Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 517 (2005) 28 – 34

www.elsevier.com/locate/ejphar

[Pt(HPxSC)Cl_3], a novel platinum(IV) compound with anticancer properties

Miloš Marković[a], Nikola Knežević[b,1], Miljana Momčilović[c], Sanja Grgurić-Šipka[b], Ljubica Harhaji[c], Vladimir Trajković[a], Marija Mostarica Stojković[a], Tibor Sabo[b], Djordje Miljković[c,*]

[a]*Institute of Microbiology and Immunology, School of Medicine, University of Belgrade, Dr. Subotica 1, 11000 Belgrade, Serbia and Montenegro*
[b]*Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia and Montenegro*
[c]*Institute for Biological Research "Siniša Stanković", Bulevar Despota Stefana 142, 11000 Belgrade, Serbia and Montenegro*

Received 14 October 2004; received in revised form 11 May 2005; accepted 24 May 2005

Abstract

There has been a continuing effort for the discovery of novel platinum(IV)-based antitumor compounds with better therapeutic performances than cisplatin. In the present work, the anticancer action of recently synthesized Pt(IV)-based complex [Pt(HPxSC)Cl_3] was investigated using rat and human astrocytoma cell lines C6 and U251. [Pt(HPxSC)Cl_3] markedly reduced the number of cultured astrocytoma cells (IC_{50}, 80 μM), as determined by crystal violet assay. The Pt(IV) complex induced apoptotic death of tumor cells, as flow cytometry analysis of the propidium iodide-stained cellular DNA revealed approx. 30% of hypodiploid cells in [Pt(HPxSC)Cl_3]-treated astrocytoma cell cultures. On the other hand, [Pt(HPxSC)Cl_3] at 200 μM did not affect the viability of rat primary astrocytes, unlike the established anticancer drug cisplatin, which displayed high toxicity toward both astrocytoma cells (IC_{50}, 15 μM) and primary astrocytes (IC_{50}, 20 μM). Moreover, [Pt(HPxSC)Cl_3] at 100 μM did not interfere with the ability of rat peritoneal macrophages to produce important antitumor molecules nitric oxide and tumor necrosis factor-α. Finally, we assessed the ability of [Pt(HPxSC)Cl_3] to restrain growth of some bacterial and yeast strains, but it showed rather limited antimicrobial activity.

Keywords: Astrocytoma; Platinum; Cancer; Apoptosis

1. Introduction

Cisplatin is a potent anticancer drug, effective against various tumors, including those of testes, ovary, and head and neck (Zhang and Lippard, 2003; Siddik, 2003). Still, its application has two main disadvantages: intrinsic and acquired drug-resistance, as well as cumulative and irreversible toxicities, particularly nephrotoxicity, ototoxicity and peripheral neuropathy (Zhang and Lippard, 2003; Siddik, 2003). Therefore, there is an enduring effort to synthesize new Pt-based drugs that would be as efficient as cisplatin, but without its side effects. The important candidates are Pt(IV)-based agents as their greater inertness comparing to corresponding Pt(II) complexes, including cisplatin, may allow the oral administration of the drugs, reduce toxicity associated with platinum-based chemotherapy, and decrease the amount of the complex lost or deactivated through reactions on the way to the target site (Hall and Hambley, 2002; Galanski et al., 2003). Development of orally available platinum drugs has focused on octahedral coordinated platinum(IV) complexes, because they are more inert to hydrolysis and other ligand exchange reactions than platinum(II) compounds and thus are less likely to be degraded in the gastrointestinal tract (Zhang and Lippard, 2003). Although some of these compounds, including

* Corresponding author. Tel./fax: +38 1 11 2657258.
E-mail address: georgije_zw@yahoo.com (D. Miljković).
[1] Present address: Department of Chemistry, Iowa State University, Ames, Iowa 50011-3111.

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.05.038

iproplatin, tetraplatin, and satraplatin have been tested in clinical trials, there are still no Pt(IV)-based therapeutics in routine clinical use (Hall and Hambley, 2002; Galanski et al., 2003).

Astrocytomas are the most common tumors of the central nervous system (CNS), usually with a poor prognosis, and extremely difficult to treat (Kleihues et al., 1995). Cisplatin, in combination with other anticancer drugs, radiotherapy and surgery, has been used for the treatment of various astrocytomas with limited efficacy and considerable toxicity (Frenay et al., 2000; Madajewicz et al., 2000; Choi et al., 2002; Mathieu et al., 2004; Brandes et al., 2004; Silvani et al., 2004; Rao et al., 2005). In addition, high level of resistance of tumor cells to the cytotoxic action of cisplatin has been observed in vitro both in primary brain tumors collected from the patients (Haroun et al., 2002) and cell lines (Jendrossek et al., 2001). Thus, exploiting new possibilities for the treatment of astrocytomas is of reasonable significance. In this paper, we report that newly synthesized Pt(IV) complex [Pt(HPxSC)Cl_3] potently destroys rat and human astrocytoma cells, but exerts only limited antimicrobial effect in vitro.

