lower total dose compared to peroral administration. In addition, the drug was more locally delivered to the tumor by administration via embolization beads than by the peroral route: After 12-14 days, sunitinib levels in the healthy liver were 150 times lower after administration via drug-eluting beads. Images of tumor and liver tissue sections showed sunitinib, which had diffused locally from beads after 1 day and farther at later time points. Interestingly, the drug diffusion seemed to be more sustained in the tumor than in the liver over 14 days, resulting in strong sunitinib fluorescence still at later time points.
Conclusions: To summarize, sunitinib combined with DC Beads® allowed for local drug delivery to liver tumor. Sunitinib is a potential drug candidate for drug-eluting bead transarterial chemoembolization of hepatocellular carcinoma.

Keywords: Drug-eluting beads, sunitinib, transarterial chemoembolization, hepatocellular carcinoma, fluorescence imaging.

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P-39

Aptamer as Targeting Ligand for the Detection of Prostate-Specific Membrane Antigen (PSMA)-Positive Cells

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Introduction: Nucleotide aptamers are short segments of DNA or RNA, which bind to a specific target molecule. Two of their major advantages are a smaller size compared to antibodies, which allows faster target recognition, and higher binding affinity compared to small molecules [1]. These properties make them interesting as specific targeting ligands for nanoparticle constructs.
Aim: The overall aim of this project is to develop prostate-specific membrane antigen (PSMA)-targeted superparamagnetic iron oxide nanoparticles (SPIONs) for detection of early PSMA-positive metastases in the lymph nodes. In this view, a PSMA targeting RNA aptamer [2] is coupled to coated SPIONs. Since RNA-based aptamers are very rapidly degraded by nucleases, notably RNases, they have to be modified in order to make them suitable for therapeutic application [1]. Additionally, a spacer is added to avoid sterical hindrance of the binding due to neighbouring coating molecules. In this study we examine the ability of the aptamer to still bind to its target receptor and to be internalized despite chemical modifications and the addition of the spacer.
Methods: A fluorophore was covalently linked to the spacer of the aptamer. PSMA-positive LNCaP cells and PSMA-negative PC3 cells as negative control were incubated with the fluorescently tagged aptamer. A third cell type of unknown PSMA expression, but of high interest for *in vivo* tests, was also incubated. As control, the same experiment was conducted using a PSMA binding small molecule [3], coupled to a different fluorophore. Binding and internalization were investigated for all 3 cell lines by confocal laser scanning microscopy imaging while keeping the cells alive, incubated at 37°C.
Results: The aptamer was successfully linked to the fluorophore by click chemistry. Confocal images show binding and rapid internalization of the aptamer comparable to the small molecule for LNCaP cells, while PC3 cells reveal no or very little internalization. For the cell line with unknown PSMA expression, internalization of the aptamer and small molecule was shown.
Conclusions: The modified aptamer, linked to a spacer, is suitable for specific targeting of PSMA positive cells. Additionally, while having a first hint of a possible expression of PSMA for the cell line of interest for *in vivo* testing, its expression will be confirmed at the gene and protein level.

Keywords: Aptamer, PSMA, internalization, LNCaP, PC3.

References:
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P-40

An Innovative and Revolutionary Mill for Pharmaceutical Research and Development

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Introduction: Frewitt has been developing and manufacturing premium-quality mills in Switzerland since 1946, mills that are critical components in the crushing and grinding processes of our renowned clientele in the pharmaceutical, chemical and food product industries. Thanks to our forward-looking orientation to the requirements of our customers, Frewitt is an expert partner for high-tech sieving, homogenization, and deagglomeration solutions for powders and granulates.
Aims: We are presenting the FREDRIVE-LAB, an innovative technology combining multiple milling system with 5 different milling processes on a single, milling platform dedicated to the pharmaceutical, fine chemical, cosmetically and food products industries.
Methods: Owing to a design enabling the use of up to 5 different interchangeable milling heads on the same base module, FREDRIVE-LAB permits conical sieve milling, hammer milling, cylindrical sieve mill, oscillating mill and pin-mill – all with a sole compact platform. The ConiWitt-Lab is a high-performance, high-quality conical sieve mill that is used for deagglomerating and sizing granular. The HammerWitt-Lab is a hammer mill that guarantees optimum milling results in the fine milling and pulverization of hard, crystalline, and fibrous products to fineness of up to 30 µm. The TurboWitt-Lab is a cylindrical sieve mill. Having a rotating sieve drum, it is well-suited for homogenizing and control-sieving granular products to a fineness down to 150 µm. The OscilloWitt-Lab is an oscillating mill used for the gentle milling and milling aiming to achieve uniform particles size up to 250 µm. The PinMill-Lab is a high power Pin-Mill for pulverizing hard, crystalline products from 2 mm to 10 µm.
Results: A pharmaceutical crystal has been milled with different milling heads. HammerWitt-Lab shows D50 21 µm and D90 95 µm. At same peripheral rotor speed, PinMill-Lab produces finer particles than HammerWitt-Lab. Higher the efficient energy applied on basic product is, finer the milled particles are.
Conclusions: The FREDRIVE-LAB has following features and functions:

- Worldwide Innovation (Patent Pending)
- Specifically engineered for drug development
- 5 different milling process integrated into 1 unique lab equipment
- Processing from 50 g batch to 30 kg/h (depending on product processed)
- Particle size down to D90 < 20 µm
- Fully scalability to pilot line, as well as production equipment FreDrive.

