Doctoral School
Pharmaceutical Sciences

KARL-FRANZENS-UNIVERSITÄT GRAZ
UNIVERSITY OF GRAZ

1st Pharma DocDay

Book of Abstracts

February 07th, 2013

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February 07th 2013

Time Schedule

9:00 - 9:15	Opening by Andreas Zimmer (Head of Doctoral School) and Kurt Schmidt (Deputy)
9:15 - 10:05	**Plenary Lecture - Dubravko Jelić - In vitro Screening in Drug Discovery & Development – Rosmarinic Acid as Anti-inflammatory Activity Example**
10:05 - 10:50	Agnieszka Lower-Nedza - Studies on Traditional Chinese Medicines Sandra Prasch (ST) - Plant-derived Modulators of Antibiotic Resistance
10:50 - 11:15	Coffee Break
11:15 - 12:15	Simone Schrank - On the Drying of Calcium Stearate Pellets: Variations in Final Dosage Form Properties Bernhard Scheicher - Influence of Protamine-Titration-Process on Self-Assembling of Secretoneurin Loaded Proticles
12:15 - 13:45	Lunch Break
13:45 - 14:30	Susanne Fink - Reducing Barriers: Migrations, Minor Ailments and Medicines Noor ul Amin Mohsin (ST) - Synthesis of New Bicyclic and Heterocyclic Substances Effective against Protozoa
14:30 - 16:00	Coffee Break and Poster Presentation
16:00 - 17:15	Elisabeth Strutzmann - Investigating the Glycan-mediated Chemokine Mode of Action Martha Gschwandtner - Expression, Purification and Characterization of Chemokines and Chemokine Mutants Christian Heine (ST) - Investigation of Anticooperative Binding Discrepancy in Nitric Oxide Synthases
17:15	Closing Remarks and Get together

ST: Short Talk

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Oral Presentations

Poster Presentations

Studies on Traditional Chinese Medicines

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Traditional Chinese medicines (TCMs) are complex mixtures containing up to hundreds different constituents but only a few compounds are responsible for their pharmacological activities. These large numbers of constituents in the TCMs make the screening and analysis of the bioactive components extremely difficult. Chromatography is one of the main techniques applied because of its powerful separation and sensitive detection.
WHO introduced the use of chromatographic methods for the standardization of medicinal plants and herbal medicinal products. Nowadays the high performance liquid chromatography (HPLC) together with thin layer chromatography (TLC) are the most common analytical methods to determine either the quality or the quantity of compounds in the phytochemical evaluation. The aim of this study was to optimize HPLC and TLC methods for the simultaneous quantification of the main compounds present in the Traditional Chinese formula Si-Miao-San and its two modifications. Representative chromatographic fingerprinting of batches of the herbal components of the formulations originated from different Chinese provinces were applied to offer integral characterization of the single herbs.
The antioxidant and anti-inflammatory effect of Si-Miao-San, its two modifications, the single herbs and the main pure ingredients were determined by the following well established and widely accepted in vitro assays: Tyrosinase assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the non enzymatical lipidperoxidation (LPO) assay.
Permeability is a major factor for orally applied drugs. Therefore permeability assay and parallel artificial membrane permeability analysis (PAMPA) were used to determine passive, transcellular drug permeability. The permeability of the well known TCM formulation (decoction of Si-Miao-San and its modifications) was evaluated in the experiment. Hexadecane/hexane and lecithin/dodecane were used to create artificial membranes. Different parameters of the methods were evaluated.
As cytotoxicity plays an important role in biochemical pathways extracts with the strongest inhibition activity were tested in a cytotoxicity assay.

Plant-derived Modulators of Antibiotic Resistance

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Tuberculosis (TB) is among the most serious infectious diseases worldwide. One third of the world population can be classified as infected with mycobacteria. Especially the arising resistances of mycobacterial strains are of concern for public health. The antibiotics which are available against TB are more and more ineffective against these bacteria and therefore threatening millions of lives every year.
The treatment against TB requires several months why many patients discontinue the therapy and therefore being the direct cause of emerging multi-resistant strains or even total-drug-resistant strains. Therefore, new antimycobacterials are in need to overcome this threat. There are only a few new compounds in the pipeline for approval and only one compound approved recently with the drawback of not changing the resistance problem.
Efflux pumps are involved in the development of antibiotic resistance due to the fact that they are transporting cytotoxic compounds out of the cells securing the survival of the cell. Therefore the administered antibiotic is not able to reach the effective concentration level. In Mycobacterium tuberculosis (MTB) there is evidence for several dozens of such efflux pumps, such as MmpL7 which is involved in isoniazid resistance [1], currently the most important antituberculotic drug. Effective efflux pump inhibitors (EPI) could be administered together with the common antibiotics returning their full effectiveness or prolongate the period until resistance develops.
Previous studies lead to identification of a number of EPIs from extracts of species of further *Zingiberaceae* plant family [2,3]. As a consequence, the aim of this study is the screening of *Zingiberaceae* plants for their potential antibacterial effects as well as their resistance modifying activities. Due to structural similarities to pungent compounds of ginger we have included *Capsicum* and Schinus sps. in our investigations. Extracts (hexane, dichlormethane and methanol) of the 24 selected plants will be screened for their minimal inhibitory concentration (MIC) and modulatory effect against Mycobacterium smegmatis (MSm). The promising ones will be fractionated and again tested for their MIC and modulation factors (MF) until having single compounds with putative antimycobacterial effects. In collaboration with a working group at the New University of Lisbon their modulatory effects on mycobacteria more closely related to MTB will be tested.

