ANTIVIRAL ACTIVITY OF PLATINUM (II) AND PALLADIUM (II) COMPLEXES OF PYRIDINE-2-CARBALDEHYDE THIOSEMICARBAZONE

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Summary. – A heterocyclic compound, pyridine-2-carbaldehyde thiosemicarbazone (HFoTsc), and its six metal coordinated bound complexes, three with platinum (II) and three with palladium (II), were studied for their activity against herpes simplex virus 1 (HSV-1) infection in cultured cells. According to their cytotoxicity the compounds were divided into two groups. Group 1 (cytotoxic compounds) included all three palladium complexes and $[Pt(HFoTsc)_2]$ Cl_2, with maximum non-toxic concentration (MNC) of 1–10 μmol/l and a 50% cytotoxic concentration (CC_{50}) of 20–100 μmol/l. Group 2 (low cytotoxic compounds) with MNC of 100 μmol/l and CC_{50} of 548–5820 μmol/l included compounds in the following order: $[Pt(HFoTsc)_2]$ Cl_2<HFoTsc<[PtCl FoTsc)]. The 50% inhibitory concentration (IC_{50}) and a selectivity index (SI) values determined during 24 hrs and 48 hrs of action were indicative of antiviral activity. IC_{50} and SI values of HFoTsc increased in parallel with the duration of action in HSV-1-infected cells. All three platinum complexes as well as $[Pd(HFoTsc)_2]Cl_2$ and $[Pd(FoTsc)_2]$ inhibited HSV-1 infection following a structure-activity relationship but only $[Pt(HFoTsc)_2]Cl_2$ expressed a significant selectivity comparable to that of HFoTsc. However, [PdCl(FoTsc)] acting 48 hrs gave a higher infectious HSV-1 titer (170%) compared to control (100%, no compound).

Key words: herpes simplex virus 1; thiosemicarbazone; platinum (II), palladium (II); cytotoxicity; antiviral activity

Introduction

The antivirals first described have been thiosemicarbazones (Tsc). They are active against a broad spectrum of human pathogens, herpes viruses included (Hutfield and Gsonka, 1964; Bauer, 1965; Shipman *et al.*, 1981, 1986; Turk *et al.*, 1986; Sidwell, 1990). The significant antiviral activity of Tsc is due to their ability to inhibit effectively viral ribonucleotide reductase (RR) (Gupta *et al.*, 1984; Turk *et al.*, 1986a,b; Chung *et al.*, 1990; Idowu *et al.*, 1992; Cory *et al.*, 1994) as well as key regulatory enzyme activities, and thus they belong to the most effective agents against DNA viruses (Miller *et al*., 1998; Liu *et al.*, 1996). Furthermore, heterocyclic Tsc are important due to their predicted beneficial biological activity (Cory *et al.*, 1995; Liu *et al.*, 1992; 1996; Klayman *et al.*, 1991; Liberta and West, 1992; Miller *et al.*, 1998, Rodriguez-Argüelles *et al.*, 1995; West *et al.*, 1993). Recently, a series of platinum (II) and neutral palladium (II) compounds containing heterocyclic Tsc have been synthesized and studied for their

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Abbreviations: BS = bovine serum, HSV-1 = herpes simplex virus 1; HFoTsc = pyridine-2-carboldehyde thiosemicarbazone; DMSO = dimethylsulfoxide; SI = selectivity index; MDBK = Madin-Darby bovine kidney; CPE = cytopathic effect; CC_{50} = concentration inhibiting cell viability by 50%, MNC = maximum non-toxic concentration; IC_{50} = concentration inhibiting CPE by 50%; RR = ribonucleotide reductase; Tsc = thiosemicarbazone

