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Overcoming cisplatin resistance using gold(III) mimics: Anticancer activity of novel gold(III) polypyridyl complexes

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ABSTRACT

Gold(III) compounds have been recognized as anticancer agents due to their structural and electronic similarities with currently employed platinum(II) species. An added benefit to gold(III) agents is the ability to overcome cisplatin resistance. This work identified four gold(III) compounds, $[Au(Phen)Cl_2]PF_6$, $[Au(DPQ)Cl_2]PF_6$, $[Au(DPPZ)Cl_2]PF_6$, and $[Au(DPQC)Cl_2]PF_6$, (Phen = 1,10-phenanthroline, DPQ = dipyrido[3,2-d:2′,3′-f]quinoxaline, DPPZ = dipyrido[3,2-a:2′,3′-c] phenazine, DPQC = dipyrido[3,2-d:2′,3′-f] cyclohexyl quinoxaline) that exhibited anticancer activity in both cisplatin sensitive and cisplatin resistant ovarian cancer cells. Two of these compounds, $[Au(DPQ)Cl_2]PF_6$ (AQ) and $[Au(DPPZ)Cl_2]PF_6$ (AZ), displayed exceptional anticancer activity and were the focus of more intensive mechanistic study. At the molecular level, AQ and AZ formed DNA adducts, generated free radicals, and upregulated pro-apoptotic signaling molecules (p53, caspases, PARP, death effectors). Taken together, these two novel gold(III) polypyridyl complexes exhibit potent antitumor activity in cisplatin resistant cancer cells. These activities may be mediated, in part, by the activation of apoptotic signaling.

1. Introduction

Platinum-containing compounds such as cisplatin, carboplatin and oxaliplatin are widely used in the treatment of a variety of cancers. Newer, second generation platinum compounds like carbo- and oxali-platin were designed to improve efficacy particularly against cell lines that demonstrate second pass cisplatin resistance [1]. Tolerance to cisplatin-induced DNA damage has been suggested as a fundamental mechanism of drug resistance [2]. Cisplatin resistance is thought to occur primarily by cells resisting DNA platination leading to the exit at G2/M phase of cell cycle and inhibiting apoptosis [3]. In addition, drug-efflux in resistant cell lines has been shown to be greater than the influx due to the up-regulation of multi drug resistance (MDR) genes and the production of antiporters such as p-glycoprotein [3]. To circumvent the problem of drug-resistance in cisplatin-resistant cells, gold(III)-based complexes have been designed as a potential alternative to cisplatin [4–8]. Gold(III) complexes exhibit isoelectronic and isostructural features with platinum (II) and have similar uptake and DNA interference activity[8]. In addition, the higher charge of gold(III) compared to platinum(II) is an added advantage for binding with DNA [5, 9].

One recent active area of interest is the design and characterization of small complexes with polypyridyl and phenanthroline ligands for use as structural DNA probes or artificial nucleases [10–15]. Since transition metal complexes of polypyridyl ligands can bind to specific DNA sequences [16], one can hope to achieve selective binding of mutated or altered DNA sequence in cancer cells compared to non-transformed cells, thereby decreasing the unwanted toxicity due to chemotherapeutics. As drug efflux is believed to be an important contributing factor in cisplatin resistance, polypyridyl ligands, owing to their stronger DNA binding capacity, may decrease resistance via cellular efflux.

While many studies report that gold(III) complexes are emerging as potential anticancer targets [17–25], few detailed mechanistic studies exist. To this end, this work evaluates the cytotoxic activity, cellular uptake, intracellular signaling cascades and DNA binding affinity of gold(III) polypyridyl complexes in cisplatin-sensitive, cisplatin-resistant and multidrug resistant ovarian cancer cells.

2. Experimental

2.1. Materials and method

The gold polypyridyl complexes $[Au(Phen)Cl_2]PF_6$, $[Au(DPQ)Cl_2]PF_6$, $[Au(DPPZ)Cl_2]PF_6$, and $[Au(DPQC)Cl_2]PF_6$ (Phen = 1,10-phenanthroline, DPQ = dipyrido[3,2-d:2′,3′-f]quinoxaline, DPPZ = dipyrido

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[3,2-a:2′,3′-c] phenazine, DPQC = dipyrido[3,2-d:2′,3′-f] cyclohexyl quinoxaline) were synthesized using analytical grade reagents and HPLC quality solvents (Fig. 1). The synthesis of the ligands and complexes has been reported elsewhere [8]. Adherent human ovarian adenocarcinoma cells (A2780) used for cytotoxicity studies were obtained from The National Cancer Institute-Frederick Cancer DCI Tumor Repository. The cisplatin-sensitive A2780 cells were exposed to cisplatin to generate cisplatin resistant A2780CP70 (IC_{50} ~70 μM) cell line (generously donated by Dr. J. Christopher States, University of Louisville College of Medicine, Louisville, KY). The Hank's balanced salt solution, Dulbecco's-phosphate buffer saline, biological grade DMSO, and Triton X-100 were purchased from Sigma (St. Louis, MO). The RPMI-1640 medium (ATCC), penicillin, streptomycin, fetal bovine serum (FBS) and trypsin-EDTA were from Invitrogen Corporation (Carlsbad, CA). RPMI-1640 medium was supplemented with 5% heat inactivated FBS, penicillin and streptomycin. Multidrug-resistant cell line HTB-161 or OVCAR-3 cell line was obtained from American Type Culture Collection (Manassas, VA). This cell line is resistant to clinically relevant concentrations of adriamycin, melphalan and cisplatin. The HTB-161 cells were propagated in RPMI-1640 Medium containing 0.01 mg/ml bovine insulin and fetal bovine serum to a final concentration of 20%. Normal ovarian cancer cell line CRL-9096 from hamster also obtained from ATCC was grown in Iscove's modified Dulbecco's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and supplemented with 0.1 mM hypoxanthine, 0.016 mM thymidine, 0.002 mM methotrexate and 10% FBS. These cells are deficient in dihydrofolate reductase and will die in the absence of hypoxanthine–thymidine. Methotrexate was added to the cell culture medium to prevent growth of revertant cells with a low resistance to the drug. Costar brand sterile, tissue culture treated 96-well black clear bottom plates were purchased from Corning (Corning, NY). The Live/Dead cell viability/cytotoxicity kit was purchased from Molecular Probes (Carlsbad, CA) and cell viability was assessed per manufacturer's protocol. The compounds were initially dissolved in DMSO, and further dilutions were made with complete medium. The final concentration of DMSO did not exceed 5% in any of the experiments and similar amounts of DMSO were added to control cells.

