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High-resolution laser ablation-inductively coupled plasma-mass spectrometry imaging of cisplatin-induced nephrotoxic side effects

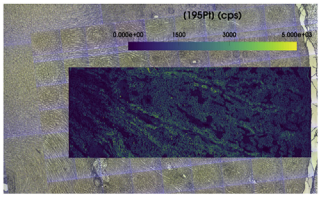
Thibaut Van Acker [a], Stijn J.M. Van Malderen [a], Marjolein Van Heerden [b], James Eric McDuffie [c], Filip Cuyckens [b], Frank Vanhaecke [a, *]

[a] Department of Analytical Chemistry, Ghent University, Campus Sterre, Krijgslaan 281 - S12, 9000, Ghent, Belgium
[b] Preclinical Development and Safety, Janssen Research and Development, Turnhoutseweg 30, 2340, Beerse, Belgium
[c] Preclinical Development and Safety, Janssen Research and Development, 3210 Merryfield Row, San Diego, CA, 92121, USA

HIGHLIGHTS

- Pt-mapping in kidney tissue with a spatial resolution down to 1 μm using LA-ICP-MS.
- Examination of the distribution of cisplatin on a (sub-)cellular scale.
- Correlation between microscopic lesions and local accumulation of cisplatin.

GRAPHICAL ABSTRACT



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ABSTRACT

Two-dimensional elemental mapping (bioimaging) via laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) was performed on 5 μm thick formalin-fixed, paraffin-embedded kidney tissue sections from Cynomolgus monkeys administered with increasing pharmacological doses of cisplatin. Laterally resolved pixels of 1 μm were achieved, enabling elemental analysis on a (sub-)cellular level. Zones of high Pt response were observed in the renal cortex, where proximal tubules are present, the epithelium of which is responsible for partial reabsorption of cisplatin. Histopathological evaluation, of hematoxylin and eosin-stained serial sections, adjacent to the sections probed via LA-ICP-MS, revealed minimal to mild cisplatin-related lesions (<100 μm) in the renal cortex. Necrotic proximal tubules with sloughed epithelial cells in their lumen could be linked directly to the areas with the highest accumulation of cisplatin, indicating a direct link between cellular concentration and toxicity, thereby providing more insight into the mechanisms through which renal damage occurs.

1. Introduction

Cisplatin (*cis*-diamminedichloroplatinum [II], CDDP) was the first platinum-containing chemotherapeutic agent and is now approved for the treatment of solid tumors, *e.g.*, testicular, cervical, colorectal, lung, ovarian, head and neck cancer [1–3]. Unfortunately, a major drawback of cisplatin use is the occurrence of severe and dose-limiting side effects, *e.g.*, vomiting, ototoxicity, neurotoxicity and nephrotoxicity [4]. Drug-induced kidney injury (DIKI) is an adverse event, in which excretion is disrupted due to exposure to toxic compounds [5]. No regulatory authority guidance regulates stand-alone kidney safety pharmacology studies. Therefore, exploratory repeat-dose DIKI studies are not routinely conducted by pharmaceutical companies, particularly not in non-human primates [6]. While the incidence of translatable DIKI is low, DIKI remains a significant cause of candidate drug attrition during the clinical drug development phase and post-marketing period [7].

* Corresponding author.
E-mail address: Frank.Vanhaecke@UGent.be (F. Vanhaecke).

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Recently, DIKI has been reported to contribute to approximately 25% of the cases of hospital-acquired acute kidney injury (AKI) events [8]. Despite restricted patient doses [9], approximately one in three patients suffers from nephrotoxicity after cisplatin chemotherapy, which endangers the kidneys' vital functions in the genitourinary and cardiovascular systems [3,4].

The renal system excretes metabolic waste products by filtering the blood and it is responsible for maintaining water, electrolyte and acid-base balances in the body. Moreover, the kidneys regulate the production of vitamin D, by synthesizing calcitriol, and the secretion of the hormone erythropoietin, which stimulates red blood cell production in the bone marrow [10]. Renal cortical and medullary tissue consists of vascular and tubular elements in close proximity to each other, with the interstitium, *i.e.* interstitial fluid and interstitial cells, in-between them [10]. In humans, a normal kidney consists of approximately 1 million of nephrons, which start in the cortex and end in the papilla [11]. Fig. 1 provides a schematic representation of a short-looped and long-looped nephron. Each nephron is subdivided in multiple tubular segments, which differ functionally and structurally from one another, starting with the renal corpuscle, *i.e.* Bowman's capsule surrounding the glomerulus, a tuft of capillary loops through which the blood flows [11]. In this structure, blood filtration occurs whereby water, electrolytes and small proteins are removed from the capillaries and enter the Bowman's capsule [11]. This filtrate flows further into the proximal convoluted tubule (PCT) and proximal straight tubule, where large amounts of water, electrolytes, glucose and amino acids are reabsorbed from the filtrate [10]. Furthermore, secretion of some administered drugs takes place in this region of the nephron [10]. The next tubular segments are the loop of Henle, distal convoluted tubule (DCT), connecting tubule, cortical and medullary collecting ducts, which are all responsible for numerous reabsorption processes and the delivery of the final urine to the minor calyx [10].