2. Materials and methods

2.1. Reagents

All the chemicals used in the experiments were from Sigma (St. Louis, USA), unless specifically stated. The complex [Pt(HPxSC)Cl_3], has been prepared as described earlier (Knežević et al., 2003). On the basis of analytical and spectroscopic data it has been proposed that the octahedral platinum(IV) complex contains monoanionic pyridoxal semicarbazone moiety coordinated in the tridentate mode and the three chlorine atoms. Dimethyl sulfoxide (DMSO) was used for dissolving the complex, and in each experiment controls with appropriate concentrations of vehicle were performed. As long-term cell cultures were used in our study, we have investigated the stability of complex [Pt(HPxSC)Cl_3] in DMSO and cell culture medium (with or without foetal calf serum) by electronic absorption spectroscopy. Electronic absorption spectra for 100 μM solutions of the complex were recorded on a GBC UV–visible Cintra 40 spectrometer. No difference was observed in the spectral patterns at the beginning and 48 h after incubation (data not shown), indicating that the complex was stable in the above mentioned conditions.

2.2. Cell cultures

Rat astrocytoma cell line C6 and human astrocytoma cell line U251 were a kind gift of Dr. Pedro Tranque (Universidad de Castilla-La Mancha, Albacete, Spain). Rat primary astrocytes were isolated from newborn Albino Oxford rats as previously described (McCarthy and de Vellis, 1980). Peritoneal macrophages were isolated from Albino Oxford rats by peritoneal lavage with cold phosphate buffered saline (PBS), followed by adherence to plastic, as previously described (Jankovic et al., 2000). The cells were cultivated in HEPES-buffered RPMI 1640 medium supplemented with 5% foetal calf serum, L-glutamine and antibiotics at 37 °C in a humidified atmosphere with 5% CO_2. For the experiments, cells were grown in flat-bottom 96-well tissue culture plates (Sarstedt, Numbrecht, Germany), except for flow cytometry analysis, where 30 mm tissue culture Petri dishes (Sarstedt) were used for cell cultivation.

2.3. Crystal violet test

Adherent cell staining with crystal violet was used for determining cell viability (Flick and Gifford, 1984). After incubation, cell cultures were washed with PBS to remove non-adherent cells, and adherent cells were then fixed with methanol and stained with 1% crystal violet solution at room temperature for 10 min. Plates were thoroughly washed with water, and crystal violet dye was dissolved in 33% acetic acid. The absorbance of dissolved dye, corresponding to the number of adherent (viable) cells, was measured in an automated microplate reader at 570 nm. The results are presented as percent of control values obtained in untreated cell cultures. Tumor cell viability in the cocultivation experiments was determined by subtracting the absorbance values of macrophage cultures from that of astrocytoma/macrophage co-cultures.

2.4. Cell cycle analysis

At the end of incubation, cells (0.5×10^6) were fixed in 70% ethanol at 4 °C for 30 min. Cells were washed twice with PBS, resuspended in PBS containing 1 mg/ml RNase and 40 μg/ml propidium iodide, and kept at 37 °C in the dark for 30 min. Red fluorescence was analyzed at FACSCalibur flow cytometer (BD, Heidelberg, Germany), using a peak fluorescence gate to discriminate aggregates. Cell distribution among cell cycle phases was determined by Cell Quest Pro software (BD, Heidelberg, Germany).

2.5. Determination of tumor necrosis factor-α (TNF-α) and nitric oxide (NO)

Concentration of bioactive TNF-α in culture supernatants was determined using cytolytic bioassay with actinomycin D-treated fibrosarcoma cell line L929, as previously described (Stosic-Grujicic et al., 2001). Nitrite accumulation, an indicator of NO production, was measured in cell culture supernatants using the Griess reagent (Green et al., 1982). Briefly, 50 μl samples of culture supernatants were mixed with an equal volume of Griess reagent (a mixture at 1:1 of 0.1% naphthylenediamine dihydrochloride and 1% sulfanilamide in 5% H_3PO_4) and incubated at room temperature for 10 min. The absorbance at 570 nm was measured in an automated microplate reader. The nitrite concentration was calculated from a $NaNO_2$ standard curve.