Another aspect of the project concerns resistance of gram-negative bacteria, particularly *Campylobacter* strains. These bacteria have the highest incidence in human gastroenteritis and show similar difficulties with multi-resistant strains. Therefore we will perform the screening also on sensitive and multi-resistant *Campylobacter jejuni* strains in collaboration with a working group at the University in Ljubljana.

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On The Drying of Calcium Stearate Pellets: Variations in Final Dosage Form Properties

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Pharmaceutical pellets intended for oral dosing are frequently prepared via the wet extrusion/spheronization technique. The last “active” step of this process is drying. Although the drying method and/or the drying conditions applied are considered to highly influence the final pellet characteristics, investigations in this field are rare.
The current study focuses on the drying behavior of calcium stearate (CS) pellets containing ibuprofen as model drug [3] in different concentrations. After spheronization three different drying methods were applied to remove the granulation liquid, i.e., 50% ethanol; i) desiccation over silica gel at ambient conditions, ii) drying in a fluid-bed (FB) apparatus at different inlet-air temperatures and iii) lyophilization. It was ensured via differential scanning calorimetry and X-ray scattering that the drying procedure did not alter the physicochemical properties of both, ibuprofen and CS.
The course of the drying process depended upon the i) drying technique, ii) the applied drying parameters and iii) the drug loading. The drying profile of the pellets dried via desiccation depended upon the ibuprofen loading, where lower ibuprofen concentrations yielded in initially linear profiles , i.e., the constant rate period. For increased ibuprofen loadings the drying profiles revealed a non-linear shape. The rate of drying decreased throughout the entire process due to shrinkage phenomena that generated a gradually decreasing pellet surface area. The drying profiles of the FB processes were again non-linear in shape independent upon the inlet-air temperature. This suggests that only the falling rate period was present, although pellet shrinkage was observed. During lyophilization shrinking was largely suppressed since no liquid was involved resulting in minimal capillary flow. The differences in the shrinking behavior correlated with the pellet size distributions. In contrast, the shape was neither influenced by the drying conditions nor by the drug loading.
Drug release studies demonstrated that the drying procedure governed the ibuprofen release rate, which decreased in the order lyophilization, FB drying (dependent upon the inlet-air temperature) and desiccation. Differences in drug liberation characteristics can be explained by the pellet micro-structure and/or the spatial drug distribution throughout the pellets. It was shown before that for high drying rates (i.e., FB drying in the current study) dissolved components are likely to be transported towards the outer shell [2]. Therefore, the ibuprofen distribution along the pellet cross sections was investigated using Raman mapping. However, ibuprofen was rather homogeneously distributed throughout all pellets despite the different ways of liquid removal. This is attributed to several reasons. First, only small amounts of ibuprofen were expected to be dissolved during pellet preparation, although ibuprofen is highly soluble in the granulation fluid (i.e., 48 g/l). Second, phase separation of 50% ethanol occurs in the presence of ibuprofen between 25 and 40 °C [3], which causes the dissolved ibuprofen to crystallize in the water phase. Third, drug transport towards the outer pellet regions is most pronounced during the constant rate period, which was not observed for all drying procedures.
The micro-structure was strongly impacted by the drying procedure. The drying conditions affected the extent of pellet shrinkage due to variations of drying regimes. Since ibuprofen release occurred based on diffusion mechanism, ibuprofen release from the CS matrix pellets was a function of the pellet micro-structure (i.e., porosity, pore size and shape). The drug release rate increased with increasing pellet porosity and was linearly proportional to the specific surface area for low ibuprofen loadings.