Keywords: Pharmaceuticals, particles, milling, process, particle size & distribution.

P-41

Designing Membrane-Interacting Peptides by Simulated Molecular Evolution

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Introduction: We compared two classes of membrane-interacting peptides: antimicrobial peptides (AMPs), which lead to disruption of membrane integrity, and mitochondrial targeting peptides (mTPs) that typically lack membrane lytic activity. Both peptide classes share common features, specifically an overall positive net charge, amphiphilic character and helical secondary structure elements [1].
Aims: We developed a computational approach for investigating the relationship between AMPs and mTPs to better understand their mutual and discriminating features, and provide a basis for the *de novo* design of synthetic membrane-interacting peptides [2].
Methods: We implemented a directed evolutionary algorithm to convert ("morph") the membrane lytic AMP protonectin into the non-disruptive membrane-targeting peptide (mTP) of mitochondrial hydroxyacylglutathione hydrolase. The mTP served as attractor point for evolutionary searching in sequence space. We designed and synthesized 26 peptides, which we tested for membrane lytic activity against four different lipid vesicle types and measured their capacity to inhibit the growth of *Staphylo-coccus aureus.* In order to identify the peptides' inducible alpha helical content we performed circular dichroism (CD) spectroscopy.
Results: We demonstrate successful morphing of the AMP into the mTP, whereby membranolytic activity non-linearly decreased with increasing distance from the start to the end peptide. Bacterial growth inhibition experiments confirmed the applicability of the directed evolutionary morphing concept to designing membrane-interacting peptides. Moreover we show that bacteriostatic activity correlates with the inducibility of the peptides' alpha helicity.
Conclusion: The results obtained in this study corroborate the directed evolutionary peptide design approach by computational peptide morphing. The study sheds light on the activity landscape of membrane active peptides and enables their future rational design.

Keywords: Peptide design, antimicrobial peptide, mitochondrial targeting peptide, evolutionary algorithm, lipid membrane.

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P-42

Quantification of Antimirs in RISC by Chemical-Ligation RT-qPCR

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Introduction: The ability to detect and quantify biomacromolecules is one of the major achievements of modern bioanalytical assays. Therapeutic oligonucleotides are often heavily modified in order to improve their pharmacokinetic and pharmacodynamic properties such as affinity, specificity and/or nuclease resistance. However, the degree of chemical modification presents analytical challenges and limitations. Indeed, chemically modified oligonucleotides are generally not detectable by conventional enzyme-based methods such as reverse-transcriptase quantitative PCR (RT-qPCR). Thus, as part of our program to develop new superior classes of chemically modified antimirs (microRNA-targeting complementary oligonucleotides), we implemented a recently developed chemical-ligation RT-qPCR (CL-RT-qPCR) method [1] to quantify intracellular amounts of chemically modified miR-122 antimirs after transfection, and to determine their delivery into the RISC (RNA-induced silencing complex).
Aims: With our novel CL-RT-qPCR method we intended to quantify antimir presence in RISC and show if it correlates with antimir activity in biological assay.
Methods: In the CL-RT-qPCR method, the antimir serves as a template to mediate coupling ligation of two DNA ligator sequences. Upon hybridization to the antimir, the ligation reaction occurs leaving a DNA ligation product which is subsequently quantified by RT-qPCR. In the absence of template (antimir) no ligation and hence no RT-qPCR takes place. Transfected antimirs were quantified in lysates and after immunoprecipitation of AGO2 protein, the principal effector protein in the RISC. We used our quantification data to rationalize the biological activity of antimirs measured in Luciferase reporter assay.
Results: Surprisingly, we found the position of the chemical modification in the antimir to be more important than its structure. Inhibitory activity was generally greatest for antimirs modified at position N3, whereas inclusion at N11 abolished activity. We hypothesized that the observed loss of biological activity was likely due to steric clashes between the chemical substituent at N11 and AGO2 protein in the RISC. Indeed, the antimir presence in the RISC, quantified by the CL-RT-qPCR, correlated with its biological potency. We rationalized these findings using recently available X-ray crystallographic data of AGO2 with a guide microRNA and a complementary RNA [2]. Our data confirmed the capacity of the CL-RT-qPCR method to detect and quantify chemically modified antimirs in cell lysates and RISC.
Conclusions: The robustness of the CL-RT-qPCR method and its template-based detection procedure offer virtually no analytical limitation in respect to the chemical format of the oligonucleotide. The biological activity of the antimirs was mainly affected by the chemical modifications in a position-dependent manner and it was correlating with its presence in RISC. These findings have broad implications for the future design of antimir drugs.

Keywords: Antimir, AGO2, RISC, chemical-ligation RT-qPCR.

