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Laser ablation inductively coupled plasma mass spectrometry for imaging of copper, zinc, and platinum in thin sections of a kidney from a mouse treated with *cis*-platin

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Abstract

Platinum complexes are used for the treatment of several types of cancer. High platinum concentrations in the target tissue and low concentrations in dose-limiting tissue structures such as renal tubules are desirable to assure selective toxicity. Microlocal analysis of platinum distribution in tissue sections may thus contribute to the optimization of platinum therapy. Scanning laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to produce images of element distribution in 14-μm thin sections of kidney tissue from a mouse treated with *cis*-platin 60 min prior to victimization. The sample surface was scanned (raster area 300 mm^2) with a focused laser beam (wavelength 266 nm, diameter of laser crater 50 μm, inter line distance 50 μm and laser power density 3×10^9 W cm^{-2}) in a cooled laser ablation chamber (about −15 °C) developed for these measurements. The laser ablation system was coupled to a double-focusing sector field ICP-MS. Ion intensities of $^{63}Cu^+$, $^{64}Zn^+$, and $^{196}Pt^+$ were measured within the tissue by LA-ICP-MS. Matrix-matched laboratory standards served for calibration of analytical data. The mass spectrometric analysis yielded an inhomogeneous distribution for Cu, Zn, and Pt in thin kidney sections. Copper was enriched in the capsule and outer cortex, zinc in the inner cortex and the platinum concentration followed a centripetal gradient with clear medullar enrichment. Thus, scanning LA-ICP-MS may be a useful tool in the preclinical development of new and less nephrotoxic platinum complexes.

Keywords: Imaging; Laser ablation inductively coupled plasma mass spectrometry; Mouse kidney; Platinum

1. Introduction

Platinum complexes are actually a major constituent in the treatment of testicular, ovarian, bladder, colon and non-small cellular lung cancer. The anti-cancer activity of *cis*-platin (*cis*-diammine-dichloro-platinum (II)) was discovered in 1969 [1]. Two additional compounds (carboplatin and oxaliplatin) have meanwhile been admitted to the market. The development of new platinum complexes [2–6] focuses on the reduction of nephrotoxicity, on the circumvention of resistance to platinum of tumor cells and on the improvement of oral bioavailability [6], and on penetration into particular tissues (e.g., brain, prostate [3]).

To date, nephrotoxicity is the dose-limiting effect for the use of platinum complexes in humans. The organic cation transporter 2 (OCT2) has been identified as a critical transporter for intracellular enrichment in proximal renal tubules [7]. Besides in vitro tests of platinum complexes on, for example, renal cell cultures, in vivo verification of in vitro results remains indispensable. Consequently, there is a need for versatile techniques to image the platinum distribution in tissue sections of small vertebrates. Previous studies used neutron activation techniques with the transmutation of ^{198}Pt (n, gamma) $^{199}Pt \rightarrow {}^{199}Au$ followed by autoradiographic detection of the β-emitting Au species [8–10]. This type of imaging has been abandoned due to the enormous experimental effort (requires nuclear reactor and special laboratories) and low accessibility. To our knowledge no other method has been described for the imaging of platinum distribution on thin section of tissues at micrometer scale.

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Several techniques for the imaging of platinum distribution on the sub-micrometer scale do exist, but they need laborious sample preparation and can hardly be applied to fields of view larger than 10 mm^2. Studies using energy dispersive X-ray microanalysis have also been reported [11–13].

In a few cases, indirect labeling of particular platinum complexes was possible: Meijera et al. [16] imaged DNA platinum adducts by antibody staining. The sub-cellular localization of fluorescein-*cis*-platin complexes has been examined by fluorescence microscopy [14]. Platinum is too heavy to allow reliable imaging by energy filtering electron microscopy [17].

Using secondary ion mass spectrometry (SIMS) [18,19], it is also possible to produce images of the element distribution with a lateral resolution in the low micrometers range and below. However, a major problem in SIMS analysis is the strong matrix dependence of the ion yields and, as a consequence, the quantification of analytical data is very difficult or is indeed impossible and only small sample areas (e.g., 250 μm × 250 μm) were analyzed [20].