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Influence of Protamine-Titration-Process on Self-Assembling of Secretoneurin Loaded Proticles

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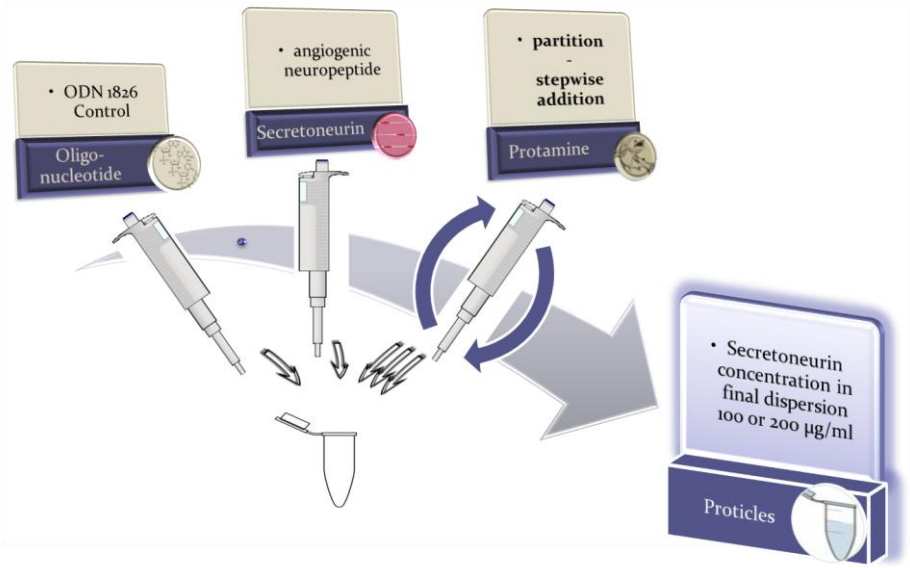
One of the major problems for the use of therapeutic peptides or proteins is their rapid degradation. In order to achieve successful treatment an applicable formulation of the peptide has to be developed. In this study the angiogenic neuropeptide secretoneurin (SN, named by Kirchmair et al.), which is a promising drug for the treatment of peripheral arterial disease and ischemic heart disease, was embedded into protamine-oligonucleotide-particles, so called Proticles. The formation of Proticles occurs by a self assembly process, previously described by Lochmann et al.
In order to include a high amount of SN, a titration process of protamine was developed, which present a new approach for the formation of Proticles. To study influences on hydrodynamic diameter (ZAve) of the particles, zeta potential and incorporation efficiency of SN, different methods of particle preparation were examined by varying i) the titration interval of added protamine and ii) the mass ratios of the components. Values for ZAve are around 130nm if the whole amount of protamine is added at once, or 1100nm if a titration process is used. Particles are positively charged and show a zeta potential between 36 and 62mV. Up to 50% of initial amount SN can be incorporated into the Proticles mainly depending on the concentration of protamine in the particle dispersion.

Graphical abstract



Acknowledgement:
Authors would like to thank piCHEM Graz for synthesizing the peptide Secretoneurin.

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Kirchmair, R., R. Hogue-Angeletti, et al. (1993). "Secretoneurin—a neuropeptide generated in brain, adrenal medulla and other endocrine tissues by proteolytic processing of secretogranin II (chromogranin C)." Neuroscience **53**(2): 359-365.

Lochmann, D., V. Vogel, et al. (2004). "Physicochemical characterization of protamine-phosphorothioate nanoparticles." Journal of Microencapsulation **21**(6): 625-641.

Reducing Barriers: Migrations, Minor Ailments and Medicines

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Objectives: (1) Provide an in-depth analysis of patients' perspectives and processes of decision making regarding the treatment of potentially self-treatable conditions among migrants of sequent generations living in the city of Graz, Austria. (2) Learn about culture-specific patterns in response towards minor ailments of people having an ethnic background in Bosnia-Herzegovina, Croatia and Turkey. (3) Examine data to compile a questionnaire and test the findings through quantitative data analysis.
Methods: A qualitative data analysis was performed, utilising inductive methods according to the ideas of grounded theory. Six semi-structured, qualitative interviews were conducted in winter 2011, following the Ethical Guidelines for International Comparative Social Science Research (MOST, UNESCO). Participants were selected by means of purposeful sampling utilising various networks and cooperating with a range of migrant organisations. Interviews were transcribed and analysed according to Hoffmann-Riem.
Results: When people need information on health issues, they tend to see a doctor rather than utilising pharmacies or health centres, although most considered themselves only seeing an M.D. if really necessary. Reading up on the internet is also common, although all participants stated that they do not trust the internet as an information source. More than half are sceptical of traditional remedies and some also stated that they had not used traditional treatments at all, although they actually had without recognising them as such.
Conclusions: Findings affirm high levels of insecurity and scepticism regarding the use of alternative and traditional treatments, irrespective of a participant's ethnicity. Results also suggest that people read up on health issues, yet do not feel competent enough to make health-related decisions or cannot differentiate between self-treatable conditions and those that are not. In order to make verifiable statements, more details on the accessibility and functionality of the present health care system in relation to the idea of Self Care are needed. Therefore, quantitative data analysis will focus on people's motivations in relation to the treatment of minor ailments.

