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Quantitative evaluation of cellular uptake, DNA incorporation and adduct formation in cisplatin sensitive and resistant cell lines: Comparison of different Pt-containing drugs

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ABSTRACT

The use of Pt-containing compounds as chemotherapeutic agents facilitates drug monitoring by using highly sensitive elemental techniques like inductively coupled plasma mass spectrometry (ICP-MS). However, methodological problems arise when trying to compare different experiments due to the high variability of biological parameters. In this work we have attempted to identify and correct such variations in order to compare the biological behavior of cisplatin, oxaliplatin and pyrodach-2 (a novel platinum-containing agent). A detailed study to address differential cellular uptake has been conducted in three different cell lines: lung adenocarcinoma (A549); cisplatin-sensitive ovarian carcinoma (A2780); and cisplatin-resistant ovarian carcinoma (A2780cis). The normalization of Pt results to cell mass, after freeze-drying, has been used to minimize the errors associated with cell counting. Similarly, Pt accumulation in DNA has been evaluated by referencing the Pt results to the DNA concentration, as measured by ^{31}P monitoring using flow-injection and ICP-MS detection. These strategies have permitted to address significantly lower Pt levels in the resistant cells when treated with cisplatin or oxaliplatin as well as an independent behaviour from the cell type (sensitive or resistant) for pyrodach-2. Similarly, different levels of incorporation in DNA have been found for the three drugs depending on the cell model revealing a different behavior regarding cell cisplatin resistance. Further speciation experiments (by using complementary HPLC–ICP-MS and HPLC–ESI-Q-TOF MS) have shown that the main target in DNA is still the N7 of the guanine but with different kinetics of the ligand exchange mechanism for each of the compounds under evaluation.

1. Introduction

The search for new chemotherapeutic drugs that overcome the limitations associated with the use of traditional treatments, such as cisplatin acquired drug resistance, is an area of continuous investigation [1,2]. In this regard, the use of alternatives such as carboplatin and oxaliplatin has been explored in numerous cancerous processes, and these therapies are nowadays in wide use [3,4]. The mechanism of action of these drugs is believed to rely first on the drug uptake by the cells followed by its transport within the cytosol to the cell nucleus where its interaction with the DNA generates the so-called Pt–DNA adducts [5]. These adducts are formed by sequential substitution of the ligands initially present within the drug structure followed by final drug–DNA binding producing several monodentate adducts, as well as many intra- and inter-strand cross links [6,7]. The intra-strand DNA complexes, where Pt bridges adjacent guanine bases, is by far the most dominant DNA adduct with both cisplatin and oxaliplatin, comprising approx. 65% of the total number of adducts [8]. The resulting adduct formation is believed to result in an inhibition of the replication and transcription leading to subsequent cell death [9]. Thus, the efficacy of a given treatment is thought to be highly dependent on the degree of formation of such adducts which is also cell-type dependent [10].

However, there are several limiting factors affecting the formation of Pt–DNA adducts [11]. The first one is related to the drug uptake from the growth media (in the case of in vitro test systems) into cells. It has been observed by some authors that the

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cellular incorporation of cisplatin (the most studied Pt–drug) is limited to approximately 1% of the total drug concentration used for exposure [12]. Interestingly, reduced drug accumulation is frequently observed in cisplatin-resistant cell lines, such as A2780cis, although the mechanism responsible for this effect remains difficult to assess [13]. It could result either from a reduced drug uptake from the culture media (due to alterations in some specific membrane transporters) [14] or enhanced drug efflux, or both. A second limiting factor is that once incorporated into the cells, the drug can be inactivated by binding to different biomolecules present within the cell cytosols (e.g., glutathione, proteins, etc.) [15]. The final outcome is that very little Pt from the initial concentration used for exposure finally reaches the target molecule, the DNA. In any in vivo system, this effect is further determined by the drug's stability in plasma. Lastly, once formed, the Pt–DNA adducts can be also repaired by specific enzymes that recognize the DNA damage [16]. The degree of tolerance to persisting (unrepaired) DNA lesions determines the fate of a given cell, which may be survival or apoptosis.

