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FROM A SYRINGE TO THE SMART, SOFT, SKIN-PENETRATORS: MODERN APPROACHES TO (TRANS)CUTANEOUS AGENT DELIVERY

Cevc G

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Mammalian skin (cutis) is nearly impermeable to most molecules, the more voluminous and hydrophilic they are the more so, notwithstanding trans-barrier pathways existence. Extensive breaching of the barrier cannot solve the problem, as it would expose a treated body to the ambient and/or cutaneous microbiota. One should, and can, therefore actively punch just a limited number (~10^2 cm^-2) of relatively wide (~10^3 nm) openings into skin barrier, e.g. by using hard, sharp objects (perforators, micro-needles), mechanical abraders, or local energy dischargers (porators). This then allows transient (~1 day) passage of small quantities (~10 mg) of a drug, including macromolecular substances (and pathogens?). Microscopic (~10^4 nm) ballistic droplets or particles can also actively insert a limited amount (~1 mg) of a drug into the upper skin region through some ~106 cm^-2 pores that such impactors typically create in skin barrier. Ballistic impactors acting as skin porators are already approved for human use; the hard nano-sized (5 nm - 10 µm) skin perforators remain to be registered for such use, however. A possible alternative are stable, but highly deformable and superficially hydrophilic, nano-sized drug carriers.* Such carriers must be designed, or at least optimised, individually and are typically composite colloids that can obtain, or retain, ability to penetrate skin barrier “softly” and thereby deliver the associated agent cargo into, and potentially across, skin barrier. If properly designed and applied, the resulting self-regulating carriers spontaneously open ~10^9 cm^-2 conduits through the primary skin barrier and transport agents through them. Being too large (~10^2 nm) to enter blood capillaries, the carriers moreover influence cutaneous drug clearance, and thus also cope with the secondary skin barrier. Cutaneous and targeted subcutaneous drug deposition or else prolonged systemic activity of the carrier-transported drugs are thus achievable. Preclinical and clinical studies have proven feasibility of agent delivery with deformable hydrophilic carriers through an intact mammalian skin barrier. Judiciously designed adaptable colloids can also bring therapeutic benefits of their own.

*Two commercial examples are Transfersome® (IDEA AG, Munich, Germany) and Sequesome® (ProBono Bio, London, UK)
SPECIFIC INHIBITION OF LEUKOCYTE SUBPOPULATION MIGRATION IN VIVO & IN VITRO

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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 early neutrophil attraction to inflammatory sites whereas CCL2 is responsible for later monocyte recruitment. Neutrophils follow a chemotactic gradient which exhibits peak concentration at the site of chemokine release. Chemokines become activated through the interaction with their specific G-protein coupled receptors (GPCRs; CXCR1 and CXCR2 for CXCL8; CCR2 for CCL2) on the respective target cells which is supported by the interaction with glycosaminoglycans (GAGs). GAGs such as heparan sulfate play a major role in inflammation by establishing the solid phase-like chemotactic gradient, thereby ensuring an optimal presentation to the GPCRs on the leukocytes [1] which leads to a direct cell infiltration and is thus pro-inflammatory.

ProtAffin Biotechnology AG has been engineering decoy proteins with enhanced glycosaminoglycan-binding affinity as well as with knocked-out (or strongly reduced GPCR receptor) binding which leads to an inhibition of cell infiltration and thus of inflammation.

We have tested several decoy mutants and their respective wild types in trans endothelial migration, chemotaxis, flow cytometric assays supported by other experiments to examine their bioactivity and function in regard of leukocyte recruitment. Furthermore, the correlation between the sulfation pattern and the regulating effect on transmigration was investigated by using selectively desulfated heparins. The effect of various GAG oligosaccharides and antibodies against GAG and proteoglycan structures on trans endothelial migration of leukocytes was also investigated. Finally, to examine these findings in vivo, we have established a mechanistic murine peritonitis for investigating cell recruitment in live animals under the influence of various inhibitors and modulators of leukocyte migration.

In our studies, we were able to identify specific sulfation patterns which are needed for leukocyte transmigration. This could be translated into specific inhibitors which influence specific leukocyte subpopulations trafficking, but also into improved engineering of our decoy mutants.