antitumor activity (Kovala-Demertzi *et al.*, 1997a,b, 1998, 1999; Offiong *et al.*, 1996; Papageorgiou *et al.*, 1997). Summarized, previously published data have shown that (i) HSV-1-encoded enzymes include both the large (UL 40) and small (UL 39) subunit of RR (Chung *et al.*, 1990; Idowu *et al.*, 1992), (ii) Tsc are effective inhibitors of HSV-1 RR, (iii) Pt(II) and Pd(II) can intercalate into DNA by cross-linking GG/CC sequences and thus inactivating DNA genomes (Butour *et al.*, 1997; Reardon *et al.*, 1999; Reedijk, 1999; Temple *et al.*, 2000), (iv) the GC nucleotides in HSV-1 DNA represent 67–69% compared to 49% in an eukaryotic cell genome (Roizman, 1990) and, (v) the antitumor activity of the platinum (II) and palladium (II) complexes of HFoTsc has been already reported (Kovala-Demertzi *et al.*, 1996). All the abovementioned studies show that the evaluation of the antiviral activity of metal complexes of HFoTsc is of interest.

In this communication we present data on the effect of a series of six metal complexes of HFoTsc, three with platinum (II) and three with palladium (II), on HSV-1 infection in cultured cells. This is the first of a series of laboratory studies focused to the antiviral activity of complexes of bivalent metals with drugs.

Materials and Methods

Preparation of compounds. Solvents were purified and dried according to standard procedures. The ligand, HFoTsc (1) (Fig. 1), and its Pt(II) and Pd(II) complexes [PtCl(FoTsc)] (2), [Pt(FoTsc)$_2$] (4), [PdCl(FoTsc)] (5), and [Pd(FoTsc)$_2$] (7) were prepared as was previously described (Kovala-Demertzi *et al.*, 1997b, 1999). Details concerning synthesis, spectroscopic study and antitumor activity of (2), (4), (5), and (7) have also been reported (Kovala-Demertzi *et al.*, 1997b). [Pd(HFoTsc)$_2$]Cl$_{2\,(6)}$ was prepared by mixing methanolic solutions of HFoTsc, and lithium tetrahalogeno-palladate(II), Li_2PdX_4 prepared *in situ* from $PdCl_2$ and LiX (1.2:1 ligand:metal molar ratio). The reaction mixture was stirred for 4 hrs at room temperature and then left at 4°C for 1 day. The powder was filtered off, washed with cold methanol and ether, dried *in vacuo* over silica gel and redried at 90°C *in vacuo* over P_2O_5. [Pt(HFoTsc)$_2$]Cl$_2$ (3) was prepared by mixing a methanolic solution of HFoTsc and a stock solution of $PtCl_4$ 1:2. The same procedure was repeated. Element analyses gave results consistent with the stoichiometry. Kovala-Demertzi *et al.* (1996, 1999) and Domopoulou *et al.* (1998) have described the chemistry of HFoTsc in more details.

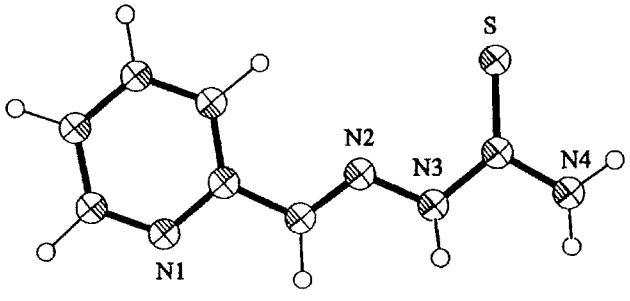

Fig. 1
Optimized PM3 geometry of neutral HFocTsc molecule in gas phase

Cells and viruses. Cells of Madin-Darby bovine kidney (MDBK) line were grown at 37°C in RPMI-1640 Medium (Gibco-BRL) supplemented with 10% of bovine serum (BS) and antibiotics. For experiments, the BS content was reduced to 5% (5% BS medium). HSV-1 Victoria strain was grown in MDBK cell monolayers. Cell cultures were harvested at full CPE, freeze thawed and stored at -70°C.

