Pt(II) *vs* Pd(II) Polyamine Complexes as New Anticancer Drugs: AStructure-Activity Study

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Received November 30, 2005: Revised January 31, 2006: Accepted February 15, 2006

Abstract: Two homologous trinuclear polyamine chelates with either Pt(II) (Ia) or Pd(II) (Ib) were screened for their anticancer properties. Their growth-inhibition activity towards a human tongue epithelioma (HSC-3) was assessed *in vitro*, and the effect of the cation alteration was determined (IC_{50}=32 μM for Ib *vs* 66 μM for Ia).

Keywords: Pt(II), Pd(II), spermidine, anticancer drugs, squamous tongue epithelioma.

INTRODUCTION

Platinum-based antitumour drugs have been the target of intense research since Rosenberg´s observation in the late sixties, of an unexpected inhibition of cell division by platinum complexes [1]. The well established anticancer drug thus discovered, known as cisplatin (*cis*-diaminedichloro-platinum(II), *cis*-$(NH_3)_2PtCl_2$, *c*DDP), is the parent of the platinum coordination compounds, which are presently used in chemotherapy. A large body of evidence indicates that the antineoplastic properties of this kind of compounds are based upon a selective interaction with DNA, after hydrolysis of the chloride atoms inside the cell. Although widely administered in the clinical practice, *c*DDP presents several drawbacks, such as severe toxicity and development of drug resistance, which cannot be neglected. Therefore, the search for structurally novel Pt(II) and Pd(II) compounds displaying antineoplastic activity is crucial, aiming at the design of more efficient and less toxic agents. Metal complexes comprising cisplatin-like moieties ($[PtCl(NH_3)_2]$ or $[Pt(NH_3)Cl_2]$) linked by variable length alkanediamine chains have generated great interest in the last few years, as third-generation *c*DDP alternatives in cancer chemotherapy [2-9]. In fact, the polyamine bridging linkers may allow a more efficient interaction of the chelate with DNA, not

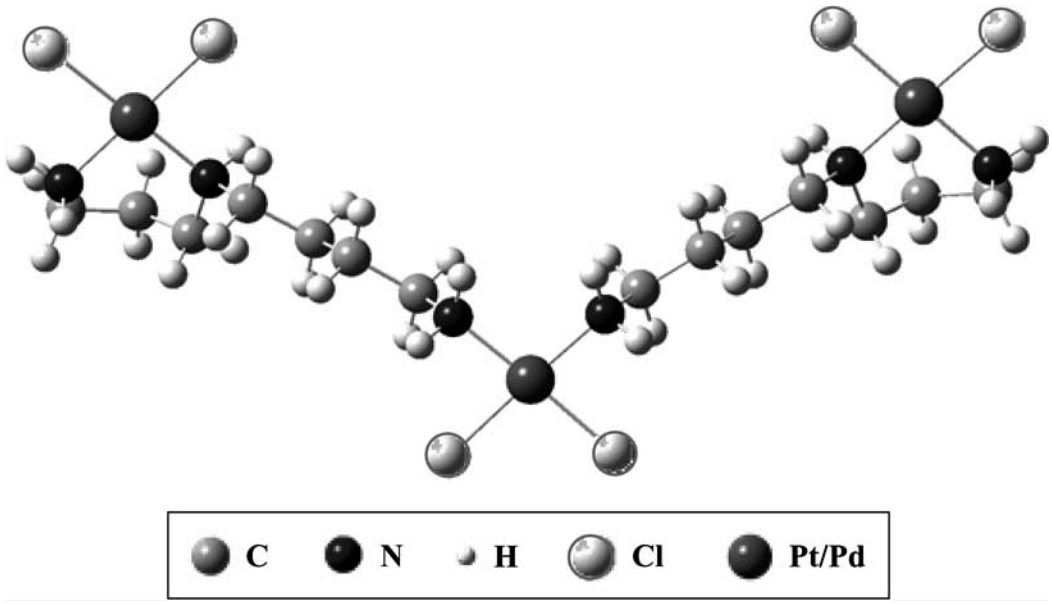

Fig. (1). Schematic representation of the structures of complexes Ia and Ib.

available to classical alkylating agents (*e.g.* formation of long-distance and/or interstrand adducts).