Synthesis of New Bicyclic and Heterocyclic Substances Effective against Protozoa

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Malaria and Trypanosoma are among the leading cause of death across the world. According to the CDC report 300- 500 million people are infected annually from this disease and more than one million die. WHO estimated that in 2010 there were 216 million documented cases of malaria and that year between 655,000 and 1, 2 million people died from this disease. Several antimalarial drugs are being used for the treatment of malaria either alone or in combination but their efficacy is decreasing due to the emergence of multidrug resistance strains of plasmodium falciparum e.g. chloroquine resistance strains of plasmodium falciparum. Even against the artemisinin and its derivatives resistance has been observed recently. Similarly about 50,000-70,000 people are infected from human African trypanosoma. Melarsoprol and other antitrypansomal drugs are being used for treating this diseases but the resistance to these available drugs is increasing. Therefore the synthesis of new antiplasmodial and antitrypanosomal drugs is of utmost importance. Bicyclo-octanes have been prepared by the reaction of benzylidene acetone and dialkylamine in a one pot reaction. They showed distinct activity against plasmodium falciparum and trypanosoma brucei rhodesiense. Later on it was investigated that a basic center is essential for the good antitrypanosomal and antifaciparum action of bicyclo-octane. Therefor several analogues of bicyclo-octanes with modified amino function have been prepared. A second basic center was incorporated into the bicyclic ring system to contribute positively to the antiplasmodial and antitrypanosomal activity. This was succeeded via Beckman or Schmidt reaction and subsequent reduction to azabicyclo-nonanes. Azabicyclo-nonanes are therefore serving as a lead compound for the further studies. In the present investigations some derivatives of azabicyclo-nonanes will be prepared with different side chain at amino position 4. In addition other heterocyclic substances will be prepared. The structures will be determined by one and two dimensional NMR and UV, IR, MS. The biological activities will be investigated at Swiss tropical and public health institute Basel, Switzerland.

Investigating the Glycan-mediated Chemokine Mode of Action

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The chemokine-mediated attraction of leukocytes to a site of inflammation is a key mechanism in inflammation. Especially CXCL8 is responsible for attracting neutrophils to inflammatory sites by establishing a chemotactic gradient. Neutrophils become activated through the interaction of CXCL8 with its specific G-protein coupled receptors (GPCRs) CXCR1 and CXCR2. In addition, binding of CXCL8 to endothelially presented heparan sulfate (HS) chains play a major role for the establishment of the solid phase-like chemotactic gradient, leading to changes in the oligomerization state of CXCL8 and ensuring an optimal presentation to the GPCRs on the leukocytes. Proteoglycans like the Syndecans and the Glypcians are the main source of HS and chondroitin sulfate (CS) on the endothelial cell surface [1]. Their expression varies in a cell type specific manner and is regulated by intracellular and exogenous stimuli. Upon stimulation with TNFα and LPS, the expression patterns of various proteoglycans in human pulmonary microvascular endothelial cells and a human umbilical vein endothelial cell line was found to change significantly [2].
In order to study the effect of chemokines and mutants with increased GAG-binding affinity on the expression of their proteoglycan co-receptors, we investigated the expression levels of the Syndecans and Glypicans using real-time PCR and flow cytometric analysis in various inflammatory models.
All data derived from the mRNA and protein level have been compared to gain a better insight into the gene expression changes following the interaction of chemokines with proteoglycans, especially the influence of chemokines on the syndecan profile of endothelial cells.

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Expression, Purification and Characterization of Chemokines and Chemokine Mutants

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Chemokines are chemotactic cytokines that are involved in various physiologic as well as pathologic processes (e.g. homeostasis, inflammation and tumorigenesis) by interacting with G-protein coupled receptors and glycosaminoglycans.
Two chemokines called secondary lymphoid-tissue chemokine (CCL21) and interferon-gamma induced protein 10 (CXCL10) are in the focus of the underlying study.
The prime goal is to establish a random mutagenesis method for generating novel chemokine mutants with altered GAG-binding affinity and improved biological functions for therapeutic use. Additionally, a deeper insight into the structure-function relationship should be gained by analysing chemokine mutants regarding their binding affinity to their receptors/ co-receptors and their biological activity.
The chemokine mutants are generated by applying site-directed mutagenesis methods and by comparing them with random mutagenesis methods (such as error-prone PCR). A set of screening assays to select the chemokine mutants which embody the desired properties is currently developed, making use of heparin-sepharose columns and methods like enzyme-linked immunosorbent assay and surface plasmon resonance. In parallel, an expression procedure for soluble and functional protein production in E.coli is established and various assays to characterize the chemokine wild types and their mutants regarding their affinity towards glycosaminoglycans are applied.
An overview of the workflow and the hitherto existing results will be presented.