2.6. Antimicrobial activity assay

In total, 17 strains of 6 different bacterial or yeast species (4 strains of *Staphylococcus aureus*, 1 of *Streptococcus pyogenes* (group A), 3 of *Enterococcus* sp., 4 of *Escherichia coli*, 2 of *Pseudomonas aeruginosa* and 3 strains of *Candida albicans*) were tested for susceptibility to [Pt(HPxSC)Cl_3]. All strains were isolated from the outpatients in our department (Institute of Microbiology and Immunology, School of Medicine, Belgrade)

and identified using standard microbiological procedures. After being obtained in a pure culture, strains were cultivated for additional 24 h in the appropriate medium (dextrose broth, Biolife, Italy) at 36 °C, cultures adjusted to the cell concentration of 2×10^6 colony forming units (CFU)/ml, 0.05 ml of which were used in subsequent experiments. The assay was performed in triplicates in the 96-well plates (Sarstedt). [Pt(HPxSC)Cl_3] was dissolved in DMSO (1:100 dilution) and added to wells. Each well contained 10^5 CFU of the microorganism and various concentration of tested complex (six 2-fold dilutions starting from 1 mM) in 100 μl. Appropriate controls with medium alone, DMSO in medium and bacteria and yeast without complex were also performed. Bacteria and yeasts were subsequently cultured for 24 h at 36 °C. The effect of [Pt(HPxSC)Cl_3] on microbial growth was determined by the measurement of the optical density in a microplate reader at 492 nm. Results are expressed as percent of inhibition of microbial growth compared to controls incubated without the complex.

2.7. Statistical analysis

The significance of the differences between various treatments was determined by analysis of variance (ANOVA), followed by Student–Newman–Keuls test. The value of $P<0.05$ was considered statistically significant.

3. Results

3.1. The reduction of C6 and U251 astrocytoma cell viability by [Pt(HPxSC)Cl_3]

In order to investigate the antitumor potential of [Pt(HPxSC)Cl_3] in vitro, the various concentrations of the complex were added to high-density cultures of rat astrocytoma C6 and human astrocytoma U251 cells, and cell number was determined after 48 h by crystal violet test. While [Pt(HPxSC)Cl_3] potently reduced the viability of both astrocytoma cell lines in a dose-dependent way (Fig. 1A, B), it was less efficient than the recognized anticancer drug cisplatin (Fig. 1A, B). Namely, the IC_{50} values calculated for complex [Pt(HPxSC)Cl_3] were 82.8 and 81.8 μM, while IC_{50} values for cisplatin were 15.4 and 15.3 μM, for C6 and U251 cells, respectively. However, [Pt(HPxSC)Cl_3] did not significantly affect the viability of rat primary astrocytes (Fig. 1C), whereas cisplatin killed primary astrocytes with the efficiency ($IC_{50}=19.1$ μM) fairly comparable to that displayed against astrocytoma cells. Cell cycle analysis after [Pt(HPxSC)Cl_3] treatment of C6 cells revealed an increase in the number of hypodiploid cells with fragmented DNA, suggesting the ability of the Pt(IV) complex to cause tumor cell apoptosis (Fig. 2). Similar results were obtained with U251 cultures (data not shown). Importantly, DMSO ($C_{max}=0.01\%$ v/v) used for dissolving the complex, did not affect the viability of either cell line. Thus, [Pt(HPxSC)Cl_3] was able to induce apoptotic death of astrocytoma cell without affecting the viability of primary astrocytes.

3.2. The influence of [Pt(HPxSC)Cl_3] on macrophages

As macrophages play an important role in antitumor immunity, we analyzed the influence of [Pt(HPxSC)Cl_3] on these cells.