LA-ICP-MS is an attractive tool for imaging (mapping) the Pt distribution in thin tissue sections due to its relatively low detection limits with a spatial resolution of 50 μm and below [21,22], simple sample preparation procedures and wide availability in analytical laboratories worldwide [23–26]. In contrast to SIMS, the LA-ICP-MS is less dependent on matrix effects [28] and allows easy quantification procedures. If no suitable standard reference material is available several strategies have been developed for quantification purposes in LA-ICP-MS, including a preparation of matrix-matched laboratory standards [21,22,24] or by using solution-based calibration [25,26]. Recently, we applied LA-ICP-MS for imaging of elements of interest (such as P, S, Cu, Zn, Th and U) in thin cross sections of rat brain samples [22] as well as human brain hippocampus [21]. The mass spectrometric analysis yielded an inhomogeneous distribution (layered structure) for P, S, Cu, and Zn in thin brain sections of the hippocampus. In the rat brain samples, small-size tumors were analyzed, where Cu and Zn deficiency in and around the tumor region was detected in comparison to the control brain tissue of the second hemisphere. Furthermore, LA-ICP-MS was extensively used for the determination of elements concentrations in selected protein spots derived from 2D gel electrophoresis [23,27,28]. Hutchinson et al. [27] used LA-ICP-MS for qualitative imaging of β-amyloid protein in brains of a transgenic mouse model of Alzheimer's disease. A correlation between the Aβ deposits and the concentration of trace elements was found. Sekaran [29] used LA-ICP-MS for 2D mapping of Cu and Zn distribution in lamb liver. They found a specific zonation of elements in the samples analyzed.

In the present study, we developed an analytical method for LA-ICP-MS imaging of platinum distribution in kidney slices from mice that were victimized 1 h after being injected with 3 mg/kg *cis*-platin. In addition to Pt the Cu and Zn distributions through the cross sections of analyzed rat kidney were measured. These distributions essentially served to underline the differential spatial distribution of different elements; however, the role of Cu and Zn in various biological processes has a great importance and has been discussed in a large number of works [21,22,27–29,32]. Solid matrix-matched standards of thin sections of tissue homogenates spiked with the elements of interest were used for their quantification.

2. Experimental

2.1. LA-ICP-MS instrumentation

A double focusing sector field ICP-MS (ICP-SFMS, ELEMENT, Thermo Electron Corporation, Bremen, Germany) coupled to a laser ablation system CETAC LSX 200 (Cetac Technologies, Inc., Omaha, NE, USA) was used for imaging of Cu, Zn, and Pt in thin renal tissue sections (thickness 14 μm). The laser ablation of thin kidney sections was performed with a frequency quadrupled Nd:YAG laser (wavelength 266 nm, repetition frequency 20 Hz, spot diameter 50 μm; laser power density 3×10^9 W cm^{-2}) in the cooled laser ablation chamber [30]. The distance between adjacent scan lines was 50 μm. The ablated material was transported by argon as a carrier gas into the inductively coupled plasma (ICP). The ions formed in the ICP were extracted in the sector field mass spectrometer and separated according to their mass-to-charge ratios. The ICP torch was shielded with a grounded platinum electrode (GuardElectrode, Thermo Electron Corporation). All measurements were performed in the low mass resolution mode of ICP-SFMS.

To obtain two-dimensional images of element distribution, the region of interest was systematically screened (line by line). The spot size of laser craters was 50 μm. ICP-MS data files for each analyzed brain section were converted into *txt* file and were used to produce 2D images of element distribution. The images were plotted using programming script in MATLAB® 6.5 computing software. Further details about the instrumentation used can be found elsewhere [21,22,30,31].