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P-43

Immobilized RGDC Peptide Nano-Formulations for Wound Healing Application

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Introduction: Dermal wound healing is a complex process, which includes four overlapping steps: inflammation, migration, proliferation and maturation [1]. In most cases, tissue repair can occur spontaneously, but depends on the size and depth of the wound. There is a growing need for the development of biomaterials promoting wound healing to improve tissue regeneration in non-healing, chronic wounds. Wound regeneration needs to be guided by biological cues, such as Arg-Gly-Asp-Cys (RGDC), a peptide known to induce cell adhesion and migration [2].
Aims: Our focus is to develop different formulations strategies for topical wound application: a sprayable suspension of nanoparticles, hydrogels and freeze-dried foams, which would hydrate upon exudate absorption. Formulations are based on the chitosan derivative O-carboxymethyl-N,N,N-trimethyl-chitosan (CMTMC) grafted with RGDC peptide.
Methods: CMTMC has been synthesized towards high carboxymethyl grafting [3] in order to allow efficient RGDC peptide grafting. The new biopolymer was assessed for its ability to induce cell human dermal fibroblasts (HDFs) adhesion using Live/Dead assay. The nanoparticle complexes are obtained by mixing via a one-shot method of oppositely charged polyelectrolytes such as chitosan derivative and chondroitin sulfate (CS). Viscous gels were obtained by mixing CMTMC and hyaluronic acid (HA) adding NaCl 0.9% or 1.2%. Foams obtained by subsequent freeze-drying can be applied as dried wound patches. Formulations cytotoxicity was evaluated *in vitro* using WST-1 assay on HDFs.
Results: The new synthesized biopolymer shows bio-adhesive and bio-compatible properties. The nanoparticle complexes were in the submicron size range. RGDC-CMTMC concentration of 0.5% and 0.3% CS led to stable, uniform nanoparticles (216.9 ± 1.3 nm, PDI = 0.1) slightly negatively charged (-40.9 ± 0.2 mV). For hydrogel and foam formation, all formulations prepared in NaCl 1.2% were brittle after lyophilization, while those prepared in NaCl 0.9% were adequate, soft foams. Further selection based on stability and viscosity criteria allowed to select a gel formulation (RGDC-CMTMC 3% and HA 2% in NaCl 0.9%) and a lyophilized foam formulation (RGDC-CMTMC 3% and HA 1.5% in NaCl 0.9%). *In vitro* results showed no toxicity on HDFs over 7 days.
Conclusions: All formulations designed for topical application showed absence of toxicity on human dermal fibroblasts over 7 days. These may be suitable peptide carriers to enhance wound healing though guiding cell proliferation and migration.

Keywords: Wound healing, chitosan, bioadhesion peptide, toxicity.

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P-44

Development of a Peroral Hydrochlorothiazide Formulation for Pediatric Use

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Introduction: There is currently no pediatric hydrochlorothiazide (HCT) product on the market. The compound is presently described as powder, mostly obtained from marketed tablets for adults and filled into hard gelatine capsules, which are then emptied and mixed into the infant's food [1]. This way of compounding is inadequate as dose adjustments are difficult and dosing errors can readily occur. Further, opening of gelatin capsules may promote inappropriate attitudes toward handling of pharma-ceutical dosage forms. Thus, liquid formulations would be desirable. Due to the very low solubility HCT, aqueous solutions are not feasible. Suspension formulations are also proposed in the literature, but issues of physical and chemical instability are important hurdles [2,3].
Aim: Development of a simple, liquid, peroral HCT formulation for pediatric use offering good chemical, physical, and microbiological stability.
Methods: Different organic solvents were assessed for achieving desired minimal HCT solubility of 5 mg/mL. Based on the Ph. Eur.- and USP-HCT monographs, a HPLC method was developed that afforded quantification of both the HCT and its related substances. With this method, the stability of HCT in glycerol, PEG 200, glycerol-PEG200 (1:1) and glycerol-PEG200-water (1:1:1) mixtures was moni-tored over 60 days. The microbiological safety of the formulations was examined by a microbiological challenge test using *E. coli* and *C. albicans*. Finally, the palatability of the formulations was tested by volunteers.
Results: Amongst the solvents used, glycerol maintained best the stability of HCT as 99.1% of the parent drug was still present after 60 days storage at 24 °C and the related compounds were not increased. When extrapolating from Arrhenius equation, 93% of intact HCT can be expected after 12 months storage at 24 °C. A similar result was obtained with the glycerol-PEG 200 mixture, but slightly more degradation occurred in PEG 200 alone. In the glycerol-PEG 200-water mixture, hydrolytic degradation became important, which lowered the HCT content after 60 days storage at 24 °C to 91%. The glycerol formulation had a strong preservative effect as the challenge with 10^5 CFU of *E.coli* or *C.albicans* fulfilled the Ph. Eur. criteria for sufficient antimicro-bial preservation. Finally, the bitter taste of HCT was well masked by the sweetness and texture provided by glycerol.
Conclusions: The HCT solution in glycerol (5 mg/mL) showed appropriate chemical stability, microbiological safety without additional preservatives, and adequate taste without any added sweetener or taste masking additives.

Keywords: Pediatric dosage form, hydrochlorothiazide stability, microbiological stability.

