Tissue interrogation using mass spectrometry based diagnostic techniques

SUMMARY

Over the last decades, mass spectrometry (MS) based diagnostic tests have become an important cornerstone in clinical decision making. The results of these tests can lead to early intervention and could thereby improve patients' outcome. Hyphenated analytical techniques such as liquid chromatography (LC) and gas chromatography (GC) coupled to MS are routinely used for the quantitative and qualitative analyses of unknown compounds in different biological samples. They show extremely low limits of detection, with good sensitivity, specificity and repeatability. Currently, the main drawback of hyphenated chromatography and mass spectrometry is related to the extensive and labour-intensive sample pre-treatment, clean-up and complicated analyses. In turn, mass spectrometry imaging (MSI) has received increasing attention. This label-free technique allows for concomitant assessment and visualisation of the spatial distribution of multiple compounds such as, drugs, lipids, proteins, and drug delivery systems in biological tissue samples. MSI also became a promising tool to support clinical decision making at various points in the clinical workflow and has proved useful for the characterisation and identification of biological tissues.

The current thesis results from translational research implementing tissue interrogation using mass spectrometry based techniques

After a general introduction on the current clinical diagnostic techniques we outlined the objectives of our research. We aim to develop and validate intraoperative MS based diagnostic techniques to guide surgical applications and to improve treatment outcome and patient safety. The gold standard method is used to objectively evaluate what the strengths and limitations are and how MS can improve these.

In Chapter I, we analysed a technique used for administration of intravenous anaesthetic drugs to achieve a user-specific plasma or effect-site target concentration. Target-controlled infusion (TCI) systems rely on pharmacokinetic/-dynamic models to predict plasma concentrations. We assessed the predictive performance of the Marsh propofol model and Minto remifentanil model for plasma and brain tissue concentrations. This was done on plasma and brain samples by comparing the propofol and remifentanil concentrations predicted by TCI with those measured using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The main result of our study was that the Minto model showed significant underestimation of remifentanil plasma concentrations but tended to overestimate brain concentrations. The Marsh model showed an overall underestimation of propofol concentrations, which was higher in the brain compared to plasma. These conflicting results show that effect-site concentrations of anaesthetic drugs during equilibrium cannot be assumed to be equal to the plasma concentration. As a consequence, under- or overdosing of anaesthetics and analgesics is a possible, but difficult to quantify, problem in anaesthesia.

In Chapter II, we evaluated the MS based technique, rapid evaporative ionisation mass spectrometry (REIMS), for the real-time detection of propofol and remifentanil in patient brain samples. REIMS connects an electrosurgical knife (iKnife) to a quadrupole coupled to a time-of-flight (QTOF) mass spectrometer and analyses the smoke evaporating from tissue during electrosurgical dissection. Notably, the procedure of sampling the smoke, transfer to the mass spectrometer, analysis and feedback of the results takes just 0.7 – 2.5 seconds, without the need for specialized sample preparation. Applying this technique during surgery would allow real-time anaesthetic monitoring, which in turn might enable individual adjustments of anaesthetic drug doses. As a

result, this could lead to a reduction in hemodynamic side effect, prevention of accidental intraoperative awareness and thereby a significant increase in the patients' health and welfare. However, using the current REIMS set-up, this technique was not sensitive enough to measure remifentanil or propofol in patients' brain samples. However, we were able to detect remifentanil in soaked muscle samples. Therefore, in the next chapter, we will further investigate REIMS for the detection of a selection of the most common drugs of abuse, as most of these are present in much higher concentrations.

In Chapter III, we aimed to screen for cocaine, diazepam, methadone and morphine in post-mortem muscle samples without sample preparation and in quasi-real time using REIMS. Human muscle samples were soaked in solutions of four drugs at different concentrations and multiple incubation times to check the feasibility of REIMS for this innovative application. The sensitivity of the REIMS technique was evaluated by extracting the samples using solid phase extraction (SPE) and quantifying them with liquid chromatography tandem MS (LC-MS/MS) using internal deuterated standards. Furthermore, a mass spectral database and multivariate statistical model of the four different drugs in muscle tissue was built. The applicability of REIMS as a screening tool was demonstrated by analysing a muscle sample from a real forensic case with a good match to the results obtained using the SPE-LC-MS/MS method.

In the next part of this PhD project, we focused on the development of real-time, intraoperative tissue interrogation for REIMS-guided glioma surgery. Since the full mass profile is available, the same REIMS set-up can be applied for the detection of drugs as well as tissue molecular markers.

In chapter IV, we evaluated the reference intraoperative tissue diagnostic techniques for glioma surgery. Significant progress has been made in the development of medical imaging modalities and brain-mapping techniques, such as magnetic resonance imaging, computed tomography or fluorescence-guided surgery (using 5-aminolevulinic acid; 5-ALA). However, in up to 65 – 80% of glioma resections some residual tumour tissue remains after surgery. Therefore, we aimed to develop the REIMS technique for molecular characterisation of gliomas. We determined the applicability of REIMS to differentiate between the different types and grades of gliomas, as well as measure the diagnostic accuracy and sensitivity of REIMS to detect tumour cells in the tumour margin of both high- and low-grade gliomas. Classification models were built by analysing biopsies from patients brain tumour or epilepsy zones (as a control). Our results indicate that REIMS is a real-time, reliable methodology that has potential to be implemented in the current surgical workflow for brain tumour removal. We demonstrate the complexity and heterogeneity of glioma infiltration and confirm the utility and potential of REIMS in the characterisation of the tumour and the tumour margins within seconds.

In chapter V, we further assessed the first 10 loadings from the glioma classification model built in chapter IV. These might determine important mass features that contributed to the differentiation of normal and cancerous tissue. Based on the exact masses obtained using MALDI-Orbitrap and REIMS tandem MS, the corresponding lipid were identified. Mass peaks that contributed to the class separation were mainly glycerophospholipids with phosphatidylethanolamines (PE) being the most commonly identified. Furthermore, phosphatidic acids (PA), and phosphatidyl inositol (PI) were also identified. Interestingly, these mass features were not exclusively present in tumour tissue, but rather showed variable intensity ratios between the cancerous and normal tissue. In a next

step, MALDI-MSI was applied to image these lipids and confirm whether these aligned with the tumour locations in the sample.

Chapter VI takes it one step further, in an attempt to understand where these difference come from. The information from molecular imaging at the cellular level, immunohistochemistry stains and scans from 5-ALA fluorescence in brain sections will be combined. Multimodal information will significantly increase our understanding of glioma biology, and could even impact the development of therapies.