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SHORT COMMUNICATION

Differential distribution of probenecid as detected by on-tissue mass spectrometry

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Abstract We demonstrate, by means of on-tissue mass spectrometry of tissue sections, that the drug probenecid can penetrate the blood–brain barrier. This method holds general promise for the detection and distribution of small molecule drugs within organ and tissue compartments.

Keywords Probenecid · On-tissue mass spectrometry · Blood–brain barrier

Introduction

Probenecid is a drug that has long played an important role but that is still of interest, e.g., in neuroprotection and cardiology (Koch et al. 2012; Colín-González and Santamaría 2013). Originally used for blocking the reabsorption of uric acid in the kidney, it has come back into focus because of its inhibitory function on pannexin hemichannels and on the ATP-receptor PTX7 (Dahl et al. 2013; Bhaskaracharya et al. 2014). Probenecid might modulate neuroinflammatory disorders, since ATP release via pannexin-1 has been shown to activate astrocytes (Orellana et al. 2013). However, can probenecid reach the tissue of the the brain? This is still not clear. The substance is hydrophilic but might cross the blood-brain barrier by using organic anion transporters. We report the implementation of a method of on-tissue detection by using mass spectrometry to address whether probenecid crosses the blood–brain barrier.

Mass spectrometry imaging (MSI) couples the *m/z*-specific measurement capability of mass spectrometry with microscopic imaging capabilities, thus providing the unique opportunity of simultaneously obtaining images from analytes detected by MS and determining their exact correlation with the physical features of the biological sample (McDonnell and Heeren 2007; Schwamborn and Caprioli 2010). During the last two decades, MSI has proven to be an extremely powerful tool for mapping the spatial distribution of small biomolecules and large proteins in biological tissues (Seeley and Caprioli 2011; Eijkel et al. 2009; Gode and Volmer 2013). The introduction of matrix-assisted laser desorption/ionization (MALDI) to MSI by Caprioli in the mid-1990s (Caprioli et al. 1997) paved the way for this powerful analytical method. Today, MALDI has become the most popular MSI technique because of its superior performance with regard to spatial resolution and sensitivity to intact biomolecules and application range, e.g., the mapping of the spatial distribution of pharmaceutical compounds in vivo, the detection of specific metabolite expression as disease biomarkers compared with controls and the demonstration of the localization of proteins in tumors and other tissue. These are the outstanding features in comparison with other imaging techniques, such as positron emission tomography (PET; Matthews et al. 2012), magnetic resonance imaging (MRI; Beckmann et al. 2004) and whole body autoradiography (WBA; Wang et al. 2012). Chemical modifications or radioactive isotope labelling of the compounds are not required for MALDI-MSI. Furthermore,

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analysis is fast and specific and enables micrometer spatial resolution. In the present work, MS was applied to the analysis of the drug probenecid and its metabolites in mouse tissue sections from liver and kidney.

Materials and methods

C57BL/6 mice were treated with probenecid (n=3; 100 mg/kg body weight) or solvent (n=4) by intraperitoneal injection once daily for a duration of 10 days. Probenecid injection solution (pH 7.3) was prepared in a total volume of 5 ml, containing 50 mg probenecid (w/v), 150 μl 2 N NaOH (v/v), 850 μl 1 M TRIS, 4 ml phosphate-buffered saline and 12.5 μl 2 M HCl. Solvent treatment was performed by injection of solvent only of the same pH. The last injection was given 2 h before the mice were killed. Brain, liver and kidney were harvested and snap-frozen for further analyses. The mice were bred and housed under standard conditions in the animal core facility at the Institute for Clinical and Experimental Surgery, Saarland University Medical Center. All animal procedures were in compliance with the German Animal Protection Law and the German Guide for the Care and Use of Laboratory Animals and were approved by the Saarland Animal Care Committee. Cryostat sections (10 μm) were taken from brain, liver and kidney and spread on dedicated ITO slides (Bruker, Germany). Tissue samples were extracted as follows: 100 mg tissue was ground and homogenized in approximately 500 μl 1 % NaCl aqueous solution. Acetonitrile (500 μl) was added to the homogenate for protein precipitation and the mixture was vortexed for 10 min. The homogenate was solubilized in acetonitrile/water/formic acid (50:50:0.1, v/v/v) for analysis by tandem mass spectrometry (MS/MS). The analyses were performed qualitatively and not quantitatively.

