SAPhS
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10th Swiss Pharma Science Day 2017

Tuesday, August 22, 2017

University Hospital – Inselspital Bern, Auditorium E. Rossi

Intention

The SWISS PHARMA SCIENCE DAY (SPhSD) is an annual event of the Swiss Academy of Pharmaceutical Sciences (SAPhS, www.saphw.ch). The 1st SPhSD was held on October 9, 2008, at the University of Bern. For congress reports 2008-2016 including all lecture and poster abstracts see www.saphw.ch. The SPhSD offers a platform to present, in form of a poster session, the latest research results of Master and PhD students, as well as Post-Docs of all the three Swiss Academic Institutions for Pharmaceutical Sciences, i.e. ETH Zurich, School of Pharmaceutical Sciences of the Universities of Geneva and Lausanne (EPGL) in Geneva and the University of Basel. Master students of the Universities of Applied Sciences, i.e. FHNW (School of Life Sciences, Muttenz) and ZHAW (Life Sciences and Facility Management, Institute of Biotechnology, Wädenswil) are also invited to participate in this event.

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

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

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

Organizing Committee:

Prof. Dr. Rudolf Brenneisen, SAPhS, Secretary General, Bern

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Prof. Dr. Gerrit Borchard, SAPhS, President

School of Pharmaceutical Sciences EPGL, University of Geneva

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Program

9:00-9:30 h	Registration, Coffee
9:30-10:00 h	Addresses of Welcome Prof. Dr. Gerrit Borchard, President SAPhS Prof. Dr. Rudolf Brenneisen, Secretary Gen. SAPhS Prof. Dr. Maurice Campagna, President Swiss Academies of Art and Sciences: «Challenges of the Swiss Academies of Art and Sciences and the SAPhS»
	Plenary Lectures Chair: Prof. Dr. Jörg Huwyler, Univ. of Basel
10:00-10:45 h	Lecture 1: Keynote Lecture, Analytics Prof. Dr. Csaba Szántay, Jr., Gedeon Richter Plc. Budapest, Hungary: «The Man and His Spectrometer: a Human-Centered Look at Structure Elucidation by NMR and MS»
10:45-11:30 h	Lecture 2: Clinical Pharmacy Prof. Dr. Christoph R. Meier, University of Basel: «Clinical Pharmacy: Much More Than a Hospital-Based Discipline»

Program (cont.)

11:30-12:15 h	Lecture 3: Natural Products Dr. Ying Wang, Novartis Pharma, Basel: «Precise Molecular-Feature Analysis (PMA) of UHPLC/Q-TOF MS for Metabolite Profiling in Synthetic Biology»
12:15-13:15 h	Lunch
13:15-14:30 h	Poster Session
	Plenary Lectures (cont.) Chair: Prof. Dr. Georg Imanidis, FHNW Life Sciences
14:30-15:15 h	Lecture 4: Nanomedicines Dr. Didier Bazile, Sanofi, Gentilly, France: «Translation of Nanomedicines to Proof-of-concept in Human – Quality Management Based on Technology Readiness Levels»
15:15-16:00 h	Lecture 5: Molecular Biology Dr. Christof Fellmann, Univ. of California, Berkeley: «Cornerstones of CRISPR-Cas in Drug Discovery and Therapy»

Program (cont.)

16:00-16:15 h	Coffee
16:15-17:15 h	Award Ceremonies
17:15-17:30 h	Closing Remarks Prof. Dr. Rudolf Brenneisen, Secretary Gen. SAPhS
17:30-18:30 h	Apéro

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Lectures

L-1

Prof. Dr. Csaba Szántay, Jr.

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CV:

Csaba Szántay, Jr. received his MSc in chemical engineering at the Technical University of Budapest (TUB) in 1982. Subsequently he worked in the NMR laboratory of TUB, where he obtained his PhD in 1986. From 1988 he worked as an NMR spectroscopist at Leeds University, UK, as a postdoctoral fellow. After returning to Hungary in 1989, he continued his work as an NMR spectroscopist in the Spectroscopic Research Department of Gedeon Richter Plc., Hungary's largest pharmaceutical company. He has been the head of this department since 1994. He and his team are dedicated to showing that a reasonable degree of original scientific research which is manifested in scientific publications can and should be done in a pharmaceutical industrial environment, and are also committed to maintaining strong ties with academia in terms of education as well as research collaborations. His main fields of resarch are the physical and mathematical theory of NMR spectroscopy and the application of high-resolution NMR in the structure determination of organic moleculs. As a result of his scientific work, he obtained from the Hungarian Academy of Sciences a "Candidature" in 1991 and a "Doctor of Sciences" title in 2000. He habilitated at the TUB in 2002, and was elected a Private Professor at the same university in 2003, where he keeps being an active lecturer.
Affiliations to scientific committees and institutions: Hungarian Chemical Society (vice president); NMR Sub-division of the Analytical Division of the Hungarian Chemical Society (president); Gedeon Richter Foundation for Chemical Education (president); Gedeon Richter Working Division of the Hungarian Chemical Society (past president); Educational Division of the Hungarian Chemical Society (member); Committee on Analytical and Environmental Chemistry of the Section of Chemical Sciences of the Hungarian Academy of Sciences (secretary); NMR Working Party of the Committee on Analytical and Environmental Chemistry (president); Scientific Committee of Magnetic Moments in Central Europe (member); Editorial Board of Concepts in Magnetic Resonance (member); Editorial Board of Analytical and Bioanalytical Chemistry (member); Doctoral Council of the Faculty of Chemical Technology and Biotechnology; Habilitation Committee and Doctoral Council of the Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics (member); George Olah Doctoral School, Budapest University of Technology and Economics (core member); Habilitation Committee and Doctoral Council of the Budapest University of Technology and Economics (member); Doctoral Council of the Faculty of Natural Sciences, Eötvös Lorand University (member); Doctoral Council of the Section of Chemical Sciences of the Hungarian Academy of Sciences (past member); Section of Chemical Sciences of the Hungarian Academy of Sciences (doctoral general assembly representative).
Awards: Academy Award of the Hungarian Academy of Sciences: 2005; Miklós Preisich Award: 2011. Up until now he has published about 130 original scientific papers, including two books.

Abstract:

"The Man and His Spectrometer: A Human-Centered Look at Structure Elucidation by NMR and MS"

The fundamental innovation behind any drug is the molecular structure of the drug substance itself. Besides that, the need to determine molecular structures (such as those of related impurities, metabolites, degradants, etc.) crops up in numerous respects during the discovery, development, clinical studies,

patenting, manufacturing and quality control of a drug substance and drug product. According to general perception, modern nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) offer an array of methods that are so powerful in terms of mapping structural details at an atomic level that from the experimental data the structures can be assembled almost mechanically and unassailably. Although this is often true, in reality situations abound where this rosy view is deceptive, leading to the sensitive question: how sure can we be about the correctness of a deduced structure? In this presentation I will take a conceptual look at the often overlooked “human element” that is inherent in structure determination even when using structure-elucidating software tools, and is closely tied to the concept of “structural confidence”. The focus will be on small molecules in an innovative pharmaceutical industrial setting. On that pretext, and in a somewhat philosophical stance, I will present a fallibility-conscious way of thinking about and practicing NMR and MS. This approach, called “Anthropic Awareness”, has been cultivated for a while in our research facility with the aim of achieving maximum structural confidence, and has proved to be highly useful not only in our everyday professional lives, but also in our nonprofessional everyday lives. The aim is to develop a keen mindfulness of how our human nature secretly influences our inferences and how this can lead even the smartest and most knowledgeable scientists to fall into what we call “Mental Traps”, resulting in mistakes ranging from widely held scientific misconceptions to faulty personal or team-level deductions. By understanding the nature of these Mental Traps, one can develop the enlightening faculty of detecting and avoiding them both in one’s own and others’ thoughts. Based on a recently published book written about this topic by our team [1], I will briefly discuss some of the most important Mental Traps that are relevant to the application of NMR and MS.

Keywords: structural confidence, NMR, MS, Mental Trap.

Reference:
Cs. Szántay, Jr, (Ed), Anthropic Awareness: the human aspects of scientific thinking in NMR spectroscopy and mass spectrometry. New York: Elsevier, 2015.

Prof. Dr. C. R. Meier

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CV:

Prof. Christoph Meier graduated as pharmacist at the University of Basel in 1987. He got his PhD at the Division of Clinical Pharmacology at the University Hospital of Basel with Prof. F. Follath, and worked then for 4 years at the Division of Clinical Pharmacology at the University Hospital of Zürich with Prof. Peter Meier-Abt. Between 1995 and 1998 he conducted a postdoctoral fellowship at the Boston Collaborative Drug Surveillance Center (BCDSP) in Lexington, MA, USA, with Professor Hershel Jick and his daughter, Professor Susan Jick, He also got a Master degree in Epidemiology at the Harvard School of Public Health in Boston, MA, and an assistant professorship at the Boston University School of Public Health, where he currently still has a faculty position as adjunct professor.
In 1998, Prof. Meier started to build up his own research unit at the Division of Clinical Pharmacology and Toxicology at the University Hospital of Basel (with Prof. S. Krähenbühl), the so-called "Basel Pharmacoepidemiology Unit". He has a close research collaboration with Prof. Hershel and Susan Jick and colleagues at the Boston Collaborative Drug Surveillance Program in Lexington, MA. In 2008 he got a "Titularprofessorship" from the Faculty of Medicine at the University of Basel, and in 2009 he was elected head of the Hospital Pharmacy at the University Hospital and professor for Clinical Pharmacy and Pharmacoepidemiology at the Department of Pharmaceutical Sciences at the University of Basel, Switzerland.
Awards: 1995–1997 Postdoctoral fellowships from the Swiss National Science Foundation and from the Harvard University, Boston, MA 1999–2005 Research grants from the Swiss National Science Foundation entitled "Pharmacoepidemiologic Research Methods in Post-Marketing Surveillance of Drugs: Assessing Risks and Benefits of Long-Term Exposure to Drugs in Cardiovascular Medicine", and "Pharmacoepidemiologic Research Methods in Post-Marketing Surveillance of Drugs: Assessing Risks and Benefits of Long-Term Exposure to Drugs" 2002 Award "Best Doctoral Thesis of the Department of Internal Medicine at the University Hospital Basel in 2002" for my doctoral student Brigitta Schlegel, MD, who conducted a study entitled "HMG-CoA-reductase inhibitors and the risk of fractures", published in JAMA in 2000 2005 Clinical Award for the best publication of the year 2005 by the Swiss Bone and Mineral Society (SBMS) for the publication "Use of beta-blockers and the risk of bone fractures", published in JAMA 2004.
Other activities: Member of the editor board of the journal Drug Safety, Member of the Human Medicines Expert Committee (HMEC) at Swissmedic.
Latest Publications:
Wilson, J Claire; Furlano, Raoul I; Jick, Susan S; Meier, Christoph R: A population-based study examining the risk of malignancy in patients diagnosed with inflammatory bowel disease, in: Journal of gastroenterology 51, 2016, H. 11, S. 1050-1062.
Spoendlin, Julia; Meier, Christian; Jick, Susan S; Meier, Christoph R: Achilles or biceps tendon rupture in women and men with type 2 diabetes: A population-based case-control study, in: Journal of diabetes and its complications 30, 2016, H. 5, S. 903-9.
Spoendlin, Julia; Meier, Christian; Jick, Susan S; Meier, Christoph R: Bisphosphonate therapy start may transiently increase the risk of tendon rupture in patients with glucocorticoid co-medication: a population-based observational study, in: Pharmacoepidemiology and drug safety 25, 2016, H. 10, S. 1116-1123.

Abstract:

“Clinical Pharmacy: Much More Than a Hospital-Based Discipline”

Numerous definitions of Clinical Pharmacy are available in the literature. The definition of the Swiss Society of Hospital Pharmacists (GSASA) is: “Clinical Pharmacy is that part of pharmacy that aims to develop and advance the appropriate and efficient use of medicines. In hospitals, the term Clinical Pharmacy is used to describe the patient centered pharmaceutical activities performed in collaboration with other health professionals. The Clinical Pharmacist has completed specific training and is responsible for his actions”. While many people still think that Clinical Pharmacy is a highly specialized niche discipline addressing only the needs of severely ill patients in tertiary care, it becomes more and more evident that efficient and safe use of medicines is not only needed in tertiary care, but even more so in the community setting. The same patient who leaves the hospital with a complex medication mix on prescription after a bone marrow transplant will show up one hour later in a community pharmacy and challenge the pharmacist. In addition, with the revision of the “Medizinalberufegesetz” in Switzerland, new challenges will emerge for pharmacists, not only in hospitals, but mainly in the ambulatory care of patients in the community. The clinical skills that pharmacists learn during their clinical pharmacy activities in hospitals in a highly interdisciplinary setting are crucial to improve their professional skills in the community pharmacy. Clinical Pharmacy as a discipline will keep growing in hospitals and must be used to teach young pharmacists on their way to become interdisciplinary professionals, capable of interacting and communicating with doctors and nurses and supporting them in providing the best possible medication to patients. The presentation will shed some light on various emerging activities of clinical pharmacists which will have a direct impact on their skills in patient-centered care in community pharmacies.

Dr. Ying Wang

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CV:

Education:
1982 B. Sc., Beijing Medical University
1985 M. Sc., Beijing Medical University
1990 Ph. D., University of Lausanne
1991 Postdoc., Biotechnology, Sandoz Pharma Ltd.
Working Experience:
1982-1983 Tonji Medical University
1985-1987 Beijing Medical University
1990 University of Lausanne
1991-1996 Preclinical Research, Sandoz Pharma Inc.
1996–now Novartis Institutes for Biomedical Research
Publications:
42 papers/book chapters have been published, e.g. in following journals: ACS Chemical Biology, Chemistry and Biology, Tetrahedron, Phytochemistry, Helvetica Chimica Acta, Molecular Biology of the Cell, EMBO Journal, Planta Medica, Journal of Ethnopharmacology, Applied and Environmental Microbiology, Journal of Chromatography A, Journal of Liquid Chromatography and Related Techniques, Journal of The American Society for Mass Spectrometry, Phytochemical review.

Abstract:

"Precise Molecular-Feature Analysis (PMA) of UHPLC/Q-TOF MS for Metabolite Profiling in Synthetic Biology"

The major goal of synthetic biology is to generate desired valuable substances with a good conversion from substrates to products by optimization of genetic and regulatory processes and pathways within cells. The secondary metabolites of microorganisms are biosynthesized in genetically encoded pathways, which involve multiple genes. These biosynthetic genes are usually clustered with regulatory and resistance genes in microbe genomes. Under laboratory cultivation conditions, most of these gene clusters are usually not expressed. Therefore, the potentially valuable "silent" pathways represent an unexploited reservoir of new secondary metabolites in microbial genome mining approaches. We use three strategies to activate these pathways, including extended cultivation, native host engineering and heterologous host expression.
To meet the challenges of metabolite profiling in synthetic biology, we have developed Precise Molecular-feature Analysis (PMA) of UHPLC/Q-TOF MS. High resolution MS, isotope pattern, ion charges, molecular-feature algorithm and retention time are used together in PMA to get cleaned mass spectra for precise identification and to integrate MS peaks for comparative quantification. Process automation with large UHPLC/Q-TOF MS data sets is realized by scripting with VBS.
In non-targeted analysis of engineered native hosts in various media, precise molecular-feature MS libraries with all metabolites of wild-type samples cultivated in same conditions are first established. In the comparative metabolite profiling, new compounds from engineered native hosts, which might be biosynthesized by activated unknown gene clusters, can be precisely identified by efficient comparison with established reference MS libraries in a fully automatic way.

For the known biosynthetic pathways in heterologous host expression, precise molecular-feature MS libraries with targeted metabolites are simulated based on adduct type, high resolution MS, isotope pattern, ion charge state and peak width (FWHM). In following PMA of heterologous expression samples, these metabolites can be quickly found by direct comparison with simulated MS libraries in targeted analysis.

In the PMA process, MS molecular-feature algorithm is used as second dimension in metabolite separation, in addition to UHPLC. This two dimension strategy has significantly increased the separation performance for co-elutes in chromatogram and decreased the background noise influence in MS spectroscopy. Besides comparative metabolite profiling, PMA-guided quantification and preparative separation has also be implemented for reproducibility monitoring in the re-fermentation and extraction process, as well as for fractionation and purification in preparative scale to get targeted metabolites or new compounds in the natural product drug discovery driven by synthetic biology.

Dr. Didier Bazile

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CV :

As the Head of Drug Delivery Technologies and Innovation at Sanofi, Didier Bazile is in charge of the initiation and the follow-up of programs and projects based on innovative Drug Delivery approaches.
He leads the interface between Technical Develoment and Discovery and supervises the implementation of Drug Delivery tools aimed at facilitating the identification of Drug Candidates and the assessment of their developability.
Didier Bazile leads a Pharmaceutical Innovation Network, managing a portfolio of products based on Drug Delivery innovation and coaching experts developing knowledge associated to (i) a route of administration (skin delivery, ear delivery, intra-articular delivery, ocular delivery) or (ii) a drug delivery approach (e.g. nanotechnologies, depot formulation) deemed critical for a variety of projects at Sanofi.
Didier Bazile started his career at Rhône-Poulenc Santé (an Aventis founder company) and hold different positions in Drug Delivery Research and Pharmaceutical Engineering. He is the author/co-author of pioneering and more recent patents and articles on PLA-PEG nanoparticles. He also had the opportunity to develop innovative Drug Delivery Systems at Laboratoires Fournier, Ethypharm and Novartis.
Didier Bazile was an alumnus of the Ecole Normale Supérieure de Cachan in Biochemistry, made a Ph.D. in Pharmacology in the Institut Gustave Roussy (University of Paris VI) and received an Accreditation to Supervise Research in Pharmaceutical Sciences (University of Paris XI).

Abstract:

“Translation of Nanomedicines to Proof-of-concept in Human – Quality Management Based on Technology Readiness Levels”

This lecture is aimed at outlining the points to consider, from an industrial perspective, when defining a quality and risks management approach for the translation of nano-carriers from Discovery to the Proof-of-Concept in human. This exercise takes advantage of the cases of nanomedicines on the market and in development, and of the regulatory documentation available in the EU and the US. Since a stepwise investment is inherent to the management of innovation, the technology Readiness Levels (TRLs) have been considered as a global framework.
In particular, the understanding and control of the drug/nano-carrier association appeared as a critical point to properly extrapolate preclinical data to human, as described for the taxanes formulations (L. Harivardhan Reddy and D. Bazile, 2014) and for PLA-PEG nanoparticles (L. Harivardhan Reddy and D. Bazile, 2016). In this context, we proposed a methodology to anticipate the fraction of nano-encapsulated drug after manufacturing, and after the dilution in blood following the administration (O. Diou et al., 2015). The proper evaluation of this fraction is of prior importance since its fate and biodistribution is thought to be significantly different from the free and protein-bound fractions investigated with standard sterile solutions. From an efficacy standpoint, the drug should stay associated to the carrier to be co-delivered in the tumor, and released according to a timescale consistent with the inhibition of cancer cells multiplication. At the same time, from a safety standpoint, the nano-encapsulated drug should not accumulate in undesired organs, in particular those known to capture the nano-carriers, such as the liver. The anticancer drug cabazitaxel encapsulated in PLA-PEG nanoparticles was chosen as a case study. We showed that the association of the drug to the nano-carrier can be satisfactorily assessed from a partition coefficient (Kp) of cabazitaxel between the PLA matrix and the suspending medium.

References:

Diou O, Greco S, Beltran T, Lairez D, Authelin J-R, Bazile D. A method to quantify the affinity of cabazitaxel for PLA-PEG nanoparticles and investigate the influence of the nano-assembly structure on the drug/particle association. Pharm Res 2015; 32: 3188–3200.

Harivardhan Reddy L, Bazile D. Drug delivery design for intravenous route with integrated physicochemistry, pharmacokinetics and pharmacodynamics: illustration with the case of taxane therapeutics. Adv Drug Deliv Rev 2014; 71: 34–57.

Harivardhan Reddy L, Bazile D. Building the design, translation and development principles of polymeric nanomedicines using the case of clinically advanced poly(lactide(glycolide))–poly(ethylene glycol) nanotechnology as a model: An industrial viewpoint. Adv Drug Deliv Rev 2016; 107: 289-332.

Dr. Christof Fellmann

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CV:

Dr. Christof Fellmann is a postdoctoral fellow in the laboratory of Dr. Jennifer A. Doudna at the University of California, Berkeley, USA, and a US National Institutes of Health (NIH) Pathway to Independence Awardee of the National Institute of General Medical Sciences (NIGMS). After under- graduate training in Switzerland and an engineering degree in biotechnology from the Ecole Supérieure de Biotechnologie Strasbourg (ESBS), France, he carried out his PhD work in the laboratory of Scott Lowe at the Cold Spring Harbor Laboratory, New York, USA. His research focuses on the molecular mechanism and therapeutic application of RNA-guided immune systems, including RNA interference (RNAi) and CRISPR–Cas tools. He is a co-founder and former Chief Scientific Officer of Mirimus Inc., a start-up company developing genetically engineered mouse models of human disease for early-stage drug discovery. Christof enjoys the outdoors and is a passionate triathlete.

Abstract:

“Cornerstones of CRISPR-Cas in Drug Discovery and Therapy”

The recent development of CRISPR–Cas systems as easily accessible and programmable tools for genome editing and regulation is spurring a revolution in biology. Paired with the rapid expansion of reference and personalized genomic sequence information, technologies based on CRISPR–Cas are enabling nearly unlimited genetic manipulation, even in previously difficult contexts, including human cells. Although much attention has focused on the potential of CRISPR–Cas to cure Mendelian diseases, the technology also holds promise to transform the development of therapies to treat complex heritable and somatic disorders. Here, we discuss how CRISPR–Cas can affect the next generation of drugs by accelerating the identification and validation of high-value targets, uncovering high-confidence biomarkers and developing differentiated breakthrough therapies. We focus on the promises, pitfalls and hurdles of this revolutionary gene-editing technology, discuss key aspects of different CRISPR–Cas screening platforms and offer our perspectives on the best practices in genome engineering.