Investigation of Anticooperative Binding Discrepancy in Nitric Oxide Synthases

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Introduction:
There are three isoforms of NOS (nitric oxide synthases): neuronal (nNOS), endothelial (eNOS) and inducible NOS (iNOS). All of them generate nitric oxide (NO) from L-arginine, O_2 and NADPH-derived electrons. NOS consist of a FAD- and FMN-containing reductase domain, an oxygenase domain that accommodates haem and tetrahydrobiopterin (BH_4) as prosthetic groups and a calmodulin (CaM)-binding region. Among other cofactors BH_4 has a unique role in NO catalysis. Formation of NO only occurs when BH_4 and the substrate L-arginine are present. In the absence of BH_4 superoxide is produced instead. Nitric oxide synthase is only active as a dimer. In its maximally active form each subunit contains 1 equivalent of BH_4, but as isolated the enzyme - particulary the neuronal isoform – usually contains only one BH_4 per dimer. Previous studies on this phenomenon suggested that the enzyme has two identical but highly anticooperative BH_4 binding sites per dimer with apparent dissociation constants of << 1 nM (high-affinity binding site) and 0,1-1 µM (low-affinity binding site). Although this hypothesis explained most of the observations quite well, some discrepancies between pteridine binding studies on the one hand and activity assays on the other, remained unsolved. Furthermore, none of the many crystal structures of NOS that have since been published appears to exhibit a difference between the mode of BH_4 binding in the two subunits. Therefore, we recently decided to reinvestigate the issue.

Preliminary results:
Binding studies with radiolabeled BH_4 generally appear to confirm the original hypothesis, since radiolabeled NOS-bound BH_4 is completely expelled by an excess of cold ligand. We observed that BH_4-free NOS displays monophasic BH_4 binding initially, with high-affinity binding developing in approximately 15 minutes. By contrast, in activity assays, we found that after preincubation with BH_4, inhibitory pteridines like 4-amino-BH_4 (4-ABH_4) and 7,8-dihydrobiopterin (BH_2) can only inhibit NOS by 50 % even after prolonged incubation. Vice versa, after preincubation with 4-ABH_4, incubation with BH_4 can only activate the enzyme to 50 % of the maximal activity. These observations are inconsistent with anticooperative binding and rather suggest (quasi-) irreversible binding in the high-affinity mode. Interestingly, we also found that BH_2, which binds to BH_4-free NOS with similar affinity as BH_4 and 4-ABH_4, does not seem to display high-affinity binding at all. Moreover, preliminary studies appear to indicate that biphasic binding may be peculiar to the neuronal isoform.

Aims:
In careful competition studies with BH_4 and a range of activating and inhibitory pteridines we aim to further investigate the apparent discrepancy between the radiolabeling studies and the activity determinations. We will also further study the nature of the conversion that creates the high-affinity site/mode in BH_4-free NOS upon incubation with BH_4. We aim to investigate the reason for the different behavior of BH_4 and BH_2 with regard to NOS binding, and to look into the cause of the apparently different behavior of nNOS and eNOS. Furthermore, we aim to elucidate the structural basis for the strikingly different affinities of BH_4 binding at high- and low-affinity.

Acknowledgements:
FWF

Investigating Chemokine Interactions and their Oligomerisation Behaviour

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Chemokines are small chemoattractant cytokines best known for their crucial role in regulating leukocyte trafficking during immune surveillance and inflammatory disorders. There is further evidence that chemokines are involved in atherosclerosis, cancer progression and HIV infection. Due to their implication in various diseases, the chemokine system has been the subject of intense investigation over the last decades for the purpose to develop novel drugs.
Despite the low sequence homology, chemokines share a highly conserved tertiary structure. However, they have been proven to be organized much more diversely than originally expected. Several chemokines form dimers and higher-order oligomers with a significant diversity sometimes resulting in unstructured aggregates. In addition, the chemokines not only form homo- but also hetero-oligomers with other chemokines. Besides interacting with their specific seven transmembrane G-protein coupled receptors (GPCRs), chemokines interact with the carbohydrate moieties (glycosaminoglycans or GAGs) of proteoglycans on endothelial cells and in the extracellular matrix. GAGs promote furthermore the formation and stabilization of oligomeric chemokines.
We are interested in studying complex formations and dynamic interactions of chemokines (mainly CXCL8 and CCL2) amongst themselves, with other proteins and with GAGs. Chemokine oligomers and interactomes are characterized by the application of a variety of biophysical and biochemical methods such as isothermal fluorescence titration, co-immunoprecipitation, affinity chromatography, 2-D gel mobility and chemical crosslink. By means of these assays we expect to identify further interacting partners of CXCL8 and CCL2 and to gain a better insight into the molecular mechanisms of chemokine bioactivity.