All in all, the interaction between chemokines and GAGs was found to be specific and to play a crucial role in acute inflammation. Therefore GAGs represent a targets with high therapeutic value.

Reference:

Acknowledgments:
FFG  
ProtAffin Biotechnologie AG
METABOLIC AND PHARMACOLOGICAL PROFILING OF THREE CLASSICAL TCM FORMULAS USED FOR CHRONIC INFLAMMATORY DISEASES WITH IMMUNE DYSFUNCTION

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Three classical TCM formulas, which have been used for chronic inflammatory diseases with immune dysfunction (allergies, asthma, morbus crohn, colitis ulcerosa), and which have clinical evidence of therapeutic activity, will be phytochemically and pharmacologically investigated in order to elucidate the mode of action, the constituents relevant for activity, and finally to establish quality control methods. The formulations to be studied are Yu Ping Feng San, Huang Qi Jian Zhong Tang and Bai Zhu Huang Qi Tang. Yu Ping Feng San, a mixture of Saposhnikoviae radix (Fang Feng, 30g), Astragali radix (Huang Qi, 60g) and Atractylodes macrocephalae rhizoma (Bai Zhu, 60g) has been phytochemically investigated. In order to compare the constituents of the single herbs with the components of the mixture and to find out whether new compounds are formed during the combined decoction of the herbs, suitable TLC and HPLC methods have been developed. \textsuperscript{n}-Hexane, dichloromethane, and methanol extracts of the single herbs and of the mixture have been prepared by Accelerated Solvent Extraction (ASE) and analysed by TLC and HPLC fingerprinting techniques. In addition, decoctions of the single herbs and of the mixture have been examined. Characteristic constituents of each herb have been identified in the extracts and assigned in the mixture using LC-DAD-MS/MS. Investigations of the immunomodulatory effects of the extracts (pro-inflammatory cytokines IL-6, IL-8, TNF-\textgreek{a}; IL-4, IL-1\textgreek{B}, IL-10, IFy expression) are in progress. Once we have this information we will correlate the phytochemical data with the immunomodulatory effects in order to identify and isolate the active compounds and metabolites.

Acknowledgments:
The project has been financially supported by the Austrian Federal Ministries of Health and of Science and Research, by China Academy of Chinese Medical Sciences, and by the China State-Funded Postgraduate Overseas Study Program.
DEVELOPMENT OF HOT-MELT COATING PROCESS AND EXCIPIENTS FOR DRUG MICROENCAPSULATION WITH MODIFIED RELEASE

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There is a growing interest in drug microencapsulation for producing improved products and to increase patient adherence to drug therapy. The controlled release is by far the main application of this technology, where these microparticles can easily be swallowed by patients with dysphagia conserving the drug release profile. On the other hand, for immediate release proposes, microencapsulation is a suitable technology by providing taste masking to unpleasant drugs.

Hot-melt fluidized bed coating technology is a relatively new but very promising and interesting concept, where melted fats, waxes or emulsifiers are spread into a drug core creating a shell. From an industrial point of view, this method avoids solvents resulting in reduced process times and costs; because several lipids excipients are considered GRAS, the time for marketing can be shorter.

However, the main components of fat excipients are triglycerides, which exhibit a complex physical behavior. The macroscopic properties of the microcapsules will therefore depend on the lipid composition, polymorphism and microstructure, being these influenced by processing conditions.

In this work, lipid microcapsules are developed using hot-melt coating technology either for immediate or controlled release proposes. The complex physical behavior of lipids is taken in consideration to develop excipients and tempering processes in order to have the desired product properties.
SYNTHESIS OF NEW BICYCLIC AMINES AND THEIR ACTIVITY AGAINST MALARIA AND TRYPANOSOMIASIS

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Malaria and trypanosomiasis are the protozoal diseases. Trypanosomiasis occurs in 36 sub-Saharan African countries. There have been several epidemics in Africa over the last century (1906, 1920, and 1970). In the year 2011 the numbers of new cases were 6743 and in 2012, 7197 people were infected\textsuperscript{(1)}. There are only few drugs available for this disease (melarsoprol and eflornithine). They are associated with some side effects and also trypanosomes are becoming resistant to these drugs. Malaria is a mosquito born infectious disease of human caused by parasitic protozoan of the genus Plasmodium. The disease is wide spread in tropical and subtropical regions of sub-Saharan Africa, Asia and America. In the year 2010, 219 million cases of malaria were reported\textsuperscript{(2)}. That year 660000 people died, most in the African region(91%). There are a number of drugs in use for the treatment of malaria but Plasmodium especially \textit{Plasmodium falciparum} develops resistance against the drugs. Therefore new compounds should be discovered for treatment of these diseases which are more effective and also act favourably at new targets where already the resistance has not yet been developed.