Posters

P-1

Nutritional Assessment in Patients Affected by Mitochondrial Cytopathy (NAMITO Study)

E. Aubry[1], C. Aeberhard[1], L. Bally[1], S. Mühlebach[2], Z. Stanga[1]

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[2] *Department of Clinical Pharmacy and Epidemiology, University of Basel, 4003 Basel, Switzerland*

Introduction: Patients suffering from mitochondrial cytopathy are at high risk for malnutrition. They often suffer from gastrointestinal symptoms (e.g. dysphagia, intestinal dysmotility, gastroparesis), which considerably influence nutritional intake and therefore deteriorate nutritional state. Literature in this regard is very sparse.
Aims: Aim of the present study was to close this gap, to evaluate a simple screening tool for protein energy malnutrition (PEM) and conduct an extended nutritional assessment to explore a potential presence of PEM in this patients' population compared to matched controls.
Methods: Prospective observational cohort study comparing outpatients with mitochondrial cytopathies to healthy controls. Nutritional screening (NRS-2002) and full nutritional assessment were conducted, including quantitative and qualitative analysis of dietary habits (7-days food recall protocol), body composition measurements (Bioimpedance analysis and anthropometrics), rest energy expenditure (indirect calorimetry) and quality of life (QoL) questionnaire (SF36v2). Blood and 24-h urine analyses were completed in the patients' group. The study was approved by the Ethics Committee (KEK-BE 242/2014) and registered on ClinicalTrials.gov (NCT02375438)
Results: Twenty-six patients were included, 11 in the patients' group (7 men, 4 women) and 15 in the control group (8 men, 7 women). No patient was screened at high risk for malnutrition according to the NRS 2002. Nutritional assessment showed, that patients had inadequate energy intake and significantly lower protein intake. Nitrogen balance and creatinine height index showed pathologic values. Body composition and function were altered as well as QoL score.
Conclusions: According to the ESPEN guidelines, all patients were malnourished. Thus, the NRS 2002 appears to be too less sensitive for outclinic chronic ill patients. There is a rational to increase protein intake and to adapt energy supply to improve disease-related symptoms and QoL. Further studies should investigate the potential positive influence of dietary management on the course of the disease.

Keywords: mitochondrial cytopathy, malnutrition, nutritional screening, nutritional assessment.

P-2

Is Testing for Postprandial Hyperinsulinemic Hypoglycemia After Gastric Bypass Necessary?

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[2] *European Center of Excellence for Bariatric and Metabolic Surgery, 3010 Bern, Switzerland*

Introduction: Bariatric surgery is the most efficient and only durable treatment of severe obesity. Standard Roux-en-Y gastric bypass (RYGB) takes an intermediate position in the risk-benefit ratio and is the operation of choice for thousands of patients. Most long-term sequels are well known and require a lifelong patient follow-up. One increasingly reported complication is the postprandial hyperinsulimic hypoglycemia (pHH) which can cause life threatening emergencies without warning symptoms. Provocative testing can detect patients at risk.
Aims: The aim of this study was to determine the prevalence of pHH after RYGB with or without symptoms of hypoglycemia.
Methods: Observational cohort study of consecutive, unselected patients 11 to 28 months after uncomplicated laparoscopic standard RYGP. In order to simulate normal habits all patients received a carbohydrate-rich standardized solid mixed meal. Insulin and glucose were measured at 30, 60, 90, 120 and 150 min thereafter. Symptoms were recorded and classified as autonomous or neuroglycopenic. Patients with hypoglycemia, defined as blood glucose of < 3.3 mmol/L (60 mg/dL), were tested a second time within a week with a protein rich standardized solid mixed meal.
Results: A total of 113 consecutive, non-selected patients were included. Total postoperative weight loss was 33.97 ± 9.3%. In 24.8% of patients glucose dropped to less than 3.3 mmol/L (60 mg/dL), 13.8% to less than 3.0 mmol/L (55 mg/dL) after the carbohydrate solid mixed meal in contrast to only one patient after a protein rich meal (0% with less than 3.0 mmol/L (55 mg/dL)). Only 40.7% showed hypoglycemic symptoms. One patient needed emergency treatment after sudden loss of consciousness 80 min after the carbohydrate meal. Asymptomatic patients carry a significant risk ($p < 0.01$) for pHH.
Conclusions: pHH after RYGB can be life threatening and occurs without warning symptoms. Therefore, testing all patients is necessary. How, when and how often remains to be investigated. A standardized solid food test is an option close to daily life situations and patients can be counselled according to the obtained results.

Keywords: post prandial hyperinsulinemic hypoglycemia, gastric bypass, provocative testing.

P-3

Intranasal Administration of Resveratrol Successfully Prevents Lung Cancer in A/J Mice

A. Monteillier, P. Furrer, E. Allémann, M. Cuendet

School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland

Introduction: Resveratrol, a phytoalexin found in various foods such as grapes, is one of the most studied natural products. It displayed several biological activities including cancer chemoprevention. However, the low bioavailability of resveratrol often limited the translation of the *in vitro* activities to *in vivo* studies. For example, oral administration of resveratrol effectively inhibited carcinogenesis in the digestive tract, but failed to protect mice from chemically-induced lung carcinogenesis [1,2]. This failure was attributed in part to the metabolism that undergoes resveratrol when taken orally. Therefore, other non-invasive administration routes must be considered to bring sufficient doses to the lungs, and the pulmonary route seems the best one.
Aim: The aim of this work was to investigate intranasal instillation of resveratrol in lung cancer chemo-prevention in A/J mice.
Methods: In order to overcome the low hydrosolubility of resveratrol, a human transposable formulation was designed using 200 mM hydroxypropyl-betadex in saline solution. After demonstrating the efficacy of both this formulation and the intranasal administration route in delivering a high amount of resveratrol into the lungs, this formulation was administered 3 times a week during 26 weeks to A/J mice having 4-[methyl(nitroso)amino]-1-(3-pyridinyl)-1-butanone (NNK)-induced lung carcinogenesis. After sacrifice, lungs were harvested, tumor were counted and measured under a dissecting microscope. *In vitro* experiments were conducted on A549 cells in the hope of understanding the mechanism of action of RES.
Results: Resveratrol-treated mice showed a 27% decrease in lung tumor multiplicity, with smaller tumors, resulting in 45% decrease in tumor load. Resveratrol administration also reduced by 57% the incidence of spontaneous tumors in non-NNK treated mice. Further *in vitro* investigations revealed a dose dependent increase in NNK-induced-H_2AX phosphorylation after resveratrol treatment, highlighting DNA repair modifications as a possible mechanism of action.
Conclusions: The present results lead to the hypothesis that resveratrol low oral bioavailability may be responsible for the lack of activity observed in former studies. Overall, this study supports the interest in intrapulmonary administration of resveratrol for further clinical development in lung cancer chemo-prevention.

Keywords: resveratrol, lung cancer, chemoprevention, intranasal instillation.

References:
[1] Saud S et al. Carcinogenesis 2014; 35 (12): 2778-2786.
[2] Hecht SS et al. Cancer Lett. 1999; 137 (2): 123-30.

Antiproliferative Activity of Compounds Isolated from *Lannea acida* Root Bark in Multiple Myeloma Cancer Stem Cells

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Introduction: In the course of the investigation of plants used in traditional medicine in Niger, 28 species were screened for their antiproliferative activity in multiple myeloma cancer stem cells (MM-CSCs). *Lannea acida* A. Rich. (Anacardiaceae) is frequently reported against various diseases such as diarrhea, nausea, amnesia, women sterility, dizziness and as a fortifier [1]. There are very few studies on the phytochemical investigation of *Lannea* spp. [2-3]. Based on this and the complete growth inhibition of MM-CSCs induced by the dichloromethane root bark extract of *L. acida* at 20 µg/mL, this extract was selected for further investigations.
Aims: The aim of this project was to isolate compounds inhibiting MM-CSC proliferation.
Methods: A bioassay-guided fractionation of the extract was performed using a normal phase semi-prep. HPLC-UV/ELSD. Fractions were tested in MM-CSCs at 20 µg/mL and were considered active when the cell proliferation was < 50%. Compounds were then isolated and identified from active fractions using MS, NMR, UV and IR. Their activity in MM-CSCs was measured at 20 µM and IC_{50} values were determined.
Results: The extract fractionation afforded 131 fractions. At 20 µg/mL, fractions 21 to 28 and 88 to 111 completely inhibited MM-CSCs proliferation. The latter were selected for further purification and yielded four compounds (**1**-**4**) (Figure 1). Compounds **1**-**4** had IC_{50} values around 10 µM.
Conclusions: Four new compounds were isolated. They presented a good activity against MM-CSCs, a type of cells resistant to conventional chemotherapy. Further investigations are ongoing to isolate other compounds and characterize the mechanism of action.

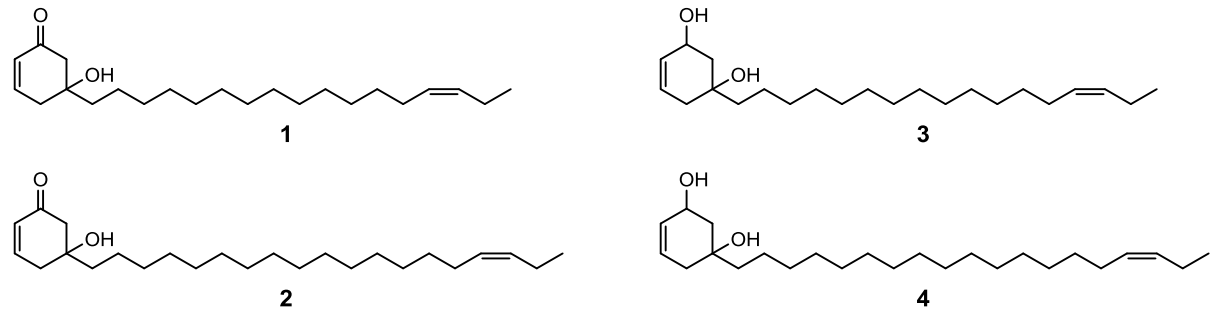

Figure 1: Compounds isolated and their antiproliferative activity in MM-CSCs.

Keywords: *Lannea acida*, multiple myeloma cancer stem cells, bioassay-guided fractionation.

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New Lead Compounds for Drug- Induced Differentiation from Blood Stream to Procyclic Trypanosome Forms Identified by Whole-Cell HTS Assays

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Introduction: *Trypanosoma brucei spp*. causes a neglected tropical disease in Africa which is lethal in humans or resulting in economic loss in livestock.
Aims: Utilizing a novel screening approach exploiting the life cycle of *T. brucei* in whole-cell HTS assays based on a transgenic trypanosome line expressing a reporter gene upon specific triggers for parasite differentiation a compound library had been screened, and 52 hits that induced loss of variant surface glycoproteins (VSG) in the low µM range had been identified [1].
Methods: To prioritize compounds for drug-likeness, and in the context of the 3Rs concept [2], we performed an *in silico* analysis of the 52 hits using ACD/labs Percepta and QikProp Schrodinger. Five compounds fulfilling Lipinski's rule of five, Veber rules, and recommended values for CNS drugs were selected for further *in vivo* pre-toxicity tests and snapshot pharmacokinetic (PK) studies in NMRI mice. Of these, 3 compounds with best PK properties were selected for *in vivo* efficacy studies.
Results: Two lead compounds strongly reduced the parasite load in *T. brucei rhodesiense* infected mice, and thereby confirmed the concept for efficacy.
Conclusions: These compounds that induce differentiation to procyclic forms could facilitate studies on the regulation of differentiation, and serve as starting points for medicinal chemistry optimization of novel treatments of human (sleeping sickness) and animal (nagana) African trypanosomiasis.

Keywords: *Trypanosoma*, whole cell HTS assay, *in silico*, snapshot PK studies, medicinal chemistry.

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Targeted Nanocarrier-Mediated Ocular Delivery of Spironolactone to Improve Corneal Wound Healing: Demonstrating Tolerability and Efficacy *In Vivo*

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Introduction: Glucocorticoids (GC) are widely prescribed to treat inflammatory and autoimmune diseases. In ophthalmology, they are used to treat post-operative ocular inflammation and to prevent corneal graft rejection after transplant surgery. However, GC can also delay normal wound healing processes leading to chronic corneal ulcers. This GC-induced delayed wound healing has been attributed to off-target over-activation of the mineralocorticoid receptor (MR). Hence, a new therapeutic strategy might involve co-administration of MR antagonists thereby preventing off-target GC binding to the MR. The aim of this study was to evaluate the tolerability and efficacy of a topically administered micelle formulation of a potent MR antagonist, spironolactone, in countering the effects of the potent GC, dexamethasone, on corneal wound healing in New Zealand white rabbits *in vivo*.

Aims: The aim of this study was to develop a nanomicellar formulation of the potent MR antagonist, spironolactone for topical application and evaluate the tolerability and efficacy of this formulation in countering the effects of the potent GC, dexamethasone, on corneal wound healing in New Zealand white rabbits *in vivo*. The biodistribution of spironolactone and its metabolites in the cornea was also investigated.

Methods: A micelle formulation of spironolactone (0.1%) made using methoxy-poly(ethylene glycol)-dihexyl-substituted-poly(lactic acid) (mPEG-hexPLA, 5.5 kDa) was developed and characterized. After induction of anaesthesia and subcutaneous administration of buprenorphine, corneal wounds were induced in the right eye of 50 New Zealand white rabbits using a scalpel blade. The rabbits were randomized into 5 groups (n=10 per group). The animals in each group were instilled using an eye-dropper (~35 µL) in their right eye 3x daily on day 0, 6x daily on days 1-4 and once on day 5 according to the following treatment protocols: animals in groups 1-3 received one drop of either 0.01% or 0.1% spironolactone micelles or 0.1% potassium canrenoate (a water-soluble precursor of canrenone, another MR antagonist), followed by Maxidex® (0.1% dexamethasone suspension). Group 4 was the positive control and animals received only PBS whereas rabbits in group 5 (negative control) received only Maxidex®. Ocular tolerability was followed with an ophthalmoscope and re-epithelialization was evaluated using fluorescein staining. At the end of the study, animals were euthanized and corneas were harvested for evaluation of biodistribution and quantification of the drug and metabolites using UHPLC-ESI-MS.

Results: The 0.1% spironolactone micelles (mean diameter ~20 nm) showed a mid-term stability of at least 6 months at 5°C. *In vivo* studies demonstrated that they were well-tolerated following multiple daily instillations over 5 days with no noticeable ocular reaction. After the 5-day treatment period, the 0.1% spironolactone micelle formulation showed a beneficial effect on the healing of dexamethasone-induced corneal wounds with a 98.2±3.9% re-epithelialization – statistically equivalent to the positive control (PBS treatment alone – 100.0 ± 0.0%); re-epithelialization of the GC-induced corneal wounds in the absence of spironolactone was 88.4 ± 14.3%. The biodistribution study demonstrated that spironolactone was metabolized to 2 active metabolites, 7α-thiomethylspironolactone and canrenone. The greater potency of the former pointed to a more important role in countering GC over-activation. *In situ* lactonization of canrenoate to canrenone was also observed.

Conclusions: These preclinical *in vivo* results highlight the effect of the co-administration of the MR antagonist, spironolactone, in off-setting GC-induced delays in wound healing. Successful translation of these results to the clinic may improve therapeutic outcomes for GC-treated patients since topical instillation of the spironolactone micelles might counter the impaired wound healing side-effects associated with routine GC therapy.

Keywords: glucocorticoids, corneal wound healing, spironolactone, polymeric nanocarriers, pre-clinical tolerability and efficacy.

Metabolite Profile and Antiproliferative Effects in HaCaT Cells of a *Salix Reticulata* Extract

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Introduction: *Salix* species (Salicaceae) have been traditionally used for their anti-inflammatory, analgesic and antipyretic properties [1]. The pro-apoptotic effect of *Salix* extracts on cancer cells has been reported [2, 3]. Such anti-inflammatory and pro-apoptotic effects would be highly desirable for skin diseases involving cellular hyperproliferation, e.g. as in psoriasis.
Aims: The aim of this work was to assess the antiproliferative effects of a MeOH extract from aerial parts of *S. reticulata* in HaCaT cells. In a further step the activity of selected constituents should be determined.
Methods: The metabolite profile of the MeOH extract was determined by HPLC-UV-MS analysis. Compounds were identified by extensive NMR analysis after targeted isolation. Cell viability was determined in an ATP assay. Cell proliferation was assessed with a BrdU incorporation ELISA assay. Time lapse assays were used for measuring cell migration.
Results: Several flavonoids, including luteolin and apigenin glycosides and catechin, two procyanidin fractions, and the phenolic glucosides picein, triandrin, and salicortin were identified in the MeOH extract. The extract reduced cell viability by approx. 60% at a concentration of 100 μg/mL. The proliferation of HaCaT cells was inhibited in a concentration dependent manner, with an approx. 50% inhibition at 100 μg/mL. In time lapse assays, the extract showed distinct inhibitory effects on cell migration at concentrations of 12.5, 25 and 50 μg/mL. Among the constituents, luteolin-7-O-β-glucuronide significantly inhibited cell proliferation at concentrations of 10 and 50 μM. In contrast, luteolin-7-O-β-glucopyranoside and a procyanidin fraction had only weak effects, while picein and salicortin did not affect cell proliferation. Luteolin-7-O-β-glucuronide (10 μM) and, to a lesser extent, the procyanidin fraction (10 μg/mL) also inhibited cell migration.
Conclusions: The MeOH extract of *S. reticulata* significantly inhibited the growth and migration of human keratinocytes *in vitro*. The activity could be attributed, at least in part, to luteolin-7-O-β-glucuronide. In psoriasis, keratinocytes seem to be resistant to apoptosis which, in conjunction with an increased keratinocyte turnover rate, results in epidermal hyperproliferation. Thus, S*. reticulata* extract or single compounds that are able to inhibit keratinocyte proliferation and induce apoptosis would have potential as a local treatment against hyperproliferation-involved skin diseases such as plaque psoriasis.

Keywords: *Salix reticulata*, luteolin-7-O-β-glucuronide, antiproliferative activity, cell migration.

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L1CAM: A Potential Molecular Marker for Ovarian Cancer Stem Cells?

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Introduction: Accumulating evidence indicates that many solid tumors, including ovarian cancer (OC), contain small populations of tumor-initiating cells, also known as cancer stem-cells (CSCs). Overlapping cell populations displaying high aldehyde dehydrogenase (ALDH) activity in combination with the expression of different cell surface markers such as CD24, CD44 and CD133, have been characterized as ovarian CSCs. These cells usually show high resistance against conventional cancer therapies and seem to be involved in metastatic process and tumor relapse [1]. An important feature of CSCs is their ability to proliferate under non-differentiating and non-adherent conditions, forming three-dimensional multicellular tumor spheroids. Indeed, the cells forming these spheroids are very aggressive in growth and show higher resistance to chemotherapeutic drugs and radiation *in vitro.* L1 cell adhesion molecule (L1CAM) has been recently shown to be a CSC-specific marker in glioblastoma [2]. L1CAM is a highly glycosylated type I transmembrane protein that plays a role in the development of the nervous system and contribute to human cancer malignancy. In cancer, L1CAM expression induces a motile and invasive phenotype, supporting aggressive tumor growth, metastasis and chemoresistance.
Aims: Based on previous findings, the aim of our research is to elucidate the biological role of L1CAM as potential ovarian CSC molecular marker.
Methods: Specific populations of cells expressing L1CAM alone or in combination with another putative CSC marker (i.e. CD133) were isolated by fluorescence-activated cell sorting (FACS) from various established OC cell lines. Plating efficiency and radioresistance were assessed by colony-forming assay. 3D cell culture has been employed to enrich for potential CSCs and therefore to investigate the spherogenic capacity of the different cell populations. Tumor take was assessed *in vivo* using CD-1 nude mice.
Results: The results indicated that L1CAM+/CD133-population has higher spherogenic and clonogenic properties in comparison to L1CAM-/CD133- cell population. We found that the cell subset defined by the expression of both L1CAM and CD133 has the highest clonogenic and spherogenic capacity. Moreover, L1CAM+/CD133+ cell population retains the highest congenic capacity after irradiation, indicating high radioresistance of these cells. Interestingly, these double positive cells showed higher tumor take in nude mice when compared to the other cell populations.
Conclusions: These results indicate that L1CAM, in combination with CD133, might define a specific population of ovarian CSCs.

Keywords: L1 cell adhesion molecule (L1CAM), ovarian cancer (OC), cancer stem cells (CSCs), fluorescence-activated cell sorting (FACS).