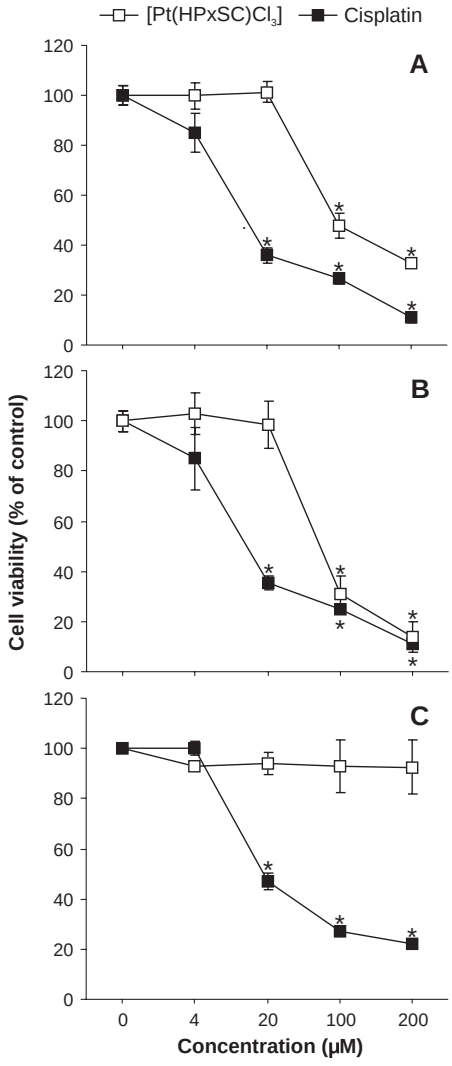

Fig. 1. The influence of [Pt(HPxSC)Cl_3] on the viability of astrocytoma cell lines and primary astrocytes. C6 cells (A), U251 cells (B), and astrocytes (C) were cultivated (5×10^4 cell/well) in the presence of various concentrations of [Pt(HPxSC)Cl_3] or cisplatin for 48 h, and their viability was determined by crystal violet assay. Data from the representative of three separate experiments with similar results are presented as mean ± S.D. of triplicate cultures (*$P<0.05$ refers to untreated cultures).

Treatment of macrophages with [Pt(HPxSC)Cl_3] did not significantly change their viability after 48 h of cultivation, as determined by crystal violet staining (Fig. 3A). Further, we tested the influence of [Pt(HPxSC)Cl_3] on antitumor properties of interferon γ (IFN-γ)-treated macrophages co-cultivated with astrocytoma cells. Both IFN-γ-stimulated macrophages and [Pt(HPxSC)Cl_3] markedly reduced viability of U251 and C6 cells, showing an additive effect when combined (Fig. 3B). We also assessed whether [Pt(HPxSC)Cl_3] could modulate production of well-known tumoricidal molecules NO and TNF-α in unstimulated and IFN-γ-stimulated macrophages. After 48 h of cultivation, IFN-γ markedly induced both NO and TNF-α release from macrophages, while [Pt(HPxSC)Cl_3] did not affect macrophage production of either NO or TNF-α, irrespective of the presence of IFN-γ (Fig. 3C,D). Thus, [Pt(HPxSC)Cl_3] apparently did not interfere with macrophage antitumor action in vitro.

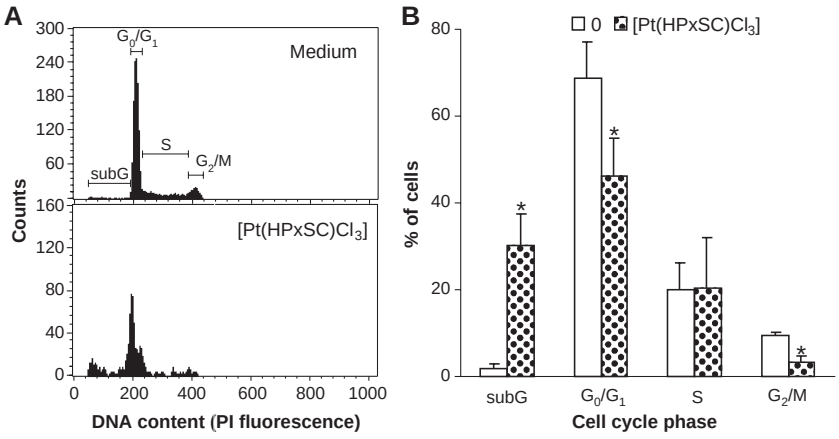

Fig. 2. Cell cycle analysis in $[Pt(HPxSC)Cl_3]$-treated C6 cells. C6 cells (5×10^5 per plate) were cultivated for 48 h in the absence or presence of 100 μM of $[Pt(HPxSC)Cl_3]$, and DNA content/cell cycle analysis was performed by flow cytometry after propidium iodide staining. The histograms from the representative experiment are presented in (A), while cell cycle distribution values (B) are means ± S.D. from three independent experiments (*$P < 0.05$ refers to untreated cultures).

3.3. Antimicrobial properties of $[Pt(HPxSC)Cl_3]$

In order to further analyze the biological activity of $[Pt(HPxSC)Cl_3]$ we have tested its influence on growth of various bacterial and yeast species (17 strains in total, see Materials and methods). $[Pt(HPxSC)Cl_3]$ did not show general antibacterial or antifungal activity in our system. However, a minor inhibitory effect on growth of some tested strains was observed. Namely, five