Bicyclooctanones are synthetic compounds prepared from benzylidene acetone and dialkylamine in a one pot reaction. They showed a pronounced activity against \textit{Plasmodium falciparum} and \textit{Trypanosoma brucei rhodesiense}\textsuperscript{(3)}. Different modifications in the bicyclooctanone showed an increase of activity. Azabicyclononanes are compounds derived from the bicyclooctanones and were found to be more active as compared to bicyclooctanones\textsuperscript{(4)}.

**Antiprotozoal activities of compounds**

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>T.B. rhodesiense $IC_{50}$ µM</th>
<th>P. falciparum $IC_{50}$ µM</th>
<th>Cytotoxicity $IC_{50}$ µmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicyclooctanone</td>
<td>8.03</td>
<td>1.19</td>
<td>26.45</td>
</tr>
<tr>
<td>Azabicyclononane</td>
<td>1.16</td>
<td>0.56</td>
<td>120.4</td>
</tr>
</tbody>
</table>

In the present investigation azabicyclononanes have been prepared with different side chains at positions 5 to see the effect of substitution and increasing lipophilicity on the biological activity. $IC_{50}$ of these compounds have been determined against above mentioned protozoan species. One compound in this series with a dibutyl side chain showed good activity and also less toxicity. So this compound is a good for the future in vivo testings. In addition a series of azabicyclononanes have been prepared with substituted piperazine rings at position 3 to investigate some structure activity relationship at this position.

References:

(1) WHO fact sheet N\textsuperscript{2} 259 updated June 2013
(2) WHO fact sheet N\textsuperscript{2} 94 reviewed March 2013
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PROTICLES AS POTENT DRUG DELIVERY SYSTEM FOR ANGIOGENIC NEUROPEPTIDE SECRETONEURIN

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Proticles represent a relatively new and potent drug delivery system for oligonucleotides and peptides. One of the major problems for the use of therapeutic peptides is their rapid enzymatic degradation followed by a short biological half-life. Therefore an efficient drug delivery system is necessary to achieve successful treatment. Proticles basically consist of DNA or RNA oligonucleotides and strongly basic peptide protamine, and have already been used as carrier system for various oligonucleotides and peptides [Pali Schöll et al. as example]. Formation of Proticles occurs within a few seconds after mixing the components by a self assembly process, previously described by Lochmann et al.

In this study angiogenic neuropeptide secretoneurin (SN, Kirchmair et al.), a promising drug for the treatment of peripheral arterial disease and ischemic heart disease, was imbedded into the matrix of Proticles. Assembled Proticles were characterized by Dynamic Light Scattering and SN loading was determined using reversed phase-HPLC. Varying i) the concentration of the components and ii) the formation process it was possible to create particles differing in size and SN loading. Size of particles was primarily influenced by the formation process, whereas SN loading was mainly depending on the concentration of the components. However no correlation between size and SN loading was observed. Up to 70\% of deployed SN could be imbedded into the matrix of Proticles with a diameter ranging from 120 nm to 1,400 nm. It was also shown that assembled proticles can successfully be lyophilized, which is an important aspect concerning long-term stability.

For application of SN it is a great benefit to have a promising drug delivery system which can protect the peptide from degradation and due to its variation in size and loading different pharmacokinetic aspects like initial dose and modified drug release can be considered.

