Cytotoxicity of Hydrophylic Silver Carboxylato Complexes

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Abstract. *Background: Gold(I) and platinum(II) d^{10} and d^8 electronic complexes such as $(Au(PPh_2CH_2CH_2PPh_2)_2)Cl$ and cisplatin, $((H_3N)_2PtCl_2)$, possess strong antineoplastic activities. Almost no information is available regarding the anticancer activity of isoelectronic silver(I) d^{10} complexes. This study examined the cytotoxicity of stable water-soluble silver(I) carboxylates. The results were related to the cytotoxicity of cisplatin and $(Au(PPh_2CH_2CH_2PPh_2)_2)Cl$. Materials and Methods: The silver carboxylates $(AgO_2CCH_2OCH_3)$, **1**, $(AgO_2C\text{-}CH_2OCH_2CH_2OCH_3)$, **2**, and $(AgO_2CCH_2OCH_2CH_2OCH_2CH_2OCH_3)$, **3**, were investigated. Cytotoxicity tests were performed on the HeLa (human cervix epitheloid) cancer cell line, resting lymphocytes and PHA (phytohaemagglutinin)-stimulated lymphocyte cultures. Cell survival was measured by means of the colorometric 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT) assay. Results: The IC_{50} (50% cell growth inhibition) values of **1-3** in the HeLa cells varied between 2.6 and 6.1 μmol dm^{-3} with being **1** the most cytotoxic silver complex. Drug activity was inversely proportional to the length of the carboxylato chain length. Complexes **1-3** were 3-5 times more cytotoxic against the HeLa cancer cells than against the PHA stimulated lymphocyte cultures. Conclusion: A drug activity-structure relationship exists in that short-chain silver carboxylates are more cytotoxic than long-chain silver carboxylates, but silver d^{10} complexes are one order of magnitude less cytotoxic than platinum(II) d^8 or gold(I) d^{10} complexes.*

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As demonstrated for cisplatin (1) and its alkynylferrocenyl-containing derivative (2), the physical properties as well as medical side-effects that inhibit the use of any potentially good chemotherapeutic drug include poor aqueous solubility, a high excretion rate from the body, development of drug resistance after continued drug dosage, high toxicity especially to the kidneys and bone marrow and, perhaps most important of all, the inability to distinguish between healthy and carcinoma cells. To combat these and other negative side-effects that many anticancer drugs possess, new antineoplastic materials are continuously being synthesised and evaluated (3, 4), combination therapies are investigated in the hope of finding synergistic effects (5), new methods of delivering an active drug to a malignant growth are developed (6-8) and new techniques over cancer treatment, such as photodynamic cancer therapy (9), are investigated.

It has been shown that free ferrocene-containing β-diketones (10) are more active than cisplatin against platinum resistant CoLo320DM (human colorectal) and COR L23/CPR (human large lung cell) cancer cell lines. In contrast, the water-soluble carboxylato complexes of ruthenium (11) were three to four orders of magnitude less active than cisplatin, but poor aqueous solubility of the paddlewheel-structured ferrocenylcarboxylato ruthenium complexes prevented their cytotoxicity determinations (12, 13). In these complexes, four carboxylato ligands are bidentate-bonded to two adjacent ruthenium atoms to form the paddlewheel. Silver(I) carboxylates of type $[AgO_2CR]$ in general form oligo- or polymeric structures in the solid state, whereby the carboxylic ligands preferentially bind in a mono- bi-, or bridging fashion (14-16) (Figure 1).

