Abstract  Background Enhanced removal of cisplatin-DNA adducts has been reported as one of main causes of cell resistance to cisplatin. This particular resistance mechanism may be circumvented by platinum complexes that bind differently to DNA. One line of work is focussed on trans platinum complexes, some of which exhibit antitumour activity similar to or even higher than that of their cis counterparts. Methods We synthesised new trans platinum complexes, trans-[PtCl₂(cyclohexylamine)(dimethylamine)] and trans-[PtCl₂(OH)₂(cyclohexylamine)(dimethylamine)], previously evaluated as cytotoxic agents towards different cancer and normal cell lines. These trans platinum compounds were highly effective against a panel of tumoral cell lines either sensitive to or with acquired resistance to cisplatin. Results In the present work we examined the mechanisms induced by these compounds to cause tumour cells toxicity. We have found that these compounds induced a complete blockade at the S phase of the cell cycle inhibiting total mRNA transcription and precluding p53 activation. Conclusion In contrast to other DNA-damaging agents, these compounds do not induce senescence-associated permanent arrest. Furthermore, only a small percentage of these cells enter into apoptosis, with most of the population dying by a necrosis-like mechanism.

Key words Antitumour drugs • Platinum anticancer drugs • Transplatin • Cytotoxic activity • Cell cycle

Introduction

Cisplatin is a small metal-containing drug, known as a DNA-modifying agent [1], which represents one of the most potent drugs available in cancer chemotherapy for several solid tumours [2, 3]. Nevertheless, some disadvantages related to the natural or frequently acquired resistance of several tumours to cisplatin, as well as severe side effects [4, 5], limit its clinical use.

During the last decades, thousands of cisplatin analogues have been synthesised, varying the nature of the leaving groups and the carrier ligands. However, these analogues often display a similar spectrum of activity [6]. This has led to the development of new platinum compounds with structures different from that of cisplatin, with the idea that a different structure will translate into a different spectrum of activity [7, 8].

It has been accepted that leaving groups (generally chloride) and two amine ligands in platinum complexes must be in the cis orientation for antitumour activity [9]. However, certain trans platinum complexes have both in vitro and in vivo antitumour activity [10–18]. In addition, some of these complexes showed the ability to circumvent the resistance to cisplatin [12, 19]. Differences in the biochemical mechanism of action between cis and trans platinum complexes could be the cause of the cytotoxicity of trans platinum compounds against cisplatin-sensitive or -resistant cell lines [3, 20]. It seems that trans platinum compounds form interstrand DNA–DNA cross-links stabilising the DNA double helix,
whereas cisplatin has an intrastrand cross-link pattern [12, 20].

The spectrum of activity of the trans platinum compounds against tumour cell lines was distinctly different from cisplatin. Analysis of the effect of trans platinum complexes on the NIH cell line panel indicated little correlation with cisplatin or carboplatin [19, 20]. More recent analysis of these data showed that the trans platinum compounds were relatively more cytotoxic in general than the conventional cisplatin class toward breast and colon tumour lines, but much less cytotoxic toward central nervous system tumour lines [21, 22]. These results raise the possibility that the trans platinum compounds may have a fundamentally different mechanism of action than the currently approved platinum-based antitumour compounds.

We recently synthesised new trans-PtCl₂ complexes with an asymmetric set of aliphatic amines as non-leaving groups [12, 15] and we reported that trans-[PtCl₂(dimethylamine)(isopropylamine)] circumvents cisplatin resistance in CH1cisR ovarian tumour cell lines endowed with mechanism of resistance due to enhanced DNA repair/tolerance. Its corresponding Pt(IV) complex, trans-[PtCl₂(OH)₂(dimethylamine)(isopropylamine)], is also able to circumvent cisplatin resistance in the pair of cell lines CH1-CH1cisR [14, 23].