References:

Acknowledgments:
Authors would like to thank piCHEM (Graz) for synthesizing Secretoneurin
HYBRID MOLECULES WITH ANTIPLASMODIAL ACTIVITY

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Malaria is one of the most dangerous infectious diseases causing approximately 630 000 deaths in 2012. Transmitted through the bite of the infected female mosquito of several anopheles species, five species of plasmodium parasites cause the disease, of which P. falciparum malaria is the most deadly form. The disease is treatable and curable, yet drug resistance against antimalarial medications in P. falciparum is a recurring problem leaving the treatment with common drugs – especially monotherapies – ineffective. Therefore, artemisinin-based combination therapy (ACT) is recommended [1]. However, combinations of artemisinins, which exhibit a very short half-life, with antimalarial agents exhibiting long half-lives can be considered as pharmacokinetically mismatched with regard to the emergence of resistance since recrudescent or new infections will encounter only one agent [2].

Hybrids are compositions of two or more pharmacophores via a linker. The hybrid molecule acts as distinct pharmacophores. The pharmacophores represent moieties with known antimalarial activity which can either share the same mechanism of action or act on different targets [3]. A possible target is the PfCRT (Plasmodium falciparum chloroquine resistance transporter). Its inhibition can reduce resistance against chloroquine in the parasites [4].

Recent studies show that hybrid antimalarials are promising future drugs capable of circumventing drug resistance mechanisms in plasmodium parasites [5]. At least one agent of hybrid antimalarials (SAR116242) is currently in preclinical development [2]. My thesis focuses on the synthesis of new hybrids with antiplasmodial activity. All newly synthesized compounds will be tested for their in vitro activity against strains of Plasmodium falciparum and their cytotoxicity at the Swiss Tropical and Public Health Institute in Basel. The structures of lead compounds will be optimized considering their physicochemical properties.

References:
Parenteral fat, also named oil or lipid, emulsions are in medical use since over 5 decades (e.g. Intralipid, approved in Europe in 1962). These emulsions are oil in water dispersions with a droplet size around 200 nm. Parenteral emulsions loaded with APIs (active pharmaceutical ingredient) can be seen as drug delivery systems (DDS). DDS focuses on the regulation of the in vivo dynamics, such as absorption, distribution, metabolism, and extended bioavailability, thereby improving the effectiveness and the safety of the drugs. Using an emulsion as DDS or by diversification of the surface of the dispersed oil droplets of emulsions a targeted increase of the concentration of the API in some parts of the human body should be achieved in this project. An active targeting via peptides linked to the surface of the so modified oil-droplets seems to be an attractive method to perform such a drug targeting. The aim of this dissertation is to achieve an improved drug delivery and drug targeting by modifying existing or developing new emulsions.

Advantage of an intravenous fat emulsion:
- ease of manufacturing
- oil droplet inside as drug reservoir
- for lipophilic drugs.

Reference:

Acknowledgments:

Graphic:
CHARACTERISATION OF NEW DESIGNER DRUGS BY CHROMATOGRAPHIC AND ELECTROPHORETIC SEPARATION TECHNIQUES

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During the last years, the spectrum of legal and illegal highs for human consumption increased enormously. These drugs of abuse are synthesized in clandestine laboratories and mainly traded over the Internet as research compounds, room odorizers, bath salts or fertilizers for plants. The progressively worldwide black market of these compounds represents a huge problem for police and drug authorities. An increasing problem is the fact that for the identification of these new substances only few analytical methods are available.

For our studies, various cathinones, cannabinoids, phenylethylamines, tryptamines and amphetamines were chosen as compound classes. Samples are purchased mainly from different Internet shops. Identity was checked by GC-MS verifying the patterns of mass fragments. When necessary, structure was elucidated by NMR.

The main goal of our work is to develop analytical methods for both separation and quantification using chromatographic and electrophoretic techniques, such as GC, HPLC and capillary electrophoresis (CE). The majority of the recreational drugs are chiral and are sold as racemic mixtures, which has been confirmed by both chiral HPLC [1] and CE experiments. However, it is not known, whether the enantiomers differ regarding their pharmacological effect. Obviously, there is a need to develop chiral separation methods for these compounds.

With respect to further method development, it should be feasible to elucidate real life samples, which are continuously provided by police and hospitals.