To overcome suppression of anticancer drug activity due to water insolubility, researchers have used water-soluble drug carriers. Thus it was shown (7) that the iron carboxylic acid $(FcCH(CH_3)CH_2CO_2H)$ with $Fc=Fe(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4)$ is at least one order of magnitude more active when

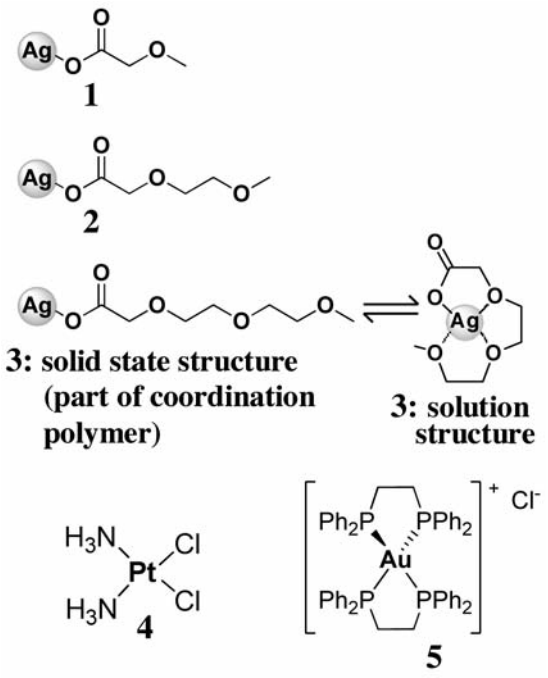

Figure 1. *Hydrophylic silver carboxylato complexes with different ethylene glycol chain lengths (**1-3**), cisplatin, **4**, and ($Au(PPh_2CH_2CH_2PPh_2)_2)Cl$, **5**.*

bound to a biocompatible and water-soluble polyaspartic acid drug carrier compared to the activity of the poorly water-soluble free acid. In this study, another way of enhancing water-solubility by choosing ethylene glycol-containing carboxylates $H(CH_2OCH_2)_nCO_2^-$ (n=1, 2, 3) as ligand to coordinate silver(I) was employed. From studies on $(AgO_2C(CH_2OCH_2)_2H)$ it is known that this transition metal complex forms in the solid state a coordination polymer, while in solution it is monomeric (14-16) (Figure 1). The question of whether partially cyclic O-chelated structures enhance or retard cytotoxicity was also addressed.

Materials and Methods

Compounds. Complexes **1-3** (Figure 1) were synthesised according to published procedures (14), complex **4** was from Aldrich (Sigma-Aldrich, St Louis, MO, USA), results for **5** were described elsewhere (2).

Sample preparation. The samples were dissolved in water giving stock concentrations of 20 mmol dm^{-3} and diluted in the appropriate growth medium supplemented with foetal calf serum (FCS) to give drug concentrations of 20-2,000 μmol dm^{-3} prior to the cell experiments.

Cell cultures. The human cervix epitheloid cancer cell line, HeLa (ATCC CCL-2) (American Type Culture Collection, Manassas, VI, USA) was grown as monolayer cultures in MEM. The growth media were maintained at 37˚C under 5% CO_2 and fortified with 10% FCS

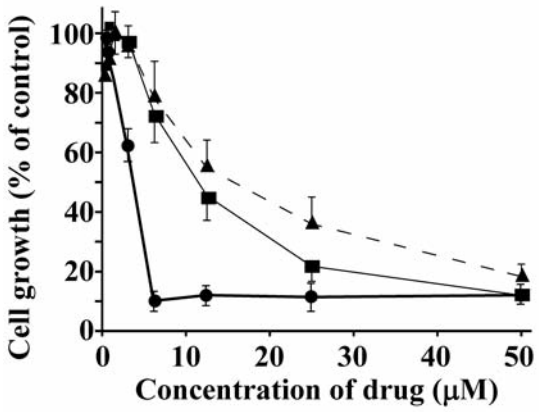

Figure 2. *Effect of concentration of 2, ($AgO_2CCH_2OCH_2CH_2OCH_3$), on the survival of HeLa cancer cells (—●—), PHA stimulated lymphocytes (—■—) and resting lymphocytes (—▲—). Data are presented as mean drug concentration±standard error of the mean of four Hela cell experiments or three lymphocyte experiments.*