Methods of determining cell growth, cell viability, MNC and CC_{50} The compounds under study were first dissolved in dimethylsulfoxide (DMSO) to a concentration of 1mol/l (stock solutions). Serial tenfold dilutions (10–0.01 μmol/l) were made from them in 5% BS medium Cells were seeded into 96-well microplates at a concentration of 1 x 10^5 cells/ml and cultured at 37°C in a CO_2 atmosphere. Confluent monolayers were washed, covered with media containing the compounds tested in concentrations from 10 μmol/l to 0.01 μmol/l and cultured at 37°C for 24, 48 and 72 hrs. Cells grown in compound-free medium served as control. CPE was read by microscopy of unstaf a compound that altered either the morphology or growth of cells.

Assay of antiviral activity of the compounds tested was done on the basis of their effects on the infectious HSV-1 titer. MDBK cells grown in 96-well plates were infected with HSV-1 in serial tenfold dilutions. After 1 hr of virus adsorption, infected cells were incubated with the compounds tested in serial tenfold dilutions (starting from MNC) at 37°C for 48 hrs. CPE and virus titer were determined for each well. The virus titer was expressed as log TCID/0.1 ml. The antiviral activity was expressed as % inhibition of virus titer as compared to that of control (infected cells incubated in compound-free medium). To see whether the effect of a compound is reversible, a compound-containing medium was replaced by a compound-free medium at 24 hrs post infection (p.i.). The virus titer was determined 24 hrs later. Dose-response relationships were constructed by linearly regressing compound concentrations against % inhibition values derived from virus titrations IC_{50} values were calculated from regression lines. In order to be able to compare the compounds on the basis of their selective inhibition of virus replication *vs.* cytotoxicity, SI values were calculated as IC_{50} to CC_{50} ratios.

Results

Antiviral activity of HFoTsc and its Pt(II) and Pd(II) complexes

The first, obligatory step in experiments with antiviral compounds is evaluation of their cytotoxicity. According to the data presented in Table 1 all three Pd(II) complexes as

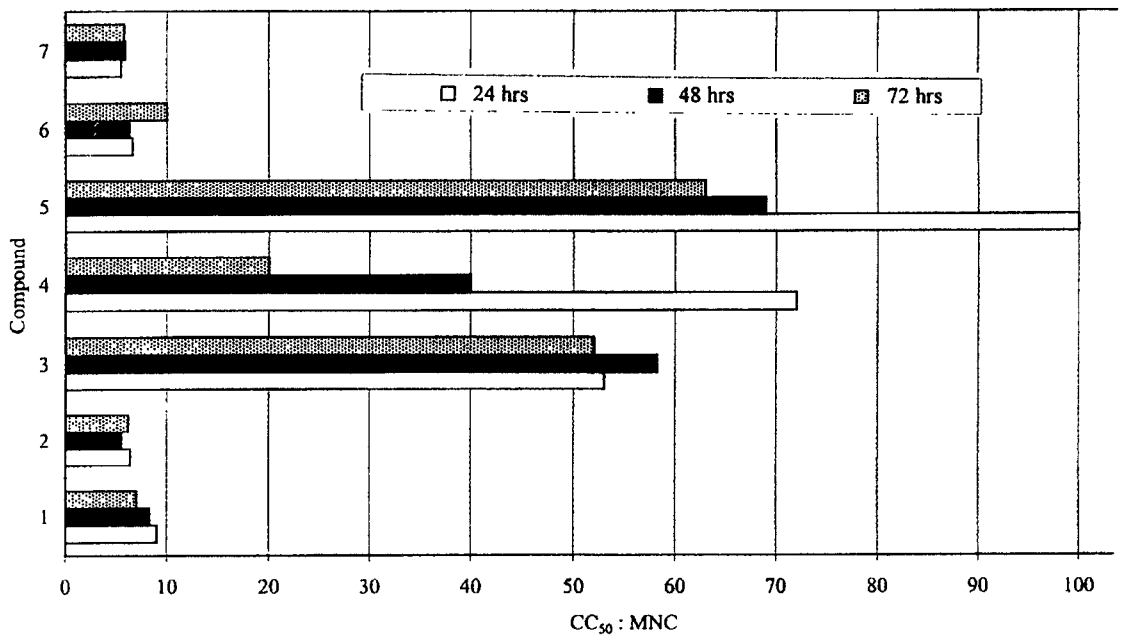

Fig. 2
The CC_{50} to MNC ratio of Pt(II) and Pd(II) complexes of HFoTsc
HFoTsc (1), [PtCl(FoTsc)] (2), $[Pt(HFoTsc)_2]Cl_2$ (3), $[Pt(FoTsc)_2]$ (4), [PdCl(FoTsc)] (5), $[Pd(HFoTsc)_2]Cl_2$ (6), $[Pd(FoTsc)_2]$ (7).

