Quantitative Mass Spectrometry Imaging (QMSI) as a tool to study a new Anticancer Drug and its Metabolites in Tumor Bearing Mice

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Introduction

Evaluation of the distribution of drugs and metabolites in whole body and targeted organs is a key parameter in drug discovery and development. Few technologies allow the study of tissue distribution of xenobiotics. QWBA is the "Gold standard" method, recognized by the regulatory agencies for such evaluations. Quantitative Mass Spectrometry Imaging (QMSI), is a new, revolutionary, label-free technique that allows the detection and tissue distribution of xenobiotics, their metabolites and endogenous compounds. In this study, the QMSI technology has been tested to detect and quantify a new anti-cancer drug candidate and its metabolites in whole body, blood and xenograft tissue in mice. The distribution was evaluated at different time points to assess potential target engagement and toxicity effects.

Material and methods

Tumor cells were subcutaneously injected in male nude mice. 5-day repeated 30 mg/kg QD oral dose of anticancer drug was initiated. One mouse per time-point was sacrificed on the 5th dosing day at 2, 4 and 8 hours after the first daily dose. Carcasses of the tumor bearing mice were snap frozen and stored at -80°C: 20 µm whole body or plasma sections were prepared on tape in a cryostat. For testis, 10 µm sections were prepared. . DHB (2,5-Dihydroxybenzoic acid) MALDI matrix spiked with the deuterium labelled drug was sprayed over the sections and dilution series of the drug using an automatic sprayer device. 7T-MALDI-FTICR was used as an analytical instrument in imaging mode at 300µm, 100µm and 30µm spatial resolution, respectively for the whole bodies, the plasma and the testes to producing MSI data. Hematoxylin and Light Green staining was performed on whole body tissue sections adjacent to the ones imaged (Hematoxylin and Eosin was used for the testis).

MSI data sets and images of the staining were loaded in Quantinetix v1.7 and Multimaging v1.0. software for analyses of the distribution and quantitation of the drug and its metabolites per organ or per histological region of interest.

With MSI, the test compound and the metabolites were detected in the main organs of the whole body, in the blood (through the heart cavity) and in the xenograft tumors. Based on this pilotstudy, the results show high concentrations of the parent drug in plasma, with a tendency to decrease over time. The ratio of the metabolite to the drug in plasma was increasing over time. Distribution of the test compound was also seen in stomach, heart and lung for the parent drug and in lung, heart, stomach, intestines and skin for its metabolites. Obviously, signals from stomach and intestine relate to the applied administration route. In addition, quantitation in the target organs by QMSI was validated by comparison with the conventional LC-MS method . High spatial resolution imaging of testis revealed that the parent drug was mostly detected in the sub capsular region at both time points, 2 and 8h after dosing . While the parent drug was observed in all histological regions of the testes, i.e. interstitial tissue, germ cell layer and tubular lumen, the strongest signal was seen in the vascular interstitial tissue whereas the signal in the germ cell layer and lumen was clearly weaker: The latter findings are probably related to the lack of blood vessels in these regions. However, since a weak signal was evident also in these compartments it cannot be excluded that parent drug penetrates the blood-testes barrier to some extent.

Conclusion

This study showed that the combination of QMSI and histology can be used to study the location of a test substance and its metabolites in a PKPD study in tissues but also in specific compartments within a specific tissue (testis) without the use of radiolabelled compounds. However it is important to note that the best analysis resolution (20µm) is yet insufficient to assess cell specific compound distribution.