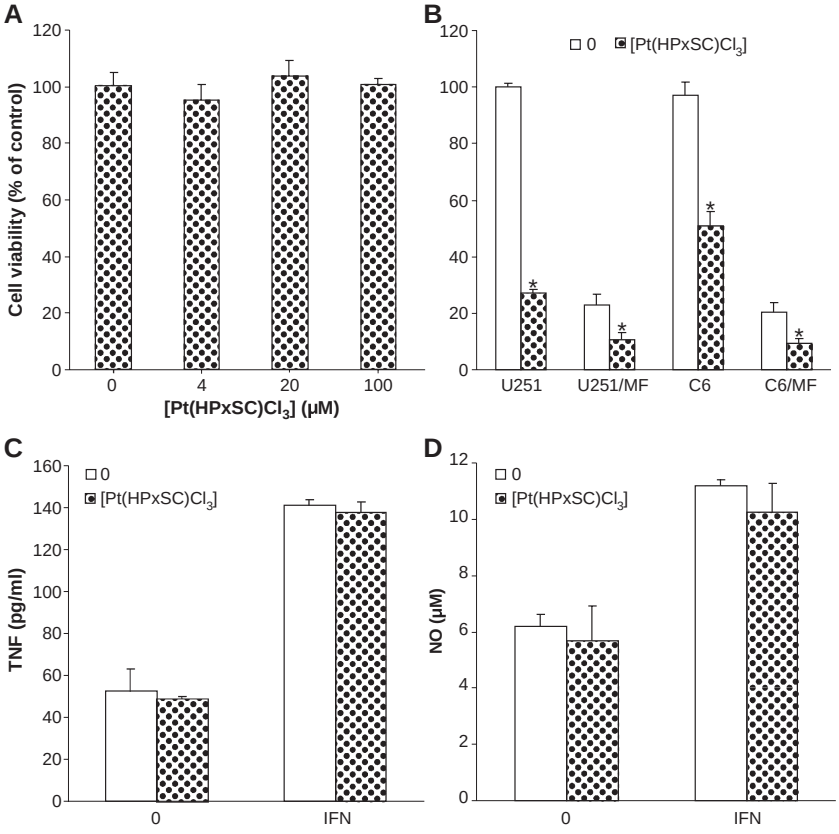

Fig. 3. The influence of $[Pt(HPxSC)Cl_3]$ on macrophage antitumor response in vitro. Rat peritoneal macrophages (5×10^4/well) were cultivated for 48 h with various concentrations of $[Pt(HPxSC)Cl_3]$ (A), or 100 μM $[Pt(HPxSC)Cl_3]$ with or without 10 ng/ml IFN-γ (C, D) and cell viability (A), TNF-α production (C), or nitric oxide production (D) were subsequently determined. (B) IFN-γ (10 ng/ml)-stimulated rat peritoneal macrophages (5×10^4/well) and U251 cells (1×10^4/well) were cultivated separately or together, in the absence or presence of 100 μM $[Pt(HPxSC)Cl_3]$, and cell viability was measured after 48 h. (A–D) Data from the representative of three independent experiments with similar results are presented as mean ± S.D. of triplicate cultures (*$P < 0.05$ refers to untreated cultures).

Table 1
The effect of [Pt(HPxSC)Cl_3] complex on the growth of various microorganisms

Microorganism strain	Concentration of [Pt(HPxSC)Cl_3] (μM)					
	31.25	62.5	125	250	500	1000
Staphylococcus aureus						
1	2±1	7±2[a]	5±4	10±1[a]	10±1[a]	18±1[a]
Enterococcus sp						
1	2±1	4±4	6±1[a]	12±1[a]	19±7[a]	33±14[a]
2	0	0	0	50±14[a]	59±14[a]	60±12[a]
Escherichia coli						
1	0	0	15±4[a]	15±3[a]	37±2[a]	62±13[a]
2	0	0	12±5[a]	14±2[a]	17±5[a]	17±10[a]
Candida albicans						
1	9±4[a]	12±2[a]	44±6[a]	36±16[a]	51±25[a]	50±11[a]

All strains were incubated in the absence or presence of various concentrations of [Pt(HPxSC)Cl_3], as described in Materials and methods. The effect of the complex on microbial growth was determined by measuring the optical density and results are expressed as percent inhibition±S.D. of triplicates ([a]$P<0.05$).

bacterial strains (one of *S. aureus*, two of *Enterococcus* sp. and two of *E. coli*) and one yeast strain (*C. albicans*) were partly inhibited in a dose-dependent way by the complex (Table 1). On the other hand, DMSO, which was used for dissolving the complex, had no observable growth inhibiting effect in concentrations used in our experiments. Still, the growth of the remaining 11 strains tested was not impaired, at least not in a dose range of complex between 31.25 and 1000 μM (not shown).

