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Elemental bioimaging of platinum in mouse tissues by laser ablation-inductively coupled plasma-mass spectrometry for the study of localization behavior of structurally similar complexes

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ABSTRACT

In the last decade, the number of applications of laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) in bioimaging has been increasing. To further extend its capability in drug development, in this study, we used this bioimaging tool to visualize deposition behavior of chemically different metallo-complexes, including two that differed only subtly in structure. A systematic approach with *in vitro* study and ICP-MS elemental analysis were included to supplement the findings.

Two chemically distinct platinum complexes (Pt-1 and Pt-2) were synthesized; their potencies were investigated first on different healthy and cancer cell lines and then on mice. The commercialized anti-cancer drug, *cis*-platin was used as a reference. In animal studies, the mice were given 4 mg/kg of the complex *via* intraperitoneal injection and sacrificed 24 h post-injection. ICP-MS analysis was performed on six organs to study the bioavailability of the complexes. Pt accumulated in the organs, from greatest to least, from liver > kidney > lung > testis > heart > brain. Among the complexes, the bioavailability showed a general trend of Pt–2 > cis-platin > Pt-1.

In LA-ICP-MS bioimaging analysis of paraffin-embedded mouse liver and kidney sections, a spatial resolution of 50 μm was adopted. Deposition trends matched the findings obtained in elemental analysis. In addition, differential deposition of Pt was observed in the kidney sections of mice treated with different complexes. The LA biomaps successfully distinguished the differential distribution of two structurally similar platinum complexes (Pt-1 and Pt-2) in mice liver and kidney. This information is of particular interest because these two Pt-based complexes can potentially be developed into anti-cancer drugs. This work demonstrates that LA-ICP-MS imaging is a valuable tool for therapeutic drug development, especially in assisting molecular modification of metal-containing complexes.

1. Introduction

Therapeutics using metal-containing complexes are one of the newest and most promising areas of medical research. For example, a gold complex has been found to be effective in treating rheumatism [1]; bismuth is being used as an antiulcer agent [2], and silver has anti-microbial applications [3]. Platinum complexes such as cisplatin, oxaliplatin and carboplatin are commonly used as anticancer drugs for different types of cancer, and other similar derivatives are currently undergoing clinical trials to evaluate their anti-cancer efficacy. However, most of these complexes have severe side effects and/or encounter drug resistance [4], which limits their use in some types of cancer. The main dose-limiting factor is nephrotoxicity [5,6], which often means that the dose required for bringing the desired anti-cancer effect cannot be administered. For patients with poor kidney functions, increase in dose and frequency in administration increases the risk of acute kid-

Abbreviations: LA-ICP-MS, laser ablation-inductively coupled plasma-mass spectrometry; FFPE, formalin fixed-paraffin embedding.

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ney injury. Given these problems with existing drugs, synthetic chemists are actively engaged in new drug design and development. Iridium- [7,8], ruthenium- [9,10] and rhodium- [11,12] containing complexes with different ligands have been synthesized and their anticancer efficacy is being studied. Alternative drug delivery is another approach to solving these problems; this means, for example, incorporating the metal complex into microspheres [13], polymeric micelles [14] or liposomes [15–17]. Nanoparticles [18,19] have also been used as carriers in attempts to control the release of the drug and thereby its toxicity.

An ideal anti-cancer drug must possess the following qualities: i) sufficient solubility to permit oral administration or subcutaneous injection; ii) good selectivity, such that it is cytotoxic to cancer cells without harming normal cells; and iii) specific accumulation, meaning that it will accumulate in the target site (organs) to exhibit its anti-cancer function while not bringing adverse effects to other parts of the body.

Cis-platin demonstrates anti-cancer ability by its complex formation with the deoxyribonucleic acid (DNA) in cancer cells. Thus, *cis*-platin drug candidates are designed with modified ligands to reduce their burden on the organs and improve their thermokinetic and thermodynamic stability, thereby achieving the above mentioned qualities of an ideal drug.

For drug development, both *in vitro* and *in vivo* studies are conducted to evaluate the cytotoxicity and the pharmacokinetics/pharmacodynamics of the complexes after administration. However, these studies are often limited to bulk analysis, which does not provide clues about the localization of the metals within the organs. Recently, elemental bioimaging by LA-ICP-MS has been utilized to map tissue sections; many successes have been reported in the study of spatial distribution in animal tissue after metallodrug administration. Moreno-Gordaliza *et al.* [20] studied the deposition of platinum in mice kidney after *cis*-platin injection. They produced biomaps of platinum, copper and zinc and investigated the relationships among the distribution profiles of these three metals. Reifschneider *et al.* [21] monitored *cis*-platin in mice cochlea, testis and kidney at 1 h and 4 days, and the results showed a significant decrease in platinum deposition over time. Pharmaceuticals containing other metals (*e.g.* Cd, Se) or heteroatoms (*e.g.* Br) have also been investigated. Hsieh *et al.* [22] assessed the deposition of CdSe in mouse lungs. One recent study of Egger *et al.* [23] includes two compounds in a LA-ICP-MS bioimaging study: the group mapped out the distribution of *cis*-platin and a ruthenium complex (KP1339) in kidney. While all the above works show the usefulness of LA-ICP-MS, all of them studied a single metallodrug or metallodrugs with different metal bases. In a recent study by Moraleja *et al.*, the different deposition of three commercialized platinum based anti-cancer complexes in rat kidney were investigated using LA bioimaging [24]. A similar study was also reported to compare the delocalization of some Pt (IV) complexes in hypoxic spheroids and xenografts by LA-ICP-MS [25]. Being able to distinguish two structurally different compounds would be of great value in pharmaceutical development, particularly in the fine-tuning of powerful drugs to reduce toxic side effects.