well as the Pt(II) complex (4) were more cytotoxic than the ligand (1) and the two Pt(II) complexes (2) and (3). Moreover, the Pt(II) complex (3) was up to 7 times less cytotoxic than the ligand (1) and up to 92 times less cytotoxic than the analogous Pd(II) complex (6) as demonstrated by CC_{50} data. A similar relationship was found between [M(Tsc)X] complexes, e.i. Pt(II) complex (2) was up to 10 times less cytotoxic than the analogous Pd(II) complex (5). Moreover, with the prolongation of action the cytotoxicity of compounds (1), (4) and (5) increased as shown by CC_{50} values. Furthermore, following the CC_{50} to MNC ratio, the compounds tested can be divided into two groups (Fig. 2). Group 1 includes compounds (1), (2), (6) and (7) with the CC_{50} to MNC ratio under 10. Group 2 includes compounds (3), (4) and (5) with the CC_{50} to MNC ratio over 2. However, the ratio for compounds (4) and (5) decreased with the prolongation of their action, while that for compounds (1), (2), (3), (6) and (7) was independent of the duration of their action.

Furthermore, the data showed that the antiviral activity of the ligand-HFoTsc (1) increased with the prolongation of its action. The dose-response relationship was manifested when (1) was applied in concentrations under 0.1 μmol/l (Fig. 3).

All three Pt(II) complexes (compounds (2–4)) inhibited the virus growth and manifested a structure-activity relationship (Fig. 4). Of these, the most effective was compound (2) during the first 24 hrs of infection (Fig. 4B). In a concentration range of 1–100 μmol/l, this compound reduced the virus titer by 94% of control. The dose-response effect was manifested by concentrations under 1 μmol/l, while no inhibition was obtained at a concentration of 0.0001 μmol/l. The antiviral activity of compound (2) decreased with prolonged action over 48 hrs (Fig. 4A). In contrast, the antiviral activity of compound (4) increased with prolonged action over 48 hrs. Of three Pt(II) complexes (compounds (2–4)) the less active against HSV-1 was compound (3), which reduced the virus titer to 80% of control (Fig. 4).

Evaluation of antiviral activity of Pd(II) complexes, compounds (5–7) also showed a structure-activity relationship. (Fig. 5). The most active was compound (7), the antiviral activity of which increased with prolonged action (Fig. 5B). In contrast, compound (6) was effective during the first 24 hrs of action only (Fig. 5). However, the antiviral effect of compound (5) needs special attention. It was found that compound (5) in concentrations of 0.001–1 μmol/l during 48 hrs action caused an increase of virus titer to 130–170% of control (Fig. 5A). On the contrary, when compound (5) was removed after 24 hrs, the virus titer for concentrations of 0.01–1.0 μmol/l decreased to 10–20% of control (Fig. 5B).