In an attempt to design new platinum compounds to overcome the above described cisplatin limitations, some authors have designed many metallodrugs, including those containing other metallic ions such as ruthenium [17] or different types of ligands in the case of Pt-based therapies [18,19]. An example of this latter case is the development of a family of anionic platinum complexes that seem to act at the cell level but avoiding penetration into the cell nucleus named phosphaplatins. Phosphaplatins [20,21] are a family of compounds containing a diaminocyclohexane core structure (similar to that of oxaliplatin) but with a pyrophosphate group as ligand instead of oxalate. These compounds have exhibited superior efficacy with reduced toxicity in several cellular models, and one member of the family, pyrodach-2, is currently in clinical development [21]. These pyrophosphate complexes seem to have different cellular and molecular antitumor mechanisms compared to conventional platinum therapeutics. In order to understand these mechanisms more completely, work regarding drug uptake, incorporation into DNA or formation of DNA–Pt adducts, in comparison to more conventional Pt–drugs in different types of cell cultures would be desirable.

The present study attempts to establish adequate analytical methodologies that permit comparison of the outcome of different chemotherapeutic treatments by monitoring parameters such as cellular uptake, DNA platination and DNA adduct formation. Methodological strategies that permit the analysis of micro-samples in combination with ICP-MS as a Pt selective detector [22] are optimized here in order to conduct the quantitative evaluation of the cellular uptake and DNA incorporation of cisplatin, oxaliplatin and a model phosphaplatin compound (pyrodach-2) (Fig. 1). The study was simultaneously conducted in three different cell lines which are model for cancer types in which Pt-therapy is almost the standard treatment: cisplatin-sensitive ovarian cancer (A2780), cisplatin-resistant ovarian cancer (A2780cis), and lung adenocarcinoma (A549). The normalization of the Pt concentration results to biological parameters such as the number of cells or the DNA concentration within the cell nucleus was evaluated. Secondly, speciation experiments have been also done in order to address the structure of the possible DNA–Pt interactions of the three drugs by applying capillary HPLC and simultaneous ICP-MS and ESI-Q-TOF MS for detection [23,24].

2. Materials and methods

2.1. Reagents

All chemicals were of analytical reagent grade or better. Ultrapure water (>18 MΩ) obtained from a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout. Working standard solutions were prepared daily by solubilization of the solid compounds in ultrapure water and 10 mM $NaHCO_3$ for pyrodach-2. Calf thymus DNA (lyophilized powder) and Nuclease S1 from *Aspergillus oryzae* were purchased from Sigma–Aldrich (St. Louis, MO, USA). Cisplatin (*cis*-diamminedichloroplatinum(II)), oxaliplatin (1,2-diaminocyclohexane oxalate platinum(II)) and pyrodach-2 (1,2-diaminocyclohexane pyrophosphate platinum (II)) were kindly provided by Phosplatin Therapeutics as highly-pure active pharmaceutical powder from a single manufacturing source (Heraeus GmbH, Hanau, Germany). For cell culture, Dulbecco's modified Eagle's medium (DMEM), PBS and trypsin were from LabClinics (Barcelona, Spain), RPMI 1640 medium and fetal bovine serum were from Invitrogen (Fisher Scientific, Madrid, Spain) and plasmocin was from InvivoGen (San Diego, USA). Nitric acid (65%, Suprapur quality) and hydrogen peroxide (30%) were

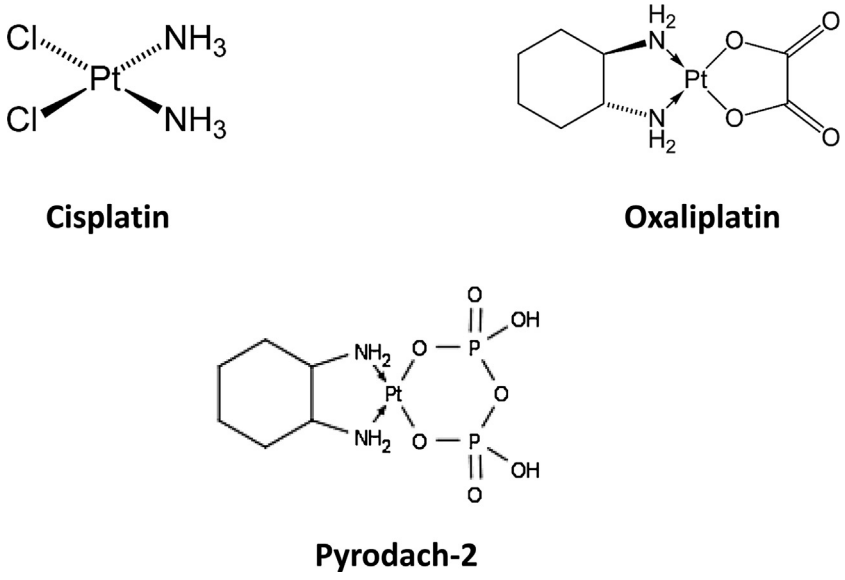

Fig. 1. Chemical structure of the different compounds used for the incubation experiments.