This paper presents a systematical approach, mainly based on elemental analysis and mapping, to investigate the distribution of two synthesized platinum complexes, Pt-1 and Pt-2 (Fig. 1), after administration to mice. *In vitro* study in cell lines was first conducted to evaluate the potencies of the complexes, followed by *in vivo* study in mice to obtain the biodistribution of the metals in different organs. LA-ICP-MS was employed to obtain the spatial distribution of the metals within mice liver and kidney and compare the localization behavior of the metal complexes. *cis*-Platin was used as a reference compound for comparison. It is believed that modification in chemical structure can influence the pharmacological behaviors of the complex, including the toxicity, accumulation, distribution and deposition in internal organs.

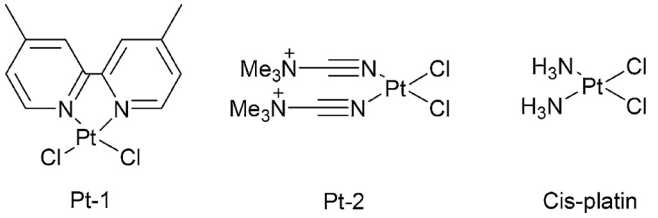

Fig. 1. Chemical structures of the platinum complexes involved in this study.

2. Experimental

2.1. Reagents and cell lines

Milli-Q water (18.2 MΩcm Millipore, Billerica, MA, USA) was used to prepare all aqueous solutions. For cytotoxicity assay, DMEM medium, RPMI-1640 medium, fetal bovine serum (FBS), penicillin and streptomycin (P/S) were obtained from Invitrogen (Carlsbad, CA). M199 medium, heparin, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromine (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA), and endothelial cell growth supplement (ECGS) was obtained from Upstate Biotechnology (USA). A. R. grade DMSO was obtained from Labscan Asia Co. (Bangkok, Thailand). Saline was prepared as 0.9% NaCl (TraceSELECT®, ≥ 99.999%, metals basis, Sigma-Aldrich). 10% formalin solution, neutral buffered (Sigma-Aldrich), Neo-Clear® xylene substitute (Merck KGaA, Darmstadt, Germany), Paraplast Plus (Leica Microsystem GmbH, Wetzlar, Germany) and ethanol (AnalaR NORMAPUR, VWR International, Radnor, Pennsylvania, USA) were used in the tissue fixation and embedding processes. Concentrated HNO_3 (Aristar, BDH, UK) and 30% H_2O_2 (Suprapur®, Merck KGaA) were used in microwave digestion. 1 mg/L platinum and thallium aqueous standard solutions were prepared by appropriate dilution from 1000 mg/L platinum and thallium stock standards (VHG, Manchester, NH, USA) respectively.

Lung carcinoma cell line (A549), human keratinocyte cell line (HaCaT), and human liver carcinoma cell line (HepG2) were obtained from American Type Culture Collection (ATCC, Manassas, Virginia, USA). Human dermal fibroblasts cell line (HDF) and human umbilical vein endothelial cell line (HUVEC) were obtained from Lonza (Walkersville, MD, USA). Human pulmonary microvascular endothelial cells (HPMEC) was obtained from ScienCell Research Laboratories (Carlsbad, California, USA). Nasopharynx cancer cell line (HK-1) was supplied by Areas of Excellence (AoE) NPC Research Tissue Bank (Hong Kong).

2.2. Metal complexes

Cis-platin (≥99.9% trace metals basis, Aldrich) was purchased from Sigma-Aldrich. Complexes Pt-1 and Pt-2 were provided by Professor Raymond, W.-Y. Wong from the Department of Chemistry, Hong Kong Baptist University. Log P values of the two synthesized complexes were calculated using the ChemDraw Ultra 8.0 (CambridgeSoft Coporation, MA, USA).

2.3. In vitro *studies of Pt-1 and Pt-2*

Three cancer cell lines (A549, HepG2 and HK-1) and four normal cell lines (HaCaT, HDF, HPMEC and HUVEC) were used. A549, HepG2, HaCaT and HDF were maintained in DMEM medium supplemented with 10% FBS and 1% P/S. HK-1 was grown in RPMI-1640 medium supplemented similarly. HPMEC and MUVEC were cul-