It is known that a simple modification in the structure of a certain compound could alter its DNA-binding patterns, thus affecting its anticancer activity [6, 24]. The cyclohexylamine ligand is a non-planar amine ligand, flexible and bulky enough to affect the kinetics and cytotoxicity, as do the aliphatic amines or the non-planar heterocyclic amine ligands (4-picoline, piperidine and piperazine) that were used by Gibson and coworkers [25].

On the other hand, the Pt(II) complex cis-[PtCl₂(NH₃)cyclohexylamine)] is the major metabolite of JM-216 [26], bis-acetato-amine(dichlorido cyclohexylamine)-platinum(IV), one of the few complexes to enter clinical trials [27].

We also synthesised and characterised several trans-Pt(II), trans-[PtCl₂(dimethylamine)(amine')] and trans-Pt(IV), trans-[PtCl₂(OH)₂(dimethylamine)(amine')], where amine' is cyclohexylamine or propylamine, with the aim of screening for potential antiproliferative and/or cytotoxic activities in different types of human cancer cells [28]. The results obtained from cytotoxicity assays show that these compounds were active in a panel of tumour cell lines at low micromolar range. Those compounds with cyclohexylamine as ligand showed higher antitumour activity being equally effective against a panel of tumoral cell lines either sensitive or with acquired resistance to cisplatin [10].

In the present work we have examined the mechanisms by which these compounds, trans-[PtCl₂(cyclohexylamine)(dimethylamine)] (compound 1) and trans-[PtCl₂(OH)₂(cyclohexylamine)(dimethylamine)] (compound 2), induced toxicity in tumour cells. However, only a small percentage of these cells enter into apoptosis, with most of the population dying through a necrosis-like mechanism. We have found that these compounds induced a complete blockade at the S phase of the cell cycle, inhibiting total mRNA transcription precluding p53 activation.

### Materials and methods

#### Cell line and culture conditions

The CNIO AZ cell line was generated from a fibrous tumour of pleura, as described in ref. [29]. It was grown in F10 culture medium (Sigma) supplemented with 10% FCS, fungizone and penicillin/streptomycin.

Cells were counted after Trypan Blue staining with a Neubauer camera and the appropriate volume containing the required number of cells (about 200,000) was calculated. Cells were seeded in 60-mm diameter Petri dishes containing final volumes of 3 ml and left to grow up to a 80% confluence before adding the drugs.

The compounds were weighed out and diluted with DMSO compound 1 and with absolute ethanol compound 2 to get them into solution to a concentration of 10 mM. A volume of 1 μl of compound solution was added per millilitre of media to obtain a final concentration of 10 μM for each drug.

#### Western blots

Media was removed from the dishes and cells were washed with 1 ml of PBS, and lysed on ice with 500 μl of 50 mM Tris–HCl buffer, pH 7.5, containing 150 mM NaCl, 1% Igpal, 1X protease inhibitor cocktail (Roche), 100 mM NaF, 2 mM Na₃VO₄ and 20 mM Na₂P₂O₇.

Cell lysates were centrifuged at 13,000 rpm for 10 min at 4 °C and the protein concentrations of the supernatants were determined by Bradford's method. Aliquots of supernatants containing equal 25 μg amounts of proteins were boiled for 5 min in Laemmli sample loading buffer, resolved by electrophoresis at 150 V in a SDS-polyacrylamide gel, and transferred for 2 h to a transfer membrane (Immobilon-P; Millipore).

The blots were blocked with 1% BSA (Sigma) in 20 mM Tris–HCl buffer, pH 7.5, with 0.9% NaCl (TBS), containing 0.1% of Tween-20 (TBST) for 2 h at room temperature. Immunodetection was conducted at 4 °C overnight in TBST containing 1% BSA. The antibodies used were: rabbit anti-p53 (FL 393) primary polyclonal Ab (1:1000 dilution; Santa Cruz Biotechnology), rabbit anti-Bax primary polyclonal Ab (1:100 dilution; Santa Cruz Biotechnology), rabbit anti-AKT primary polyclonal Ab (1:1000 dilution; Santa Cruz Biotechnology)