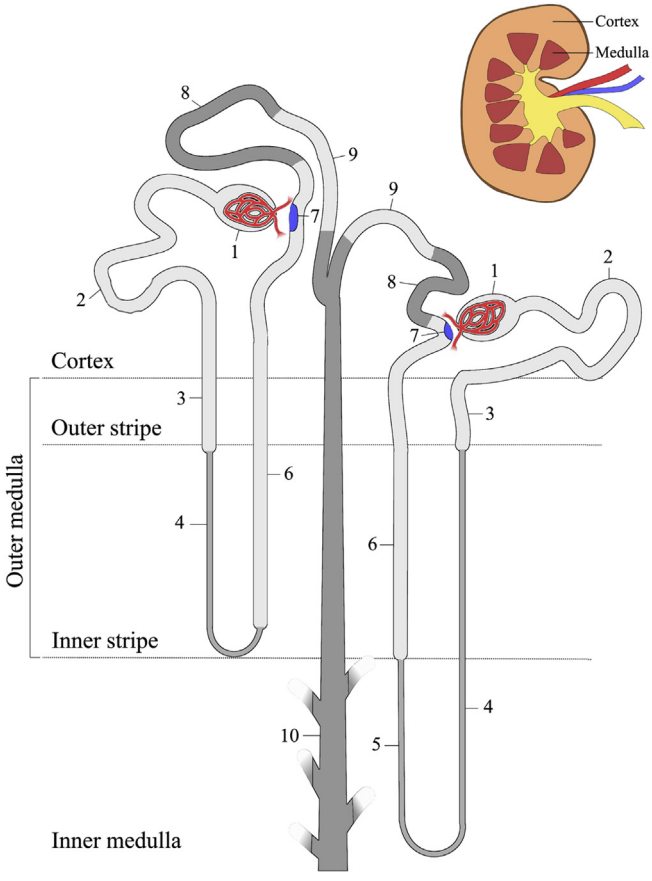

Fig. 1. Schematic representation of the nephrons, the functional units of the kidney. The regions of the cortex, outer and inner stripe of the outer medulla and the inner medulla are separated by the dashed lines. The numbers are indicating different tubular segments: (1) renal corpuscle, (2) proximal convoluted tubule, (3) proximal straight tubule, (4) thin descending limb, (5) thin ascending limb, (6) thick ascending limb, (7) macula densa, (8) distal convoluted tubule, (9) connecting tubule and (10) collecting duct. The nomenclature is based on standard nomenclature for structures of the kidney [12].

In the case of nephrotoxicity, cisplatin accumulation can occur locally in renal tissue [13], where cisplatin is taken up in cortical tubular epithelial cells of proximal and distal tubules via passive diffusion and via copper transporters, P-type copper-transporting ATPases, multi-drug extrusion transporters and organic cation transporters (OCTs) [3,4,14,15]. In renal tissue, especially OCT2 plays a major role in cisplatin nephrotoxicity as being a kidney-specific transporter protein, it is omnipresent in proximal tubular epithelium at the basolateral membrane [3]. The identification of the molecular mechanisms involved is ongoing. Once cisplatin enters cells, it binds to nucleophilic molecules, *e.g.*, proteins, RNA and DNA, which may trigger DNA cross-linking, apoptosis and necrosis [1,4]. Subsequently, this phenomenon may lead to severe renal tissue damage, decreased glomerular filtration rate and ultimately, renal failure [4]. Because the mechanisms responsible for nephron segment-specific DIKI remain poorly understood, there is a great need for sensitive, label-free methods for visualizing and quantifying the distribution of candidate drugs in the kidney to support investigational studies of nephrotoxicity during drug research and development.

Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) is a well-known analytical technique capable of performing quantitative spatially resolved analysis of elemental distributions with μm lateral resolution. The main features of LA-ICP-MS include minimal sample preparation, wide linear dynamic range, multi-element capability and low limits of detection. Bioimaging via LA-ICP-MS was first proposed by Wang et al. [16] and has gradually gained interest in the bioanalytical field and has been utilized to visualize the distribution of endogenous metals in numerous tissues, *e.g.*, mouse brain, heart and spinal cord tissue [17–20], human brain, lung, lymph node tissue and kidney stones [21–24] and metal-labelled antibodies designed to target specific antigens [25–31]. Analysis of the distribution of administered pharmaceutical agents such as cisplatin in target and non-target tissues has been performed in many studies [32–42], providing profound insights into depth of tissue penetration, metabolic fate *in vivo*, and serious adverse effects which, in turn, can enhance the success rate of utilizing cisplatin as a prototype to develop LA-ICP-MS methods that may be adapted to further evaluate candidate drugs or their metabolites with known kidney safety liabilities, particularly during the early stages of the drug development process [32].

Accurate quantification protocols in LA-ICP-MS require matrix-matched standards, online solution addition calibration, or isotope dilution [43–45]. A recent review by Limbeck et al. [46] summarized calibration strategies and approaches for the preparation of standards, including sections of spiked tissue homogenates [21], spiked blood [47], spiked polymethylmethacrylate spin-coated on quartz slides [48], printed standards [49–52], and dried droplet standards [53]. Based on their density and water content, spiked gelatin standards are pseudo-matrix-matched to biological tissues and can be applied as an inexpensive and simple alternative [34,54–56]. In this work, spiked gelatin droplet standards, with In as an internal standard, were prepared for quantification purposes. A low dispersion mixing bulb, ARIS [57], which was developed at Ghent University and is commercialized by Teledyne CETAC