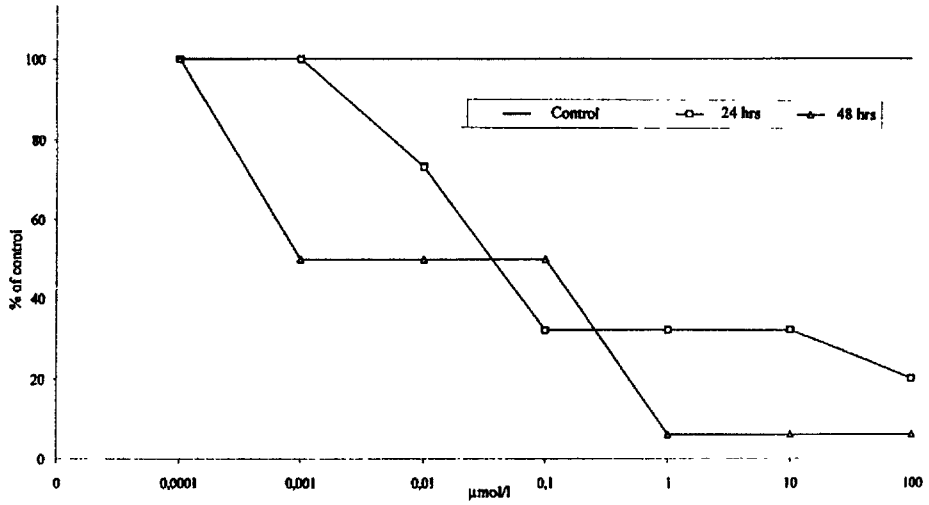

Fig. 3
Activity of HFoTsc against HSV-1 infection in cultured cells

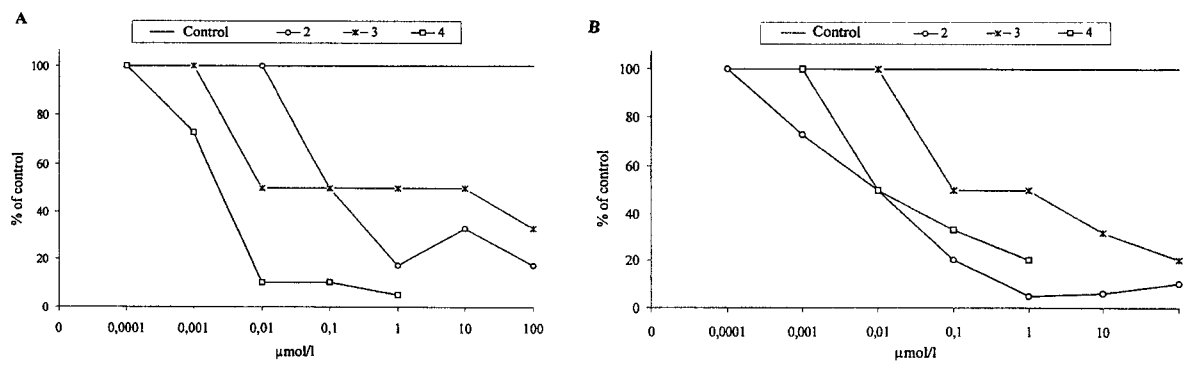

Fig. 4
Activity of Pt(II) complexes of HFoTsc against HSV-1 infection in cultured cells
A. Action during 24 hrs. B. Action during 48 hrs. For the legend see Fig. 2.

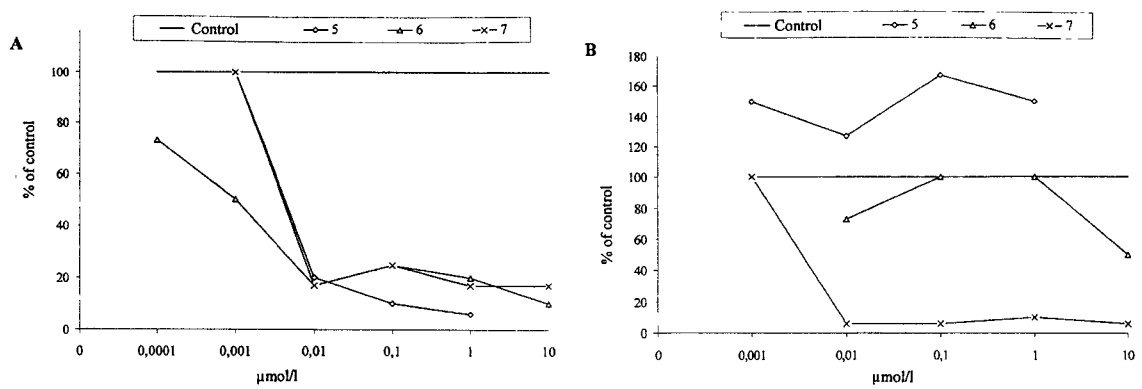

Fig. 5
Activity of Pd(II) complexes of HFoTsc against HSV-1 infection in cultured cells
A. Action during 24 hrs B. Action during 48 hrs. For the legend see Fig. 2.