Reference:
DESIGN AND APPLICATION OF MONOA MINE OXIDASE MODIFIED BIOSENSORS FOR THE DETECTION OF BIOGENIC AMINES

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⁴University of Graz, Institute of Chemistry, Graz (Austria)

Biogenic amines (BA) and especially among them the neurotransmitters dopamine, norepinephrine and serotonin play important roles in numerous physiological processes in the human body. The level of these BAs is regulated by two enzymes, monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B). A dysfunction of this regulation leads to neurodegenerative diseases such as Morbus Parkinson, Morbus Alzheimer and depression.

Beside endogenous biogenic amines produced in different tissues, exogenous amines are absorbed from food such as fish, meat, wine, cheese and vegetables. The ingestion of high amounts of these biogenic amines can result in allergic reactions, rash, dysfunctional blood pressure, tachycardia, headaches and nausea. [1]

That implies that there is a strong need of diagnostic and analytical devices for the detection of BAs. For this purpose biosensors are appropriate devices as they produce a direct signal, often do not require a complex sample preparation and are therefore not time-consuming and economic.

The developed biosensor is designed on the basis of MnO2 modified carbon paste electrodes and is part of a flow injection analysis system. The sensor enables the determination of total biogenic monoamine content using human monoamine oxidase B as biological recognition element and a dialysis membrane for immobilisation of the enzyme.

MAOs catalyse the oxidative deamination of biogenic amines in the presence of oxygen and water. In the course of this reaction hydrogen peroxide is produced beside the corresponding aldehyde and ammonia which is shown in the following equation.

\[
\text{MAO} \quad R-\text{CH}_2\text{-NH}_2 + \text{O}_2 + \text{H}_2\text{O} \rightarrow R-\text{CHO} + \text{NH}_3 + \text{H}_2\text{O}_2
\]

The functional principle of the sensor is based on amperometry. The enzymatically produced H2O2 is determined at the above mentioned MnO2 modified carbon paste electrode with a fixed potential of +0.4V vs. Ag/AgCl. Sörensen phosphate buffer pH 7.5 serves as media [2].

The described sensor was successfully tested in commercial fish sauce showing a standard deviation of 4.0%.

References:
BIOPHYSICAL APPROACHES TO CHARACTERIZE GAG-CHEMOKINE INTERACTIONS

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The CXC-chemokine Interleukin-8 (IL-8) plays a crucial role in many acute and chronic inflammatory diseases, e.g. arthritis and COPD that are characterized by strong neutrophil infiltration into the tissue. The binding of IL-8 to heparansulfate (HS) glycosaminoglycans (GAGs) on the surface of endothelial cells is important for the recruitment of neutrophils to an inflammatory site. In order to treat inflammatory disorders, ProtAffin has developed an IL-8-based decoy protein (PA401) which shows increased binding affinity to specific GAG structures combined with a knock-out of the chemokine's GPCR activity [1]. Based on these properties, PA401 is proposed to compete in vivo with wtIL-8 for binding to specific GAG sites, thereby disrupting the GAG-mediated, solid-phase chemotactic gradient and thus decreasing neutrophil infiltration into the inflamed tissue. The strong anti-inflammatory effect of PA401 has been shown in various animal models [2]. For characterizing our decoy proteins it was indispensable to establish dedicated methods for quantitating the GAG-decoy interactions. Here we compared four different in vitro methods: surface plasmon resonance (SPR), isothermal fluorescence titration (IFT), isothermal titration calorimetry (ITC), and an in-house developed competition assay (ELICO). This assay is based on classical ELISA, by which the competition potential (IC50 value) of PA401 with respect to proteins/chemokines pre-bound to GAGs is determined. For this purpose, GAGs like heparin or HS were coated onto microwell plates, which were then incubated with biotinylated target proteins (such as wtIL-8). The PA401 decoy was subsequently added at increasing concentrations and the remaining bound biotinylated protein was detected via streptavidin-HRP and quantitated in an ELISA-like set-up using tetramethyl benzidine. IC50 values are compared with respective Kd values and are discussed for different GAGs. Advantages and disadvantages of applying these 4 methods are compared.