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P-45

Phytochemical Heme Oxygenase Induction: A Biotherapeutic Tool for Prevention and Management of Chronic Inflammatory Disease

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Introduction: Major symptoms of many severe chronic diseases feature sustained high expression of pro-inflammatory cytokines, interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-), interleukin-1 beta (IL-1). Inflammatory mediators interact with many cell types to impair expression of cytoprotective heat shock proteins (HSP), causing tissue damage, pain and debilitation. Current pharmacological counter-measures to the resulting syndromes typically rely on small molecule drugs which interfere with inflammatory signaling cascades in ways that may transiently ameliorate pain, but fail to remediate underlying causes of a disease and are often debilitatingly toxic.
Aims: The clinical investigations on which the present report is based [1-3], were conducted to restore healthy containment of inflammation to appropriate biological roles. Cell culture models and human osteoarthritis (OA) patients were used, by pharmacologically amplifying expression of heme oxygenase-1 (HO-1, aka HSP-32), a powerful endogenous antioxidant expressed inducibly by all tissues. Treat-ments were conducted to specifically inhibit CD3+ T cell inflammatory cytokine production, which is a primary cause of pain and tissue damage in inflammatory diseases.
Methods: Peripheral blood mononuclear cells (PBMC) from 12 type 2 diabetes (T2DM) patients, 11 rheumatoid arthritis (RA) patients and 8 matched healthy control subjects for each group, were cultured 24 h with 100 ng/mL lipopolysaccharide (LPS), co-incubated with 0.5–100 mg/mL sour cherry seed (SCSE), an inducer of HO-1. Cultures were evaluated by two-color flow cytometry for percent represen-tation of CD3+ T cells activated to express inflammatory cytokines. In related experiments, 20 OA patients with knee joint involvement and 10 matched healthy controls were administered topical SCSE twice daily across kneecaps for 2 months, then assessed for clinical and laboratory correlates of OA.
Results: SCSE addition to cell cultures significantly suppressed LPS-increased CD3+TNF-α+ and CD3 +IL8+ representation from all participants ($p<0.05$), with greater pharmacological effect in suppression of CD3 +TNF-α + noted in cells from T2DM and RA patients versus healthy control subjects. These effects correlated with increased HO-1 expression in SCE-treated PBMC from all subjects ($p<0.05$). SCSE topical treatment dramatically abated joint pain and decreased serum (CRP - a biomarker for inflammation) and peripheral blood representation of CD3+ T cells activated to express inflammatory cytokines. These effects occurred in an HO-1 dose-dependent manner.
Conclusions: SCSE-mediated, HO-1 induction is a potent, side effect-free, preventive and therapeutic for chronic inflammatory disease.

Keywords: Inflammation, inflammatory cytokines, T lymphocytes, biotherapeutic, sour cherry.

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P-46

Dual Functional Nerve Conduits Promote Directional Axonal Outgrowth

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Introduction: Peripheral nerve injuries often result in life-long disabilities due to lack of efficient treatment measures. Development of biologically functional nerve conduits (NCs) with topographical guidance and neurotrophic factors (NTFs) support may address some of the limitations associated with currently available treatment modalities, i.e., nerve grafting and artificial nerve conduits (NCs) [1].
Aims: This study aimed at developing dual functional collagen nerve conduits (C-NC) for trophic and topographical guidance support. For this, glial cell line-derived neurotrophic factor (GDNF) was engineered with collagen binding domain (CBD) resulting in CBD-GDNF. Further, C-NC was functio-nalized with aligned PLGA nanofibers. Dual functional scaffolds were obtained by enriching the nanostructured C-NC with CBD-GDNF.
Methods: Coding sequence for recombinant CBD-GDNF was cloned into eukaryotic vector pCDNA3, expressed in mammalian 911 cell-line and purified by immobilized metal affinity chromatography. Recombinant CBD-GDNF or native GDNF (50 ng/NC) was loaded into nanostructured C-NCs fabricated by electrospinning and spinning mandrel technique. For preparing aligned nanofibers, 8% (w/w) PLGA (85:15) in chloroform was prepared and electrospun. Release kinetics were studied *in vitro* over 28 days from nanostructured C-NC. Bioactivity of released growth factors was assessed by using Neuro-2A cell line. Furthermore, the ability of nanostructured C-NC scaffolds to promote axonal outgrowth was tested *in vitro* using dorsal root ganglions (DRGs) and spinal cord (SC) explants isolated from 9 days old chicken embryos [2].
Results: GDNF was engineered with collagen binding domain resulting in CBD-GDNF, and its expres-sion and purity are characterized by SDS PAGE analysis. The *in vitro* release of GDNF and CBD-GDNF from the nanostructured C-NC was sustained over 28 days. Interestingly, CBD-GDNF mediated slow and low release with significantly reduced initial burst release when compared to native GDNF. Incuba-tion of Neuro2A cells with release medium containing NTFs, resulted in neuronal differentiation and axonal outgrowth. The topography of C-NC scaffolds loaded with GDNF or CBD-GDNF determined the direction and extent of axonal outgrowth from DRG and spinal cord explant tissues. Axonal outgrowth of sensory neurons was perfectly (99.9%) in line with the aligned fibres, but randomly oriented on non-aligned fibers. Similar results were obtained from spinal cord motor neurons, too.
Conclusions: Engineered GDNF with collagen binding domain (CBD-GDNF) was successfully produced. Immobilization of NTFs in C-NCs by fusion with collagen-binding domain is an appealing strategy for controlling release kinetics with reduced burst release and for sustaining bioactivity of NTFs. Importantly, PLGA nanofibers showed potential for guiding peripheral axonal regeneration. Thus, the PLGA aligned nanofibers and CBD-GDNF were integrated into C-NC for harnessing dual functions, i.e., trophic and guidance cues. Future studies will assess the beneficial effects of dual functional C-NC in a rat nerve gap model.