Table 1. MNC, CC_{50} and IC_{50} of HFoTsc and its Pt (II) and Pd (II) complexes

Compound	Duration of action of compounds							
	24 hrs			48 hrs			72 hrs	
	MNC	CC_{50}	IC_{50}	MNC	CC_{50}	IC_{50}	MNC	CC_{50}
HFoTsc (1)	100	900	0.04	100	830	0.001	100	700
[PtCl(FoTsc)] (2)	100	640	0.01	100	548	0.1	100	620
$[Pt(HFoTsc)_2]Cl_2$ (3)	100	5300	0.1	100	5820	0.01	100	5200
$[Pt(FoTsc)_2]$ (4)	1	72	0.01	1	40	0.006	1	20
[PdCl(FoTsc)] (5)	1	100	0.004	1	69	>1.0*	1	63
$[Pd(HFoTsc)_2]Cl_2$ (6)	10	66	0.001	10	63	10	10	100
$[Pd(FoTsc)_2]$ (7)	10	55	0.004	10	59	0.06	10	58

MNC, CC_{50} and IC_{50} are expressed in μmol/l.
*Virus titer for compound (5) was higher than that for control.

IC_{50} and SI of HFoTsc and its Pt(II) and Pd(II) complexes

Based on the data from dose-response curves we calculated the IC_{50} and SI values of compounds tested. As shown in Table 1, IC_{50} of compound (1) sharply decreased with the prolongation of action. The same phenomenon was found for its Pt(II) complexes (3) and (4). The IC_{50} values of compounds (2), (6) and (7) increased, however, with prolonged action. Virus titer for compound (5) was even higher than that of control, leading to IC_{50} over 1.0 μmol/l.

As shown in Fig. 6, SI of compound (6) increased with prolonged action. Of six metal complexes tested, only the Pt(II) complex (3) expressed a significant selectivity against HSV-1 infection in cultured cells, which increased with prolonged action. Moreover, it was found that the SI value of compound (3) with 24 hrs of action was 2 times higher than that of compound (1). The SI values of other five metal