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P-9

The Risk of Incident Osteoarthritis of the Hand in New Users of Statins: A Propensity Score-Matched Sequential Cohort Study

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Introduction: Preclinical evidence suggests a protective effect of statins on the risk of hand osteoarthritis (HOA) presumably via pleiotropic anti-inflammatory mechanisms. However, evidence from observational studies to assess if such a potential effect translates into clinical practice is scarce and inconclusive.
Aims: We aimed to investigate the association between new statin use and incident HOA.
Methods: We performed a propensity score-matched sequential cohort study using data from the UK-based Clinical Practice Research Datalink, a large and well validated primary care database. Statin users had ≥1 statin prescription between 1996 and 2015 and were matched 1:1 on their propensity score to non-users within 10 sequential 2-years cohort entry blocks. Patients were aged 45-84 years and had a ≥3 years statin-free active history prior to cohort entry. After a 180-days run-in period (allowing statin users to reach maintenance dose and excluding all patients with a follow-up ≤180 days), patients were followed in an "as-treated" approach until a recorded diagnosis of HOA or until censoring (change in exposure status, maximum follow-up 5.5 years). We applied Cox proportional hazard regression to calculated hazard ratios (HR) with 95% confidence intervals (CI) overall and in subgroups of age, sex, daily statin dose, statin agent, absence or presence of hyperlipidaemia, and duration of follow-up. Moreover, we ran all analyses with negative control outcomes (psoriasis, tinnitus) and positive control outcomes (myopathy, composite cardiovascular outcome) to validate our findings and study population, respectively. We further performed the overall analysis with an active comparator approach (investigating the association of HOA and new statin use when compared to new topical glaucoma therapy use) and thereby assessed potential surveillance bias.
Results: Among 237'864 statin users and the same number of non-users, we observed an overall HR of 0.98 (95% CI 0.88-1.09). The observed null result remained unchanged in all subgroups. Results were highly similar in negative control outcomes and showed known associations with positive control outcomes. Also the active comparator analysis showed a null result with a HR of 0.85 (95% CI 0.56-1.29), though lacking power due to limited number of glaucoma therapy initiators (n=18,571).
Conclusions: The results of this large observational cohort study do not suggest a protective effect of statins on the risk of HOA. Given that previous studies observed different results, our study highlights the need for rigorous control of confounding when studying statins in large electronic databases.

Keywords: HMG-CoA reductase inhibitor, osteoarthritis of the hand, propensity score, control outcomes.

P-10

Therapeutic Protein Injected Subcutaneously: Understanding *In Situ* Aggregate Formation

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Introduction: While more and more biologicals are reaching the market, extensive stability studies remain essential to improve the formulation of new candidates as the aggregation tendency is a protein-dependent phenomenon. Once the optimal formulation is found, long-term stability issues still remain. *In situ* aggregates can be formed at the injection site due to changes in the protein microenvironment: pH, temperature, ionic interactions, etc. [1]. Moreover, these aggregates, due to their size, shape and the epitopes they expose could be recognized as foreign bodies by the innate immune system, leading to various adverse events such as injection-site reactions.
Aims: Our aim is to better understand the formation of these protein aggregates in the subcutaneous space and their interaction with immune cells.
Methods: For this purpose, we chose a model therapeutic protein injected subcutaneously three times per week, called rhIFNalpha2b and commercialized under the trade name Intron A®. We performed a stability study applying five different stress conditions to the native protein. We monitored biophysical characteristics, including conformational changes, with orthogonal methods such as: circular dichroism, anisotropy and intrinsic fluorescence spectroscopy, dynamic light scattering, and UV-visible spectroscopy. The protein biological activity was also tracked prior to and after forced aggregation using an antiviral proliferation assay with human lung epithelial cells (A549) challenged with vesicular stomatitis virus tagged with green fluorescent protein. Finally, a three-dimensional cell culture model mimicking the human subcutaneous tissue was developed. Therefore, we selected various hydrogels into which dendritic cells were embedded. Cells were maintained in culture for three days and their viability was monitored daily using cell proliferation reagent WST-1. Hydrogels' elastic Young's moduli in compression were correlated to *ex vivo* human subcutaneous tissue samples using a TA-XT*plus* Texture Analyzer.
Results: Results obtained from the orthogonal methods previously mentioned were combined in seven-axes radar charts where each axis corresponds to one parameter defined as representative of the degree of conformational change. The various patterns obtained on the radar charts were stress- and concentration-dependent. Regarding the bioactivity assay, we observed that only aggregates obtained after thermal stress exposure lost their antiviral ability. Results indicate a good cytocompatibility of the hydrogels. Measured elastic Young's moduli on human subcutaneous fatty tissue were in accordance with the literature [2] and our measurements on hydrogels.
Conclusions: Further studies are needed to combine the injection of protein aggregates within hydrogels containing dendritic cells, in order to follow the uptake of these aggregates and the pro-inflammatory cytokines released in response by the cells.

Keywords: therapeutic protein, aggregation, immunogenicity.

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In Vitro Effect on Myometrial Contractility of a Combination of *Bryophyllum pinnatum* Press Juice and Atosiban

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Introduction: The herbal medicine *Bryophyllum pinnatum* has been used as a tocolytic agent in anthroposophic medicine [1] and, recently, in conventional settings alone or as an add-on medication with tocolytic agents such as atosiban.
Aims: Our aim was to investigate the effects of *B. pinnatum* leaf press juice and atosiban, alone and in combination, on the contractility of myometrial tissue obtained from women undergoing Caesarean section.
Methods: Myometrial biopsies were collected during elective Caesarean section. Myometrial strips were placed under tension into a myograph chamber, and spontaneous contractions were recorded. After a 30-min period of regular contractions, *B. pinnatum* press juice (0.08 or 0.25%, i.e. corresponding to 0.8 and 2.5 µg/mL, respectively), atosiban (0.53 or 0.27 µg/mL), or the combination of both (Comb1= 0.08% *B. pinnatum juice* + 0.53 µg/mL atosiban; Comb2= 0.25% *B. pinnatum juice* + 0.27 µg/mL atosiban) were added to the chamber. Area under the curve (AUC) of contractions was determined as a measure of contractions strength. Results are expressed as percentage of initial value.
Results: All test substances inhibited myometrium contractility, i.e. they led to significantly lower AUCs compared to control (in all cases $p < 0.05$). When Comb1 was tested, contractions intensity lowered to 55.6 ± 13.0% (n = 15). This was not significantly different from atosiban effect alone (46.3 ± 20.8%; n = 14). When Comb2 concentrations were tested, *B. pinnatum* press juice decreased contractions down to 73.0 ± 8.8%, atosiban to 86.0 ± 4.9%, and the combination to 55.2 ± 12.6%. Thus, the inhibitory effect of Comb2 was significantly higher than atosiban alone at the correspondent concentration ($p < 0.05$).
Conclusions: *B. pinnatum* and atosiban inhibit spontaneous myometrial contractions *in vitro*. The inhibitory effect of their combination is dependent on the concentrations used, and at a high *B. pinnatum* to atosiban ratio (Comb2), the effect is significantly higher than that of atosiban alone. Therefore, a combination of both substances in the clinical practice appears promising.

Keywords: *Bryophyllum pinnatum*, atosiban, myometrium, tocolysis, *in vitro.*

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Withanolide D Exhibits Similar Cytostatic Effect in Drug-resistant and Drug-Sensitive Multiple Myeloma Cells

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Introduction: Despite recent progress in the treatment of multiple myeloma (MM), this malignancy remains incurable. This incurability has been attributed to the existence of a resistant cancer cell subpopulation termed cancer stem cells (CSCs). One CSC characteristic is high expression of ABC efflux transporters, a feature that renders these cells resistant to virtually all conventional therapies. Thus, the identification of compounds with antiproliferative activity independent of efflux may help tackling MM-CSCs. Recent evidence suggests that withanolides, a group of steroidal lactones found in *Withania somnifera*, are capable of targeting resistant CSCs in a variety of tumor models.
Aims: The aim of this project was to investigate the cytostatic effect of withanolide D (WND) in drug-resistant and drug-sensitive MM cells.
Methods: Highly tumorigenic MM-CSCs ($CD44^+$, $CD166^+$ and $CD138^-$), dexamethasone-resistant (MM.1R), RPMI-8226, and dexamethasone-sensitive (MM.1S) myeloma tumoral cells were used to study the antiproliferative effect of withanolide D using MTT/XTT assays. In MM-CSCs and RPMI 8226 cells, the antiproliferative effect of WND with or without the P-glycoprotein inhibitor verapamil was examined using MTT/XTT assays. The direct and indirect effect of WND on P-gp efflux was also examined using the rhodamine 123 efflux assay and mRNA expression, respectively.
Results: WND exhibited similar IC_{50} values in drug-resistant and drug-sensitive MM cells, and the use of verapamil did not significantly lower the IC_{50} values of WND in resistant MM cells. WND did not inhibit rhodamine 123 efflux, nor did it affect P-gp mRNA expression at relevant cytostatic doses.
Conclusions: WND similarly inhibited the proliferation of drug-resistant and drug-sensitive MM cells, and this effect was likely independent of the P-gp efflux activity. WND may not inhibit the activity of P-gp directly or indirectly. These results warrant further investigation of WND in additional MM-CSCs models and potentially in *in vivo* models.

Keywords: multiple myeloma, withanolide D, cancer stem cells, resistance.

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Targeted Iontophoretic Delivery of Buflomedil Hydrochloride for Oral Submucosal Fibrosis

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Introduction: Constant-current buccal iontophoresis is a controlled non-invasive technique used to increase the delivery kinetics and bioavailability of hydrosoluble ionisable drugs in the buccal mucosa. We have previously demonstrated that short duration iontophoresis can be used for the buccal delivery of chemotherapeutics for the treatment of head and neck cancer [1].
Aims: The aim of the present study was to investigate the topical iontophoretic delivery of buflomedil hydrochloride (BUF) to the buccal mucosa for the local treatment of oral submucosal fibrosis. Although oral administration of BUF in conjunction with adjunct therapy has been shown to relieve symptoms of the disease there is a serious risk of systemic side-effects [2].
Methods: Transport experiments were conducted using porcine oesophageal mucosa, which is an excellent surrogate for human buccal mucosa [3]. Full thickness mucosa (~1.5 mm) was clamped in vertical Franz diffusion cells (area = 2.0 cm^2). Unbuffered BUF solution (20 mM, pH~5.5) or BUF gel (20 mM, 2% HEC gel, pH~5.8) was placed in the donor compartment and 12 mL PBS(pH 7.4) was filled in the cathodal(receiver) compartment. A constant current density of 1 mA/cm^2 was applied for 10 min using a power supply (Kepco®, Flushing, NY) and Ag/AgCl electrodes. After 10 min, mucosa in contact with the formulation was punched out, snap-frozen and cryotomed using a Microm HM 560 Cryostat (Thermo Scientific; Walldorf, Germany) to obtain a series of 8 lamellae (thickness = 40 µm). The lamellae and the remaining mucosa were extracted overnight with MeOH:H_2O (75:25 v/v). BUF was quantified by UHPLC-MS/MS using a Waters Acquity® UPLC® Xevo® TQ-MS system. Gradient separation was achieved using Waters XBridge® BEH C18 (50 x 2.1 mm, 2.5 µm) RP column (temp.: 40°C). The mobile phase consisted of 0.1% formic acid in acetonitrile and 0.1% formic acid. The limit of quantification was 5 ng/mL and the run time was 2 min.
Results: Passive deposition of BUF in the epithelium and lamina propria (connective tissue) of the mucosal tissue after a 10 min application of the 20 mM solution or 2% HEC gel formulations was 16.2 ± 5 and 3.6 ± 1.9 $\mu g/cm^2$, respectively, indicating superior delivery from solution. Anodal iontophoresis for 10 min at 1 mA/cm^2 resulted in significant increases in BUF deposition from the two formulations, 305.2 ± 101.0 and 315.2 ± 24.1 $\mu g/cm^2$, respectively, with no statistically significant difference between the two formulations (Student's t-test). Passive BUF deposition in the epithelium, which is the target compartment, was 13.7 ± 4.6 and 2.6 ± 1.8 $\mu g/cm^2$ from the solution and gel formulations, respectively. The quantity of BUF deposited in the epithelium increased significantly after iontophoresis, with 201.9 ± 99.5 and 198.4 ± 19.3 $\mu g/cm^2$ for solution and gel formulations, respectively.
Conclusion: The results demonstrated that short duration anodal iontophoresis significantly increased local buccal bioavailability of BUF and produced a more homogeneous and deeper drug penetration than simple passive diffusion. The presence of the electric potential gradient in addition to the concentration gradient clearly worked to enhance BUF delivery. In addition to improving bioavailability and efficacy, topical delivery decreases risk of systemic exposure and side-effects, which could also improve patient compliance.

Keywords: iontophoresis, buccal mucosa, oral submucous fibrosis.

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LST-3TM12 is a Splice Variant of OATP1B3 and a Functional Transporter

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Introduction: Membrane proteins facilitating cellular entry and efflux are key determinants of pharmacokinetics. Two uptake transporters extensively studied for their role in hepatocellular handling of drugs, are OATP1B3 and OATP1B1. These two transporters are encoded by *SLCO1B3* and *SLCO1B1,* those genes are located on chromosome 12. Between *SLCO1B3* and *SLCO1B1* another transporter gene is located, namely *SLCO1B7*. This gene locus is considered to be a pseudogene, since no function of the encoded protein OATP1B7 has been reported. However, there are two previously published mRNA sequences, namely *LST-3TM12* (NCBI: AY257470) and *LST-3b* (NCBI: A442325.1), linked to *SLCO1B7*.
Aims: It was the aim of this study to further investigate *SLCO1B7* (OATP1B7), *LST-3TM12*, and *LST3-b* by *in silico* analysis. Furthermore, expression of related protein products was studied in human tissue. Finally, functionality of the putative transporters OATP1B7 and LST-3TM12 was assessed by heterologous expression assays.
Methods: *In silico* analysis of *SLCO1B7*, *LST-3TM12,* and *LST-3b* was performed by sequence alignment, and prediction of 2D- and 3D-models. Expression was determined by real time PCR, Western blot analysis and immunohistological staining. Functional assessment of OATP1B7 and LST-3TM12 transporter was performed by heterologous expression assays in HeLa-cells.
Results: Our *in silico* analysis revealed that *LST-3TM12* and *LST-3b* have the same coding sequence, and that they are a product of splicing of the mRNAs encoding for *SLCO1B3* and *SLCO1B7*. 2D- and 3D-prediction models suggested that OATP1B3 and LST-3TM12 consist of 12 transmembrane domains (TMDs), while OATP1B7 exhibits only 11 TMDs. Real-time PCR showed mRNA expression of *SLCO1B7/LST-3TM12* in human liver. LST-3TM12 transporter and/or OATP1B7 are expressed in perivenous hepatocytes as shown by immunohistological staining. In heterologous expression assays of OATP1B7 and LST-3TM12 transporter we found that LST-3TM12 transports dehydroepiandrosterone. No transport activity of the herein tested substrates was observed for OATP1B7.
Conclusions: Taken together, we report that *LST-3TM12* is a splice variant of *SLCO1B3* and *SLCO1B7* and that the resulting mRNA is expressed in human liver. The LST-3TM12 transporter and/or OATP1B7 are mainly expressed in human liver tissue. LST-3TM12 functions as a transporter of dehydroepiandrosterone. Future studies are warranted to further characterize the role of this transporter in drug metabolism.

Keywords: SLCO1B3, SLCO1B7, LST-3TM12, solute carriers, organic anion transporting polypeptides.

In Vitro Effect on Myometrial Contractility of a Combination of *Bryophyllum pinnatum* Press Juice and Nifedipine

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Introduction: Herbal preparations of *Bryophyllum pinnatum* [1] have been used as tocolytic agent in anthroposophic medicine and, recently, in conventional settings as an add-on medication with tocolytic agents such as nifedipine.
Aims: Our aim was to investigate the effects of *B. pinnatum* leaf press juice and nifedipine, alone and in combination, on the spontaneous contractility of human myometrial tissue *in vitro*.
Methods: Myometrial biopsies were collected during elective Caesarean section. Four myometrial strips were placed under tension into a myograph chamber, and spontaneous contractions were recorded. After a 30-min period of regular contractions, Krebs solution (control; two strips) or nifedipine (final concentration 9 nM; two strips) was added and contractility was recorded for 30 min. To measure the effects of *B. pinnatum* alone and of the combination, *B. pinnatum* press juice (final concentration 0.25% corresponding to 2.5 µg/mL) was then added to all chambers, and contractions were recorded for 30 min. After a washout period, vitality of strips was observed for additional 30 min. Area under the curve (AUC) and amplitude of contractions were determined as a measure of the strength of contractions. Results are expressed as percentage of initial value.
Results: All test substances lowered the strength of myometrium contractility, i.e. they led to significantly lower AUC and to lower amplitude compared to control (in all cases $p<0.05$). Nifedipine lowered AUC to 71.9 ± 6.59%, and *B. pinnatum* decreased it to 78.7 ± 6.49%. The combination of *B. pinnatum* and nifedipine lowered the AUC to 36.5 ± 8.30%, which was significantly different from the effect of *B. pinnatum* or nifedipine alone. Nifedipine lowered the amplitude to 87.1 ± 8.63% of initial contraction, and *B. pinnatum* to 93.4 ± 6.36%. The combination of *B. pinnatum* and nifedipine lowered the amplitude to 63.0 ± 9.29%, which was significantly different from *B. pinnatum* or nifedipine alone.
Conclusions: *B. pinnatum* and nifedipine, alone or combined, exert inhibitory effects on the strength of spontaneous myometrial contractions *in vitro*. At the concentrations tested, the effect of the combination was significantly stronger than the effects of the *B. pinnatum* alone and of nifedipine alone. A combination of both substances in the clinical practice thus appears promising.

Keywords: *Bryophyllum pinnatum*, nifedipine, myometrium, tocolysis, *in vitro.*

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Estrone 3-Sulfate is Not a Substrate of the Rat Organic Anion Transporting Polypeptide 2 b1 (Oatp2b1)

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Introduction: Animal models are commonly used to determine the role of drug transporters *in vivo*, but often little is known about the rodent orthologues of the human transporters. Thus, it remains questionable if these models are fully adequate to predict human transporter-mediated drug disposition. One family of drug transporters that has been studied for their implication in pharmacokinetics is the family of organic anion transporting polypeptides (OATPs). The family member OATP2B1 exhibits broad tissue distribution and is known to transport various substrates including estrone 3-sulfate and statins. OATP2B1 is assumed to contribute to absorption and elimination of its substrates. In this context, various *in vivo* studies analyzed the impact of the rodent orthologue Oatp2b1 on drug distribution. However, hitherto no study has focused on the species specific differences comparing the rodent and human transporter.
Aims: Our study aimed to compare the rat and human orthologues, analyzing expression and function of both transporters.
Methods: In order to study the function of rat Oatp2b1, the cell line MDCK-Oatp2b1 was generated and presence of the protein was validated by Western blot analysis and immunofluorescence microscopy. Transport activity was quantified in uptake studies using radiolabeled compounds such as estrone 3-sulfate (E_1S). Different humanized Oatp2b1 plasmids were generated to analyze the impact of specific regions of the rat protein on transport function. Therefore, proteins were transiently overexpressed in HeLa cells using a vTF7 based expression system [1]. Protein expression was validated by immunofluorescence and by Western blot analysis after biotinylation of cell surface proteins.
Results: Even if we observed a transport of the OATP substrate bromosulfophthalein, comparing E_1S there was no significant uptake in presence of Oatp2b1. Contrary, human OATP2B1 significantly enhanced the intracellular accumulation of both substrates. For both transporters expression was validated by Western Blot analysis and immunofluorescent staining. Similar results were obtained after transient overexpression of both transporters in HeLa cells. Stepwise “humanization” of specific regions, namely transmembrane domain (TMD) 9, TMD10, or extracellular loop 5 of Oatp2b1 did not significantly change transport activity. Only the overexpression of the chimera Oatp2b1-OATP2B1 showed a slightly higher uptake of E_1S compared to control-transfected cells, nevertheless, not comparable to the transport activity of the human OATP2B1. Transport by the humanized Oatp2b1-variants was neither observed for E_1S, nor for dehydroepiandrosterone sulfate, another known OATP2B1 substrate. However, Western blot analysis of cell surface proteins and immunofluorescent staining showed an expression of all proteins.
Conclusions: Our study demonstrated that rat Oapt2b1 does not transport the known OATP2B1 substrate estrone 3-sulfate. Even though previous studies suggested that TMD9 and TMD10 may be responsible for substrate recognition of E_1S by OATPs we were not able to show this relation.

Keywords: OATP2B1/Oatp2b1, estrone-3 sulfate.

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Absolute Configuration of Sesquiterpene Lactones with Potent Immunosuppressive Activity

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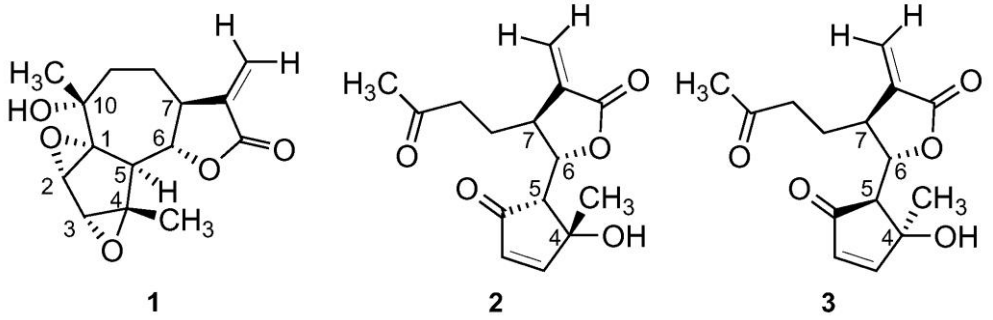
Introduction: In a screening of *Artemisia argyi* (Asteraceae) and subsequent HPLC-based activity profiling canin **1** and the stereoisomeric seco-tanapartholides **2** and **3** were identified as compounds with potent immunosuppressive activity *in vitro*. These isoprenoids were first discovered in 1969 and 1982, respectively, and have been extensively studied in the past for various biological activities [1]. Their molecular structure and relative configuration has been studied by means of NMR and X-ray crystallography [2], but the absolute configurations remained unresolved.
Aims: To establish the absolute configurations of compounds **1-3** by a combination of electronic circular dichroism spectroscopy (ECD) and vibrational circular dichroism spectroscopy (VCD).
Methods: The relative stereochemistry of compounds **1-3** was, as far as possible, determined by NOESY correlations. ECD spectra of **1-3** were measured and compared to spectra calculated *ab initio* for different possible stereoisomers. For compounds **2** and **3**, VCD spectra were recorded and compared to calculated spectra of possible stereoisomers.
Results: The absolute configuration of **1** was established as (1R,2S,3R,4S,5S,6S,7S,10R)-canin. For compounds **2** and **3** the ECD data lowered the number of possible configurational isomers to 4 stereo-isomers. The absolute configuration was finally established by VCD. Compound **2** was identified as (4R,5R,6S,7S)-seco-tanapartholide, and **3** as (4S,5S,6S,7S)-seco-tanapartholide.
Conclusions: The combination of ECD and VCD can be considered as a powerful approach for resolving the absolute configuration of conformationally flexible molecules.