and 1% penicillin and streptomycin. Purified mononuclear leukocytes were prepared from whole blood collected from three healthy adult volunteers by density centrifugation on Histopague-1077 (Sigma-Aldrich) cushions at 400 ×*g* for 25 min at ambient temperature. The lymphocyte band was washed and the cells resuspended in RPMI 1640 supplemented with 10% FCS. The cells were seeded (at 5000 cells/well in the case of cancer cells and 4×10^5 cells/well in the case of mononuclear leukocytes) in 96 well microtiter plates in a final volume of 200 μl of growth medium in the presence or absence of different concentrations of the experimental drugs. Appropriate solvent control systems were included. To some of the wells a mitogen (phytohaemagglutinin, PHA, Remel Europe Ltd, Dartford, Kent, UK) was added at a concentration of 2.5 μg/ml.

All the HeLa cell studies were performed utilising seven days of drug exposure to the cells in accordance with previous studies (2, 7). After incubation at 37˚C for 7 days in the case of the cancer cells and 3 days in the case of the mononuclear leukocytes, cell survival was measured by means of the colorometric 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazodium bromide (MTT) assay (17). The plates were read on a spectrophotometer at 570 nm with a reference wavelength of 630 nm. Wells without cells and with cells but without drugs were included as controls. Survival curves were plotted (Figure 2) as a function of drug dose and the drug concentration that caused 50% inhibition of cell growth (IC_{50}) was estimated by extrapolation.

Results

The cell growth inhibitory properties of **1-5** expressed as IC_{50} values in the HeLa cells are summarised in Table I. The silver(I) complex IC_{50} values ranged between 2.6 and 6.0 μmol dm^{-3}. The lowest IC_{50} values correspond to the more active compounds. The most active silver drug was found to be complex **1**. The d^8 square planar platinum(II) and d^{10} tetrahedral gold(I) complexes **4** and **5** were at least one order of magnitude more cytotoxic than the silver complexes

Table I. *Chemosensitivity of complexes **1-5** expressed as IC_{50} values after 7 days of incubation with the HeLa cancer cell line.*

Compound	IC_{50}[a] (μmol dm^{-3})
1 ($AgO_2CCH_2OCH_3$)	2.60±0.5
2 ($AgO_2CCH_2OCH_2CH_2OCH_3$)	4.96±1.4
3 ($AgO_2CCH_2OCH_2CH_2OCH_2CH_2OCH_3$)	6.10±1.2
4 cisplatin, ($(H_3N)_2PtCl_2$)	0.19±0.02
5 ($Au(PPh_2CH_2CH_2PPh_2)2)Cl$	0.14±0.01

[a]Data are presented as mean drug concentration causing 50% inhibition of cell growth±standard error of the mean (SEM) of four experiments.

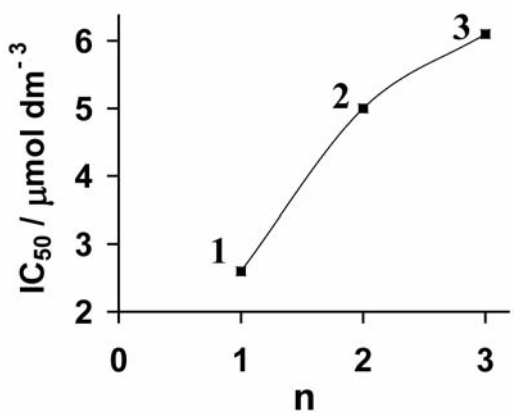

Figure 3. *Structure-reactivity relationship of the silver complexes ($AgO_2C(CH_2OCH_2)_nH$) (n=1, 2, 3). Compound numbers are next to each data point.*

($AgO_2C(CH_2OCH_2)_nH$) with n=1 (complex 1), 2 (complex 2) or 3 (complex 3). A structure-activity relationship was observed in that silver complex **1** with the shortest carboxylate ligand was most reactive, and complex **3** with the longest chain carboxylate ligand was the least reactive (Figure 3). Tumour specificity calculated as IC_{50} of PHA stimulated lymphocytes divided with IC_{50} of HeLa cancer cells was between 2.1 (for **2**) and 4.7 (for **1**). The IC_{50} values for the resting lymphocytes were between 19±6 (compound **2**) and 5.9±0.2 μmol dm^{-3} (compound **1**).