The present work reports the study of two homologous Pt(II) or Pd(II) trinuclear chelates with the biogenic amine spermidine ($H_2N(CH_2)_3NH(CH_2)_4NH_2$). Their antitumour effect towards a human cancer cell line, squamous tongue

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1570-1808/06 $50.00+.00

epithelioma, HSC-3, was evaluated and compared, using the sulphorhodamine-B-based assay to assess inhibition of cell proliferation. The results thus obtained will hopefully yield reliable information for further insight into the molecular basis of cytotoxicity for this kind of complexes.

RESULTS

The spermidine trinuclear (2,2,2/c,c,c) complexes, $(MCl_2)_3(spd)_2$, spd= spermidine, M=Pt(II) (Ia) and Pd(II) (Ib) (Fig. (**1**)), are identified by vibrational spectroscopy (FTIR, Raman and Inelastic Neutron Scattering) and elemental analysis (including Cl).

Figure (**2**) comprises the dose- and time-response plots, HSC-3 cell density as a function of the incubation time with different concentrations of the drug, for the two complexes studied. The results obtained clearly evidence that the Pd(II) chelate displays a higher cytotoxicity towards HSC-3 than its Pt(II) analogue: although this difference is rather small for a 10 μM concentration, it is already statistically significant for a 20 μM dose. Inhibition of cell proliferation is very evident for 30 μM and 50 μM concentrations, a *ca.* threefold effect being measured for 50 μM-Pd(II) *vs* 50 μM-Pt(II). Moreover, for a 24 h incubation period with complex Ib, there is already a measurable cell density decrease, as opposed to Ia (Fig. (**2**)).

The values of 50% inhibitory concentration after a 48 h incubation period (concentration of drug yielding a 50% decrease in either cell density or cell viability, IC_{50}) were determined for these two compounds. Complex Ib showed to have a much higher antiproliferative activity against the HSC-3 cell line as compared to Ia (IC_{50}=32 μM (0.026 mg/mL) for Ib *vs* >50 μM (0.071 mg/mL) for Ia) and may be regarded as an efficient anticancer agent according to the threshold set by Farrell and co-workers [10]. Cisplatin and carboplatin, in turn, yield IC_{50} values of 7 μM and 3 μM, respectively.

The Ia and Ib chelates differ solely in the nature of the metal centre; although Ia contains Pt(II), Ib is a Pd(II) compound (Fig. (**1**)). Consequently, the distinct behaviour observed for these two complexes for their anticancer activity can only reflect this difference, a change from a second to a third Group cation. Interestingly enough, this unique variation significantly affected the cytotoxic properties of these polyamine chelates. This can reflect a distinct interaction of the Pt(II) and Pd(II) spermidine complexes with the DNA double helix, which seems to be enhanced for the latter. If, as it is commonly accepted, this type of alkylating agents bind to the nitrogen atoms of the DNA purine bases (*e.g.* N_7 of adenine and/or guanine), this binding is favoured for Pd(II) over Pt(II) for the spermidine complexes investigated in this work. In fact, the Pd(II) compounds are known to be more water soluble and kinetically more labile than their Pt(II) counterparts, which may lead to a faster hydrolysis of the chloride moiety inside the cell and consequently, to a more pronounced antitumour activity.

The preliminary results now reported constitute an unequivocal proof that the cytotoxic properties of polyamine metal complexes are closely determined by their structural characteristics (such as the nature of the metal ion), and may thus be tailored in order to suit specific applications or biological targets (*e.g.* type of cancer). The knowledge that a simple change in the coordinating metal can lead to such a relevant increase in antineoplastic activity as the one presently observed is of utmost importance for understanding