Keywords: Nerve regeneration, growth factors delivery, nerve conduits, nanofibers.

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P-47

Intravenous Delta-9-Tetrahydrocannabinol to Prevent Postoperative Nausea and Vomiting: A Randomized Controlled Trial

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Introduction: Evidence suggests that cannabinoids can prevent chemotherapy-induced nausea and vomiting. The use of delta-9-tetrahydrocannabinol (THC) has also been suggested for the prevention of postoperative nausea and vomiting (PONV), but evidence is very limited and inconclusive.
Aims: To evaluate the effectiveness of intravenous THC in the prevention of PONV.
Methods: This double-blind, randomized, placebo-controlled trial with patient stratification according to the risk of PONV was performed with IRB approval and written informed consent. Forty patients at high risk for PONV received either 0.125 mg/kg intravenous THC or placebo at the end of surgery before emergence from anesthesia. The primary outcome parameter was PONV during the first 24 h after emergence. Secondary outcome parameters included early and late nausea, emetic episodes and PONV, and side effects such as sedation or psychotropic alterations. Our hypothesis was that THC would reduce the relative risk of PONV by 25% compared with placebo.
Results: The relative risk reduction of overall PONV in the THC group was 12% (95% confidence interval, −37% to 43%), potentially less than the clinically significant 25% relative risk reduction demonstrated by other drugs used for PONV prophylaxis. Calculation of the effect of treatment group on overall PONV by logistic regression adjusted for anesthesia time gave an odds ratio of 0.97 (95% confidence interval, 0.21 to 4.43, P = 0.97). Psychotropic THC side effects were clinically relevant and mainly consisted of sedation and confusion that were not tampered by the effects of anesthesia. The study was discontinued after 40 patients because of the inefficacy of THC against PONV and the finding of clinically unacceptable side effects that would impede the use of THC in the studied setting.
Conclusions: Because of an unacceptable side effect profile and uncertain antiemetic effects, intrave-nous THC administered at the end of surgery before emergence from anesthesia cannot be recommen-ded for the prevention of PONV in high-risk patients.

Keywords: Postoperative nausea and vomiting, tetrahydrocannabinol.

P-48

A Polyphenol Enriched Fraction of *Rose Oil Distillation Water* Inhibits Proliferation in HaCaT Cells and Induces Apoptosis

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Introduction: Water steam distillation of rose flowers (*Rosa damascena*) separates the essential oil from the polyphenol containing rose oil distillation waste water (RODW). While the essential oil represents the desired liquid for the cosmetic industry, the polyphenol containing RODW is in the center of our interest. Recently, a strategy was developed to separate RODW into a polyphenol depleted water fraction and a polyphenol enriched fraction [RF20-(SP-207)]. Polyphenols are known to have a wide spectrum of biochemical and pharmacological effects, such as antioxidant, anticancer, anti-inflammatory and numerous other activities. In particular, rose petals are known to contain compounds with potential anti-proliferative activity, such as flavonoids, gallic and protocatechuic acids, and tannins.
Aims: Medicinal regiments for hyper-proliferation involved diseases such as psoriasis and cancer are strongly limited up to now. In the present study, it was of interest to investigate possible antiproliferative effects of RF20-(SP-207) and fractions thereof F(I)-(IV) as a comparison in immortalized human keratinocytes (HaCaT). Additionally, we examined apoptosis-inducing actions of RF20-(SP-207) and F(IV).
Methods: Cell viability was assessed by the MTT-assay. The BrdU cell proliferation assay was used to measure cell proliferation. Cell migration was elucidated by time lapse microscopy. Vascular endothelial growth factor (VEGF) was measured in cell supernatants by a commercial ELISA assay. Apoptotic processes were analyzed by fluorescence microscopy after 488 annexin V conjugate staining.
Results: The data demonstrated that from all tested fractions only F(IV) revealed a dose dependent anti-proliferative activity which is comparable to RF20-(SP-207) (IC_{50} of approx. 10 µg/mL). This effect is stronger than the two positive controls LY294002 10 µM (PI3K-inhibitor, 30% inhibition) and NVP-BEZ235 100 nM (dual PI3K/mTOR-inhibitor, 30% inhibition) and clearly exceeded the anti-proliferative action of quercetin 50 µM (approx. 20% inhibition). Time lapse microscopy detected a significant impairment of cell migration under influence of F(IV) and RF20-(SP-207). At a concentration of 10 µg/mL of both extract and fraction, cell motion was strongly retarded, at 100 µg/mL it totally ceased. The retardation effect was comparable to LY294002 and NVP-BEZ235. Fluorescence microscopy images confirm the qualitative increase of pro-apoptotic actions under influence of RF20-(SP-207). The VEGF-level was reduced by RF20-(SP-207) at 10 and 100 µg/mL in a concentration-independent manner (approx. 50% compared to the untreated control). In contrast, F(IV) only at 10 µg/mL was able to significantly decrease the VEGF-level.
Conclusion: In conclusion, RF20-(SP-207) has been shown to be very promising in terms of controlling cell fate and could be developed as a supportive, local therapy against hyperproliferation-involved diseases such as plaque psoriasis.