4. Discussion

As astrocytomas are the most common tumors of CNS, with no efficient therapy available (Kleihues et al., 1995), an effort for discovering new potential anti-astrocytoma drugs has been continuous. We have recently reported synthesis and chemical characterization of novel Pt(IV)-based octahedral complex (Knežević et al., 2003). In this paper, we present [Pt(HPxSC)Cl_3] as a powerful antitumor molecule that exerts pro-apoptotic activity against astrocytoma cells in vitro, without affecting the viability of primary astrocytes and macrophages, or macrophage anti-astrocytoma action. Although antibacterial and/or antifungal effects of cisplatin and other Pt(II) and Pt(IV) complexes were previously reported (Cardenas et al., 1999; Watabe et al., 2001; Kushev et al., 2002; Gumus et al., 2003), [Pt(HPxSC)Cl_3] did not show significant antimicrobial effect against the analyzed strains. Such results do not justify further experiments of antimicrobial characteristics of our complex and exclude it as a candidate for an antimicrobial agent.

It should be noted that [Pt(HPxSC)Cl_3] was considerably less effective against astrocytoma cells in comparison with prototypical Pt-based drug cisplatin. However, while cisplatin was highly toxic to rat primary astrocytes cells in our experiments, as well as in previous studies (Hardej and Trombetta, 2002; Wick et al., 2004), we did not observe any significant alteration of primary astrocyte viability upon [Pt(HPxSC)Cl_3] treatment. One of the reasons for this tumor cell-selective toxicity of [Pt(HPxSC)Cl_3] might be at least partly due to the fact that, unlike astrocytoma cells, confluent primary astrocytes do not extensively divide in culture. Namely, the existence of positive correlation was described between cell proliferation rate and susceptibility to apoptosis induction in certain settings (Wang et al., 1999; Laitinen et al., 2000; Los et al., 2001). Whereas putative selective killing of tumor cells makes [Pt(HPxSC)Cl_3] a plausible candidate for a relatively "safe" anticancer drug, it is on future studies to examine whether beneficial/side effect ratio of this novel Pt(IV) complex would compare favorably to that of presumably more toxic, but also more efficient cisplatin.

Importantly, our experiments demonstrate that [Pt(HPxSC)Cl_3] cooperates with macrophages in their antitumor actions in vitro. Although this cooperation is rather additive than synergistic, it could still be significant as it would presumably allow combination with some immune-based anticancer strategies. Moreover, we have shown that [Pt(HPxSC)Cl_3] does not reduce macrophage production of important antitumor molecules TNF-α and nitric oxide. While TNF-α induces rapid cell death in susceptible tumor cells and blocks tumor angiogenesis (Hagen and Eggermont, 2004), NO produced by macrophages is efficient against tumor cells in vitro (Miljkovic et al., 2004) and contributes to the elimination of tumors in vivo (MacMicking et al., 1997). Thus, it seems reasonable to assume that inability of [Pt(HPxSC)Cl_3] to reduce TNF-α and NO production in macrophages could be a valuable therapeutic property.

Finally, an important concern about the potential use of [Pt(HPxSC)Cl_3] for astrocytoma treatment arises from the fact that the complex might not easily pass the blood–brain barrier. However, the role of the blood–brain barrier in the chemotherapy of brain tumors is controversial (Stewart, 1994), and intravenous administration of cisplatin was fairly efficient in some clinical trials for astrocytoma treatment (Choi et al., 2002; Brandes et al., 2004; Silvani et al., 2004). Moreover, glioma treatment with intravenous cisplatin resulted in 10-fold higher concentration of the drug in the tumor as compared with that in normal brain tissue (Van den Bent et al., 1998), supporting the hypothesis on the relative absence of the blood–brain barrier within brain tumors. In addition to clinically-proven intra-arterial administration of platinum-based chemotherapeutics (Madajewicz et al., 2000), there is an expanding field of innovative strategies for improved drug-delivery into the brain, including nanoparticles, temporary disruption of the blood–brain barrier, or various systems for intracranial administration (Kreuter, 2001; Kemper et al., 2004). It should be also noted that the influence of [Pt(HPxSC)Cl_3] was not restricted to astrocytoma cell lines, as this Pt(IV) complex potently inhibited the viability of mouse L929 fibrosarcoma cells (unpublished observa-

tion), indicating that it could be potentially interesting for its antitumor properties in general.

To conclude, [Pt(HPxSC)Cl_3] seems to be very efficient against astrocytomas in vitro, possibly acting as an pro-apoptotic agent. Further investigation about its mechanism of action, as well as exploration of its capability to restrain tumor formation in vivo would be our future goals.

Acknowledgements

This work was supported by the funds of The Ministry of Science and Environmental Protection of the Republic of Serbia (Grants No. 1253, 1664, and 2020). The authors would like to thank Dr. Stanislava Stošić-Grujičić from the Institute for Biological Research, Belgrade for thorough reading of the manuscript and helpful suggestions.