2.2. Samples and sample preparation

NMRI (nu/nu) mice from the university's breeding stock were kept in type III filter top cages (Tecniplast, Hohenpeissenberg, Germany) under specified pathogen free (SPF) conditions according to the FELASA recommendations for the species mouse (cf., www.felasa.org). Drinking water and laboratory animal chow (V1244; ssniff Spezialdiaeten, Soest, Germany) were provided ad libitum. Water and food as well as caging and bedding materials (H1505-01; ssniff Spezialdiaeten, Soest, Germany) were autoclaved before use. The physical data of the animal room were: temperature 22 ± 2 °C, relative humidity 55 ± 5%; 15 air changes per hour; 12-h light/12-h dark cycles. A single female mouse at the age of 8 weeks was separated for a single intraperitoneal injection of *cis*-DD-platin (3 mg/kg of body weight, dissolved in sterile polyethylene glycol 300/0.9% NaCl, v/v). Exactly 60 min after injection the mouse was killed by cervical dislocation. The kidneys were removed immediately and quick-frozen in liquid nitrogen. Serial sections (14 μm) were cut in a cryostat (Leica, CM3050S) at −20 °C, thaw-mounted on glass slides and air dried. An adjacent section was routinely stained with hematoxyline and eosine for visualization of microanatomy.

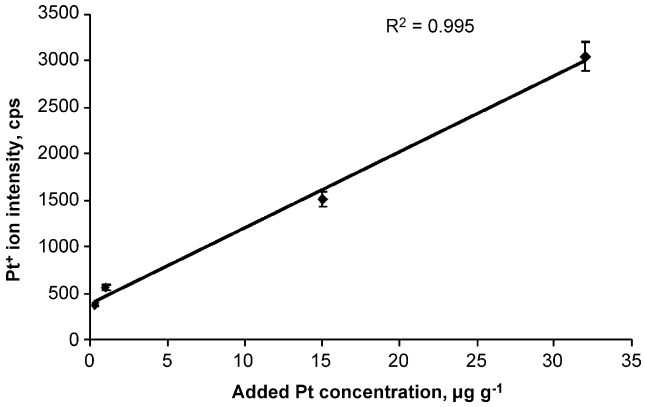

Fig. 1. Calibration curve for quantitative determination of Pt in thin cross sections of mouse kidney measured on synthetically prepared matrix-matched standards by LA-ICP-MS. Tissue homogenates from kidneys of two untreated control mice were spiked with water or dilutions of a standard solution, respectively, with a final mass fraction of added liquid of 10%.

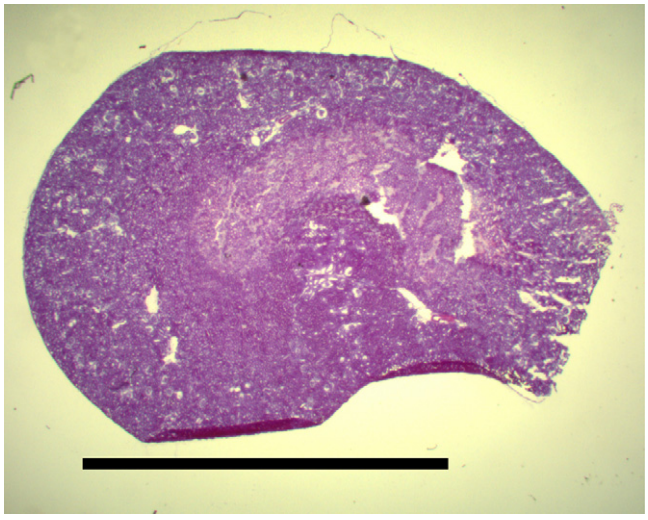

Fig. 2. Tissue stained with hematoxylin eosin demonstrating general architecture of mouse kidney. From the periphery to the center the following can be discriminated: the connective tissue capsule, the cortex containing glomeruli and tubules and the medulla containing distal tubuli and the collecting ducts. Scale bar 5 mm.