Keywords: Rose oil distillation water, cell proliferation, apoptosis, VEGF.

P-49

Development of an Oral Pediatric Formulation Based on Hydrochlorothiazide

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Introduction: Few specialty drugs are adapted for children, and drugs are often used off-label or unlicensed [1]. Esidrex® is the only specialty drug based on hydrochlorothiazide available in Switzerland in 25-mg tablets. Hydrochlorothiazide (HCT) is an antihypertensive thiazide diuretic, very largely used in pediatric departments of Swiss hospitals [2]. It is a white bitter crystalline powder that is very slightly soluble in water (0.06 g/100 mL). It is an electrostatic powder that sticks to glass or plastic containers rendering difficult an accurate dosing. At the present time, HCT tablets are formulated as capsules in Swiss German hospitals whereas the tablets are grinded and suspended in a commercial vehicle Ora-® from Perrigo Company (USA). But the vehicle is not intended exclusively for pediatric use and for the formulation of hydrochlorothiazide.
Aims: The aim of this study was to develop an oral suspension based on hydrochlorothiazide that is stable, can be delivered to neonates and infants and can be prepared in hospital pharmacies.
Methods: A black list of excipients that should not be used in pediatrics for oral suspensions was established and compared to the ingredients present in the commercial vehicles Ora-Sweet®, Ora-Plus® and Ora-Blend®. Oral suspensions were prepared either from Esidrex® tablets or from the pure substance HCT with different selected excipients including wetting, suspending, thickening, buffering and sweetening agents, as well as fillers, salts and preservatives. The suspensions were characterized in terms of organoleptic properties, density, pH, osmolality, viscosity and zeta potential. The chemical stability of HCT was assessed at different temperatures (5, 25, 40 and 60°C) by HPLC. The physical stability of the suspensions was tested by centrifugation taking as a reference the suspension prepared in hospital with Ora-Blend®.
Results: A suspension of HCT 5 mg/mL stable for 7 days was obtained by grinding Esidrex® tablets in a vehicle made of glycerol, sorbitol, carmellose sodium, lactose, xanthan gum, sucrose, calcium chloride, citric acid, sodium phosphate, and potassium sorbate in purified water. The properties of the formulated suspension were similar to the reference prepared in Ora-Blend®: pH 3.8, non-Newtonian pseudoplastic rheological behavior, osmolality 1690 [mml/kg], zeta potential of -34.6 ± 0.8. The content uniformity of the administered doses complies with the Swiss and European regulation. The preparation of the suspension from the pure powder gave not reproducible content uniformity due to the sticking and electrostatic properties of the powder.
Conclusion: A new formulation of HCT was successfully developed to enable an accurate administra-tion of the drug in neonates or infants. The suspension is stable for 7 days at room temperature and has a pleasant taste.

Keywords: Oral suspension, hydrochlorothiazide, pediatrics.

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P-50

Documentation of Drug Related Problems in Community Pharmacies

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Introduction: Drug related problems (DRPs) can induce negative clinical and financial consequences [1]. Therefore, pharmacists should play an essential role in their management. To bring out this role, an instrument based on a classification system of DRPs is required [2]. Several systems were developed, but only some were validated. The community pharmacy department of Ambulatory Care & Community Medicine developed its own tool which is published in 2008 [3]. It is used in-house for several years without being validated.
Aims: To update and validate an instrument to document pharmacists' management of DRPs while dispensing medicines in community pharmacies and to asses its acceptability by community pharma-cists in the French-speaking part of Switzerland.
Methods: An update was performed by taking into account the main classification systems validated and published since 2007, especially those from the PCNE (Pharmaceutical Care Network Europe) and the University of Basel. It allows to classify DRP according to: type of DRP (e.g. clinical: adherence problem or contraindication; e.g technical: reimbursement problem or drug temporary unavailable), clinical consequences, pharmacist's intervention and other people involved in the DRP management (e.g. prescriber or patient's relative). A prospective observational study including 10 community pharmacists from the French-speaking part of Switzerland (convenience sampling) was conducted to validate the tool. Each pharmacist received an online training at the beginning of the study. Inter-rater reliability was assessed using 24 standardised cases documented by pharmacists and by calculating Fleiss's Kappa coefficients. One month later, intra-rater reliability was similarely assessed using 10 standardised cases. These cases were randomly selected among the 24 cases and modified to present a situation involving a similar DRP. To assess internal validity, each pharmacist was asked to document 15 DRPs detected in daily practice and during dispensing procedure. Acceptability and feasability were assessed by a satisfaction survey (11 questions) using a 5-point Likert scale and based on similar surveys used in previous studies.
Results: A new version of the tool was developed and validated. The reliability of the tool was demon-strated as Kappa coefficients were above the minimal accepted value of 0.40: 0.53 for inter-rater reliability (moderate agreement between raters) and 0.62 for intra-rater reliability (substantial agreement for the same rater). Among the 131 documented cases, each type of DRP could be documented and every drugs' class (according to ATC code) could be introduced which demonstrate its internal validity. According to the feedback from the pharmacists, the implementation of this instrumenin community pharmacies seems possible.
Conclusions: The validation of this instrument was successfully conducted and demonstrated its reliability. To our knowledge, this is the second classification system, among those published to document DRPs in community pharmacies, to be validated according to its intra-rater reliability. It showed to be usefull to document any kind of DRP.