Acknowledgements:
ProtAffin Biotechnolgie AG
FFG

P. falciparum – GAG Interactions as a Key Role for Future Malaria Treatments

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The importance of highly negatively charged glycosaminoglycans (GAGs) for forming a major part of the extracellular matrix thereby acting as a first interacting partner for exogenous infective particles on endothelial cells is a generally accepted fact. Among the vast diversity of possible interaction partners, especially signaling and surface attached microbial and parasitic proteins provide an even more interesting research field.
When it comes to the Malaria proteome, the number of potential GAG interacting proteins is outstanding, even more so due to the parasite's "journey" along and through different surface structures including human endothelia, mosquito gut and salivary glands epithelia.
The traditional medical treatments provide a resource of low molecular weight compounds aiming at different structures in the biosynthetic and energy providing structures within malaria infected hepatocytes and erythrocytes. These drugs show, at least in combination, good results despite the increasing cases of resistant strains and the enlarging endemic areas which highlights the need for a novel class of anti-malaria drugs and vaccines.
The molecular mechanisms of Pregnancy associated malaria, caused by Plasmodium falciparum, are quite well investigated. The genetic conservation of the major surface molecule found on the infected erythrocyte, the PfEmp 1 var2CSA protein, gives a good model for getting better insights into the interactions between GAGs and surface expressed proteins of the infected erythrocytes.
We have recently initiated the investigation of such interactions using a variety of biophysical methods including Isothermal Fluorescence titration, circular dichroism as well as anisotropy measurements which was preceded by bacterial expression and purification of the recombinant major GAG- binding domains of PfEmp 1 var2CSA. First biophysical data as well as expression and purification results will be presented.

Acknowledgements:
FFG

Design, Optimization and Characterization on Monoamine Oxidase modified Biosensors for the Determination of Biogenic Amines

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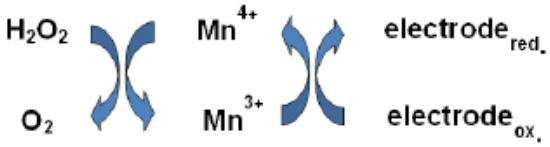
Biogenic amines (BA) and especially among them dopamine, norepinephrine and serotonin play a key role in brain metabolism. The regulation of these biogenic amines is carried out by monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B). A disruption of this regulation leads to neurodegenerative diseases such as Morbus Parkinson, Morbus Alzheimer and depression. Therefore there is a strong need of diagnostic and analytical devices for the determination of Bas. Biosensors are interesting tools in analytical chemistry and diagnostics as they produce a direct signal, are not time-consuming and economic.

In this thesis highly selective and sensitive analytical devices for monitoring biogenic amines in complex matrices should be developed using a flow injection analysis system. To achieve this goal biosensors should be designed on the basis of MnO_2 modified carbon paste electrodes with human MAO-A and MAO-B as biological recognition elements. According to the following quation MAOs catalyse the oxidative deamination of biogenic amines in the presence of oxygen and water. The products of this reaction are hydrogen peroxide, ammonia and the corresponding aldehyde.

$$R\text{-}CH_2\text{-}NH_2 + O_2 + H_2O \xrightarrow{MAO} R\text{-}CHO + NH_3 + H_2O_2$$

The enzymatically produced H_2O_2 is detected at the MnO_2 modified carbon paste electrode at a potential of +0.4 V vs. Ag/AgCl by using Sörensen phosphate buffer pH 7.5 as media [1]. The functional principle of the transducer is shown in the following scheme [2].



The fixation of the enzyme is a crucial step in preparing biosensors. It has been performed with different types of dialysis membranes.
The behaviour, sensitivity and stability of the produced biosensors were investigates and will be discussed in the poster.

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Metabolomic and Pharmacological Profiling of Lonicera Species

Ortmann S.[1], Sturm S.[2], Binder M.[3], Atanasov A.G.[4], Malainer C.[4], Heiss E.H.[4], Stuppner H.[2], Dirsch V.[4], Bochkov V.[3], Zhao Z.M.[5], Miao J.[5] Bauer R.[1]

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For this pilot study we used the upper parts of four different Lonicera species (*Lonicera dasystyla* Rehd., *Lonicera confusa* (Sweet) DC., *Lonicera hypoglauca* Miquel, *Lonicera macranthoides* Hand.-Mazz.). Leaves and stems of these species have been investigated separately. Extracts were made using ASE (Accelerated Solvent Extraction) with ethanol as solvent. The extracts have been analysed by NMR and LC-MS analyses. The NMR- and LC-MS-data was analysed with SIMCA P+ and the gained results indicated a clean discrimination between the species as well as between the extracts from leaves and stems. In addition the anti-inflammatory activity of these extracts has been studied in various assays: Inhibitory effect on NO production in LPS-/IFN-γ-induced RAW 264.7 mouse macrophages, interleukin-8 expression in TNF-α- and LPS-induced HUVEC cells and NF-κB transactivation activity as well as activation of peroxisome proliferation activated receptor β/δ (PPAR β/δ) in HEK293 cells have been investigated. The pharmacological results showed differences in the anti-inflammatory activity between the species. Furthermore it could be shown that the leaves are more active than the stems. There also seems to be discrimination between active and non-active species when combining the NMR-data with the pharmacological activity. For the prediction of active compounds more samples will be investigated.