Reference:

Graphic:
HOT MELT COATING WITH TRIACYLGLYCERIDES – THE EFFECT OF TEMPERING ON DRUG RELEASE

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Lipid microcapsules are a multi-particulate delivery system with potential to increase patient adherence and to personalize doses at a low cost production. However, lipids exhibit a complex physical behaviour with different polymorphs and structure affecting drug release. Using hot-melt coating technology, citric acid crystals were coated with molten tristearin to 50% (w/w). Process temperatures were selected to either crystallize the unstable α-form, or tempering, crystallizing the stable β-form from the melt. Additionally, a sample from the α-form product was subjected to heat treatment to transform into a stable coating comprising the β-form as well. The two β-coatings with different thermal histories and the α one were characterized by DSC, XRD, PSD, SEM and the drug release was studied over 24 hours.

Three tristearin 50% (w/w) coatings with different properties could be produced: a α coating releasing 35.5% (R² = 0.841 for zero order kinetic); a coating with the β-form generated by a heat treatment of coated sample with the α-form, exhibiting fractal structures on the surface, releasing 39.1% (R² = 0.824 for zero order kinetic); and a β coating obtained from tempering during the process, without fractal structures, releasing 18.3% (R² = 0.963 for zero order kinetic).

In this work it is shown that triglycerides can be tempered for pharmaceutical purposes creating a valuable coating for extended drug release.

Reference:
CAPSAICINOIDS OF CAPSICUM FRUTESCENS AS MODULATORS OF EFFLUX PUMPS IN MYCOBACTERIA

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Efflux pumps (EP) play an essential role in resistance development in various diseases such as cancer or infectious diseases. These ubiquitous membrane transport proteins are responsible for the efflux of xenobiotics out of the (bacterial) cell. Thus the concentration of an administered pharmaceutical is below the effective concentration and cells become resistant against this specific drug. [1] In case of tuberculosis (TB) many EP are known to be important for resistances against first-line drugs in TB-treatment. For instance, MmpL7 found in Mycobacterium tuberculosis mediates resistance against isoniazid, the most important TB drug at present. [2]

The aim of this study is the finding of efflux pump inhibitors (EPI) to overcome the vicious circle. These compounds are able to block EP with the consequence of achieving the effective concentration and therefore kill the cell. [3]

For this purpose we screened 24 plants for their antimycobacterial and modulatory effect on Mycobacterium smegmatis. In this screening Capsicum frutescens showed promising modulatory activity. The hexane extract of the fruits was devoid of antimycobacterial activity (minimal inhibitory concentration of 512 mg/l), however, it revealed a notable modulation factor (MF) of 8 for ethidium bromide, a substrate of many efflux pumps. Interestingly, both the hexane and dichloromethane extract showed good MF. We performed GC/MS experiments in order to evaluate the content of capsaicinoids in these two extracts. The results emphasized that the pungent capsaicinoids are possible active components. To evaluate which of these capsaicinoids is mainly responsible for the modulatory activity of Capsicum extracts a preparative HPLC experiment was performed to isolate the 4 major capsaicinoids which were then again tested on Mycobacterium smegmatis.

![Capsaicin](image)

References:


INHIBITION OF COX-2 AND NF-KB1 EXPRESSION IN THP-1 CELLS BY SESQUITERPENE LACTONES FROM ONOPORDUM ACANTHIUM

Pan S¹, Lajter I², Vasas A², Bauer R¹, Hohmann J²

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COX-2 and NF-κB1 are well known mediators of inflammation and are highly expressed in various diseases like rheumatoid arthritis, asthma, chronic obstructive pulmonary disease, and other autoimmune diseases, like Morbus Crohn and Colitis ulcerosa. Since many anti-inflammatory agents on the market treating these diseases cause severe side effects, it is crucial to discover novel lead compounds from natural sources targeting COX-2 and NF-κB1.

Onopordum acanthium L. (Asteraceae) is known for its traditional use as an anti-cancer and anti-inflammatory agent. Based on this knowledge, extracts prepared from the roots and aerial parts of the plant have been investigated for their COX-2 and NF-κB1 gene expression inhibition in PMA differentiated THP-1 macrophages stimulated with LPS. At a concentration of 10 µg/mL several extracts showed +/- 50% COX-2 mRNA inhibition. Two isolated constituents, the sesquiterpene lactones 4β,14-dihydro-3-dehydro-zaluzanin C (1) and zaluzanin C (2), dose dependently inhibited COX-2 (IC₅₀ = 2.6 µM for 1 and IC₅₀ 0= 5.4 µM for 2) and NF-κB1 gene expression. Furthermore, the XTT-assay showed that the inhibitory effect is not due to cytotoxicity.