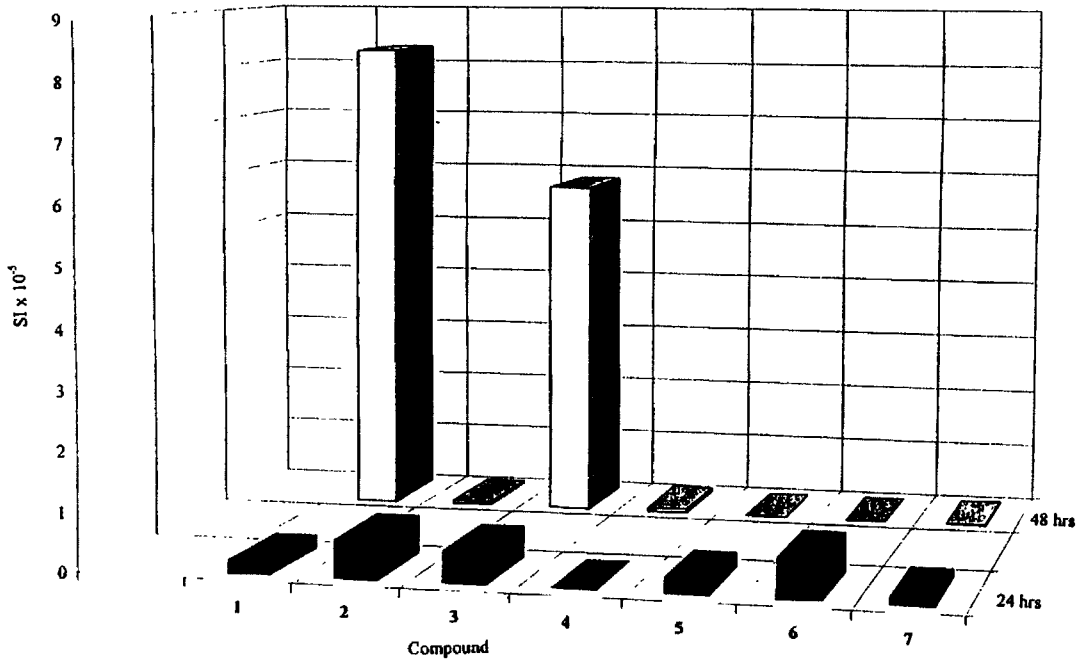

Fig. 6
SI values of Pt(II) and Pd(II) complexes of HFoTsc
SI is ratio of IC_{50} to CC_{50}. For the legend see Fig. 2.

complexes were higher than that of compounds (1) and (3). However, the SI value of compound (4) increased with prolonged action while those of compounds (2), (5), (6) and (7) sharply decreased (Fig. 6).

Discussion

The *in vivo* antitumor activities of Pt(II) and Pd(II) complexes of HFoTsc have been already demonstrated by Kovala-Demertzi and coworkers (Kovala-Demertzi *et al.*, 1997, 1998, 1999). Based on this and taking into consideration the fact that Tsc are known effective inhibitors of HSV-1 infection *in vivo* (Bauer, 1965; Cory *et al.*, 1994; Gupta *et al.*, 1984) we decided to test whether these complexes are active against HSV-1 infection also in cultured cells.

In the first group of experiments two tests were used in parallel to determine the cytotoxicity range of the compounds tested. Both MNC and CC_{50} were evaluated simultaneously by morphological and cell survival criteria. Based on their cytotoxicity, the compounds were divided into two groups. Group 1 of cytotoxic compounds included Pd(II) complexes (compounds (5–7)) and the Pt(II) complex of the deprotonated ligand, compound (4). The cytotoxic concentration ranges obtained by MNC (1–10 μmol/l) and CC_{50} (20–100 mmol/l) were similar. Group 2 included low cytotoxic compounds with MNC of 100 μmol/l and CC_{50} ranging from 0.5 to 5.8 mmol/l. Group 2 included HFoTsc (compound 1) and the two chloride containing Pt(II) complexes (compounds (2) and (3)) in the following order: (3)<(1)<(2). Thus, of seven compounds tested the least cytotoxic was Pt(II) complex of neutral protonated ligand, compound (3). According to CC_{50} values compound (3) was 19 times less cytotoxic that the ligand, compound (1).

Furthermore, on the basis of the data from cytotoxicity experiments, we calculated the CC_{50} to MNC ratios. This ratio characterizes the tolerable concentration range in which the particular compound could be applied avoiding significant cell damage. According to this ratio, the compounds followed the order (7)<(2)<(6)<(1)<<(4)< (3)<(5). Three metal complexes, two Pt(II) and one Pd(II), were characteristic by a high cell tolerable concentration range. These were the less cytotoxic compound (3) with a concentration range seven times higher than that of compound (1) and the highly cytotoxic complexes of deprotonated ligand, compounds (4) and (5). In solution, $[M(FoTsc)_2]$ complexes (compounds (4) and (5)) dissociate to metal ion and FoTsc. Upon entering the cell the metal ion, Pt(II) or Pd(II), and FoTsc direct their action to two different cell targets simultaneously. These are the RR and the genomic DNA (Liu *et al.*, 1997; Mansuri-Torshizi *et al.*, 1992; Reardon *et al.*, 1999; Reedijk, 1999; Temple *et al.*, 2000). As deprotonated FoTsc gives an extensive network of hydrogen bonds several other metabolic pathways including DNA polymerase could also be affected (Byushkin *et al.*, 1987; Miller *et al.*, 1998), resulting in an increased cytotoxicity as we already determined. However, the structure of compound (3) corresponds to $[M(HFoTsc)_2]X_2$ (Kovala-Demertzi, 1997). Obviously, the protonated neutral ligand in zwitterion form HFoTsc decreases the cytotoxicity of Pt(II) but not of Pd(II) complex. The role of the ligand in the cytotoxicity of the particular complex was well demonstrated by CC_{50} values according to which Pt(II) complexes followed the order (3)<<(2)<<(4). However, the lower cytotoxicity of compound (3) is accompanied by a significant anti-leukemia activity (Kovala-Demertzi *et al.*, 1998, 1999). A cell specific response induced by compound (3) is also possible. Experiments directed to determine such specificity are in progress.

