Ruprecht-Karls-Universität Heidelberg
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Dissertations-Kurzfassung

In-situ pharmacological analysis of small molecule drugs by MALDI-TOF mass spectrometry imaging

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In drug discovery and development, a thorough understanding of drug distribution and its pharmacological efficacy are fundamental. Tissue distribution, however, is usually not homogeneous and plasma concentrations do not reflect actual drug and metabolite concentrations in target tissues. Especially in the brain and in carcinomas, where physiological barriers and transporters limit drug entry, drug targeting to sub-compartments has to be assessed to enable a targeted and effective therapy. Matrix assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) is an evolving tool for fast tissue analysis that has, in contrast to the current gold standards in drug metabolism and pharmacokinetics (DMPK) studies, the capability of distinguishing between drug and corresponding metabolite masses while preserving spatial information. Most importantly, this new technology offers the possibility to simultaneously monitor endogenous molecules *in-situ*. Consequently, the correlation of drug distribution and pharmacodynamic (PD) biomarkers in the targeted pathway as well as the simultaneous detection of drug and disease state biomarkers in target tissue by MSI or with medical imaging techniques would have a major impact on the development of future medications. This thesis therefore had the following aims:

1. Provide proof-of-concept that PK-PD studies are possible with MALDI MSI by developing a method for the simultaneous measurement of small molecule drug distribution and drug effect biomarkers (pharmacodynamic biomarker) in tissue sections *in-situ*.
2. Development of a MSI method for the identification of physiological disease state biomarkers. Subsequent application of the identified markers in MSI drug distribution studies in target carcinoma tissue for the interpretation of drug efficacy data measured a) by MALDI MSI or b) in collaboration by PET/CT imaging.
3. Development of a MALDI MSI-based relative quantification method for drug distribution analysis using an internal standard.

The main results of this dissertation are:

- Proof-of-concept that the developed MALDI MSI method could simultaneously visualize the distribution of the Alzheimer´s Disease drug donepezil and the drug effect on its PD biomarker acetylcholine in targeted brain tissue. PK-PD MS imaging was further applied to the tryptophan 2,3-dioxygenase inhibitor 680C91 in a murine glioma xenograft model. Brain penetration of the inhibitor and efficient drug distribution to the carcinoma were demonstrated. The proximal biomarkers tryptophan and kynurenine, however, revealed partial drug response in liver but not in the glioma xenograft.
- The detection of donepezil pseudo-metabolites in tissue led to the investigation of on tissue in-source decay as a so far unrecognized source of MALDI imaging artefacts.
- Tumour profile analysis of murine glioma and gastric cancer tissue identified the putative phosphatidylcholine biomarkers PC(34:2)+K^+ and PC(34:1)+K^+, respectively, thus enabling the evaluation of carcinoma targeting by the anti-tumour agents 680C91 and fasudil, correspondingly.
- Gastric cancer drug distribution of the kinase inhibitor fasudil revealed inefficient tumor penetration of the compound. Importantly, fasudil distribution measured by MSI was effectively combined with PET/CT gastric tumour imaging enabling the mutual investigation of drug tumour penetration *ex-vivo* and drug efficacy *in-vivo*.
- Relative quantification of the tyrosine kinase inhibitor dasatinib in mouse kidney sections was shown successfully using a deuterated compound analogue as an internal standard revealing an approximately ten-fold compound accumulation in the outer medulla in comparison to the inner medulla and pelvis region.

In summary, this study demonstrates that MALDI MSI holds a high potential for the analysis of PK-PD relationships and for biomarker identification. This technology could therefore become an important complementary tool in the drug discovery process.