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# Development of an Automated MALDI Mass Spectrometry Assay for Direct Analysis of Cellular Drug Uptake via the Organic Anion Transporting Polypeptide OATP2B1

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Cells as living organisms need to be in constant exchange with the environment to keep up their normal function. For that cause, transport processes across the cell membrane are essential. Those processes include passive diffusion, facilitated permeation via channels and also transport proteins. Besides ATP binding cassette (ABC) transporters, there are also solute carrier transporters (SLC) active, which are mainly focused on drug uptake and are secondary active, mostly energised through an electrochemical gradient. The organic anion transporting polypeptide 2B1 (OATP2B1) is one of about 400 members of the SLC family and is an important drug target, which still needs more focused research. Transport proteins have caused a stir over the last few years due to their involvement in human disease. The dysfunction of several transporters is connected to neurological disorders, diabetes, gout and elevated blood pressure. But there is also evidence for their association with cancer. Apart from that, transport proteins are also prominent in drug-drug interactions (DDI), since their inhibition by one drug may change the pharmacokinetics of other drugs. As the population is aging and elderly people tend to take several drugs concomitantly, this problem will increase over time. Evidence of the involvement of transport proteins in DDI also led to their inclusion in drug development guidelines published by the Food and drug administration (FDA). Currently, most transporter assays are based on either radioisotope- or fluorescence-based assays. Both, however, have pitfalls: Even though radioisotope assays are sensitive and simple in detection, the big problem is the waste management and exposure to the working personnel. The fluorescence-based assays are compatible with high-throughput sequencing (HTS), but prone to false-negatives and –positives due to quenching and autofluorescence effects. Both methods are also dependent on a label. Matrix assisted laser desorption/ionisation (MALDI) mass spectrometry (MS) is a label-free method, which is automatable and therefore HTS compatible. Thus, this thesis had the following aims:

Using the transport process of the SLC transporter OATP2B1 as an example, development of a MALDI MS workflow that enables sensitive and reproducible measurement of an OATP2B1 substrate in whole cells

Incorporation of the developed MALDI MS workflow into pharmacologically relevant assays and assay automation

Screening of 300 marketed drugs and discovery of OATP2B1 inhibitors (and possible DDI) by MALDI MS and comparison of screening results with a fluorescence-based reference assay

The main results of this dissertation are:

A cell-based MALDI MS uptake assay for Estrone-3-Sulfate (E3S) through OATP2B1 was developed and optimised in terms of matrix and solvent composition, matrix crystallisation, normalisation and cell number.

The assay was automated by usage of a pipetting platform and the method was applied to the evaluation of transport kinetics. A time- and concentration dependence of the E3S uptake could be determined.

Through a screening of 294 marketed drugs, 67 inhibitors of the uptake were identified and analysed regarding their concentration-dependent response. The inhibitory concentration (IC50) determination revealed 14 potent inhibitors with a pIC50 ≥ 6. As a reference assay, a fluorescence-based assay was established based on the uptake of dibromofluoresceine (DBF). 47 overlapping inhibitors were found with both methods, the reason for differences were discussed as differences in the methodological setup and the use of another substrate.

In conclusion, the proof of principle was done, that MALDI MS cell-based assays are a feasible and useful technique in transporter research. On top of that, also the identification of candidates for clinically

relevant DDIs was successful. While the developed fluorescence-based method acts as a good reference method, the newly developed MALDI MS method represents a completely new way to analyse substrate and inhibitor of transporters. With its ease and speed in handling and most notably the label-free approach, the MALDI MS method is an indispensable tool for transporter characterisation and DDI analysis.