Keywords: Drug related problems, classification system, validation, community pharmacy.

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P-51

Toll-Like Receptor 2 Stimulation Enhances Barrier Function in Normal and Asthmatic Bronchial Epithelial Cells

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Introduction: The airway epithelium acts as a crucial physical and immunological barrier against several inhaled antigens and allergens. Tight junctions between these cells form a highly regulated and imper-meable physical barrier. Disruption of airway epithelial tight junctions has been reported in asthmatic bronchial biopsies. This suggests higher susceptibility of airway immune cells to inhaled particles and pathogens and hence asthmatic aggravation.
Aim: To investigate the regulation of Toll-like receptor 2 (TLR2) in augmenting epithelial barrier integrity in normal and asthmatic human primary bronchial epithelial cells.
Methods: Transepithelial electrical resistance (TEER) and paracellular flux experiments were used in parallel to RT-PCR, Western blotting and immunofluorescence experiments to study the tight junctional regulation by TLR2.
Results: We have previously shown that TLR2 stimulation enhances the tight junction associated epithelial barrier function of human bronchial epithelial cell monolayers by increasing the expression of claudin-1 and ZO-1 tight junction proteins via atypical protein kinase C Zeta in Calu-3 cells. In the current study we investigated the effect of TLR2 stimulation in Mucilair™ primary human bronchial cells and airway epithelial cells obtained from asthmatic, COPD and heavy cigarette smoker patients. TLR2 stimulation by peptidoglycan led to significant increase in TEER and a decrease in paracellular flux of FITC 3 kDa in primary human bronchial epithelial cells and cells from asthmatic individuals. Immuno-fluoresence analysis indicated an increase in the expression levels of tight junction proteins claudin-1 and ZO-1in normal and asthmatic epithelial cells. These effects were not noticed in cells from COPD and heavy cigarette smokers.
Conclusions: TLR2 ligation could be a promising therapeutic strategy for asthma and other epithelial barrier disorders.

Keywords: Tight junctions, toll-like receptor 2, barrier function, protein kinase C.

P-52

A Liposomal Florescence Assay to Predict Intestinal Drug Absorption

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Introduction: Passive lipid bilayer permeation of drug-like compounds with weak acidic or basic functionality can be studied with our recently introduced liposomal fluorescence permeation assay [1]. Drugs added to the liposomes permeate the lipid bilayer preferentially in their neutral form and the subsequent proton release/ capture of an acid/ base leads to a change in luminal pH, which can be monitored with the encapsulated pH-sensitive fluorescent dye HPTS (8-hydroxy-pyrene-1,3,6-trisulfonic acid).
Aims: The liposomal fluorescence permeation assay was optimised with regard to improved fluorescence signal intensity and to reduced lipid concentration. We aimed to evaluate the potential of the optimised assay to predict intestinal human absorption of drug-like compounds.
Methods: Palmitoyl-oleoyl-phosphatidylcholine (POPC) liposomes containing the pH-sensitive fluorescent dye HPTS in their lumen were prepared by thin lipid film hydration and extrusion. Extraliposomal HPTS was replaced with buffer pH 6.0 by size exclusion chromatography. Liposomes were mixed in a stopped-flow apparatus with basic or acidic drugs and the change in fluorescence was recorded. Permeation coefficients were calculated from the kinetics and corrected for predicted paracellular permeation [2]. These values were compared with published human intestinal perfusion data [3].
Results: An increase in the luminal HPTS concentration improved fluorescence signal intensity and reproducibility of the permeation measurements and allowed a reduction of the lipid concentration. The permeation coefficients of structurally diverse orally bioavailable drugs obtained with the optimised assay and adjusted for paracellular permeation ranged from 6.0×10^{-7} cm/s (furosemide) to 5.0×10^{-4} cm/s (verapamil) and correlated with published human intestinal perfusion data with a correlation coefficient of $R^2 = 0.90$ (N = 12). Predicted human intestinal absorption of piroxicam based on this correlation was in agreement with human effective permeability.
Conclusions: Our liposomal assay can be used as a model of the intestinal drug barrier and is ready to predict intestinal absorption of compounds with weak acidic or basic functionality. We aim to transfer it to a plate-reader compatible format (see abstract P-53) and furthermore to adapt it for the prediction of blood brain barrier passage.

Keywords: Intestinal absorption, bioavailability, liposomes, prediction, permeation.