Discussion

In this study, oligomeric ethylene glycol fragments as part of the carboxylate ligand were used to solubilise the otherwise water-insoluble silver(I) species. Another way of making complexes more water-soluble is to convert them to ionic compounds, and the charged complex ($Au(PPh_2CH_2CH_2 PPh_2)_2)Cl$, **5**, was also investigated for cytotoxicity. Like the gold(I) core of ($Au(PPh_2CH_2CH_2PPh_2)_2)Cl$, **5**, the silver(I) centre of complexes **1-3** also possesses a d^{10} electronic configuration, although it exhibits in solution a mononuclear arrangement in which the silver(I) ion is stabilized by O-chelation by the ethylene glycol fractions of the $H(CH_2OCH_2)_nCO_2^-$ ligands. Thus comparisons of the intrinsic relative cytotoxicity of the two d^{10} metallic systems (Au^+ and Ag^+) lying underneath each other in group 11 of the periodic table of the elements and the d^8 Pt^{2+} species which lies to the left of Au at the bottom of group 10 in the periodic table were made. The ionic gold(I) complex **5** having IC_{50}=0.14 μmol dm^{-3} was slightly more cytotoxic than cisplatin, **4** (IC_{50}=0.19 μmol dm^{-3} under identical conditions) and at least one order of magnitude more cytotoxic than the silver complexes **1-3**. This clearly showed a tendency of increased cytotoxicity going down in group 11 of the periodic table.

A structure-activity relationship was observed in that silver complex **1** with the shortest carboxylate ligand was most reactive, and complex **3** with the longest chain carboxylate ligand was the least reactive. The solution structure of silver complexes **1-3** merits further thought. Although the ethylene glycol moieties of the carboxylate ligands instilled water-solubility to the silver complexes, the solution structures of **1-3** are probably not oligomeric or polymeric as found in the solid state (14), for example, the silver(I) ion Ag^+ of **3** was found to be coordinated by the oxygen atoms of the ethylene glycol carboxylic unit. Thus the Ag^+ ion is relatively unprotected from interacting with any biological target molecules (*e.g.* DNA) in **1**, more shielded in **2**, while in **3** it is masked at the most from its surroundings. It was also found that 1 was the most cytotoxic (and most tumour specific, the tumour specificity of 1 compared well with the HeLa tumour specificity of cisplatin (6.9) under similar conditions (10)) and 3 the least cytotoxic antineoplastic drug. This combined result was consistent with the solution structure of **1-3** impairing the capability of these silver complexes to interact with biologically relevant target molecules more effectively, the more completely the ligand surrounds (encapsulates) the central silver ion. Complete silver ion encapsulation, and therefore the most deactivation by the carboxylato ligands, only occurs in **3**, having the longest carboxylato chain length.

Conclusion

The cytotoxicity of the d^{10} gold(I) ($Au(PPh_2CH_2CH_2PPh_2)_2)Cl$ and d^8 platinum(II) ($(H_3N)_2PtCl_2$) complexes are comparable, while silver(I) ($AgO_2C(CH_2OCH_2)_nH$) (n=1, 2 or 3) complexes, also d^{10} species, are at least one order of magnitude less cytotoxic, consistent with cytotoxicity increasing from top to the bottom of group 11 of the periodic table of the elements. The inversely proportional structure-reactivity relationship of the ($AgO_2C(CH_2OCH_2)_nH$) complexes is attributed to their geometry in solution where longer chain ethylene glycol-based carboxylato ligands can surround the silver(I) ion thereby inhibiting its interaction with relevant target biological molecules.

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