In the second group of experiments the antiviral activity of HFoTsc compounds was evaluated against HSV-1 infection in cultured cells. Based on the data from dose-response curves, IC_{50} and SI it is evident that pyridine-2-carbaldehyde thiosemicarbazone (HFoTsc, compound (1)), is an irreversible and selective inhibitor of HSV-1 infection in cultured cells when applied in a non-cytotoxic concentration range of 0.01–100 μmol/l. Moreover, with the prolongation of its action the selectivity of the compound increased as was manifested by 28-times increased SI at 48 hrs of action as compared to that obtained at 24 hrs of action. These results are in accordance with the well known fact that, apart from the broad spectrum of activities, Tsc and its derivatives including HFoTsc are also effective antivirals. Their activity against HSV-1 infection is based on the selective inhibition of a key virus-specific enzyme, RR (Gupta *et al.*, 1984; Klayman *et al.*, 1991; Turk *et al.*, 1986a,b). However, according to the data published by Roizman (1990) GC nucleotides in HSV-1 genome represent 67% *vs.* 48% in the eukaryotic genome. Furthermore, it is well known that Pt(II) and Pd(II) bind preferentially to N7 of guanine (N7G) and cross-link GG/CC base pairs thus inactivating the genome (Butour *et al.*, 1997; Liu *et al.*, 1997; Mansuri-Torshizi *et al.*, 1992; Mital *et al.*, 1991; Reardon *et al.*, 1999; Reedijk 1999; Temple *et al.*, 2000; Zou *et al.*, 1994).

The data from our antiviral experiments show that Pt(II) and Pd(II) complexes of HFoTsc are able to affect HSV-1 infection following a structure-activity relationship. Thus, among three Pt(II) complexes, compound (2) suppressed the first cycle of virus replication only while the antiviral activity of compound (4) increased with the prolongation of its action in HSV-1-infected cells. Both are complexes of deprotonated ligand. Obviously, chloride ions participate in the structure of compound (2) but not (4), and predetermine both the close tolerable concentration range (manifested by the CC_{50} to MNC ratio under 10) and the reversible antiviral

efficacy of compound (2). Affecting different time intervals, compounds (2) and (4) are non-selective antivirals as was shown by their SI. Of six metal complexes tested only compound (3) (Pt(II) complex) selectively inhibited HSV-1 infection in cultured cells as was demonstrated by SI comparable to that of the ligand (compound (1)). We propose that the selective anti-HSV-1 activity of compound (3) is due to the simultaneous influence of at least two viral targets, RR and DNA. This is based on the fact that, in solution, $[M(HFoTsc)_2 X_2]$ complexes convert to metal complex [MCl(FoTsc)] and ligand H_2FoTscCl 1:1 Thus, being influenced by H_2FoTscCl and [MCl(FoTsc)], virus replication in cultured cells is suppressed by their simultaneous action on the virus-specific RR and the synthesis of new viral genomes. Furthermore, the role of the metal ion in the realization of antiviral activity of the particular complex was demonstrated when Pd(II) complexes, similar to Pd(II) ones, were applied in the same experimental conditions. Thus, all three Pd(II) complexes selectively inhibited the first cycle of HSV-1 replication in cultured cells. This was demonstrated by the data from the dose-response curves and confirmed by IC_{50} and SI values. However, with the prolongation of action, compounds (6) and (7) progressively lost their anti-HSV-1 activity, while compound (5) caused an increase of virus titer compared to control. These results need special attention, because there are to date no literature data showing an increase of HSV-1 (virus) growth under the influence of a metal complex.

Summarized, the data obtained here show that when HFoTsc is coordinately bound to a metal ion the effect of the particular complex against HSV-1 infection in cultured cells is predetermined by both the metal ion and the ligand specificities.

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