1 **2** **3**

Keywords: Sesquiterpene lactones, absolute configuration, ECD, VCD

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P-18

In-Depth Metabolome Investigation of a Multi-Herb Formula Used in Traditional Chinese Medicine

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Introduction: In Traditional Chinese Medicine (TCM), the preparations consist generally in a mixture of several herbs. The quality control for such a multi-herb mixture is challenging because every single herb already contains hundreds of constituents. The TCM selected for this study is used to treat atopic dermatitis (atopic eczema), a common inflammatory skin disorder, treated in Western medicine by topical corticosteroids and emollients [1]. Recently, an open label clinical study showed the efficacy of this new TCM formula based on a mixture of ten herbs [2].
Aims: The aim of this work was to design an innovative strategy for a comprehensive and rational standardisation. It is based on an in-depth high resolution metabolite profiling of the TCM formula, localisation of chemical markers by data mining and targeted preparative isolation of standards directly from the complex TCM mixture.
Methods: For this investigation, the TCM formula and all single plant extracts were analysed by UHPLC-PDA-ELSD-HRMS. Potential chemical markers were selected by data mining. For a rational and rapid isolation, the TCM formula was fractionated after an enrichment procedure at large scale by MPLC. For this, the analytical HPLC-PDA-ELSD conditions were transferred geometrically to the preparative MPLC-UV-ELSD using chromatographic calculations, ensuring the same selectivity at both scales [3]. At-line high throughput UHPLC fingerprint of all fractions merged in a MPLC x UHPLC plot enabled the localisation and further purification of the target markers.
Results: Since the UHPLC-HRMS metabolite fingerprints of all crude extracts allow the detection of several thousand *m/z* features, a strategy was elaborated to filter all this information and select key biomarkers for further quality control studies. Peak picking was performed on all single plants and on the TCM formula extracts. A differential analysis of the highlighted features provided an efficient way to correlate them to each herb in the mixture. ELSD detection was used as a filter for a selection of the more abundant markers of each plant. The markers were dereplicated using a combination of HRMS molecular formula assignment and of data dependant MS/MS interpretation. Seven potential selected markers were then isolated at the preparative scale.
Conclusions: These different steps allowed an in-depth metabolome characterisation of the TCM and provided all the necessary data for a selected isolation of specific markers. This provided all the necessary information for the development of the quality control method that could thus efficiently take into consideration the complexity of the multi-herb preparation and give a rational for standardisation.

Keywords: UHPLC-PDA-ELSD-HRMS, multi-herb quality control, standardisation, Traditional Chinese Medicine, dereplication.

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Evaluation of the Impact of Physicochemical Characteristics in Iron Sucrose and Iron Sucrose Similars on Clinical Outcomes

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Introduction: Iron sucrose (IS) is a complex colloidal drug used to reestablish iron levels in patients suffering from severe anemia. During the last decade, several pharmaceutical companies introduced "intended copies" of IS in various markets. Nevertheless, retrospective and prospective studies proved that patients treated either with IS or iron sucrose similars (ISSs) experienced divergent clinical outcomes [1, 2]. It is not clear as to what physicochemical characteristics might be attributed to these clinical differences. Recently, the European Medicines Agency (EMA) published a reflection paper addressing the need for new assays to prove quality, efficacy and safety of both IS and ISSs [3]. Moreover, the European Directorate for the Quality of Medicines and Healthcare (EDQM) is currently working on the elaboration of a monograph on IS to clearly identify the key characteristics of this compound. Evaluation of equivalency of complex drug products, such as iron colloids and liposomes, continues to remain as one of the US Food and Drug Administration (FDA) regulatory science priorities in 2017 [4].
Aims: IS and several other commercial ISSs were studied for their physicochemical attributes. All the assays were designed to mimic the clinical injection of the drugs in the blood stream.
Methods: Size and shape of the colloidal particles were investigated by Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) and Low Voltage Electron Microscopy (LVEM). Stability of the drugs was evaluated through zeta potential measurements and the amount of labile iron (potentially causing toxic side effects) was estimated using a colorimetric procedure. *In vitro* dissolution kinetics of IS and ISSs were elucidated in relevant media over time.
Results: Significant differences were identified between IS and other commercial ISSs for all assays performed. DLS results revealed that IS presented a monomodal distribution by Intensity with an average size of 12 nm, whereas all of the ISSs showed a bimodal distribution with the presence of aggregates of micron size. TEM and LVEM images showed significantly divergent arrangements of particles in the different products. Zeta potential measurements proved that both IS and ISSs are stable colloidal suspensions with average values of around -30 mV. The amounts of labile iron identified in both IS and ISSs were significantly different, with values ranging between 3 and 6%. Finally, the *in vitro* dissolution kinetic studies showed different degradation pathways for IS and ISSs.
Conclusions: Results from this ongoing study suggest that there might be differences between IS and ISSs that can be discerned through physicochemical characterization and advance our understanding of differences between originator and its follow-on drugs. Moreover, the protocols developed might be used to create a list of most appropriate assays to lay the basis for testing equivalency between IS and ISSs.

Keywords: iron sucrose, physicochemical characterization, iron sucrose similars.

Disclaimer: The views expressed in this poster do not necessarily represent those of the US Food and Drug Administration.

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^{44}Sc-PSMA-617 - A Diagnostic Match to ^{177}Lu-PSMA-617

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Introduction: The prostate-specific membrane antigen (PSMA) is an attractive target for imaging and therapy of prostate cancer (PCa). A DOTA-functionalized PSMA ligand, PSMA-617, is used in clinics worldwide for PET imaging (^{68}Ga) and radionuclide therapy (^{177}Lu) of PCa.
Aims: In this study, PSMA-617 was labeled with cyclotron-produced ^{44}Sc ($T_{1/2}$ = 4.04 h) and investigated pre-clinically for its use as a diagnostic match to ^{177}Lu-PSMA-617.
Methods: ^{44}Sc was produced by irradiation of ^{44}Ca targets at the research cyclotron at Paul Scherrer Institut (PSI) and used for radiolabeling of PSMA-617. Subsequently, ^{44}Sc-PSMA-617 was evaluated *in vitro* and compared to its ^{177}Lu- and ^{68}Ga-labeled matches, as well as ^{68}Ga-PSMA-11. PSMA-positive PC-3 PIP and PSMA-negative PC-3 flu PCa cells were used for this purpose. ^{44}Sc-PSMA-617 and its diagnostic and therapeutic counterparts were injected into mice bearing PC-3 PIP/flu tumor xenografts for biodistribution studies and nuclear imaging.
Results: PSMA-617 was labeled with ^{44}Sc at high radiochemical purity (>97%) and specific activities up to 10 MBq/nmol. Compared to ^{177}Lu- and ^{68}Ga-labeled PSMA-617, ^{44}Sc-PSMA-617 revealed similar *in vitro* properties and bound specifically to PSMA-expressing PC-3 PIP tumor cells, while binding to PC-3 flu cells was not observed. This was in agreement with the *in vivo* data, which showed high tumor uptake of ^{44}Sc-PSMA-617 (51.9 ± 4.05% IA/g at 4 h p.i.) as well as fast renal excretion. This enabled distinct visualization of PSMA-positive PC-3 PIP tumor xenografts shortly after injection, with increasing tumor-to-background contrast over time. The tissue distribution of ^{44}Sc-PSMA-617 was almost identical to that of ^{177}Lu-PSMA-617, while the ^{68}Ga-labeled ligands, in particular ^{68}Ga-PSMA-11, showed different distribution kinetics.
Conclusions: ^{44}Sc-PSMA-617 is a promising diagnostic match to be used in tandem with ^{177}Lu-PSMA-617 as both radioligands have equal *in vitro* and *in vivo* characteristics [1]. A centralized production of ^{44}Sc-PSMA-617 and transport to satellite PET centers would be feasible due to the almost four-fold longer half-life of ^{44}Sc relative to ^{68}Ga. These features make ^{44}Sc-PSMA-617 particularly appealing for clinical application.

Keywords: ^{44}Sc, PSMA-617, PSMA-PET, radionuclide theragnostics.

Reference:
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Oxidative Stability of Biomarkers: A Neglected Issue in Determination of Small Molecule Biomarker in Daily Clinical Routine and Metabolomics Studies

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Introduction: Pre-analytical factors play an important role in the correct determination of blood concentrations of biomarkers and drugs in the clinical environment. In the context of daily clinical routine and clinical studies, oxidative stability *in vitro* should be carefully considered.
Aims: Our present work represents a closer examination of the pre-analytical complexity of vitamin C determination and suggests a convenient method for vitamin C processing during clinical routine work.
Methods: Different blood matrices were analyzed by LC-UV. Different handling-, time- and storage conditions were investigated in detail.
Results: Determination of vitamin C concentration is highly dependent from sample matrix and pre-analytical handling conditions. Oxidative protection significantly prevented degradation under different storage conditions. Separation of stabilized plasma from blood cells showed tremendous effects on vitamin C concentration. In addition, long-term storage of vitamin C was positively influenced by the addition of oxidative protection agents, however this effect was limited to 6 months if samples were stored at -20 °C.
Conclusions: It is important for physicians working in clinical routine and in clinical studies to have a value of biomarker representative of the actual pathophysiological state and not a mirror image of the concentration after storage. Oxidative degradation could affect not only vitamin C but also other small molecule metabolites and should therefore be intensively evaluated before method implementation in daily clinical routine as well as in metabolomics studies.

Keywords: stability, oxidative protection, vitamin C, clinical studies, pre-analytical factor.

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PDMS-*b*-PMOXA Polymersomes: Hepatocyte Targeting and Assessment of Toxicity

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Introduction: Many promising therapeutic compounds suffer from disadvantages like low bioavailability, rapid clearance, and high systemic toxicity. To overcome these challenges, nanoparticles can be used as promising drug delivery systems. Nanomedicines include different classes of formulations such as drug-protein conjugates, drug-polymer conjugates, liposomes, micelles and polymersomes. Polymersomes are hollow vesicles formed by self-assembly of amphiphilic block co-polymers such as poly(dimethylsiloxane)-*b*-poly(2-methyloxazoline) (PDMS-*b*-PMOXA). Polymersomes formed by these block co-polymers are promising since the individual polymer blocks have been reported to be biocompatible. Herein we present the formulation of polymersomes based on PDMS-*b*-PMOXA.
Aims: Formulation of PDMS-*b*-PMOXA polymersomes with differently modified surfaces. Comparison of cellular uptake of targeted and non-targeted polymersomes. Analysis of the biocompatibility of the polymersomes.
Methods: The polymersomes were prepared by thin-film rehydration. Characterization of the polymersomes included measuring size by dynamic light scattering and visualization by (Cryo)-Transmission Electron Microscopy. We incorporated carboxyfluorescein in order to examine release behavior *in vitro*. In addition, we studied asialoglycoprotein receptor specific uptake of asialofetuin-modified PDMS-*b*-PMOXA polymersomes by HepG2 cells using fluorescent activated cell sorting and confocal microscopy. Finally, we were assessing the potential toxicity of various PDMS-*b*-PMOXA polymersome formulations *in vitro* and *in vivo* using the zebrafish-model.
Results: We formulated a variety of differently modified PDMS-*b*-PMOXA polymersomes, which were of similar size and had a small size distribution. The PDMS-*b*-PMOXA polymersomes formed hollow spheres that could be used for drug encapsulation. Slow and sustained drug release into a neutral buffer was temperature dependent. PDMS-*b*-PMOXA polymersomes were functionalized with a targeting moiety and could be used for specific cell targeting. Functionalization of PDMS-*b*-PMOXA polymersomes with asialofetuin or fetuin did not alter cell viability of our polymersomes in HepG2 cells. Biocompatibility was further investigated using the zebra fish model. Preliminary data indicate no significant effects of PDMS-*b*-PMOXA polymersomes on the development of zebra fish embryos.
Conclusions: We achieved a targeted drug delivery system that is biocompatible *in vitro* and presumably also *in vivo*. The possibility for targeting and drug encapsulation makes it a promising tool for potential clinical applications.

Keywords: PDMS-*b*-PMOXA, polymersomes, targeted drug delivery, asialofetuin, zebrafish.

Reference:
Kiene K et al. Eur J Pharm Biopharm 2017; in press, doi: 10.1016/j.ejpb.2017.07.002

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Effect of Withanolide D and its Analogs in Combination with Radiation on Breast and Prostate Cancer Cells

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Introduction: Radiation treatment is one of the most widely used in cancer therapy. To improve the tumor control while minimizing normal tissue radiotoxicity, the combination of radiation with radiosensitizing agents is a new approach that has been explored in recent years. The withanolides, a family of natural compounds, have recently emerged as potential anticancer drugs. Indeed, many studies have already investigated the cytotoxic and anti-tumor activities of withaferin A (WFA), the most studied withanolide. Interestingly, this compound also showed radiosensitizing properties, suggesting withanolides as promising compounds for identifying new radiosensitizers.
Aims: Investigation of withanolide D (WD) as a radiosensitizing agent in prostate (PC3) and breast (MCF7) cancer cell lines.
Methods: Using MTT and clonogenic assays, cell proliferation and radiosensitivity were assessed in MCF7 and PC3 cells after treatment with WFA or WD and exposure to X-ray irradiation. A gene expression assay was then performed with a panel of 10 genes known as drug- and radiation-responsive to investigate the molecular mechanisms of withanolide-radiation combination. The effect of a combination between WFA and WD was also studied to determine if they could have a synergistic effect on radio-sensitization.
Results: WFA and WD reduced cell proliferation and clonogenic survival in both cell lines. WD decreased HIST1H3D and RFC4 gene expression in both MCF7 and PC3 cell lines compared to the control and WFA-exposed cells, suggesting a different molecular mechanism of action between these 2 compounds. The drug combination did not enhance the effect on clonogenic survival. However, supplemental experiments showed that some analogs of WFA and WD containing additional hydroxyl groups could improve the radiosensitivity, suggesting that this could play an important role in the radiosensitizing effect of withanolides.
Conclusions: This study showed that WFA and WD could act as effective radiosensitizing agents in breast and prostate cancer cells and their analogs containing additional hydroxyl groups could be more efficient radiosensitizers. This paves the way for future studies on withanolides in radiation and cancer research.

Keywords: radiosensitizer, withaferin A, withanolide D, MCF7, PC3.

EMILIA – e-Medication Plan After Hospital Discharge in Aargau: Design of an Observational Study

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Introduction: Hospital discharge is an important transition in a patient's care, as changes in medication regimen frequently occur during admission, as well as at discharge [1]. After discharge community pharmacists may play a pivotal role in patient care through medication reconciliation, solving medication discrepancies and improving patient knowledge about their medicines [2,3]. An electronic medication plan accessible for all health care professionals may help to overcome problems at transition of care points.
Aims: To acquire information about a) the acceptance and satisfaction of patients, healthcare professionals and carers with the new e-medication plan, b) the number of discrepancies between discharge prescription and dispensed medicines in the community pharmacy, and c) the number and type of interventions performed by the community pharmacists filling the hospital discharge prescription.
Methods: A prospective observational study will be performed in the region of Baden AG and in collaboration with eHealthAargau. One hundred adult patients discharged from the Cantonal Hospital Baden to home, with > 1 chronic medication before hospital entry or > 1 new medication for a minimum of 3 months will be asked by a clinical pharmacist on the ward to participate in the study. Patients confirming participation get access to the individual e-medication platform, where an individual e-medication plan will be deposited after hospital discharge. The clinical pharmacist will generate the first version of the e-medication plan with the hospital discharge information (discharge prescription, medication plan). The patients will fill the hospital discharge prescription in their community pharmacy. Community pharmacists are instructed to assess all discrepancies between the prescribed versus dispensed medicines, and to document all interventions performed during filling the prescription with the pharmDISC tool. Lastly, they will update the e-medication plan with the dispensed medicines. Thirty days post-discharge, the clinical pharmacist will contact the patients to assess their current medication and eventual unplanned hospital admissions or GP visits, same as the acceptance and satisfaction with the new e-medication plan.
Results and Conclusions: The evaluation of the new e-medication plan in this study would provide valuable information for implementation and further improvement of the electronic tool in view of the release of the electronic patient record in Switzerland by 2020. Furthermore, the data about the interventions and discrepancies in patients filling a hospital discharge prescription in the community pharmacy are essential for the development of a new pharmacist-led cognitive service for patients following discharge in the community pharmacy.

Keywords: e-medication plan, interventions, hospital discharge, medication discrepancies, seamless care.

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P-25

Anticonvulsant Agents from *Boswellia sacra* Identified by Zebrafish Bioassay-Guided Fractionation

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Introduction: Epilepsy is a chronic CNS disorder characterised by recurrent seizures. It is the most prevalent neurological disease worldwide, with over 50 million people afflicted [1]. Despite the availability of over 25 approved anti-epileptic drugs (AEDs), a third of treated epilepsy patients experience serious side effects to these medications, and another third does not respond at all and is referred to as being "pharmacoresistant". Thus, there is a clear need for new AEDs.
Aims: We investigated the resin of *Boswellia sacra* for its anticonvulsant constituents with the zebrafish epilepsy assay. Also known as the Sacred Frankincense, this resin has been used as a medicinal drug in many parts of the world for thousands of years.
Methods: The resin has been extracted by solvents with increasing polarities and screened with the zebrafish epilepsy model with seizures induced by the $GABA_A$ antagonist pentylenetetrazole (PTZ) [2]. In order to easily isolate and identify the active compounds, the analytical HPLC-PDA-ELSD conditions were transferred geometrically to a preparative medium-pressure liquid chromatography column (MPLC-UV-ELSD) using chromatographic calculations [3]. This gradient transfer ensured that the same selectivity and elution order was kept between the analytical and the preparative scale and provided an efficient isolation of the active compounds at the mg-scale. Fractions were analysed for their anticonvulsant activity, purified by semi-preparative HPLC, and screened again.
Results: The hexanic extract of *B. sacra* resin exhibited significant anticonvulsant activity and decreased 85% of PTZ-induced seizures. The bioassay-guided fractionation provided the isolation of 7 terpenes including a new diterpene, of which 2 exhibited *in vivo* anticonvulsant activity and reduced up to 67 % of epileptic seizures.
Conclusions: The present work reports for the first time the anticonvulsant activity of extracts and isolated compounds from *Boswellia sacra* and confirmed the CNS activity reported in *Boswellia* species.

Keywords: ethnopharmacology, *Boswellia sacra*, epilepsy, anticonvulsant, zebrafish.

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3D Printing of Tailored Drug-Eluting Oral Device

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Introduction: Despite the burgeoning interest in 3D printing for the manufacture of customizable oral dosage formulations, including a FDA-approved tablet (Spritam®) notwithstanding, the full potential of 3D printing in pharmaceutical sciences has not been realized. To demonstrate the feasibility of 3D printing for the manufacture of personalized drug delivery devices, the process of developing custom-made "pharma-inks" based on clinically approved materials and a model drug for the 3D printing of a tailored drug-eluting mouthguard is investigated.
Aims: The primary objective is to develop a 3D printed drug-eluting personalized mouthguard prototype in an iterative process of material selection, *in vitro* characterizations and 3D design. Another aim is to control the drug release profile of the prints by tuning the polymer composition.
Methods: A model drug, clobetasol propionate **(**CBS), was added to poly(D-lactic acid) PLA and poly(vinyl alcohol) PVA powder blends at various ratios to yield an homogenous mixture, which was then extruded under high temperatures to produce drug-loaded filaments (pharma-inks). The CBS loading in filaments was first quantified by HPLC. The release kinetics was assessed by immersing filaments in simulated saliva buffer at 37°C and withdrawing aliquots at regular time intervals to determine the CBS concentration. Subsequently, selected filaments were characterized in terms of the thermal, structural and mechanical properties by differential scanning calorimetry, X-ray diffraction, scanning electron microscopy and texture analysis respectively. The pharma-inks provided the feedstock for fused deposition modeling (FDM)-based 3D printing of the mouthguard prototypes, based on software modified models. The CBS loading and release kinetics of the printed prototypes were evaluated by aforementioned methods. The compression strength before and after release was examined by texture analysis.
Results: Pharma-inks were produced by incorporating CBS in PLA/PVA blend filaments through hot melt extrusion. The loading of CBS increased with increasing PLA:PVA ratio. The *in vitro* release study revealed that an increase in the water soluble PVA phase in the filament matrix led to a concomitant increase in CBS release rate. Two blends with favorable release profiles were selected for further characterizations. CBS was observed to be largely dispersed in the blended polymer filaments in an amorphous state. Upon the addition of PVA to PLA in the filament matrix, the filament surface became rougher and granular while the tensile strength increased. Dual-layered (drug-loaded and drug-free) mouthguards were designed and 3D printed using the pharma-inks. Minimal CBS loss was detected during the printing process. The release profiles of the printed prototypes mirrored that of the filaments. The mechanical strength of the mouthguards decreased after the release study, probably due to the dissolution of PVA.
Conclusions: Pharma-inks based on the model drug CBS-loaded PLA/PVA blend filaments were produced by extrusion, which were subsequently used to 3D print drug-eluting mouthguards. The control of drug release rate was attained by varying the ratio of PLA:PVA in the filament matrix. Overall, this study strongly supports 3D printing as an emerging platform for the manufacture of personalized drug delivery devices.

Keywords: 3D printing, personalized oral device, mouthguard, tunable release.