References

Brandes, A.A., Basso, U., Reni, M., Vastola, F., Tosoni, A., Cavallo, G., Scopece, L., Ferreri, A.J., Panucci, M.G., Monfardini, S., Ermani, M., Gruppo Italiano Cooperativo di Neuro-Oncologia, 2004. First-line chemotherapy with cisplatin plus fractionated temozolomide in recurrent glioblastoma multiforme: a phase II study of the Gruppo Italiano Cooperativo di Neuro-Oncologia. J. Clin. Oncol. 22, 1598–1604.

Cardenas, M.E., Cruz, M.C., Del Poeta, M., Chung, N., Perfect, J.R., Heitman, J., 1999. Antifungal activities of antineoplastic agents: Saccharomyces cerevisiae as a model system to study drug action. Clin. Microbiol. Rev. 12, 583–611.

Choi, I.S., Lee, S.H., Kim, T.Y., Bang, J.S., Paek, S.H., Kim, S., Kim, I.H., Heo, D.S., Bang, Y.J., Kim, D.G., Jung, H.W., Kim, N.K., 2002. Phase II study of chemotherapy with ACNU plus cisplatin followed by cranial irradiation in patients with newly diagnosed glioblastoma multiforme. J. Neurooncol. 60, 171–176.

Flick, D.A., Gifford, G.E., 1984. Comparison of in vitro cell cytotoxic assays for tumor necrosis factor. J. Immunol. Methods 68, 167–175.

Frenay, M., Lebrun, C., Lonjon, M., Bondiau, P.Y., Chatel, M., 2000. Up-front chemotherapy with fotemustine (F)/cisplatin (CDDP)/etoposide (VP16) regimen in the treatment of 33 non-removable glioblastomas. Eur. J. Cancer 36, 1026–1031.

Galanski, M., Arion, V.B., Jakupec, M.A., Keppler, B.K., 2003. Recent developments in the field of tumor-inhibiting metal complexes. Curr. Pharm. Des. 9, 2078–2089.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R., 1982. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal. Biochem. 126, 131–138.

Gumus, F., Pamuk, I., Ozden, T., Yildiz, S., Diril, N., Oksuzoglu, E., Gur, S., Ozkul, A., 2003. Synthesis, characterization and in vitro cytotoxic, mutagenic and antimicrobial activity of platinum(II) complexes with substituted benzimidazole ligands. J. Inorg. Biochem. 94, 255–262.

Hagen, T.L., Eggermont, A.M., 2004. Tumor vascular therapy with TNF: critical review on animal models. Methods Mol. Med. 98, 227–246.

Hall, M.D., Hambley, T.W., 2002. Platinum(IV) antitumor compounds: their bioorganic chemistry. Coord. Chem. Rev. 232, 49–67.

Hardej, D., Trombetta, L.D., 2002. The effects of ebselen on cisplatin and diethyldithiocarbamate (DDC) cytotoxicity in rat hippocampal astrocytes. Toxicol. Lett. 131, 215–226.

Haroun, R.I., Clatterbuck, R.E., Gibbons, M.C., Burger, P.C., Parker, R., Fruehauf, J.P., Brem, H., 2002. Extreme drug resistance in primary brain tumors: in vitro analysis of 64 resection specimens. J. Neurooncol. 58, 115–123.

Jankovic, V., Samardzic, T., Stosic-Grujicic, S., Popadic, D., Trajkovic, V., 2000. Cell-specific inhibition of inducible nitric oxide synthase activation by leflunomide. Cell. Immunol. 199, 73–80.

Jendrossek, V., Kugler, W., Erdlenbruch, B., Eibl, H., Lang, F., Lakomek, M., 2001. Erucylphosphocholine-induced apoptosis in chemoresistant glioblastoma cell lines: involvement of caspase activation and mitochondrial alterations. Anticancer Res. 21, 3389–3396.

Kemper, E.M., Boogerd, W., Thuis, I., Beijnen, J.H., van Tellingen, O., 2004. Modulation of the blood–brain barrier in oncology: therapeutic opportunities for the treatment of brain tumours? Cancer Treat. Rev. 30, 415–423.

Kleihues, P., Soylemezoglu, F., Schauble, B., Scheithauer, B.W., Burger, P.C., 1995. Histopathology, classification, and grading of gliomas. Glia 15, 211–221.

Knežević, N.Ž., Leovac, V.M., Jevtović, V.S., Grgurić-Šipka, S., Sabo, T.J., 2003. Platinum(IV) complex with pyridoxal semcarbazone. Inorg. Chem. Commun. 6, 561–564.

Kreuter, J., 2001. Nanoparticulate systems for brain delivery of drugs. Adv. Drug Deliv. Rev. 47, 65–81.