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Total Synthesis of New Semiquinone-derivates Based on Jacaranone

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Because of the increasing drugresistences of Plasmodia against common antimalaria agents, new antiplasmodial compounds based on Jacaranone are synthesized in this work.
The essential precompound is 2-[4-(Thexyldimethylsilyloxy)phenyl]ethanol (Thexyl-Tyrosol), which is prepared from Methyl-2-(4-hydroxyphenyl)acetate by silylating with the protecting group Thexyldimethylchlorosilane (TDSCl) followed by the reduction of the ester with LAH to an alcohol.
The ethanolic chain is the most important target for further reactions as described below.
On the one hand, the amino acid Boc-Leucine is esterified with Thexyl-Tyrosol, followed by the cleavage of the Silylgroup and followed by oxidation. The last step is the cleavage of the Boc-group to obtain a free amino group.
On the other hand the Thexyl-Tyrosol is used for the Mitsunobu reaction with cyclic imides, for the N-Alkylation of amines with the primary alcohol, for the conversion of the ethanolic chain into an alkylhalide (bromide and iodide) followed by N-alkylation to its corresponding amine.
The subsequent synthesis step is used for the cleavage of the Silyl protecting group to obtain the free phenolic hydroxyl group.
The oxidation with Phenyliodinediacetate (PIDA) is the last step, which finally leads to the Jacaranonederivative.
The antiplasmodial activity and cytotoxicity of these new compounds will be tested by the Swiss Tropical and Public Health Institute in Basel.

Applications of Hypervalent Iodine Reagents in the Preparation of Synthetic Quinoids

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Jacaranone and its glucosides as well as other naturally occurring quinoids are very interesting chemical leads with several biological activities. [1]
To prepare these promising compounds a number of procedures were found in the literature, but most of them suffer from limitations that are manifested in low yields, mixtures of products or various side reactions. [2]

As part of a program directed at the discovery of new antiprotozoal agents, we were interested in the facile and reproducible construction of the quinol skeleton out of *para*-substituted phenols.
In this context the environmentally benign hypervalent iodine(III) reagents, such as $PhI(OAc)_2$ (PIDA), $PhI(OCOCF_3)_2$ (PIFA) and the recently published µ-oxo-bridged phenyliodine trifluoroacetate are a versatile oxidation tool meeting the concept of green chemistry due to their low toxicity, easy handling, safety and ready availability. [3]

Our investigations especially referred to the oxidizing behaviour of the "µ-oxo-bridged-dimer". The use of this dimer instead of PIDA and PIFA for oxidative de-aromatizations could produce the appropriate *para*-quinones in comparable or somewhat better yields.
A protected glucosidic ester of the preliminary stage of jacaranone and derivatives of commercial available *para*-substituted phenols acted as starting materials in order to evaluate the comparison between the different hypervalent iodine(III)-mediated phenolic oxidations.

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Dexamethasone-loaded Lipid Nanoparticles for Pulmonary Application: SLN VS NLC

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Pulmonary drug delivery is a non-invasive application form that stands out for local treatment of airway diseases by reaching the epithelium directly, circumventing first pass metabolism and avoiding systemic toxicity. Lipid nanoparticles (LNP) are an innovative nanoparticulate carrier system in which solid lipid particles are stabilized by surfactants in an outer aqueous phase. There are two types of LNP, i.e. the Solid Lipid Nanoparticles (SLN) and the Nanostructured Lipid Carriers (NLC). These two types of LNP distinguish themselves by the composition of the lipid matrix. The matrix of SLN consists of a solid lipid whereas the matrix of NLC is composed of a blend of a solid and a liquid lipid. The benefit of this blend is the possibility of a higher drug load and the minimisation of drug leakage during storage. Lipid nanoparticles possess several advantages for pulmonary application, e.g. deep lung deposition, retention in the lung and low toxicity. However, LNP have to fulfill the same requirements as other aqueous formulations for pulmonary application, i.e. sterility, isotonicity, good tolerability and good aerosolization properties. In this study two isotonic and sterile Dexamethasone-loaded LNP formulations, i.e. Dexamethasone-loaded SLN and Dexamethasone-loaded NLC were developed and their suitability for pulmonary application investigated.
Dexamethasone-loaded LNP were produced by hot high pressure homogenization applying 5 homogenization cycles at 500 bar and 65°C leading to SLN with a particle size of about 120 nm and NLC with a particle size of about 200 nm. The polydispersity index (PI) showed a narrow particle size distribution of both formulations. Zeta potential, melting point, entrapment efficiency (E.E.) and pH-value were of the same order of magnitude for SLN and NLC and confirmed suitability of the developed LNP for pulmonary application. Tonicity of SLN and NLC was adjusted by adding glycerol 85%. SLN were sterilized by sterile filtration whereas sterility of NLC was obtained by steam sterilization. Adjustment of tonicity and sterilization led to no or only minor changes in particle size, PI, E.E., zeta potential, melting point and pH-value of LNP. Cytotoxicity of Dexamethasone-loaded SLN and NLC was assessed by LDH and MTS assay using A549 cells. No cytotoxic effects could be found in dose relevant concentrations approving good tolerability of Dexamethasone-loaded LNP.
LNP dispersions were nebulized using two different types of nebulizers, i.e. the jet stream nebulizer Pari Boy and the vibrating membrane nebulizer Beurer IH 50. SLN and NLC could be nebulized via Pari Boy but not via Beurer IH 50 due to clogging of the mesh membrane. Laser diffraction measurements of the aerosol generated nebulizing Dexamethasone-loaded LNP via Pari Boy showed monomodal volume distribution curves with an aerosol droplet size being favourable for deep lung deposition. The particle size of SLN and NLC after nebulization was found to be of the same order of magnitude as before nebulization, thus indicating good resistance of Dexamethasone-loaded LNP against shear forces during the nebulization process.
In summery it was possible to develop isotonic, sterile and well tolerable Dexamethasone-loaded LNP which are stable during jet stream nebulization and show good aerosolization properties. Therefore both types of LNP, i.e. SLN and NLC are promising carrier systems for pulmonary delivery of Dexamethasone.