P-27

Identification of Novel Inhibitors of Vascular Calcification for Chronic Kidney Disease Patients

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Introduction: Calcification is a highly regulated process localized to bone and dental structures in healthy individuals. Precipitation of calcium phosphate crystals in the blood is usually prevented by their association with serum proteins and subsequent formation of soluble nanoparticles termed primary calciprotein particles (CPPs). Disruptions to this homeostatic state can lead to calcification of the vasculature or soft tissue. Vascular calcification (VC) is highly prevalent in chronic kidney disease (CKD) patients and is associated with an increased cardiovascular risk and mortality. The prevalence of CKD is increasing worldwide and to this date there is no approved therapy for the pharmacological treatment or prevention of VC [1].
Aims: A test for measuring serum calcification propensity, called T_{50} assay, has recently been established for clinical use, and is increasingly recognized as marker for mortality in CKD patients [2]. Here, we present data from screening a library of small molecules in an adapted version of this assay in order to identify a pharmaceutical lead capable of reducing serum calcification, and hence ultimately lowering pathological VC in CKD.
Methods: The screening library consisted of 10 analogues of *myo*-inositol hexaphosphate (IP6), a highly anionic natural molecule, with covalently attached poly(ethylene glycol) (PEG) chains. For the T_{50} assay, human serum was spiked with ionic calcium and phosphate to induce CPP formation. Increasing concentrations of test compounds were added, and the time required for amorphous primary CPPs to develop into larger, crystalline secondary CPPs was detected by time-resolved changes of light scattering. In order to test stability in serum, compounds were incubated in human serum and thereafter tested in the T_{50} assay. Potential calcium chelating capacity, which is related to toxic effects, was investigated by incubating increasing concentrations of test compounds in human serum and measuring free ionic calcium with the *o*-cresolphthalein-complexone method. Susceptibility to enzymatic metabolism was assessed *in vitro* by incubating the test compounds with phytase and monitoring the kinetics of phosphate hydrolysis by detection of a malachite green-phosphomolybdate complex.
Results: The compounds' activity was defined as the concentration necessary to delay T_{50} to 350 min, termed c350. The bis-PEG molecule INS-3041 was 10-times as potent as the unmodified IP6. INS-3001, a bis-PEG with lower molecular weight, displayed the highest activity per unit mass. Both compounds showed higher serum and phytase stability and lower calcium chelating propensity compared to IP6 and are considered for further investigations.
Conclusions: We identified PEGylated IP6 analogues as novel inhibitors of serum calcification. Subsequent *in vitro* metabolism studies revealed the bis-PEGs INS-3001 and INS-3041 as most promising candidates for drug development.

Keywords: ascular calcification, calciprotein particle, inositol hexaphosphate, PEG.

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Targeted Stimulation of Lymphatic Vessels: A Novel Approach to the Treatment of Chronic Skin Inflammation

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Introduction: The lymphatic vasculature is vital for tissue fluid homeostasis, immune surveillance and dietary lipid absorption. It undergoes extensive remodeling in inflamed tissue and plays an important role in regulating the inflammatory response by influencing drainage of extravasated fluid, inflammatory mediators and leukocytes. Transgenic overexpression, local injection and viral delivery of Vascular Endothelial Growth Factor (VEGF)-C, one of the main regulators of lymphatic vessel formation, have been shown to alleviate chronic inflammation in several disease models. While these techniques are suitable for research purposes, a clinically feasible method of VEGF-C administration is currently not available.
Aims: The project's goal was to design, produce and evaluate the therapeutic potential of a fusion protein capable of achieving inflammation-site specific delivery of VEGF-C after systemic application.
Methods: We produced F8-VEGF-C, a fusion protein consisting of human VEGF-C linked to the F8 diabody specific to the alternatively-spliced, angiogenesis marking extradomain A (EDA) of fibronectin. The protein's biodistribution profile was investigated by autoradiography and its biological activity assessed *in vivo* and *in vitro* using primary human lymphatic endothelial cells (LECs). The therapeutic activity of F8-VEGF-C was tested in 2 mouse models of chronic, psoriasis-like ear skin inflammation: one induced by the TLR7/8 ligand imiquimod and one mediated by the over-expression of VEGF-A. Important study parameters included extent of ear edema, histological evaluation of the vasculature, lymphatic drainage function and flow cytometric analysis of inflammatory cell populations.
Results: F8-VEGF-C induced the proliferation of LECs and selectively accumulated in inflamed tissue after intravenous injection. In both mouse models, the fusion protein significantly reduced ear edema when compared to an untargeted control construct. Furthermore, F8-VEGF-C caused a marked increase in the lymphatic vasculature and clearance assays revealed that the fusion protein improves lymphatic drainage, indicating that the newly formed vessels are functional. Numbers of leukocytes, regulatory T cells and γδ T cells in inflamed ears were significantly reduced upon F8-VEGF-C treatment.
Conclusions: Our results reveal that the inflammation site-specific induction of lymphatic vessels by targeted delivery of VEGF-C in the form of F8-VEGF-C represents a new and promising approach for the treatment of chronic inflammatory diseases.

Keywords: chronic skin inflammation, targeted drug delivery, lymphatic vasculature, vascular endothelial growth factor C, fibronectin.

P-29

Calcium Phosphate Nanoparticles for the Dual Delivery of Bisphosphonates and pDNA

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Introduction: Although the delivery of therapeutic genes has promising medical potentials, their clinical translation is slowed down by unfavorable biodistribution profiles and poor intracellular bioavailability. Bisphosphonates (BPs), such as alendronate (Ale), are inhibitors of the Farnesyl Pyrophosphate Synthase (FPPS), an enzyme part of the mevalonate pathway and a promising target in cancer therapy. However, BPs show rapid distribution to bones and insufficient membrane permeability. Therefore, both nucleic acids and BP need an efficacious and biocompatible delivery system to overcome their limitations. In the present work, a calcium phosphate (CaP)-based nanocarrier, with high adsorptive capacity for both nucleic acids and BPs, was investigated for the dual delivery of Ale and pDNA.
Aims: Preparation and *in vitro* evaluation of pDNA loaded Cap nanoparticles, stabilized with different poly(ethylene glycol) (PEG)-Ale chelators.
Methods: Stable and pH-sensitive PEG-Ale chelators were synthesized and characterized. The pH-sensitive conjugate was obtained by using a maleic anhydride derivative as cleavable linker. pDNA loaded CaP nanoparticles were prepared by a well-established co-precipitation method and stabilized by non-covalent PEGylation [1,2]. The particles were characterized in terms of pDNA encapsulation efficiency, physical stability, cellular uptake, transfection efficiency and cytoxicity on a B16F10 murine melanoma cell line and on J774.2 murine macrophages.
Results: The pH cleavable PEG-Ale conjugated prodrug exhibited tunable release of the bisphosphonate that was enhanced under acid conditions, such as those found in the endosomal compartment. PEG-Ale stabilized pDNA loaded CaP nanoparticles of 140-200 nm with narrow polydispersity and about 90% pDNA encapsulation efficiency were prepared. They remained stable for at least one week, and demonstrated pH-dependent dissolution. The CaP NPs were taken up by both cancer cells and macrophages, and exhibited moderate and sustained ability to transfect cancer cells while displaying low toxicity.
Conclusions: An innovative platform based on bioresorbable PEG-Ale stabilized CaP nanoparticles, that demonstrated potential for *in vitro* gene delivery was developed. Further investigations are needed to assess inhibition of the mevalonate pathway in macrophages following the uptake of PEG-Ale stabilized CaP nanoparticles.

Keywords: non-viral gene delivery, calcium phosphate, nanoparticles, alendronate, *in vitro* transfection.

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Phospholipid Modified Functionalized Calcium Carbonate for Oral Delivery of Poorly Water-Soluble Drugs

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Introduction: Formulation of poorly water-soluble drugs as solid lipid-based drug delivery systems has been a core subject in pharmaceutical research. Lipid-based formulations are attractive candidates due to their ability to produce a lipophilic microenvironment in the gastrointestinal tract (GI) and delivering low soluble drugs to their absorption sites. However, formulating phospholipids as solid dosage forms requires sophisticated formulation approaches. Utilizing porous material such as functionalized calcium carbonate (FCC) as carriers for encapsulation of both the lipid phase and the active ingredient is one of the strategies for addressing this challenge. FCC is an inorganic material with a highly porous surface and internal structure. It is biocompatible, non-toxic and has high loading efficiencies.
Aims: The aim of the current project is to incorporate poorly water-soluble drugs together with phospholipids into FCC and produce oral solid formulations that allow effective delivery of these drugs to the GI and result in increased bioavailability of such compounds.
Methods: Dipalmitoylphosphatidylcholine (DPPC) was loaded into FCC particles using a solvent evaporation method. Changes in pore size and surface area of the carrier, as well as its structure after loading were studied. Loading efficiency was evaluated by quantifying the amount of the encapsulated phospholipid using thermo-gravimetric analysis. Maximum loading capacity was determined by SEM imaging.
Results: Results from SEM and porosity measurements showed effective loading of the phospholipid. Thermo-gravimetric measurements determined quantitative amount of loaded DPPC and highest loading capacity was decided to be 38% *w/w* phospholipid-to-carrier ratio.
Conclusions: FCC shows promising results as a solid carrier for incorporation of phospholipids with high loading capacity and favorable physicochemical characteristics. As the next step, loading and dissolution of a poorly water-soluble model drug will be studied. Effect of GI conditions on absorption of the drug will be determined and studies concerning the nature and characteristics of this drug delivery system will be carried out.

Keywords: functionalized calcium carbonate, porous carriers, poorly water-soluble drugs, oral lipid-based drug delivery.

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P-31

A Microparticulate-Based Formulation for the Oral Treatment of Phenylketonuria

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Introduction: Phenylketonuria (PKU) is a hereditary disorder affecting the metabolism of phenylalanine (Phe) due to a deficiency in the enzyme phenylalanine hydroxylase. It is characterized by elevated blood Phe concentration, which can lead to severe intellectual disability in newborns. The current strategy to prevent this devastating consequence is limited to a strict, life-long, Phe-restricted diet, which implies major lifestyle disturbances. To this end, two strategies for PKU treatment have been developed: Kuvan, a synthetic enzyme cofactor only active on milder forms of PKU, and Pegvaliase, an enzyme replacement therapy intended for subcutaneous administration showing significant drawbacks, e.g. development of antibodies against the enzyme and polymers of the formulation [1].
Aims: The aim of this project is to design an efficient Phe-metabolizing system suitable for oral delivery that can bypass the issues arising with injectable enzyme replacement formulations.
Methods: Mesoporous silica particles (MSPs) were synthesized following a procedure previously described, using a Poloxamer P-123 - mesitylene mixture as template and tetraethylorthosilicate as silica precursor [2]. To enlarge the pore diameter a hydrothermal treatment at 170°C for 5 h in teflon lined autoclaved was performed. The obtained particles were characterized in term of size, zeta potential, morphology, surface area, and pore diameter. Phenylalanine ammonia-lyase (PAL, 310 kDa) was encapsulated in MSPs by impregnation method. The encapsulation efficiency, drug loading and residual activity of the loaded enzyme were determined. Finally, the particles were coated with chitosan in order to shield the payload from intestinal protease.
Results: MSPs with a hydrodynamic volume of 16 ± 3 µm and a zeta potential of -15 ± 3 mV were obtained. By means of hydrothermal treatment, the pore diameter could be enlarged to 34 nm (MSP-L) starting from 13 nm (MSP-S) resulting in a greater enzyme loading of about 5% and 2%, respectively. PAL encapsulation in MSP-L could be further improved by raising the PAL:MSP mass ratio. In fact, 1:10, 2:10 and 4:10 ratios led to a loading of 5%, 16% and 38%, respectively. Upon encapsulation, PAL enzymatic activity was maintained. The formation of the coating was visually assessed by using rhodamine-conjugated chitosan, which did not result in a significant increase in particle size.
Conclusions: MSPs with a size in the µm-range and large pore diameter were designed. PAL was efficiently encapsulated in the formulated particles and its activity was preserved. Therefore, a Phe-metabolizing system could be obtained. However, further investigations are needed to identify the optimal coating conditions to allow the permeation of Phe into the carrier and at the same time to hinder the permeation of intestinal digestive enzymes.

Keywords: phenylketonuria, mesoporous silica particles, enzyme encapsulation.

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P-32

Findings in the Development of an Enzymatically Triggered Nanoformulation

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Introduction: Targeted cancer therapy includes both identification of drug targets specific to cancer cells or changes in kinetic parameters leading to accumulation of commonly used anti-cancer drugs in the surrounding of malignant transformed cells. The latter strategy is aiming at the enrichment of drug concentration at the tumor site whereby healthy cells get spared and adverse events decrease. Tumor-cell derived enzymes could be exploited as a trigger for site specific drug release. One family of enzymes shown to be highly expressed in the surrounding of proliferating cells is the gelatinase matrix-metalloproteinase 9 (MMP-9). MMP-9 is an enzyme responsible for collagen degradation in connection with cell migration and proliferation. Thereby, MMP-9 is a marker for enhanced tumor malignancy and could be used for prodrug activation.
Aims: The aim of our study was to synthesize a self-assembling nanoformulation consisting of a MMP-9-labile peptide coupled to an anti-cancer drug and to test the formulation for functionality in a tumor entity identified to exhibit enhanced levels of MMP-9.
Methods: To identify a suitable cancer target for our novel nanoformulation we quantified expression of MMP-9 in a commercially available tissue collection by multiplex real-time PCR and confirmed our observation using renowned databases (GEO-database, Human Protein Atlas). Immunohistochemistry was performed on tissue samples to validate MMP-9 protein expression. Using bioconjugate chemistry methods, paclitaxel was coupled to a MMP9-labile peptide forming amphiphilic molecules leading to self-assembling nanoparticles. The nanoparticles were characterized by dynamic light scattering and transmission electron microscopy. Their effects on cancer was tested by exposing cancer cell lines to the nanoparticles.
Results: Significantly increased mRNA expression of MMP-9 comparing normal to malignant transformed tissue was observed in several tumor entities, which is corroborated by immunohistochemistry and database analysis. Ultimately brain tumor, particularly glioblastoma multiforme, was identified as a tumor entity where MMP-9 could be used to trigger drug release. Commonly used brain cancer cell lines, namely LN-18 and U87-MG, were characterized for MMP-9 expression and activity. *In vitro* characterization of the synthesized nanoformulation was performed in the selected cell lines comparing its response to paclitaxel.
Conclusions: Taken together, we verified enhanced expression of MMP9 in glioblastoma multiforme. Commonly used brain cancer cell lines were characterized prior to *in vitro* studies on MMP-9 triggered drug release. Further studies are required to fully characterize the nanoformulation and understand its effects *in vitro* and *in vivo*.

Keywords: triggered drug release, matrix-metalloproteinase 9, nanoparticle, bioconjugate.

P-33

An Endocannabinoid Uptake Inhibitor from Black Pepper Exerts Pronounced Anti-Inflammatory Effects in Mice

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Introduction: Guineensine is a dietary *N*-isobutylamide widely present in black and long pepper (*Piper nigrum* and *P. longum*). Black pepper is one of the most popular spices worldwide employed for its culinary (i.e. spicy) but also medical properties. The outstanding bioactivity of guineensine *in vivo* was only recently reported and the most potent biological activity showed so far is the inhibition of the endocannabinoid uptake process (IC_{50} = 290 nM for anandamide in U937 cells). This effect was matched by indirect activation of CB1 receptor and overall cannabimimetic effects in mice. Furthermore, the endocannabinoid system is involved in regulating numerous biochemical processes related to cellular stress and homeostasis, hence modulates fundamental pathophysiological processes, including inflammation and pain.
Aims: Given the emerging link between nutrition and the endocannabinoid system, here we studied the anti-inflammatory and analgesic effects of guineensine. In addition, guineensine was profiled *in vitro* in order to explore its polypharmacology.
Methods: For inflammation and pain evaluation, BALB/c mice received intraperitoneal (i.p.) injection of guineensine (2.5, 5.0 or 10.0 mg/kg) and were evaluated through the formalin test, carrageenan-induced paw edema and LPS-induced hypothermia. In LPS-induced hypothermia, locomotion was evaluated in the open field test and some extrapyramidal behaviors were evaluated in the tetrad test. In addition, pro-inflammatory cytokine levels were measured. For the polypharmacological screening, guineensine was tested *in vitro* in 45 CNS-related receptors, ion channels and transporters.
Results: At 2.5 mg/kg i.p. significant anti-inflammatory effects were observed and at 5.0 mg/kg i.p. analgesia. Moreover, guineensine inhibited pro-inflammatory cytokine production in endotoxemia. Intriguingly, guineensine and LPS independently induced catalepsy but in combination this effect was abolished. Both hypothermia and analgesia were blocked by the CB1 receptor inverse agonist rimonabant but the pronounced hypolocomotion was CB1 receptor-independent. The profiling screening revealed apparent interactions of guineensine with the dopamine transporter DAT, 5HT2A and sigma receptors, uncovering its prospective polypharmacology.
Conclusions: Our data showed that upon i.p. administration, guineensine exerts significant anti-inflammatory effects in different mouse models, including endotoxemia. Moreover, we suggest additional CNS targets for this dietary natural product, which may explain, at least in part, the CB1 receptor-independent extrapyramidal effects.

Keywords: endocannabinoids, inflammation, guineensine, pepper, polypharmacology.

Discovery of Natural Products Targeting Aberrant Proliferative Signaling in Melanoma

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Introduction: The incidence of melanoma, the most fatal dermatological cancer, is dramatically rising. In more than 50% of malignant melanomas the BRAF V600E mutation is present, leading to aberrant MAPK signaling and uncontrolled proliferation. Vemurafenib, a specific inhibitor of the V600E mutated form of B-Raf, has been approved in 2011 for the treatment of metastatic melanomas. Despite spectacular initial results, patients treated with vemurafenib relapse within 2-18 months due to drug resistance. Studies on the mechanism of drug resistance suggest that the development of combination therapies is required to address this problem. Novel inhibitors targeting aberrant proliferation signaling (MAPK and PI3K pathways) in melanoma are therefore urgently needed.
Aims: To identify novel compounds targeting MAPK or PI3K pathways.
Method: We combined our natural product lead discovery platform with an innovative high-content screening (HCS) assay (Fig. 1) that quantifies aberrant MAPK/PI3K signaling in a melanoma model system with patient-relevant oncogenic mutations. The screen involved imaging of aberrant ERK/AKT signaling activities with the aid of genetically-encoded biosensors. Coupled with automated capturing and analysis of microscopic images, this approach allows to analyse thousands of single cells and conditions.

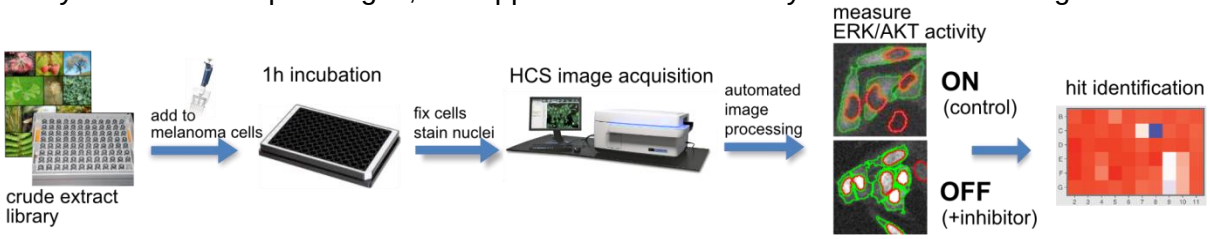

Figure 1: High-content screening (HCS) workflow

Results: The HCS assay was first validated with known specific inhibitors, prior to its use for an initial screening of 88 plant extracts. An EtOAc extract from leaves of *Casearia arborea* was found to inhibit both the MAPK and PI3K pathways in A2058 melanoma cells. HPLC-based activity profiling combined with an efficient dereplication strategy identified Cucurbitacins Q and B to be responsible for the activity in the extract. These results were obtained in analytical scale using less than 5 mg of extract. A scale-up isolation will allow for the full structural assignment of the compounds, and for further characterization of mechanisms of action.
Conclusions: The current results provide a proof-of-concept for our approach that will be used for the screening and profiling of a library of > 800 EtOAc plant extracts.

Keywords: high-content screening, natural products, melanoma, targeted therapy.

Automated Comparative Processing and Data Clustering of Large Numbers of LC-ESIMS Datasets

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Introduction: The technological development of LC-MS instrumentation has led to significant improvements of performance and sensitivity. Complex samples, such as plant extracts, can now be analyzed in high-throughput mode. Software tools allow efficient deconvolution of LC-MS chromatograms to obtain comprehensive information on single constituents in a complex mixture. However, the systematic and unbiased comparison of large numbers of complex LC-MS chromatograms remains a challenge. Some software tools for comparative processing have been developed, but they have certain limitations, such as black-box approach, lack of user friendliness, or limited options for data sharing.
Aims: To develop a workflow for processing a large number of LC-MS datasets in a comparative and comprehensive way.
Results and Conclusions: We developed a two-step processing protocol (Fig. 1), comprising a parallel comparative processing integrated in ACD/Labs, and a web platform developed in R language designed for clustering and visualization of chromatographic data. The latter tool was named FreeClust and is freely accessible on the web.