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P-53

Permeation of Drugs Across Phosphatidylcholine Bilayers: Influence of Lipid Chain Saturation

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Introduction: Our *in vitro* liposomal fluorescence assay [1] provides a rapid, well-defined and low-cost tool to measure lipid bilayer permeation. The fluorescent pH-indicator 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) is loaded in the luminal compartment of liposomes, changing its fluorescence upon permeation of a weak acid or base. Measurements with 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine (POPC; C16:0,C18:1) liposomes revealed a good correlation with human intestinal absorption kinetics (see abstract P-52). However, the fast kinetics of permeation into POPC liposomes requires the use of a stopped flow instrument.
Aims: We envisaged to slow down permeation kinetics by introducing saturated lipid chains in order to allow to transfer the assay to a multi-well plate reader format that can be used for screening in early drug development to predict intestinal absorption.
Methods: Liposomes including HPTS were prepared from fully saturated 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine (DPPC; C16:0,C16:0) and mixtures of DPPC with 7 to 17 % POPC, respectively, by lipid film hydration and extrusion. External HPTS was replaced with buffer pH 6 by size exclusion chromatography. Liposomes were mixed with drugs and HPTS fluorescence was followed in a plate reader below (37°C, 15 drugs) and with a stopped flow apparatus above (48°C, 7 drugs) the transition temperature of DPPC. Permeation coefficients were compared with *in vivo* human intestinal [2] and POPC permeation coefficients.
Results: Permeation coefficients in DPPC liposomes were higher at 48 than 37°C. At both temperatures, the values were distributed in a narrow range and did not correlate with human *in vivo* data or results from POPC liposomes. Addition of up to 17 % POPC at 37°C did not significantly affect the values. While POPC permeation coefficients correlated with the hydrogen bonding donor capacity of the drugs, DPPC permeation coefficients were independent of hydrogen bonding properties and molecular weight.
Conclusions: Acyl chain saturation is a major determinant for lipid bilayer permeation. While permeation across partially unsaturated phospholipids correlates with *in vivo* kinetics, the dependence on drug physicochemical properties gets lost in fully saturated lipids, independent of whether they are in the liquid-crystalline or gel state. We hypothesize that the permeation-determinant mechanism is different in partially unsaturated (POPC) and saturated (DPPC) lipid bilayers. Further lipid compositions are currently tested to slow down permeation by keeping the correlation with *in vivo* data. Our results are in addition of high interest for molecular dynamics simulations with lipid bilayers, where DPPC is generally used as a model lipid.

Keywords: Drug permeation, lipid acyl chain saturation, correlation, DPPC.

References:
[1] Eyer K et al. J Control Release 2014; 173: 102-9.
[2] Dahlgren D et al. J Pharm Sci; in press (doi: 10.1002/jps.24258).

P-54

Preclinical Imaging of Atherosclerotic Plaque Inflammation with the CD80/CD86-Specific ^{111}In-DOTA-Belatacept

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Introduction: Atherosclerotic plaque vulnerability is related to underlying inflammatory processes with antigen-presenting cells playing a central role. We identified the T-lymphocyte activation antigens CD80 and CD86 expressed on antigen-presenting cells as promising targets for the imaging of plaque inflammation. The expression of both costimulatory molecules is increased in human vulnerable compared to stable plaques [1].
Aims: In this proof-of-principle study, the fusion protein belatacept (Nulojix®), containing the extracellular portion of CTLA-4 as binding motif, was labeled with indium-111. The binding of this radiolabeled probe to human and murine atherosclerotic plaque tissue was investigated *in vitro* and/or *in vivo* to evaluate the potential of this tracer for atherosclerosis imaging.
Methods: Commercially available belatacept was conjugated with *p*-SCN-Bn-DOTA, radiolabeled with In-111 and purified by FPLC. Binding of the radioactive probe to human carotid plaques was evaluated by *in vitro* autoradiography. CD1 nude mice bearing Raji xenografts and ApoE KO mice were injected i.v. with 10 MBq ^{111}In-belatacept (25 µg, baseline). Blockade animals received additionally unlabeled belatacept (500 µg). *In vivo* and *ex vivo* SPECT/CT scans and biodistribution studies were performed 48 h post injection. ApoE KO mice were fed a high fat diet to promote atherosclerosis development. Oil red o staining was performed with excised blood vessels to visualize plaque lipids.
Results: ^{111}In-belatacept was successfully produced in 73-78% radiochemical yield. Radiotracer binding to human atherosclerotic plaques was determined by autoradiography and the specific binding correlated with the number of immune cells within the plaque and the size of the lipid/necrotic core. *In vivo*, ^{111}In-belatacept accumulated in CD80/CD86-positive Raji xenografts, lymph nodes and salivary glands and a reduced accumulation was determined under blockade conditions. These results were confirmed by biodistribution experiments revealing a displaceable binding to Raji xenografts and salivary glands. *Ex vivo* SPECT scans of the aortic arch and the carotids of ApoE KO mice showed a high accumulation in atherosclerotic plaques and a reduced signal under blockade conditions. Tracer accumulation and plaque localization determined by oil red o staining were consistent.
Conclusions: The T-lymphocyte activation antigens CD80 and CD86 are promising targets for non-invasive imaging of activated antigen-presenting cells. ^{111}In-belatacept specifically accumulates in CD80- and CD86-positive human-derived Raji xenografts and binds to murine as well as human atherosclerotic plaques.

Keywords: Imaging, atherosclerosis, inflammation, SPECT.

Reference:
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