Development of an intestinal 3D in vitro Permeability Model to investigate the Transport of Polystyrene Nanoparticles

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Intestinal epithelial cell culture models (i.e., Caco-2 cell line, FAE 2D model) are commonly used to asses absorption of drug molecules and transcytosis of nanoparticles across the intestinal mucosa. However, currently there is no *in vitro* model available that implies all cell types of the intestinal epithelial barrier. Hence, the objective of our study was the development of an alternative *in vitro* permeability model based on a triple culture: Caco-2 cells, mucus-secreting goblet cells and M cells. Therefore, Caco-2 cells and mucus secreting goblet cells were co-cultured on transwells following a cultivation of 2 weeks. Next, Raji B cells were added to stimulate differentiation of M cells. The 3D *in vitro* model was characterized regarding the surface morphology, integrity and altered expression of M cell markers (down-regulation of alkaline phosphatase activity and an increased binding of wheat germ agglutinin). Goblet cells were identified due to the presence of mucus components stained with Alcian Blue and Acridine Orange. However, with the development of the 3D *in vitro* permeability model we are able to mimic the human intestinal epithelium considering both the role of M cells and mucus-secreting goblet cells.

Investigations concerning the Interactions of Neutral Nanoparticles with the Buccal Mucosa

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The buccal mucosa shows a variety of functions of which the protection of the underlying tissue and thus, avoidance of the penetration/permeation of xenobiotics, is the most important one [1]. However, the buccal mucosa is also a promising and accessible site for drug delivery that is not associated with the first pass metabolism. Previous studies demonstrated that spherical viruses (38 nm and 55 nm) are able to diffuse through the gastric and nasal mucus barriers and infect the underlying epithelia. This phenomenon is attributed to the specific neutral surfaces of viruses, which are equally coated with positive and negative charges [2]. To evaluate whether neutral spherical nanoparticles are small enough to cross external or epithelial barriers in the oral cavity, plain polystyrene (PP 25 nm - 200 nm) particles were used as model particles for buccal permeability studies. Their physicochemical properties (i.e., hydrodynamic size and zeta potential) in (biological) media were measured using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) and the surface hydophilicity/hydrophobicity was determined with the Rose Bengal adsorption method. The permeability studies through excised porcine buccal mucosa were performed at 37°C with static Franz diffusion cells. Additionally, all permeability experiments were performed at 4°C to differentiate between active and passive transport mechanisms. Particle uptake into oral TR 146 cells was recorded with fluorescence microscopy and cell damage was evaluated. To study the dynamics of the particles, the diffusion coefficients were evaluated based on images acquired from the ex-vivo studies. The results demonstrated that in MQ water dispersion was best and smallest sizes were recorded. Particles, dispersed in saliva, increased in size and decreased in surface charge due to aggregation/formation of a protein corona. PP particles exhibited hydrophilic surface properties. Thereby, the largest particles appeared less hydrophilic (K=0.09 ml/mg) than the smallest particles (K=0.02 ml/mg). All particles permeated the mucus layer and penetrated into the buccal epithelium with uptake rates of 2.69% ± 0.15% for the smallest and 9.25% ± 2.64% for the largest. Decreasing the temperature to 4°C reduced the uptake of all PP particles markedly. The 200 nm particles showed a diffusion coefficient of $9.09 \cdot 10^{-14}$ m²/s. In contrast, for the smallest particles the diffusion coefficient was 172-fold lower ($5.28 \cdot 10^{-16}$ m²/s).
In summary, PP particles avoided adhesion to mucin fibres as well as steric inhibition of the dense fibre mesh and thus, were able to permeate the mucus layer. Compared to 25 nm particles, 200 nm PP particles penetrated more rapidly into deeper regions of the epithelium, indicating that in the oral cavity the size of neutral particles determines the uptake rate. The transport mechanisms of neutral particles are governed by two routes, the passive diffusion and the endocytotic pathways.

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