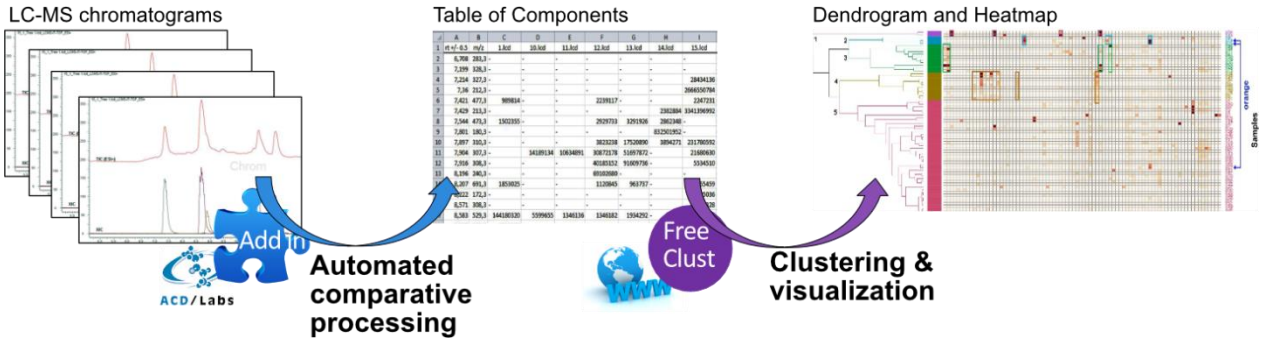

Figure 1: Two-step data mining workflow

In a first step, all relevant chromatographic and spectroscopic data are automatically extracted and assembled in an Excel spreadsheet containing retention time, molecular ions with the respective ion abundance, and sample names. In a second step, the matrix is loaded into an online web application where the user has the possibility to compare and cluster the data according to similarity of metabolic patterns. Various statistical algorithms and options for visualization of data in intuitive 2D heatmaps are available. As a proof-of-concept, we applied this processing workflow to a set of LC-ESIMS profiles obtained with 69 honey samples. The LC–ESIMS chromatograms were deconvoluted, and a table of components present in all samples was completed within a few hours of calculation. Statistical functions grouped honey samples in clusters and sub-clusters, thereby highlighting correlations between metabolic patterns and origins of honeys. Implementation in the ACD/Labs software package enables ulterior integration of other analytical data and *in silico* prediction tools for modern drug discovery.

Keywords: LC-ESIMS, comparative processing, add-in tool, ACD/Labs, clustering.

Mouthfeel Study of Orally Disintegrating Tablets

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Introduction: Orally disintegrating tablets (ODT) are a safe and convenient solid oral dosage form that can be administered without water. ODTs disintegrate rapidly in the oral cavity and therefore taste masking and mouthfeel enhancement are key aspects in the development of ODT. In the literature, Functionalized Calcium Carbonate (FCC) is already reported to be suitable for ODTs [1]. These tablets disintegrate within seconds, therefore a suitable method to measure disintegration time is needed.
Aims: In this work we investigate the applicability and test the acceptability of FCC as a main excipient in mouthfeel enhanced ODT. Moreover, we present a novel model to analyze the kinetics of tablet disintegration *in vitro*, as the method according to Ph.Eur. delivers no information about the kinetics during disintegration.
Methods: To test the acceptability of the FCC-based ODT an *in vivo* study was performed, where 20 healthy volunteers put an ODT on the tongue. The *in vivo* disintegration time was measured and eight questions were answered using a 10-step visual analogue scale. The *in vitro* disintegration time was measured using a Tensiometer Krüss K100 and a custom made grid. With a novel mathematical model, the kinetics of water sorption and disintegration were assessed.
Results: We showed that the tablet was well accepted and the taste and feeling in the mouth was rated very positive. This is supported by the value for skewness and mode which indicate that the questions are shifted towards the most positive answers. The *in vivo* disintegration time was 23 sec, which is reasonable for the patient. With the *in vitro* measurements we show that the amount of liquid needed for complete tablet disintegration is around 0.3 mL. This amount is available in the mouth of an adult, certainly when stimulated with taste sensation [2].
Conclusions: Functionalized calcium carbonate as a main ingredient for ODT is well accepted by healthy volunteers and the *in vivo* disintegration time of the ODT is reasonable for the patient. It was shown that the amount of saliva necessary for complete disintegration is available in the mouth of an adult. These findings suggest a high potential for FCC to be used for the further development of age-appropriate ODTs.

Keywords: mouthfeel enhancement, orally disintegrating tablet, functionalized calcium carbonate.

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Bioactive Potential and Role of Secondary Metabolites Within the Microorganism Community of the Sea Grass *Posidonia oceanica*

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Introduction: *Posidonia oceanica,* a dominant herb of marine grassland from the Mediterranean, has been selected for this project as an unique model system for studying its microbiota. This species is known for its longevity and is potentially the host for a large endophyte community.
Aims: The aim of the project was to comprehensively profile the endophyte community of this peculiar marine plant, use it as a model for deciphering microbiome interactions and investigate if this can inspire the discovery of new antibacterial agents.
Methods: The diversity of endophytic microbial communities from leaves and rhizomes was assessed by high throughput sequencing approaches and 23 strains of fungi and bacteria were isolated and identified. These strains were cultivated and extracted by ethyl acetate for metabolite profiling and antimicrobial activity screening. All samples were dereplicated by UHPLC-HRMS/MS and molecular networking for a preliminary survey of their secondary metabolite composition.
Results: Among all fungal strains *Lulwoana* sp. were found very specific to the marine environment. Their metabolite profiles reveal the presence of original corymbiferan lactones and their targeted isolation reveal the presence of a new methylated derivative. Extensive profiling in different cultivation media also demonstrated the diversity of such compounds in these endophytes. In parallel the extracts were screened for their antimicrobial activity against *Staphylococcus aureus*, *Propionibacterium acnes* and *Pseudomonas aeruginosa. Penicillium* and *Fusarium* spp. strains were found to display interesting antimicrobial activities and a correlation between their chemical profiles and activities will be presented.
Conclusions: All the results obtained provide a first survey of chemical and bioactivity profiles in view of studying interactions between the members of this unique community.

Keywords: marine, *Posidonia oceanica*, endophyte, fungi, bioactivity, antibacterial.

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Pharmaceutical Sciences in the Community Pharmacy: Impact of the Pharmacist on Public Health

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Introduction: At the end of his/her scientific education, the community pharmacist's activity is often shared between health sciences and the necessities of the pharmacy management. The evolution of pharmacy in Switzerland therefore increasingly refers to "*patientèle*" as an association of the terms patient and client. PharmaSuisse has recently developed numerous interventions for and with the community pharmacist, allowing him/her to become an essential partner in the health system - notably in therapeutic education and motivational interviews [1,2]. Recent revisions on the law on therapeutic products (LPTh) and on the law on medical professions (LPMed) specify the new role of pharmacists in basic healthcare.
Aims: It is highly pertinent to point out the role of community pharmacists in the perspective of current challenges and needs in public health. This presentation gives an overview of a few pharmaceutical interventions and their impact on patient care.
Methods: In this presentation we document some pharmaceutical interventions that took place during the 8-months internship in community pharmacy required for the Master in Pharmacy, and we relate them to the teaching received in pharmaceutical sciences. The main points discussed concern: health prevention, therapeutic follow-up, opioids replacement therapy, medical advice and development in complementary medicines, but also pharmaceutical preparations.
Results: The following projects were carried out:
- Prevention and vaccination campaigns
- Implementation of patient triage protocoles (NetCare)
- Interprofessional collaboration for treatments of patients suffering with addictions
- Development and adaptation of formulations to address the lack or shortage of commercially available essential medicines - particularly in paediatrics
- Follow-up and medical advice for sleep disturbances or benzodiazepine use.

Conclusions: The community pharmacy, as a privileged place of contact with patients, offers great opportunities of research and development in public health. Through their enlarged competences the community pharmacists are able to adapt to the specific needs of patients, and the pharmaceutical interventions conducted contribute to respond to the challenges of today and tomorrow.

Keywords: pharmaceutical sciences, community pharmacy, public health, pharmaceutical interventions.

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P-39

3D Printed Bioceramics for Personalized Drug Loaded Osteoconductive Bone Implants

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Introduction: 3D printing reveals opportunities for the fabrication of personalized bone grafts and applications in bone regeneration [1]. Patient-specific 3D printed implants can be produced from computed tomography datasets with complex geometries and full functionalities. However, the incorporation of active pharmaceutical ingredients into 3D printed scaffolds represents a challenge as drug stability and activity, appropriate drug loading capacity and desired controlled release profiles need to be attained [2].
Aims: The aim of this study was to develop a novel 3D drug delivery system combining powder-binder jetting based 3D printing of bioceramics with controlled drug release of simvastatin.
Methods: An industrial powder printer Voxeljet (VX200, Voxeljet AG, Friedberg, Germany) was used to 3D print the bioceramic materials. Bioceramics consisted of porous beta-tricalcium phosphate (β-TCP) granules (Degradable Solutions AG, Schlieren, Switzerland). For drug incorporation β-TCP granules were coated with poly(D,L-lactide-co-glycolide) containing the drug simvastatin as previously described [3]. Drug loading and *in vitro* drug release were measured by an UPLC-MS method.
Results: Bone implants were generated by digitally-controlled layer-by-layer deposition of bioceramic materials under low temperature conditions to create freeform geometries with adequate mechanical stability. Drug loading was achieved by incorporating simvastatin into the polymer coating of the TCP granules, which served as starting material for the 3D printing process. Implant properties were optimized by varying binder to active substance composition. Simvastatin was released *in vitro* from bone grafts in its bioactive form, simvastatin acid, over the course of several weeks.
Conclusions: 3D printed bioceramic bone grafts hold promise as combinatory 3D drug delivery system to improve osteoconductive and eventually osteoinductive scaffolds properties. Further, cell assays and preclinical evaluation will be needed to elucidate its potential for personalized treatments and its applicability for the delivery of drugs to bone tissue.

Keywords: powder-binder jetting based 3D printing, drug delivery systems, bioceramics, bone implants, osteoconductive.

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P-40

Improved Cutaneous Delivery of Pentoxifylline Using P.L.E.A.S.E.® Fractional Laser Ablation

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Introduction: Pentoxifylline (PTX) is indicated for radiation-induced fibrosis [1]. However, therapeutic efficacy of PTX after oral or intravenous administration is limited and dose-related side effects are common. To-date there are no topical formulations of PTX.
Aims: The aim of this study was to develop topical formulations of PTX, to investigate cutaneous delivery of the drug and to determine its biodistribution in the skin. Passive delivery was compared to that after fractional laser ablation using the P.L.E.A.S.E.® (Precise Laser Epidermal System) device. This is an Er:YAG fractionally ablative laser that can be used for controlled microporation and the creation of transport channels in the skin.
Methods: PTX solution (200 mM), hydrogel (with equivalent loading of 5.56% *w/w*) and a PVP patch (again with a loading of 5.56% *w/w*) were prepared. *In vitro* studies were conducted using porcine ear skin. Micropore depth was determined by full field optical coherence tomography using a Light-CT Scanner. After microporation, skin samples were mounted in vertical Franz diffusion cells (area = 2.0 cm^2). The donor and receptor compartments were filled with PBS at pH 7.4. After equilibration, buffer solution in the donor compartment was replaced by the PTX formulation. One-mL aliquots were withdrawn from the receiver compartment at predetermined intervals (formulations were applied for 8 h). Upon completion of the experiments, the skin surface was cleaned and the porated area in contact with the formulation punched out (area = 0.5 cm^2), snap-frozen and cryotomed using a Microm HM 560 Cryostat to obtain a series of 8 lamellae (thickness = 40 µm). The lamellae and the remaining skin piece were extracted overnight with MeOH:H_2O (50:50). PTX was quantified by a validated UHPLC-MS/MS method.
Results: A 18-fold increase in delivery efficiency was observed after laser microporation for both of PTX solution and hydrogel formulations (from 1.90 ± 0.99% to 34.97 ± 9.82% and 1.63 ± 0.40% to 29.56 ± 7.03%, respectively). Decreasing the amount of hydrogel applied, so as to approximate finite dose conditions, resulted in a further increase in delivery efficiency to 62.93 ± 17.11%. Total delivery and delivery efficiency of PTX from hydrogel formulation increased as a function of fluence (energy applied per unit area to create the pores). PTX delivery efficiency from the PVP patch (each 2 cm^2 patch contained 11.14 mg PTX) was increased from 1.08 ± 0.26% to 27.97 ± 3.61% with laser microporation (fluence of 88.9 J/ cm^2), corresponding to a 28-fold increase.
Conclusions: Fractional laser ablation enabled a significant increase in PTX delivery to the skin. The amounts delivered could be controlled by modulating the laser fluence. Therefore, fractional laser ablation may be an effective, targeted and minimally invasive alternative for the topical treatment of radiation-induced skin fibrosis.

Keywords: radiation-induced fibrosis, pentoxifylline, fractional laser ablation, hydrogel, patch.

Reference:
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P-41

Bioprospecting for Anti-Chagas Medicinal Plants in Bolivia and Biological Evaluation

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Introduction: In Latin America, an estimated 8 million people are infected with *Trypanosoma cruzi*, the etiologic agent of Chagas disease [1]. The current chemotherapy regimen is limited to the acute phase of the disease and is often associated with severe side effects. Bolivia has the world's highest infection rates and primarily the lowest socioeconomic population is affected, which relies on traditional medicine for the treatment of many infectious diseases.
Aims: To carry out an extensive ethnobotanical and ethnopharmacological fieldwork in Bolivia and to assess the *in vitro* anti-trypanosomal potential of anti-Chagas medicinal plants with the aim to identify novel bioactive natural products. The working hypothesis was that the ethnomedical context of the disease leads to a higher hit rate in anti-Chagas plant extracts.
Methods: We conducted an ethnobotanical study in selected parts of Bolivia with the aim to study the consensus on the efficacy of anti-Chagas plants and to collect plant material in situ for subsequent extraction and *in vitro* testing. 135 plant extracts of the collected material were screened for general cytotoxicity in different cell lines and for anti-trypanosomal activity in *Trypanosoma cruzi* epimastigote forms and *Trypanosoma brucei* procyclic forms. Moreover, 583 medicinal plant extracts described in Dioscorides' *De Materia Medica* were screened with the aim to compare results of our ethno-directed approach with a random screening. Mechanistically, we focus on plant natural products that inhibit the *T. cruzi* fatty acid amide hydrolase (FAAH), which is crucial for the generation of ethanolamine in the parasite. The parasite appears to take the FAAH substrates (N-acylethanolamines) from the host cells. We hypothesize that the active site of human and trypanosomal FAAH are sufficiently different to make it a potential drug target. To that aim, we have established the biochemical assays to screen for this target using our active extracts and focused libraries.
Results: Ethnobotanical fieldwork was carried out using open and structured interviews in different regions and in different ethnic groups of Bolivia, including the Chaco region. Altogether, a total of 95 plant species (traditionally used against Chagas disease or its symptoms) have been deposited and identified at the National Herbarium of Bolivia. Different plant species showed apparent toxic selectivity for trypanosomes over the screened mammalian cells and will be followed up in the *T. cruzi* amastigote stage. Our findings indicate that the ethno-directed approach did not lead to the identification of a significant larger percentage of species with anti-trypanosomal activity. First insights into the potential role of *T. cruzi* FAAH were obtained.
Conclusions: Despite no ethnobotanical correlation of anti-trypanosomal species, plant natural products nonetheless constitute an interesting starting point for anti-Chagas drug discovery.

Keywords: *T. cruzi*, Chagas, medicinal plants, in vitro screening, drug discovery.

Reference:
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Cyclopeptidic Photosensitizer Prodrugs for Selective Photodynamic Therapy of Cancer

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Introduction: Photodynamic therapy (PDT) combines light, oxygen and a photosensitizer (PS) to treat locally cancers. It can be considered as "selective" if the prodrug can be specifically accumulated and activated in the tumor area. Lipophilic PSs are usually delivered thanks to a carrier, by encapsulation or covalent coupling, in order to increase the overall hydrophilicity and improve the pharmacokinetic profile. In addition, a high initial PS loading would ideally quench the photophysical properties of the prodrug, thus resulting in a better contrast and signal/noise ratio when reaching the tumor. To achieve these goals, mostly polymeric carrier are used [1].
Aims: Here, we used cyclopeptidic templates as starting carriers in order to synthesize conjugates with well-defined structure including accurate knowledge of the number and position of loaded PS(s), black hole quenchers (BHQ), and PEG chain(s). The aim of the present study was to demonstrate their activation by enzymatic reaction, to achieve PDT *in vitro* and to assess their fate *in vivo* on the chorioallantoic membrane (CAM) model [2] and in tumor-bearing mice.
Methods: Three conjugates ($cPPP_{4/5}$, $cPPP_{4/5}^2$ and uPA-$cPPQ_{2+2/5}$) and two reference conjugates ($cPPP_{1/5}$ and $_D$-$cPPP_{D4/5}$), differing by their loading of PSs, BHQs and PEG chains, were first incubated with a model (trypsin) and a targeted enzyme (urokinase-like plasminogen activator, uPA) to assess restoration of the fluorescence. Secondly, they were incubated with prostate cancer cells (PC-3) and PDT was performed at different light doses. Pharmacokinetics and biodistribution were assessed on the CAM model having lung cancer cells (A549) nodules. Finally, BALB/c mice inoculated with breast cancer cells (4T1) enabled us to investigate further the optimal window for further PDT thanks to an alternate conjugate, namely CathB-$cPPQ_{2+2/5}$.
Results: $cPPP_{1/5}$ and $_D$-$cPPP_{D4/5}$ showed no noticeable fluorescence increase upon enzymatic and cellular incubation, while $cPPP_{4/5}$ and uPA-$cPPQ_{2+2/5}$ were activated by the enzymes, with a 80-fold increase in fluorescence emission for uPA-$cPPQ_{2+2/5}$ after 2 h of uPA digestion. PDT was successfully conducted with $cPPP_{1/5}$, $cPPP_{4/5}$ and uPA-$cPPQ_{2+2/5}$. The CAM assay showed significant increase of fluorescence in the tumor area with the di-PEGylated $cPPP_{4/5}^2$ (see Figure 1) while the mono-PEGylated $cPPP_{4/5}$ showed no significant increase overtime. Finally, *in vivo* experiments in tumor-bearing mice confirmed an optimal treatment window after 8 h of accumulation of CathB-$cPPQ_{2+2/5}$ in the tumor area.

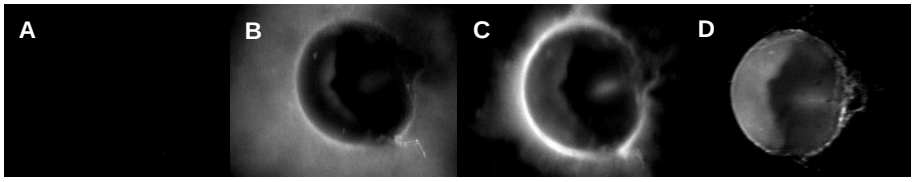

Figure 1: Microscope images of A549 nodules incubated with $cPPP_{4/5}^2$ after 0 (A), 1 (B), 2 (C), and 6 h (D)

Conclusions: This design offers well-defined prodrugs, initially quenched. They may be selectively activated by enzymatic reaction, and PDT can be performed at low PS concentration. Pharmacokinetic and biodistribution studies revealed that a double PEGylation of the constructs increases its retention time and allows a better accumulation in the tumor nodules.

Keywords: cyclopeptide, photodiagnosis, photodynamic therapy, photosensitizer.

References:

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DynaStI: An Expert-Curated Dynamic Retention Time Database for Steroidomics

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Introduction: Hundreds to thousands of metabolites can be simultaneously monitored in biological matrices using untargeted LC-MS experiments. Unambiguous compound identification remains mandatory to draw relevant biological conclusions from the data. As several hits can match a molecular formula, unique molecular identity can be difficult to obtain. Retention time constitutes an essential information to complement HRMS and MS^n spectra for positional and constitutional steroid isomers identification [1]. Thus, an automatic steroid annotation based on retention time and HRMS is developed.
Aims: A web application was developed for automatic steroid annotation using the annotation level established by the MSI/COSMOS initiatives [3]. DynaStI(***dyna****mic* ***st****eroid* ***I****dentification*) is an expert-curated endogenous steroid database designed for LC-MS steroidomic studies.
Methods: Experimental LSS measures were obtained from a previous work [1]. The database runs on a MySQL server and a web application front-end/back-end was developed for steroid annotation. DynaStIcollects experimental and *in silico* (Quantitative Structure Retention Relationship) linear solvent strength (LSS) parameters to predict dynamically the retention time of steroid in any gradient conditions. To date, the database contains 198 endogenous molecules and each steroid entry includes key chemical information (IUPAC name, CAS number, a human curated SMILES, the most abundant ion detected in HRMS) as well as links to major databases, *i.e.*HMDB, LipidMaps and SwissLipids. DynaStI was validated using a case study involving the H295R reference cell line incubated with forskolin, a compound known to stimulate steroidogenesis [2].
Results: DynaStI is a web application developed using a JavaScript/HTML front-end and a PHP/C++ back-end. The front-end allows to access to the database, browse/edit/add steroids and upload LC-HRMS features. Compound annotation is managed by a C++ software, DynMetId, which predicts retention times using LSS parameters (log k_W and S), compares monoisotopic masses, molecular adducts, isotopic pattern and assigns annotation levels according to COSMOS/MSI standards [3]. DynaStI can be applied after LC-MS raw data processing with Progenesis, Compound Discoverer, MZmine or XCMS. DynaStI thus converts unknown feature lists into a subset of identified steroid giving the possibility to get sounds biologically insights from the data. DynaStI allowed a deeper understanding of mechanistic effects involved in steroidogenesis stimulation by forskolin in H295Rcells.
Conclusions: A dynamic retention time prediction database was implemented using a user-friendly web application.The database was developed to be publicly available and durable overtime.

Keywords: database, steroidomics, Retention Time Prediction, QSRR, UHPLC-HRMS.