Kushev, D., Gorneva, G., Enchev, V., Naydenova, E., Popova, J., Taxirov, S., Maneva, L., Grancharov, K., Spassovska, N., 2002. Synthesis, cytotoxicity, antibacterial and antitumor activity of platinum(II) complexes of 3-aminocyclohexanespiro-5-hydantoin. J. Inorg. Biochem. 89, 203–211.

Laitinen, K.L., Soini, Y., Mattila, J., Paakko, P., 2000. A typical bronchopulmonary carcinoids show a tendency toward increased apoptotic and proliferative activity. Cancer 88, 1590–1598.

Los, M., Stroh, C., Janicke, R.U., Engels, I.H., Schulze-Osthoff, K., 2001. Caspases: more than just killers? Trends Immunol. 22, 31–34.

MacMicking, J., Xie, Q.W., Nathan, C., 1997. Nitric oxide and macrophage function. Ann. Rev. Immunol. 15, 323–350.

Madajewicz, S., Chowhan, N., Tfayli, A., Roque, C., Meek, A., Davis, R., Wolf, W., Cabahug, C., Roche, P., Manzione, J., Iliya, A., Shady, M., Hentschel, P., Atkins, H., Braun, A., 2000. Therapy for patients with high grade astrocytoma using intraarterial chemotherapy and radiation therapy. Cancer 88, 2350–2356.

Mathieu, N.T., Genet, D., Labrousse, F., Bouillet, P., Denes, S.L., Martin, J., Labourey, J.L., Venat, L., Clavere, P., Moreau, J.J., 2004. Pre-irradiation chemotherapy for newly diagnosed high grade astrocytoma. Anticancer Res. 24, 1249–1253.

McCarthy, K.D., de Vellis, J., 1980. Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. J. Cell. Biol. 85, 890–902.

Rao, R.D., Krishnan, S., Fitch, T.R., Schomberg, P.J., Dinapoli, R.P., Nordstrom, K., Scheithauer, B., O'Fallon, J.R., Maurer, M.J., Buckner, J.C., 2005. Phase II trial of carmustine, cisplatin, and oral etoposide chemotherapy before radiotherapy for grade 3 astrocytoma (anaplastic astrocytoma): results of North Central Cancer Treatment Group trial 98-72-51. Int. J. Radiat. Oncol. Biol. Phys. 61, 380–386.

Siddik, Z.H., 2003. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 22, 7265–7279.

Silvani, A., Eoli, M., Salmaggi, A., Lamperti, E., Maccagnano, E., Broggi, G., Boiardi, A., 2004. Phase II trial of cisplatin plus temozolomide, in recurrent and progressive malignant glioma patients. J. Neurooncol. 66, 203–208.

Stewart, D.J., 1994. A critique of the role of the blood–brain barrier in the chemotherapy of human brain tumors. J. Neurooncol. 20, 121–139.

Stosic-Grujicic, S., Maksimovic, D., Badovinac, V., Samardzic, T., Trajkovic, V., Lukic, M., Mostarica Stojkovic, M., 2001. Antidiabetogenic effect of pentoxifylline is associated with systemic and target tissue modulation of cytokines and nitric oxide production. J. Autoimmun. 16, 47–58.

Van den Bent, M.J., Schellens, J.H., Vecht, C.J., Sillevis Smit, P.A., Loosveld, O.J., Ma, J., Tijssen, C.C., Jansen, R.L., Kros, J.M., Verweij,

J., 1998. Phase II study on cisplatin and ifosfamide in recurrent high grade gliomas. Eur. J. Cancer 34, 1570–1574.

Wang, L.D., Zhou, Q., Yang, W.C., Yang, C.S., 1999. Apoptosis and cell proliferation in esophageal precancerous and cancerous lesions: study of a high-risk population in northern China. Anticancer Res. 19, 369–374.

Watabe, M., Kai, M., Asanuma, S., Yoshikane, M., Horiuchi, A., Ogasawara, A., Watanabe, T., Mikami, T., Matsumoto, T., 2001. Platinum(IV) complexes with dipeptide. X-ray crystal structure, 195Pt NMR spectra, and their inhibitory glucose metabolism activity in *Candida albicans*. Inorg. Chem. 40, 1496–1500.

Wick, A., Wick, W., Hirrlinger, J., Gerhardt, E., Dringen, R., Dichgans, J., Weller, M., Schulz, J.B., 2004. Chemotherapy-induced cell death in primary cerebellar granule neurons but not in astrocytes: in vitro paradigm of differential neurotoxicity. J. Neurochem. 91, 1067–1074.

Zhang, C.X., Lippard, S.J., 2003. New metal complexes as potential therapeutics. Curr. Opin. Chem. Biol. 7, 481–489.