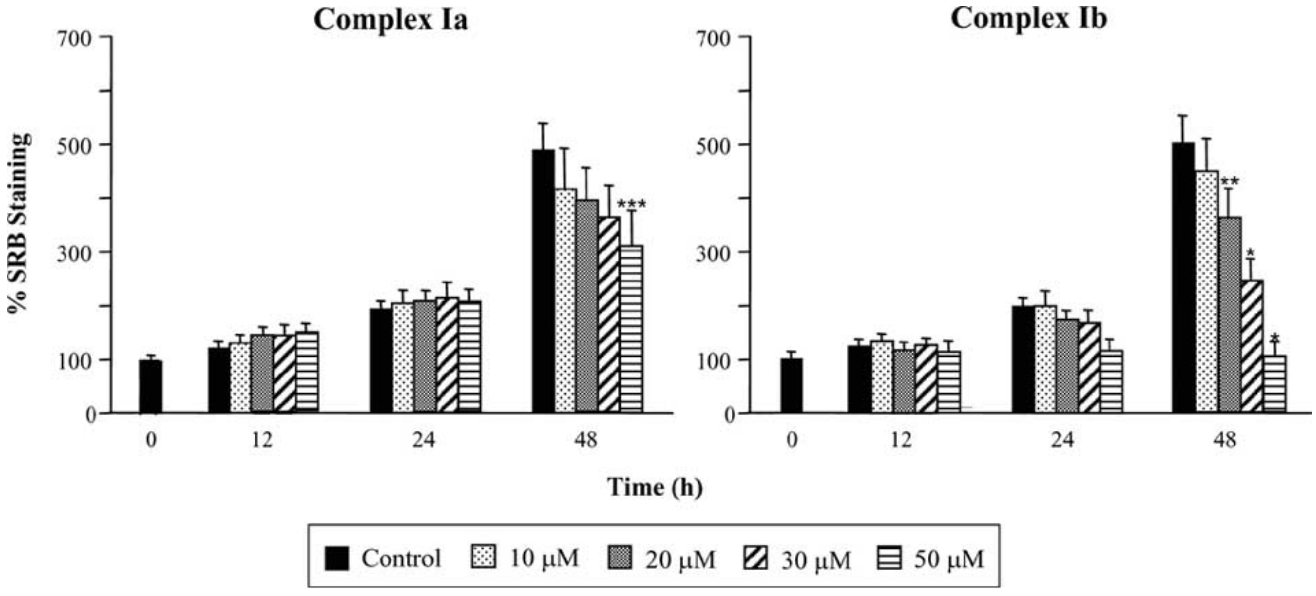

Fig. (2). Time and dose-dependence plots for the antiproliferative effect of compounds Ia and Ib against human squamous tongue epithelioma (HSC-3). Cells (5.0 x 10^4 cells/ml) were incubated with the drugs for time periods of 12 to 48 h. The data is expressed as a percentage of the control for time-zero (100%) and represent the average ± standard error of the mean from six independent experiments. (Statistical analysis: one way ANOVA with Newman-Keuls multiple comparison test. * P<0.001, ** P<0.01, *** P<0.05).

the structure activity relationships (SAR´s) ruling these kinds of systems.

The information gathered by this type of studies will hopefully lead to the design of new, third-generation anticancer drugs. These should comply to some relevant criteria, namely, good water solubility, stability under physiological conditions, specificity of the mechanism of action and an optimised hydrolysis process within the cell. Tissue specificity and low toxicity towards non-neoplastic cells are also goals expected to be achieved in the near future.

EXPERIMENTAL

Synthesis

The complexes studied in this work, $(MCl_2)_3(spd)_2$, (M=Pt(II) or Pd(II), spd=spermidine), were synthetised according to [11], with slight modifications.

Cell Lines

The epithelial-like adherent human cell line from squamous tongue epithelioma (HSC-3) was purchased from the American Type Culture Collection (ATCC, USA).

Cell Growth-Inhibition Evaluation

Cytotoxicity and cell density evaluation following drug exposure for drug concentrations ranging from 10 to 50 μM were assessed, using the sulphorhodamine-B method as described in [12]. The clinically used drugs cisplatin and carboplatin were always considered in the biological experiments for comparison purposes. For this particular cancer cell line (HSC-3), carboplatin is known to be a more suitable chemotherapeutic agent than cisplatin.

ACKNOWLEDGEMENTS

Financial support from FCT (Portugal) – Project POCTI/47256/QUI/2002 (co-financed by the European Community fund FEDER). SF also acknowledges PhD scholarship SFRH/BD/17493/2004.

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