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HbA_{1c} Levels, Body Weight Change, and Risk of Pancreatic Cancer Among Patients With Long-Standing Diabetes Mellitus: A Case-Control Study

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Introduction: Screening for sporadic pancreatic cancer (PaC) needs the upfront characterization of high-risk groups. New-onset diabetes mellitus and weight loss represent two important criteria for identifying people at increased risk of PaC in the general population.
Aims: To assess whether similar criteria (i.e., HbA_{1c} levels and body weight change) may render it possible to identify patients at high risk of PaC within the long-standing diabetes mellitus population.
Methods: Using data from the UK-based Clinical Practice Research Datalink, we conducted a matched (1:10) case-control study. Cases were patients, aged 30 to 89 years, with an incident diagnosis of PaC (index date) and preceding diabetes mellitus present for >2 years at the index date. We matched the cases with controls, i.e., patients without a diagnosis of PaC, by various variables including diabetes duration. We categorized HbA_{1c} levels according to different time intervals before the index date, each divided into quartiles. Weight change specified the relative change from baseline (i.e., >3 years before the index date) until the index date. Applying multivariable conditional logistic regression, we compared HbA_{1c} levels as well as weight change between cases and controls.
Results: We found 476 cases and 4724 controls. Compared with HbA_{1c} levels ≤47.5 mmol/mol, HbA_{1c} levels ≥64.0 mmol/mol were associated with odds ratios (ORs) for PaC of 4.94 (95% CI 3.52-6.94) and 2.66 (95% CI 2.00-3.54) within 6 months and >1-2 years before the index date, respectively. Weight loss ≥15.0% was associated with an OR of 15.40 (95% CI 10.65-22.26) compared with no weight change. 14.7% of cases and 0.5% of controls showed both weight loss ≥15.0% and an HbA_{1c} level ≥64.0 mmol/mol (within 2 years before the index date). The OR for PaC associated with presence of both characteristics was 60.97 (95% CI 35.87-103.65), when compared with patients showing none of them.
Conclusions: High HbA_{1c} levels and weight loss appeared to be helpful criteria for identifying patients at high risk for PaC among patients with long-standing diabetes mellitus. Studies on the exact course of worsening in glycemic control and weight loss at a patient level are needed. Efforts to define high-risk groups should focus on both patients with new-onset diabetes mellitus and patients with long-standing diabetes mellitus.

Keywords: pancreatic cancer, diabetes, weight loss, HbA_{1c}, CPRD.

P-45

In Silico Adaptive Design of Peptides with Selective Anticancer Activity

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Introduction: Membranolytic anticancer peptides (ACPs) are a promising strategy in the fight against cancer. Their receptor-independent mechanism of action is thought to hinder the development of resistances. Until now, hundreds of ACPs have been identified and collected in specialized databases [1]. However, data on ACP selectivity towards non-cancer cells is not readily available and there is a lack of computational tools for the design of selective ACPs.
Aims: We aimed to develop a computational pipeline for predicting the activity of *de novo* generated ACPs and improving their selectivity towards non-cancer cells.
Methods: A support vector machine model was constructed and used to analyse three different peptide libraries which we generated *in silico* [2]: (i) amphipathic peptides with varying hydrophobic arcs, (ii) peptides with a hydrophobic gradient along the sequence, and (iii) peptides with the amino acid composition of known alpha-helical ACPs. The evolutionary algorithm was employed to optimize the selectivity of the most active ACPs [3]. ACPs were synthesized with solid phase peptide synthesis and their potency was tested against the MCF-7 and A549 cancer cell lines, the HDMEC non-cancer cell line and human erythrocytes.
Results: A selection of 12 predicted active and inactive peptides from each library were experimentally validated. 10 out of the 12 predictions turned out to be correct. The most active peptide was chosen as a parent for the selectivity optimization runs with the Vespa evolutionary algorithm. After the first iteration of peptide maturation, we observed a 10-fold improvement in selectivity with regard to non-cancer cells, and a 15-fold improvement with regard to human erythrocytes.
Conclusions: The results of the present study provide proof-of-concept for the applicability of machine learning and evolutionary algorithms to designing and optimising innovative ACPs.

Keywords: anticancer peptide, machine learning, evolutionary algorithm, selectivity.

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A Mechanistic Model to Facilitate Process Development of Hot-Melt Extrusion to Produce Solid Dispersions for Increased Bioavailability of Low Water-Soluble Drugs

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Introduction: Oral drug delivery in form of amorphous solid dispersion can increase bioavailability of low-soluble active pharmaceutical ingredients (API). Hot-melt extrusion has become a recognized, solvent-free, and continuous method to produce amorphous solid dispersions of API in polymers. The large number of material properties and process parameters and thereof resulting combinations make a fully experimental process optimization impossible. To tackle this problem, we propose a mechanistic model that predicts product properties and therefore has the potential to significantly decrease the number experiments. This may reduce time, costs and risks associated with process development and therefore promotes hot-melt extrusion as an attractive method to increase API bioavailability.
Aims: The present study aims to create and validate a mechanistic model that enables for rational material selection and process design by linking those variables to the product properties of hot-melt extrudates for pharmaceutical use.
Methods: The theory of the scalable model is based on the mean residence time (MRT) of the material in the extruder and the time to dissolution (TTD) of the API in the molten polymer during the extrusion process. The MRT was calculated based on an extended model of Gao et al. [1] in combination with the model of Potente et. al. [2]. The TTD was modeled by combining the Flory-Huggins theory for polymer-solvent miscibility and the approximated solution of the dissolution time of a solid sphere in an unbound, stagnant liquid by Rice et. al. [3], which we extended by a variable solute concentration. The diffusion coefficient of the API in the molten polymer was obtained by calculating the mean square displacement of API molecules in molten polymer simulated by molecular dynamics. The overall MRT and MRT of compartments was measured by tracer pulse experiments. The exit die pressure and the pressure decay along the screw axis was measured with a pressure sensor at the die and a known point of zero pressure. We measured the TTD by a series of extrusions at defined MRTs and quantitative x-ray powder diffraction of the resulting extrudates as well as hot stage microscopy. Molecular dynamics were performed using Desmond in Maestro using the OPLS_2005 force field. The model computation was done in Wolfram Mathematica.
Results: Comparison of measured and modelled data support the mechanistic concepts on which the MRT and the TTD models are based. When looking at absolute predicted values, all compartments of the MRT model were predicted with good accuracy (correlation factor of 0.996 to 0.892 with slopes 1.04 to 1.39). The modelled overall MRT deviates from the measurement (correlation factor 0.828 and with slope 0.44). Further work will be necessary to improve this part of the model. The experimental results for TTD of a system Terbinafine in Soluplus showed good agreement with modelled values. Further findings include that the MRT at a certain temperature seems to be the responsible determinate that affects TTD and not screw speed when working with constant geometries.
Conclusions: It was possible to develop a sophisticated model for hot-melt extrusion in the pharmaceutical context as well as to establish experimental methods to prove the model.

Keywords: hot-melt extrusion, amorphous solid dispersion, process modeling, bioavailability.

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Establishment and Validation of Counterflow as a Method to Detect Substrates of OATP2B1

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Introduction: The ubiquitously expressed uptake transporter, organic anion transporting polypeptide (OATP) 2B1, is assumed to play a central role in pharmacokinetics and is one of the transporters previously recommended to be included in the assessment during the process of drug development. However, *in vitro* methods currently applied to identify transporter interaction as substrate or inhibitor are based on radiolabels or highly sensitive mass spectrometry. In the late 1950s a method called competitive counterflow was introduced allowing substrate identification without direct quantification of the studied compound. This method has recently been applied for the characterization of a substrate of the Organic Cation Transporter (OCT) [1].
Aims: It was the aim of this study to test whether competitive counterflow can be used for substrate identification in OATP2B1 overexpressing cell systems. Therefore, we compared three currently used cellular expression systems, a stably transfected MDCKII cell line, a vTF7 based expression system, and an adenoviral system, the last both performed in HeLa cells for transient expression of OATP2B1.
Methods: Experiments were performed in MDCKII cells, which are stably expressing OATP2B1 and in HeLa cells, which were either transfected with plasmids encoding for the transporter using the vaccinia vTF7 or transduced with an adenovirus. Transport studies with all three expression systems were performed using [^{3}H]-estrone 3-sulfate (E_1S), a known OATP2B1 substrate. Therefore, cells were treated with radiolabeled estrone 3-sulfate until saturation was reached. Then, [^{3}H]-E_1S was removed and cells were exposed to the same concentration of radiolabeled E_1S with or without a test compound until saturation was reached for the second time. The 40 test compounds used are widely prescribed drugs, clinically used plant compounds, and chemicals used as probe substrates in transport studies. Intracellular [^{3}H]-E_1S accumulation was measured by scintillation counting. The uptake was quantified and the impact of the test compound was compared to that of the solvent control.
Results: The first and second time-point of saturation of OATP2B1 in MDCKII cells as well as in the vTF7 based expression system using radiolabeled E_1S was reached at 30 min and 90 sec, respectively. However, using the adenoviral expression system the time-point of reaching saturation the second time could not be determined. The counterflow experiments testing the capability of the test compounds on E_1S transport showed that compounds including atorvastatin or glibenclamid are substrates of OATP2B1 which have been already described in previous studies [2,3]. Furthermore, we identified compounds as non-substrates of OATP2B1 e.g. the beta-blocker metoprolol. However, some of the tested compounds seemed rather to be inhibitors than substrates of OATP2B1.
Conclusions: Regarding our results we assume that competitive counter flow can be used as an alternative method for simplified detection of substrates of membrane transporters. Nevertheless, further studies are needed to prove the reliability of this method.

Keywords: OATP2B1, transporter, competitive counter flow, substrate.

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Anticancer Peptide Maturation Towards Greater Cytotoxicity

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Introduction: Membranolytic peptides, such as antimicrobial peptides (AMPs) and anticancer peptides (ACPs), represent a new class of therapeutic agents, because they target and interact with lipid membranes without the need for receptors [1]. This mechanism of action potentially hinders the development of resistance [1]. The drawback of the ACPs is they often act in the high micromolar range.
Aims: On that account, we started from known and active ACPs developed in our group, and generated new sequences by a simulated molecular evolution technique (SME) [2] in order to obtain higher activity against the cancer cell line MCF7.
Methods: In SME, a series of new sequences (offspring) are generated by mutating a parent peptide. The amino acids are not randomly mutated but in a well-defined way based on the property similarity principle [2]. For this study, the Grantham matrix [3] was used to define amino acid similarity and the transition probabilities. With this approach, we explored the activity landscape around our chosen parent peptides while retaining overall structural features. For every cycle, the offspring were synthesized with solid phase peptide synthesis and tested against MCF7 cells with the MTT cytotoxicity assay.
Results: Two computationally designed parent peptides were chosen based on their high activity and acceptable selectivity. Starting from these templates, peptide cytotoxicity against MCF7 cells was systematically optimized leading to new and significantly more potent synthetic peptides.
Conclusions: The presented study validates the use of the SME technique to optimize ACP activity and thereby discovered more potent synthetic ACPs. Furthermore we will be able to learn more about features which are important for active and selective ACPs.

Keywords: anticancer peptide, simulated molecular evolution technique, MCF7 cells, activity maturation, Grantham matrix.

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Controlling the Release of Macromolecular Drugs from Silk-based Drug Delivery Systems via Purification Process and Chemical Modification

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Introduction: Silk sericin removal is the critical step in silk fibroin (SF) purification process, resulting in structural changes of the SF. Besides the changes in SF integrity, the charge of the encapsulated drug affects the release. In this study, we investigated the influence of the purification process (degumming time) on the structure of SF and encapsulated differently charged, high molecular weight (10 kDa) dextrans to compare their release behavior. Additionally, sustained release was achieved by chemical modification via CuAAC (copper (II)-catalyzed azido alkyne cycloaddition) of the tyrosine residues of SF.
Aims: This work focuses on controlling the release of differently charged model compounds by silk fibroin purification process and aiming a targeted delivery with a biodegradable crosslinker.
Methods: SF was prepared as previously described [1]. The silk cocoons were degummed for 0.5, 1 and 2 h in 0.02 M sodium carbonate solution. SF films were prepared, mixed with the differently charged dextrans derivatives (15 mg/mL) and release studies were conducted in phosphate buffered saline (PBS) at 37°C. The differently degummed SF solutions were analyzed by SDS PAGE and size exclusion chromatography with static and dynamic light scattering. Film structure was analyzed by scanning electron microscope and model compound distribution was analyzed by confocal laser scanning microscopy and FT-IR mapping. The tyrosine residues of the silk solution were coupled with different equivalents of aniline derivative to form a diazonium salt [2]. Then the aniline derivative was allowed to react with an alkyne-poly (ethylene glycol)-alkyne (1000 Da) for 4 h to crosslink the tyrosine residues.
Results: Drug release was shown to be driven by diffusion through the SF matrix [1]. Longer degumming times, resulting in a looser SF network, lead to a more pronounced burst release for the uncharged dextran. Interestingly, the negatively charged dextran showed an inversed effect. Longer degumming times lead to a slower release due to the degradation of the hydrophilic blocks of SF which carry a negative charge at physiological pH. Therefore, electrostatic repulsion is increased within shorter degumming times, leading to a more pronounced burst release. The positively charged dextran constituted an exception, since the burst release was all over faster than the uncharged and negatively charged dextran and in between the different degumming times, no significant differences were observed. With confocal microscopy and FT-IR mapping phase separation of the positively charged dextran was observable, whereas uncharged and negatively charged dextran were homogeneously distributed in the SF films. Not only the degumming time but also chemical modification of SF was investigated. By crosslinking of SF the release rate of the dextrans was significantly decreased.
Conclusions: The silk fibroin purification is an important parameter to control the release. The degumming time affects the molecular weight distribution as well as the charge distribution in the SF matrix. As a result, these characteristics of the polymer influence the release of differently charged model compounds. On the other hand, by crosslinking the tyrosine residues of SF the release rate was significantly decreased, leading to a sustained release. These tools provide the opportunity to tailor the release with regard to aim a bioresponsive drug delivery system.

Keywords: silk fibroin, biopolymer, controlled release, click chemistry.

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Interaction of Peptides and Proteins with Lipid Membranes Studied on a Microfluidic Device

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Introduction: Peptides and proteins interact with lipid membranes in various ways, e.g. they can permeate across lipid membranes, partition into it and form pores, or induce membranolysis. To systematically study these processes, we developed a microfluidic device that allows for trapping, treatment, and analysis of up to 192 individual giant lipid vesicles (GVs). Here, we demonstrate the versatility of our microfluidic method in different applications where we exposed the GVs to chemical treatments like penetrating peptides, drugs, lysis buffers, toxins, or staining dyes for a precisely controlled duration while they are constantly monitored with microscopic or spectroscopic methods.
Aims: We want to gain a deeper understanding of processes like permeation and pore formation to finally predict the interactions of peptides with lipid membranes.
Methods: A 2-layered microfluidic device was fabricated using poly-(dimethylsiloxane) (PDMS), and covered with a glass slide. Next, a suspension of giant vesicles was prepared and supplied into the device. Vesicles (with diameters of 8 to 25 µm) were immobilized in so-called hydrodynamic traps and could be isolated from the flow in a small volume by use of integrated round valves. Fast exchanges of chemical solutions were accomplished without washing away the vesicles.
Results: We supplied peptides or toxins to the vesicles that penetrate across the membrane, lyse the membrane, or form pores in the membrane. (1) Permeation studies were done with short polypeptides. The HIV-1 trans-acting activator of transcription (TAT) domain and the nona-arginine (Arg-9) peptide possess the ability to cross natural cells as well as artificial membranes. Permeation of the fluorescence-labeled peptides into GVs was then characterized with fluorescence correlation spectroscopy (FCS), which provides information on the intra- and extra-vesicular concentrations. We found that the composition of the membrane (anionic lipids and lipids that induce a negative curvature in combination with cholesterol and neutral lipids) influences its permeability for the tested cell-penetrating peptides (CPPs). (2) Pore formation and rupture of vesicles was observed for the antimicrobial peptide Lavracin using confocal microscopy. Binding of Lavracin to a vesicle membrane that mimics a bacterial cell led to the growth of membrane pores after the accumulation of peptide on the partially multilamellar GV surfaces and rupture of the GV within minutes. (3) Moreover, we analyzed the binding and pore formation of a cholesterol-dependent cytolysin, pneumolysin (PLY), in GV-membranes as a function of the cholesterol composition. In addition, we used ternary lipid mixtures to investigate PLY binding to lipid rafts. GVs featuring a high cholesterol concentration within the lipid bilayer showed fast binding and homogenous distribution of PLY in the membrane and pore formation within seconds. Furthermore, we found a phase-selective binding of PLY to lipid rafts.
Conclusions: Our microfluidic trap array constitutes a valuable platform for drug or toxin screening and can be used to visualize interactions of molecules with an artificial cell membrane.

Keywords: artificial membranes, giant vesicles, cell-penetrating peptides, membranolytic peptides, pneumolysin.

Collapse Temperature Modifiers in Freeze-Dried Protein Formulations

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Introduction: Freeze-drying of biopharmaceuticals is a lengthy and therefore expensive process step used to improve product stability. Hence process optimization mainly focuses on reducing cycle time which can be achieved by e.g. increasing primary drying temperature. However, product temperatures above the glass transition temperature (T'_g), which is relatively low for common bulking agents like sucrose, may lead to cake collapse. Collapse can be detrimental due to lack of elegance and potential influence on product quality attributes.
Aims: The aim of the present study was to investigate excipients to increase the collapse temperature of protein formulations and their effect on product quality.
Methods: Dextran 1, 40, 150 and 500 kDa, HES, and HPβCD formulations were prepared with BSA (10 mg/mL) with a total solid content of 80 mg/mL at various sucrose/modifier ratios. The formulations were screened for T'_g and $T_{collapse}$ by DSC and Freeze-Dry Microscopy. The formulations were freeze-dried and visually inspected. To further characterize the product quality, residual moisture, specific surface area, and reconstitution time were determined.
Results: A significant increase in $T'_g/T_{collapse}$ and subsequent elimination/reduction of collapse/dents was already observed with 20% modifier. However, some modifiers showed very long reconstitution times at concentrations of 40% or higher.
Conclusions: All modifiers investigated significantly reduced the occurrence of cake collapse. Good correlation was found between cake appearance and DSC results. The investigated modifiers will be further screened with different proteins and tested for protein stability.

Keywords: lyophilization, freeze-drying, protein formulation, dextran, collapse.

Optimization of Lipid-Based Gene Delivery Systems Towards Improved Transfection of Hepatocytes

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Introduction: Despite advances in medical research, liver diseases remain one of the few diseases with increasing incidence rates in western countries over the past decades. Although many drugs are taken up and cleared from the blood by hepatocytes, there is still a lack of efficient delivery strategies specifically targeting hepatocytes, e.g. for gene delivery [1].
Aims: To elucidate important factors for successful gene delivery to hepatocytes using lipid-based drug delivery systems, we have used a design of experiment.
Methods: Novel lipid-based nanoparticles and commercially available transfection (TFX) reagents were investigated *in vitro* and the influence of various factors on the transfection efficiency was evaluated, i.e. amount of DNA, TFX reagent-to-DNA ratio or type of plasmid. Transfection efficiency was determined by introduction of green fluorescent protein (GFP) into cells and subsequent confocal microscopy and flow cytometry 24 h after transfection.
Results: The highest percentages of transfected cells were achieved at low to medium ratios, high amounts of DNA, and the use of a miniaturized DNA vector.
Conclusions: The present study represents a promising starting point for further improvement of hepatocyte specific gene delivery vehicles.

Keywords: gene delivery, nanoparticles, non-viral, DNA.

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Targeted DNA Therapeutics - Challenges and Opportunities for Hepatic Diseases

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Introduction: Hepatic disorders affect millions of people around the globe and incidence rates are further increasing. While survival rates have improved for most diseases during recent decades, liver diseases still represent a considerable public health burden. Current therapies for diseases of hepatocytes are limited and in most cases only treat symptoms. Therefore, improved therapeutic technologies are urgently needed. Targeted nanomedicines for the delivery of therapeutic genes have the potential to overcome the lack of satisfactory and alternative treatment options [1].
Aims: This research project focuses on the design of functional nanomedicines for targeted nucleic acid delivery (i.e. plasmid DNA) to liver parenchymal cells.
Results: One strategy is the active drug delivery via the asialoglycoprotein receptor (ASGPR), which is predominantly expressed on hepatocytes. However, the success of such technologies depends on the expression level of the ASGPR [2] and the targeting ability of the gene delivery system [3,4]. To evaluate the applicability of our approach in the clinic, we have evaluated the expression level of the major receptor subunit ASGR1 in human tissue samples. In order to mediate the selective intracellular delivery of drugs such as nucleic acids to parenchymal liver cells, we have developed a ligand-modified liposomal drug delivery system.
Conclusions: Our study highlights the importance to validate potential targets for their clinical relevance and develop nanoparticulate drug delivery systems accordingly. With our targeting strategy, we have the possibility to address an unmet medical need. Non-viral gene delivery may offer well tolerated therapeutic options for diseases of the liver such as Crigler-Najjar syndrome, where a single gene defect leads to severe clinical manifestations. This research project is the first step towards a future therapeutic intervention for this and other orphan monogenetic liver diseases.

Keywords: nanomedicine, gene delivery, active targeting, hepatocyte, asialoglycoprotein receptor.

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Gate Keeper Nanoparticles and Their pH Dependent Cytotoxicity in Hepatocellular Carcinoma Cell Lines

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Introduction and Aims: Recently, nanoceria has attracted enormous interest as a natural enzyme with superoxide dismutase and catalase mimetic activity. This phenomenon is based on its ability to reversibly switch from Ce^{3+} to Ce^{4+} and high oxygen mobility at its surface in natural and acidic pH. Importantly, these nano-particles are able to increase reactive oxygen species (ROS) levels in acidic pH. Since it is known that the pH environment is different in cancer as compared to normal healthy cells, this factor can be used as stimuli-response trigger.
Methods: In this study, cytotoxic effects of free nanoceria and nanoceria coated mesoporous silica nanoparticles were investigated in HCC derived cancer cell lines (HepG2, Hep3B and Huh7) at pH 7.4 and pH 5. Nanoparticle characterization was performed by scanning and transmission electron microscopy, dynamic light scattering, Fourier transform infrared spectroscopy, thermal gravimetric analysis, and UV-visible spectroscopy. MTT assay and ROS content measurement were used to investigate cytotoxic effects.
Results: The results showed that nanoceria effectively coated the surface of mesoporous silica nano-particles and their enzymatic activity was initiated in acidic pH resulting in increased ROS levels in cancer cells.
Conclusions: We conclude that the use of nanoceria as gatekeeper is a promising strategy to avoid drug leakage from porous materials and in addition specifically kill cancer cells.