Taken together, we could demonstrate for the first time that sesquiterpene lactones from Onopordum acanthium exert strong anti-inflammatory effect in-vitro by inhibiting COX-2 and NF-κB1 expression.

Acknowledgments:
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EFFICIENT NITROSATION OF GLUTATHIONE BY NITRIC OXIDE

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Nitrosothiols are increasingly regarded as important participants in a range of physiological processes, yet little is known about their biological generation. Nitrosothiols can be formed from the corresponding thiols by nitric oxide in a reaction that requires the presence of oxygen and is mediated by reactive intermediates (NO₂⁻ or N₂O₃) formed in the course of NO autoxidation (Scheme 1)²,³,⁴. Since the autoxidation of NO is second order in NO, it is extremely slow at submicromolar NO concentrations, casting doubt on its physiological relevance. In this paper we present evidence that at submicromolar NO concentrations the aerobic nitrosation of glutathione does not involve NO autoxidation but a reaction that is first order in NO. We show that this reaction produces nitrosogluthathione efficiently in a reaction that is strongly stimulated by physiological concentrations of Mg²⁺. These observations suggest that direct aerobic nitrosation may represent a physiologically relevant pathway of nitrosothiol formation (Scheme 2)¹.

Scheme 1: Nitrosation of thiols via autoxidation

\[
\begin{align*}
2 \text{NO} + \text{O}_2 & \rightarrow 2 \text{NO}_2 \\
\text{NO}_2 + \text{NO} & \rightarrow \text{N}_2\text{O}_3 \\
\text{N}_2\text{O}_3 + \text{RSH} & \rightarrow \text{RSNO} + \text{H}^+ + \text{NO}_2^- \\
\text{NO}_2 + \text{RSH} & \rightarrow \text{RS}^- + \text{H}^+ + \text{NO}_2^- \\
\text{NO} + \text{RS}^- & \rightarrow \text{RSNO} 
\end{align*}
\]

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INVESTIGATING THE IP-10 GLYCOSAMINOGLYCAN INTERACTION BY DIFFERENT BIOPHYSICAL METHODS

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The interaction of chemokines (chemotactic cytokines) with heparan sulphate proteoglycans (such as Syndecans or Glypicans) is involved in many pathologic processes [1]. One of these processes is the participation of Interferon-gamma inducible protein 10 (IP-10, CXCL10) in the devastating and so far incurable disease Idiopathic Pulmonary Fibrosis (IPF), which is characterized by exaggerated extracellular matrix deposition and aggressive structural remodelling [2]. In IPF the interaction of Syndecan-4 on fibroblasts with IP-10 serves to inhibit fibroblast recruitment and subsequent fibrosis [3]. Therefore this interaction of IP-10 with glycosaminoglycans/ proteoglycans represents a potential therapeutic route towards IPF and is worth a detailed investigation.

IP-10 V19W (fluorescence engineering necessary for use in fluorescence methods) was expressed in E. coli, refolded and purified applying FPLC and HPLC separation techniques. The identity was confirmed by SDS-PAGE and subsequent western blotting as well as by MS analysis. Structure and folding were investigated by applying a chaotrope-induced fluorescence shift assays as well as circular dichroism measurements. The glycosaminoglycan binding affinity towards different types of glycans, like heparin, heparan sulphate and dermatan sulphate, was investigated by applying biophysical interaction studies such as surface plasmon resonance, isothermal fluorescence titration and isothermal titration calorimetry yielding a dissociation constant of the molecular interaction. The results of the interaction studies of IP-10 V19W towards glycosaminoglycans will be presented.

References:

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EXPRESSION, PURIFICATION AND CHARACTERISATION OF NOVEL IP-10 RECEPTOR KNOCK-OUT MUTANTS

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The interaction of chemokines, their G-protein coupled receptors and their co-receptors, the glycosaminoglycans (GAGs), play a crucial role in numerous pathophysiological processes. Many diseases center around an imbalance of these macromolecules. In the focus of the underlying study is the chemokine interferon-gamma induced protein 10 (CXCL10, IP10). Examples of diseases, where IP10 is misregulated are e.g. COPD, cystic fibrosis or lung cancer. One approach to palliate or even cure these diseases is to interfere with the chemokine network via the application of macromolecular decoys or antagonists targeting IP-10 and its receptors. The future goal of the current work is the production of a library of IP10-mutants with altered GAG-binding as well as receptor activation ability.