2.3. Calibration procedure

Matrix-matched laboratory standards with well-defined concentrations of the elements of interest were prepared for the calibration of analytical data. For this purpose, aliquots of tissue homogenates from two untreated control mice were spiked with standard solutions containing known concentrations of selected elements (Cu, Zn, Pt) with a final mass fraction of added liquid of 10%. The added concentrations of elements in the prepared standard tissues after spiking were 10, 5, and 1 $\mu g\,g^{-1}$ for Cu and Zn, and 0.32, 1, 15 and 32 $\mu g\,g^{-1}$ for Pt. Another aliquot of tissue homogenate was used for blank correction. Further, these suspensions were frozen, cut on a cryostat and mounted onto glass slides in the same way as the samples. The calibration curve for platinum measured with these standards yielded a correlation factor of $R^2 = 0.9950$ (see Fig. 1). The details of the preparation of matrix-matched standards have been reported elsewhere [21,22].

In addition, for the evaluation procedure a defined volume of 10 $\mu g\,g^{-1}$ Cu and Zn standard calibration solution was applied as a line mark onto the slides beside the tissue section. This step was performed to correlate the time scale between laser ablation line scans and ICP-MS measurements during evaluation procedures.

3. Results and discussion

In Fig. 2, an adjacent section stained with hematoxylin and eosin (HE) is presented that shows all the structures constituting the general anatomy of a kidney. These include the connective tissue capsule, the cortex containing glomeruli and tubules, and the medulla in the innermost part composed of distal tubules and the collecting ducts.

To obtain two-dimensional imaging of element distributions in the analyzed samples, the mouse kidney tissue sections were systematically screened (line by line) with the focused laser beam with a diameter of 50 μm. This spot size was shown to be adequate to obtain sufficient sensitivity and precision of the developed procedure. By LA-ICP-MS measurements an inhomogeneous distribution with a layered structure of analyzed tissue was found for all elements measured. An artificial buckling of the tissue parallel to the microtome blade can be seen on the upper and lower edge zones. In this region higher ion intensities for all elements due to the thicker tissue layer were measured.

In Figs. 3 and 4, the quantitative images of copper, zinc and platinum distributions in two adjacent cross sections of renal tissue determined by LA-ICP-MS are presented. The concentrations of Cu, Zn and Pt were found to be in the range of 3–7, 2–6 and 10–25 $\mu g\,g^{-1}$, respectively. The limits of detection (3σ of the blank ion intensity divided by sensitivity of the analyte) were calculated using synthetically prepared matrix-matched laboratory standards. For the Pt, Cu and Zn the LODs using LA-ICP-MS were determined to be 0.8, 0.34 and 0.14 $\mu g\,g^{-1}$, respectively. The precisions (relative standard deviation, R.S.D.) for the selected single line scans were in the range of 20–30%.

The element distributions in thin tissues of mouse kidney correlated very well between the two adjacent sections measured. Copper was enriched in the capsule and external part of the cortex (about 6 $\mu g\,g^{-1}$) coinciding with the glomeruli while zinc was enriched (up to 5 $\mu g\,g^{-1}$) in the inner part of the cortex composed of tubules. There seemed to be renal lobes with higher zinc concentrations than others, reflecting the congruence of structural and functional organization.

Platinum concentration was maximal (about 26 $\mu g\,g^{-1}$) in the medulla, moderate (about 14 $\mu g\,g^{-1}$) in the inner part of the cortex and progressively decreased (6–10 $\mu g\,g^{-1}$) to the periphery.

Of all the organs the kidney possesses a unique capillary perfusion which follows the path from the periphery to the center. Glomeruli in which the primary urine is filtered from the blood and tubules where this filtrate is concentrated are connected in series. Urine concentration follows a centripetal gradient.