2.2. In-vitro cytotoxicity assay

All the cell lines were cultured in a humidified atmosphere of 5% CO_2/95% air, adhesion of cells (~50%) to the culture surface occurred within the first few hours after seeding. Rapid growth was observed thereafter following overnight incubation. The cells were allowed to reach about 70% confluence at which time the cells were washed with Hank's balanced salt solution and harvested using standard trypsin-EDTA method. The harvested cells were seeded in 96-well black clear bottom tissue culture plates and allowed to grow to about 90% confluence in supplemented growth medium. At this stage, the medium was aspirated and the cells were washed with Dulbecco's-phosphate buffered saline (D-PBS). Subsequently, the cells were incubated for 2 h in D-PBS solution containing varying concentrations of $NaAuCl_4 \cdot 2H_2O$, polypyridyl ligand, Au-ligand complex or the standard drug cisplatin in DMSO at the concentrations. Control wells containing similarly grown cells were treated with 5% DMSO. In some experiments cells were incubated with a 5% Triton-X100 as a positive control to assess cell death. All wells were treated with Live/Dead viability/cytotoxicity reagent and the dye concentration and incubation time optimized. After incubation for 2 h, 50 μL of Live cell reagent (1 μL calcein AM/1 mL of D-PBS) was added to wells and then incubated for further 2 h. Fluorescence was measured at excitation and emission wavelengths of 485 nm and 525 nm respectively using a 515 nm cut-off filter (Spectramax, Gemini Microplate Spectrofluorometer, Molecular Devices, Sunnyvale, CA). From the fluorescence intensities (obtained from the weighted average of twelve wells from quadruplet trials), the % inhibition was calculated. The inhibiting concentration (IC_{50}) was determined by plotting log (conc.) of test compounds vs. % inhibition. The % inhibition was also cross checked using a standard MTT (MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

2.3. Drug treatment, total protein determination and sample preparation for inductively coupled plasma emission spectroscopy (ICP) analysis

The gold polypyridyl complexes $[Au(Phen)Cl_2]PF_6$, $[Au(DPQ)Cl_2]PF_6$, $[Au(DPPZ)Cl_2]PF_6$, and $[Au(DPQC)Cl_2]PF_6$ (10 μM) were added to cells during their growth-phase and incubated for 2 h. The cell monolayers were washed three times with cold PBS and harvested by trypsination and was completely eluted from the plate. The cell suspension was centrifuged at 1000 *g* for 5 min at 4 °C and the pellet was resuspended in D-PBS and was centrifuged at 1000 *g* for 5 min and was repeated once and the pellet was stored at −80 °C until use. The pellets were resuspended in mQ water to obtain a homogeneous cell suspension. Aliquots were removed and treated with radioimmunoprecipitation assay (RIPA) buffer sonicated and protein concentration of the lysate was determined by bicinchonic acid (BCA) assay. The remaining cell suspension was transferred into a glass tube containing 50% HNO_3 and was mineralized until complete drying at 120 °C. Dry gold containing materials were dissolved in 2 mL of 2% HNO_3 and the clear solution obtained was analyzed for total gold. Sonicated samples were assayed by BCA-method for their protein quantification.

2.4. Determination of reactive oxygen species (ROS)

The presence of intracellular reactive oxygen intermediates was measured based on the ability of cells to oxidize fluorogenic dyes to their corresponding fluorescent analogs. The non-ionic, nonpolar H_2DCF-DA crosses cell membranes and is hydrolyzed enzymatically by intracellular esterases to nonfluorescent DCFH. In the presence of

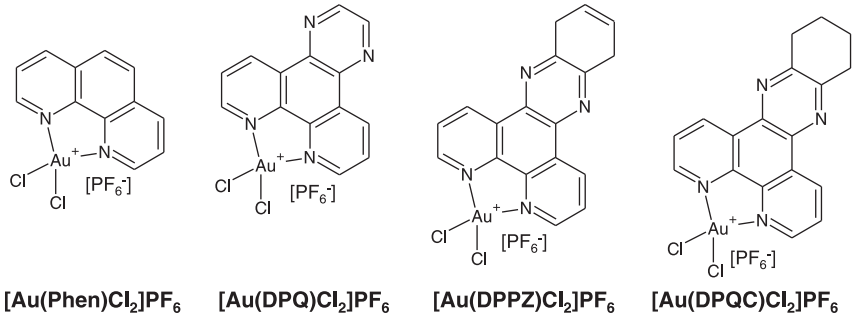

Fig. 1. Gold(III) polypyridyl complexes. Proposed structures of the gold(III) polypyridyl complexes.