A Bruker solariX 7 Tesla Fourier transform ion cyclotron resonance mass spectrometer (FTICR-MS) was used for the measurement. Tissue extracts were analyzed first by direct infusion in both positive and negative modes and electrospray ionization (ESI) MS/MS was applied for validation of the compound. In MS/MS mode, precursor ions were first isolated in the quadrupole, externally accumulated in the hexapole for 0.1–0.5 s and then 3–18 eV collision energy was applied for CID and 16 individual transients were collected and added for each spectrum to enhance S/N (Qi and O'Connor 2014). Peak assignment was based on the matching of both theoretical mass and isotopic patterns; spectra were internally calibrated by using a linear calibration function (Qi and O'Connor 2014). In MSI mode, homogeneous MALDI matrix layers were deposited onto the tissue surface by using a Bruker ImagePrep system with cyano-4-hydroxycinnamic acid (CHCA) solution (7 mg/ml; 50/50/0.2 acetonitrile-water/trifluoroacetic acid). The pixel size in imaging experiments was 50 μm and positive ions were monitored in a narrow m/z range of 280–290.

Results and discussion

The plasma concentrations of probenicid that are necessary to enable passage through the blood–brain barrier remain unclear, as does whether probenicid can indeed cross the barrier at all. In our treatment protocol, the drug was applied intraperitoneally once daily over a period of 10 days at a dosage of 100 mg/kg body weight. Interestingly, probenecid was clearly detectable in the brain (Fig. 1). In comparison with the liver, the relative concentration of the drug seemed to be much lower in the brain tissue. However, a tissue-specific calibration for the actual tissue concentration was not performed; despite being elaborate, this calibration is, in principle, possible. The finding that, although treatment continued for over a week, only very small traces of probenecid were detectable, indicates that the compound does not easily pass the blood–brain barrier. Detectable spots were seen in approximately one third of the section area, highlighting the presence of negative areas and that probenecid was not distributed homogeneously. Furthermore, the visible spots could feasibly be linked in some way to the content, the wall, or the vicinity of the blood vessels, which could not be differentiated with the present instrument. As MSI techniques are still in a developmental phase, resolution will undoubtedly improve over the next few years,

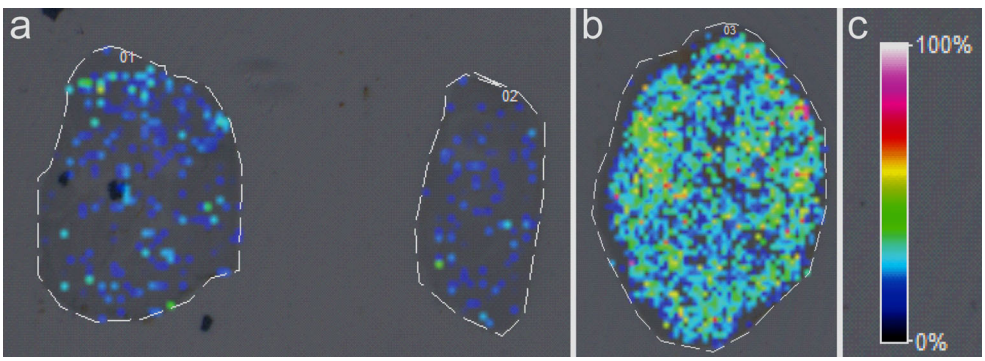

Fig. 1 Representative mass spectrometry (MS) imaging. Section in the frontal and sagittal planes (**a**) of the brain and a section through the liver (**b**) demonstrating the relative content of probenecid (**c**)

permitting enhanced visualization of morphological details. On-tissue mass spectrometry suggested that probenecid was present in brain tissue after a 10-day treatment. Thus, probenecid treatment of neuroinflammation might have not only peripheral but also central targets. The information on the exact pattern of occurrence of this drug within tissue would have been lost if the tissue samples had been first extracted and then analyzed. Importantly, we suggest that metabolites of probenecid can be active compounds, e.g., $C_{10}H_{13}NO_4S$ or $C_{13}H_{19}NO_5S$. The two metabolites were detected on the slides with very low signal intensities (data not shown). An interesting further problem is the way in which probenecid distribution changes in the presence of a leaky blood-brain barrier, as seen, for example, in trauma of the central nervous system (CNS), stroke, or neuroinflammtion. This will be the focus of future studies.

In conclusion, on-tissue mass spectrometry has an evolving role not only in functional analysis during in vivo studies in general but also in CNS studies in particular.

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