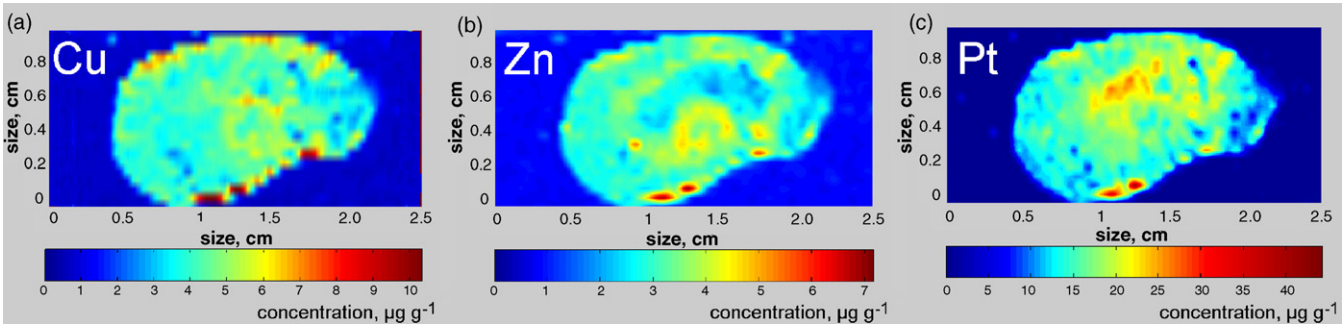

Fig. 3. Lateral distributions of Cu, Zn and Pt on thin cross section of mouse kidney (Section 1) measured by the LA-ICP-MS.

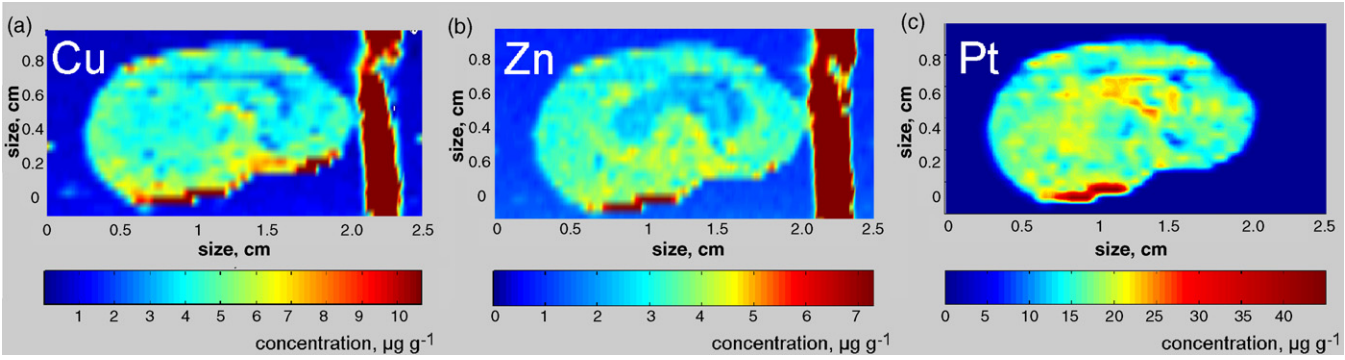

Fig. 4. Lateral distributions of Cu, Zn and Pt on thin cross section of mouse kidney (Section 2) measured by the LA-ICP-MS. A vertical line, visible on the right-hand side of the section in the Cu (a) and the Zn (b) images, was drawn with standard solutions of Cu and Zn and was used for *x*–*y* position purposes during evaluation procedure.

Apparently the concentration of platinum reflects this gradient. The contribution of specific platinum retention in a given zone due to protein binding or cellular enrichment in this early phase of elimination seems negligible. The copper distribution may correlate to some degree with the distribution of blood volume as copper is known to be highly concentrated in the blood compartment compared to other tissues. It would be very interesting to monitor the renal platinum distribution at later points in time or after prolonged exposure to platinum. Enrichment in proximal tubules has been reported in the latter case [15].

In future, scanning LA-ICP-MS can also be applied for the analysis of Pt in other biological tissues (e.g., human brain cross sections) or hairs. The latter would allow a continuous monitoring of platinum concentrations in humans during clinical studies in addition to routine analysis of liquid plasma samples or urine by conventional ICP-MS.

4. Conclusion

An analytical method was developed for two-dimensional imaging of platinum distribution in tissues in the 50 μm range. In contrast to other surface analytical techniques, LA-ICP-MS has the possibility of rapid multielemental screening of a relatively large analyzed area (depending on the size of the ablation chamber) as well as requiring hardly any sample preparation steps. The platinum distribution can be mapped throughout multiple organs or even in whole body sections of mice. Scanning LA-ICP-MS may be helpful in the development of new platinum complexes for the treatment of various cancers.

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