In a first step, we generated a fluorescently engineered IP-10 variant which was subjected to bio comparability studies with the native, non-fluorescent protein. Compared to the wild type, the mutant exhibited similar GAG-binding affinity as well as chemotactic potential. In a next step, we designed and expressed fluorescent chemokine receptor-knock-out mutants. All mutants were designed based on molecular modelling studies. The identity and purity as well as the dimerization behaviour of our mutants was tested by gel electrophoresis (under native and reducing conditions) and by western blotting. Their chemotactic potential was verified using Boyden Chamber assays. Secondary structure and unfolding of our IP-10 mutants was studied by far-UV Circular Dichroism Spectroscopy and by applying Gua.HCl-induced denaturing conditions.

An overview of the workflow and major results will be presented.
ADVANCED INTESTINAL IN VITRO TRIPLE CULTURE PERMEABILITY MODEL TO STUDY TRANSPORT OF NANOPARTICLES

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PURPOSE: Oral uptake of drugs shows a poor bioavailability, mainly due to their lack of stability and their low mucosal permeability in the hostile intestinal environment. Promising strategies to improve oral drug delivery are nano-carrier-systems. To design such systems, simplified biological models are necessary so that the mechanisms of interest can readily be studied. Currently there is no in vitro model available that implies all cell types of the intestinal barrier. Hence, the objective of our study was to develop, characterize and validate an alternative in vitro permeability model based on a triple culture including Caco-2 cells, mucus-secreting goblet cells and M cells.

METHODS: Caco-2 cells and mucus-secreting goblet cells were co-cultured for 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. Goblet cells were identified due to the presence of mucus components stained with Alcian Blue and Acridine Orange. Furthermore we correlated data from in vitro investigations with a commonly used ex vivo method (Using chambers, porcine intestine) and compared the results with in vivo data from literature. The validity of the in vitro model was reflected in the ability to predict the permeability of a model drug but also of reference nanoparticles.

RESULTS: The presented 3D in vitro permeability model shows high resemblance to the physiological and biochemical properties of the human small intestine. M cells were identified due to an altered expression of potential human M cell markers (down-regulation of alkaline phosphatase activity and an increased binding of wheat germ agglutinin). Additionally, mucus homogeneously covered the monolayer.

CONCLUSIONS: Mucus formation in the 3D model contributes a significant barrier to drug transport. However, in vitro permeability values for the reference nanoparticles/model drug correlated well with data obtained from ex vivo results and permeability data listed in literature. Hence it can be concluded, that this model will be an efficient tool to study intestinal uptake of particulate systems and should be taken into account for the rational design of (nano-)carriers.
IDENTIFICATION OF ACTIVE COMPOUNDS IN LONICERA SPECIES BY MEANS OF METABOLIC PROFILING WITH LC-MS

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Lonicera japonica Thunb. (Caprifoliaceae) is widely used in traditional Chinese medicine for its antibacterial and anti-inflammatory properties [1]. Based on this knowledge, and following the results of a screening of a large number of Chinese medicinal plants, we analysed ethanolic extracts from 35 different Lonicera samples. The extracts were tested for inhibition of NO production in LPS-/IFN-γ-stimulated RAW 264.7 mouse macrophages [2]. At 50µg/ml concentration some extracts showed a very good inhibitory effect (up to 88% inhibition referred to control) on the NO production. Furthermore, the extracts were analysed by LC-MS. The LC-MS data was screened for potential candidates in an untargeted manner by MZmine 2[3]; peaks were verified and quantified by Lipid Data Analyzer [4]. The abundance of the peaks was linked to the pharmacological data using SIMCA 13[5]. This multivariate data analysis led to the identification of, so far, ten compounds which seem to be relevant for the inhibitory effect on the NO-production.

References:
[1] Xiaofei Shang, et al., Lonicera japonica Thunb.: Ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine, J. Ethnopharmacol. 138 (2011), 1-21

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