Keywords: mesoporous silica nanoparticles, nanoceria, gate keeper, hepatocellular carcinoma, reactive oxygen species.

HPTLC Coupled Estrogenic Activity Assessment of *Trifolium pratense* L.

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Introduction: Plant-derived estrogens, known as phytoestrogens, are present in numerous phytopharmaceuticals and dietary supplements and marketed as natural alternatives to estrogen replacement therapies [1]. Moreover, they are discussed to be indicated as complementary therapy for different types of cancers. Genistein, a naturally occurring phytoestrogen from soy bean and red clover, acts as a potent anticancer agent. However, in some types of breast cancer, the intake of genistein may be contra-indicated, since the isoflavone may promote cancer cell proliferation [1].
Aims: The rapid planar-YES assay designed for low concentrations of target endocrine disruptors in environmental samples [2] has been adapted for the screening of phytoestrogenic compounds in red clover (*Trifolium pratense* L.) supplements. The study aims to evaluate the application for bioactivity fingerprinting for multicompound estrogenic active ingredients for R&D and QC.
Methods: HPTLC Silica 60 plates (Merck, Darmstadt), chromatographed on automated equipment (CAMAG, Muttenz). Reporter-gene Assay yeast estrogen screen (YES) adapted to be coupled to HPTLC [2].
Results: HPTLC-separated extracts of red clover raw material show pronounced fluorescent zones of genistein and biochanin A indicating estrogenic activity. The half maximal effective concentration (EC50), measured by quantitative intensity evaluation of the zones of genistein and biochanin A in different concentrations was 2.1 and 3.3 ng/band, respectively. Moreover, the same compounds were identified in separated samples from commercially available red clover capsules and tablets. Excipients originating from tablet and capsules matrices such as microcrystalline cellulose or polyvinyl pyrrolidone did not interfere with the method. Daidzein only showed weak estrogenic activity, notably that a specific intestinal microbe in human is needed to bioconvert daidzein to its active metabolite equol that was not tested in our study [1].
Conclusions: The planar-YES assay can be used as a screening tool for phytoestrogens, however a limitation is given by the extrapolation of the results to effects in human cells, since they might differ in respect to cellular substance uptake. Studies elucidating such differences need to be undertaken in the future.

Keywords: phytoestrogens, endocrine testing, yeast estrogen screen, bioautography, HPTLC.

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Thermoresponsive Self-Assembled Hyaluronic Acid-Based Nanoparticles

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Introduction: Fast degradation, need for repeated injections, and limited efficacy of hyaluronic acid (HA) call into question its use for joint lubrication and dermatological applications [1].
Aims: The aim of the present study was to synthesize an injectable HA hydrogel able to form spontaneously nanostructures under heating from room to body temperature, which would render HA less sensitive to enzymatic degradation and prolong residence time at the injection site.
Methods: The steps of the study were, (i) to synthesize and characterize a new thermoresponsive copolymer (HA Nano) composed of HA and pNiPAM, able to form spontaneously nanoparticles (assessed by NMR, SEM, DLS, NTA), (ii) to synthesize a fluorescent HA Nano for performing intravital imaging, (iii) to evaluate the injectability, stability, hyaluronidase degradation (by texture analyzer, rheology and DLS respectively), (iv) to determine cytotoxicity of HA Nano on human synoviocytes (MTT test), (v) to assess *in vivo* biocompatibility, (vi) to follow the residence time and distribution in tissues of HA Nano after subcutaneous and intra-articular injection in mice, and (vii) to investigate *in vivo* the activity of the particles on osteoarthritic (OA) mice having a destabilization of the medial meniscus (DMM) (evaluated by X-ray CT, Multiplex ELISA and histology) [2].
Results: HA Nano was synthesized by green chemistry. This hydrogel spontaneously transformed into nanoparticles with a size range of 50 to 250 nm at 31 °C (Fig. 1). HA Nano appeared to be injectable, stable, biocompatible and biodegradable, with a significantly prolonged residence time at the injection site in the skin (Fig. 2) and knee joints of mice compared to conventional HA. It was less sensitive to enzymatic hyaluronidase at 37 °C. Finally, HA Nano had a protecting effect on the epiphysis and cartilage of the medial tibia and reduced serum levels/expression of vascular endothelial growth factor and cytokines (IL-1β, TNFα) in DMM model.

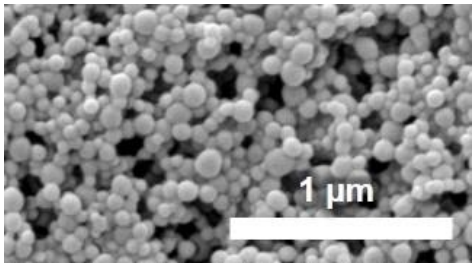

Fig. 1. SEM micrograph of HA Nano at 37 °C.

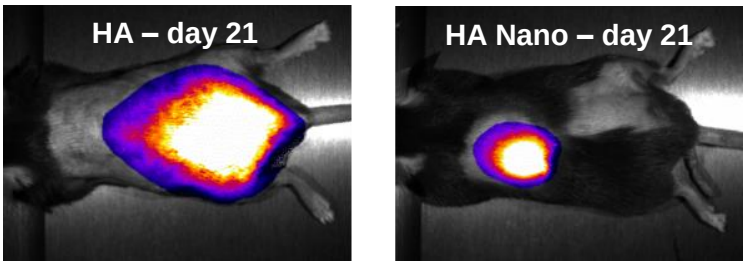

Fig. 2. Intravital fluorescence after subcutaneous injection in mice.

Conclusions: HA able to form nanoparticles spontaneously at body temperature was successfully developed to offer an extended HA treatment lifetime. Compared to conventional HA, HA Nano is less sensitive to enzymatic degradation, easier to inject, and more persistent *in vivo*. As shown in an OA mice model, it has a protecting effect on bone and cartilage, and reduces expression of OA biomarkers. HA Nano shows promise for lubricating joints, as a tissue filler in various medical applications, and potentially as a drug delivery system.

Keywords: hyaluronic acid, thermoresponsive, nanoparticles, joint lubrication, dermatological.

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Nano-Micelles – a Blood Pool Contrast Agent for MRI

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Introduction: Efficient cardio-vascular imaging procedures are needed to enable the accurate diagnosis of related diseases, such as stroke, thrombosis or heart failure for example. Magnetic Resonance Imaging (MRI) is a non-invasive technique providing a clear benefit compared to other imaging techniques by avoiding ionizing radiation for signal creation. However, MRI suffers from poor inherent sensitivity. To improve the quality of images MRI contrast agents (CAs) are frequently injected before imaging. Unfortunately there are no CAs currently on the market that allow imaging of central blood compartment (blood-pool agents). These CAs circulate in the vasculature without rapid diffusion into tissues and kidney elimination. Commercially available CAs diffuse rapidly into tissues and do not allow the addition of targeting moieties for targeted molecular imaging.
Aims: Recently, we have synthesized a macromolecule, acting as a building block for a self-assembly nano-micelles containing a DOTA-chelate loaded with gadolinium (Gd) (Figure 1). The aim of the present work was to fully characterize the nano-micelles and evaluate their potential as blood-pool CA for MRI.

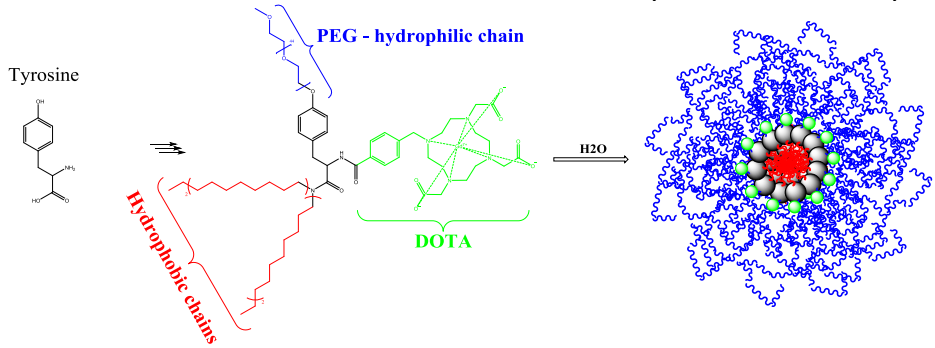

Figure 1: Building blocks and Nano-micelle formation

Methods: Full characterization of the nano-micelle building block has been reported. In this work we have produced the nano-micelles in water by nanoprecipitation and analyzed the parameters such as size and size distribution by dynamic light scattering (DLS), stability at room temperature (T=20 °C) over 4 weeks, cytotoxicity on HT1080 cells and relaxivity by Nuclear Magnetic Resonance (NMR). Finally, the micelles were injected in the tail vein of C57BL/6J mice for first *in vivo* imaging of the whole body.
Results: The nano-micelles have a mean size of 10 ± 3 nm and remain stable for at least 4 weeks. They do not show any significant cytotoxicity in HT1080 cells with the viability higher than 80% at the concentration required for imaging. First *in vivo* MR images show that they retained in the central vascular compartment for more than 4 h.
Conclusions: Compared to marketed CAs our nano-micelles seem to enable cardiovascular imaging due to their nano-micellar structure, size, and high Gd-loading [1]. As a consequence of these carefully engineered parameters, the nano-micellar CA remains in the vascular compartment for prolonged periods of time. They are therefore promising candidates as blood pool CA for vascular- and cardio-MRI [2]. Additional *in vivo* experiments are ongoing to confirm the encouraging first results. In the future, ligands will be added to the surface of MRI nano-micelles to allow targeted molecular imaging.

Keywords: MRI, contrast agent, blood pool, cardio-vascular, micelles.

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Combination of Analytical Methods for a Database of Reference Compounds in Metabolomic Studies

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Introduction and Aims: Metabolomic sciences aim at the study of low molecular weight molecules in different biological matrices. Although the investigation of the whole metabolic content can be achieved by means of untargeted approaches, the annotation of relevant metabolites is necessary for an optimal interpretation of the biological information present in the studied systems [1]. In order to make possible the identification of features, a chemical library containing above 630 standard metabolites (Mass Spectrometry Metabolite Library of Standards, Sigma-Aldrich), was investigated in our laboratory with generic HILIC and RPLC methods hyphenated to MS in positive and negative acquisition modes.
Methods: LC methods were developed on a Waters H-Class Acquity system, using Waters Acquity UPLC® BEH Amide (HILIC) and Phenomenex® Kinetex C18 (RPLC) stationary phases. MS analysis was conducted on a Bruker maXis 3G QTOF. Both methods were chosen owing to their orthogonal retention properties [2]. The quality of the chemical information delivered by each method was carefully evaluated, as well as the overlap among them based on Derringer's desirability functions, established on different criteria such as retention, sensitivity and peak shape.
Results: Computed scores allowed to spot the best method(s) for the analysis of every detected compound. Furthermore, the coverage and complementarity of the methods was further evaluated. As a result, the combination of all LC methods and ionization modes led to an overall coverage of 80% of the proposed library. It was found that when only 2 out of the 4 analytical methods were carefully chosen, the obtained coverage decreased by only 17%. Finally, the most informative set of LC methods and ionization modes was applied to the investigation of biological matrices, and the annotation of metabolites was carried out by using the constructed database.
Conclusions: The ranking of the analytical methods allowed to choose the optimal combination of them rendering the most valuable chemical information with higher throughput. The exhaustive database constructed enabled the annotation, with the highest possible confidence, of metabolites in neural cell cultures.

Keywords: metabolomics, database, desirability, metabolites annotation.

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Layer-By-Layer Coating of Whole Inactivated Influenza Virus (WIV): a Single-Shot Vaccine Approach?

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Introduction: In many situations, particularly during influenza pandemics, the requirement to administer multiple doses of a vaccine to induce immunity is an issue. It would be very beneficial to develop vaccine formulations allowing for a single dose of a vaccine to induce immunity equal to what is observed after several immunizations.
Aims: In this project, we propose to use a pandemic H5N1 whole inactivated influenza virus (WIV) [1] formulated with adjuvants, to induce a high and long-lasting immune response after a single immunization. In this context, we have explored the adjuvant capability of a layer-by-layer (LbL) polyelectrolyte coating [2] of WIV. The *in vivo* efficacy of the newly developed formulation was investigated in parallel with two adjuvants: the saponin extract "QS-21" and a squalene based oil-in-water emulsion (SWE).
Methods: LbL formulations were prepared by the successive deposition of oppositely charged poly-electrolyte coatings onto the WIV surface, namely DEAE-dextran (cationic) and sodium alginate (anionic). For this purpose, we have explored alternative polymers, concentrations of coatings, incubation times and washing methods (ultrafiltration (UF) and ultracentrifugation (UC)). The compatibility of the WIV in combi-nation with the different adjuvants was assessed in extensive characterization and stability studies (size by dynamic light scattering (DLS), zeta-potential (ZP), pH, transmission electron microscopy (TEM) imaging, and micro bicinchoninic acid (BCA) protein assays). Finally, the immunogenicity of the adju-vanted WIV was tested *in vivo* in mice, by measuring the humoral response against the vaccine by hemagglutination inhibition (HI) assay after one injection.
Results: The addition of consecutive layers of polymers onto the WIV surface for WIV/LbL formulations was confirmed by the reversal of the ZP between -30 mV and +30 mV, and a particle size increase from 150 nm to 200 nm after deposition of 4 layers. Then, the loss of WIV detected during the UF washing step (80-90% loss) was effectively reduced by switching to UC (20-30% loss). In parallel, the size of the WIV combined to SWE remained stable at 140 nm for at least 1-2 weeks (at 4 °C), but increased to 153 nm after 3 months storage. Furthermore, we observed that the hydrodynamic diameter of the WIV/QS21 particles is QS21 concentration dependent. Combined with 30 µg HA /mL WIV, the particle size was 160 nm at 20 µg/mL QS21 and 190 nm at 90 µg/mL QS21. After 1 month at 4 °C, a slight size increase of approximately 10 nm was observed, confirmed by TEM imaging where the disruption of the virus started to be observed. Preliminary *in vivo* results showed that after a single injection of WIV/SWE a strong HI titer was induced, similar to what was obtained after 2 injections of WIV alone. The subsequent *in vivo* evaluation of the other vaccine formulations is still under investigation, but initial results (2 weeks after the injection) indicate promising HI titers, as WIV/LbL induced similar HI titer to the WIV/SWE adjuvant formulation.
Conclusions: This study has confirmed the feasibility of LbL technology applied to WIV. Nevertheless, further characterizations of this formulation are still required to have a better understanding of the physico-chemical interactions between the different layers of polymers. Finally, the *in vivo* results indicate the potential of WIV/LbL as an adjuvanted vaccine formulation, and encourages further investigations for its use as a single-shot vaccine approach.

Keywords: influenza, single-shot, adjuvants, layer-by-layer.

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Evaluation of a Rat Prostate Cancer Cell Line for Targeting of PSMA-Bearing Metastases

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Introduction: Switching from *in vitro* tests to more complex *in vivo* studies represents an important step in the development of a pharmaceutical preparation. The choice of a suitable animal model, adapted to the addressed research questions, is crucial for the quality and the outcome of the study, but can be challenging, especially in case of complex formulations.
Aims: To detect prostate cancer (PC) metastases in lymph nodes, iron oxide nanoparticles, developed for magnetic resonance imaging (MRI) and magnetic hyperthermia [1] were functionalized with an aptamer targeting the prostate specific membrane antigen (PSMA). To test the performance of these particles in a clinical MRI scanner *in vivo*, a rat pc cell line (Mat LyLu), which is known to metastasize to lymph nodes, was tested for binding of the PSMA-targeting aptamer *in vitro*.
Methods: First, specific binding and internalization of the aptamer functionalized nanoparticles in PSMA-positive, human LNCaP cells and human, PSMA-negative PC3 cells was investigated by Prussian Blue assay and transmission electron microscopy (TEM). To test specific binding of the PSMA-targeting aptamer to the rat PC cell line, fluorescence microscopy and confocal laser scanning microscopy imaging, as well as saturation and competitive binding assays were performed. Additionally, we tested the presence of PSMA RNA in the rat PC cells by RT-PCR.
Results: Aptamer-functionalized nanoparticles showed a superior binding to PSMA-positive LNCaP cells in comparison to PC3 cells and scrambled aptamer particles. Internalization was confirmed by TEM. As for PSMA-positive LNCaP cells, the aptamer was binding to and internalized by the investigated rat PC cells within 1 h as shown by fluorescence imaging. Results from the saturation and competitive binding assays support the concept of interaction with a specific binding site. In addition, the presence of PSMA mRNA was detected by RT-PCR, to a lower extent compared to LNCaP cells however.
Conclusions: We developed aptamer-functionalized iron oxide nanoparticles specifically targeting PSMA-expressing cells. Our *in vitro* findings hold promise for the use of the rat PC cell line as a tumor model for PSMA targeting. *In vivo* studies using a rat allotransplant model are ongoing to confirm this hypothesis and to test our PSMA-targeted nanoparticles for detection of PC lymph node metastases.

Keywords: iron oxide nanoparticles, active targeting, PSMA, metastases.

Reference:
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Adverse Events Profile of Oral Corticosteroids Among Asthma Patients in the UK

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Introduction and Aims: Previous studies have linked oral corticosteroid use in asthma patients to various adverse events. This study aimed to assess in more depth than has previously been done the toxicity profile of oral prednisolone among adult asthma patients.
Methods: Using data from the UK-based Clinical Practice Research Datalink, we conducted a series of cohort studies, each with a nested case-control analysis, to quantify the risk of 11 different potential corticosteroid-related adverse events.
Results: Incidence rates per 1000 person-years of potential corticosteroid-related adverse events in patients with new current use of oral prednisolone ranged from 1.4 (95% confidence interval [CI], 1.0-1.8) for peptic ulcer to 78.0 (95% CI, 74.8-81.2) for severe infections. After adjusting for confounding, current oral prednisolone use was most strongly associated with an increased risk of severe infection (odds ratio [OR] 2.16; 95% CI, 2.05-2.27) compared with non-use of prednisolone. There were smaller elevated risks of peptic ulcer (OR 1.47; 95% CI, 1.12-1.92), affective disorders (OR 1.47; 95% CI, 1.32-1.63), herpes zoster (OR 1.32; 95% CI 1.19-1.48), cardiovascular events (OR 1.33; 95% CI 1.18-1.49), diabetes mellitus type 2 (OR 1.35; 95% CI 1.22-1.49), bone related conditions (OR 1.27; 95% CI 1.17-1.37), and cataract at higher cumulative doses (cumulative dose >2000mg: OR 1.43; 95% CI 1.17-1.73), compared with non-use of prednisolone. We did not observe an association between current oral prednisolone use and glaucoma, chronic kidney disease, or hypertension. Past use of oral prednisolone was not associated with any of the study outcomes. We observed possible dose-response relationships between current oral prednisolone use and the risk of cardiovascular events, affective disorders, bone-related conditions, severe infections, diabetes mellitus type 2, and cataract, but not the other investigated outcomes.
Conclusions: Oral prednisolone use is associated with an increased risk of infections, gastrointestinal, neuropsychiatric, ocular, cardiovascular, metabolic, and bone-related complications among adult asthma patients. The risk is associated with current but not past use of oral prednisolone use, and for some outcomes with the prescribed dose of oral prednisolone.

Keywords: clinical practice research datalink, asthma, corticosteroids, adverse events, observational study.

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Which Anion is the Right One? - Optimization of Electrolyte Therapy in Hospitalized Patients

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Introduction:A crystalloid solution, which is used for electrolyte and fluid replacement, must correspond in its composition to the electrolyte fraction of the patient's plasma. Ringer´s solution, the first balanced solution on the market, has been constantly developed over the past years. Meanwhile, there are different suppliers seeking to improve the original product. For the daily work in the hospital, the optimal solution for electrolyte replacement must be found among the variety of products currently in the market, in particular, in regard to critically-ill patients and their needs.
Aims: The goal of our research was to find a balanced solution, which is the most suitable solution for electrolyte and fluid substitution from a physiological point of view. Ideally, this solution can be used for intensive care patients as well as patients with restricted organ function ultimately leading to a streamlined product range.
Methods: A literature research was performed to provide an overview of the available solutions. Particular attention is paid to the composition of the organic anions and their metabolism in the body.
Results: The organic anions lactate, malate, acetate and gluconate are metabolized to bicarbonate under varying oxygen consumption. Lactate appears to be disadvantageous due to its oxygen balance; in addition, the risk of lactic acidosis cannot be ruled out. Its use can be problematic in patients with tissue hypoxia and metabolic acidosis; furthermore lactate is metabolized exclusively renally and hepatically. The chloride content of most solutions is above the physiological range, which could possibly lead to renal damage. Not all products have the same compatibility data (concomitant administration of other medications) [1-3].
Conclusions: According to our research, it is advisable to use a solution which contains acetate exclusively as an organic anion. In addition, it should have the most physiological chloride content. The availability of compatibility data is a relevant criterion for the selection of the product. Besides the availability, another important selection criterion is the quality and handling of the containers. The example of the electrolyte replacement solution demonstrates impressively how practical questions occurring during the hospital-pharmacy workday can be satisfactorily answered when analyzed scientifically.

Keywords: electrolyte balance, ringer acetate, metabolic acidosis, crystalloids, balanced solution.

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