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Cellular pharmacological properties of gold(III) porphyrin 1a, a potential anticancer drug lead

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Abstract

The development of gold(III) complexes as potential anticancer drugs with higher cytotoxicity and fewer side effects than existing metal anticancer drugs has been actively pursued in recent years. In this study, we explored the cellular pharmacological properties of gold(III) porphyrin 1a, an anticancer drug lead we previously described. The cytotoxicity study of gold(III) porphyrin 1a by naphthol blue black (NBB) staining assay demonstrated that the higher cytotoxicity of gold(III) porphyrin 1a was not related to its photosensitizing activity. Serum dependent test revealed that serum proteins exhibited lesser effects on the activity of gold(III) porphyrin 1a. In addition, *in vivo* and *in vitro* binding assays showed that gold(III) porphyrin 1a acted on DNA noncovalently, which was differently from cisplatin. Flow cytometric study indicated that gold(III) porphyrin 1a inhibited cell growth partly through abrogating cell cycle at G_0–G_1, and induced apoptosis in SUNE1 cells. The enhanced expression of p53, a cell cycle-controlling and apoptosis-related protein, further demonstrated that the cell cycle arrest and apoptosis induced by gold porphyrin 1a were p53 dependent. Our results highlighted the potential of gold(III) porphyrin 1a as an anticancer drug.

Keywords: Gold compound; Cytotoxicity; Anticancer; DNA binding

1. Introduction

Medicinal applications of metal complexes as therapeutic drugs have a history of at least 5,000 years (Orvig and Abrams, 1999). The major classes of anticancer metal-based drugs include platinum (Zhang and Lippard, 2003), ruthenium (Wang et al., 2005), gold(I) (Garcia-Orad et al., 1993), and gold(III) compounds (Buckley et al., 1996).

It is generally believed that the cytotoxic effects of metal complexes are the consequence of binding to DNA. The goal is to cause DNA damage specifically in cancer cells, leading to cell death. Evidence suggests that cisplatin exerts its anticancer effect through preferentially binding to quinine N-7 of cellular DNA (Boudsocq et al., 2005). Some preliminary data suggesting a direct interaction of gold(III) complexes with DNA as the basis for their cytotoxic effects were also reported (Crooke and Mirabelli, 1983; Mirabelli et al., 1985). The major limitations of cisplatin are related to its side effects (nephrotoxicity, neurotoxicity, and emetogensis) and drug resistance (Wong and Giandomenico, 1999). Resistance to cisplatin is multi-factorial, most cases consist of mechanisms limiting the formation of DNA adducts or operating downstream of the cisplatin-DNA interaction to promote cell survival (Aird et al., 2002). The major limitation of gold(III) complexes is that few exhibit good stability under physiological conditions, due to the reduction of gold(III) to gold(I) (Shaw, 1999). However, low cisplatin cross resistance has been observed in gold complexes (Zhang and Lippard, 2003). There is therefore considerable interest in the development of tumor-selective and stable gold drug leads.

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The design and testing of gold complexes with anticancer activity over the past few decades were based on three rationales (Haiduc and Silvestru, 1989; Sadler et al., 1984; Shaw, 1994): (a) analogies between square planar complexes of platinum(II) and gold(III), both of which are d^8 ions; (b) analogy to the immunomodulatory effects of gold (I) antiarthritic agents; and (c) complexation of gold(I) and gold(III) with known anticancer agents to form new compounds with enhanced activity. Buckley et al. (1996) first reported some organogold(III) complexes endowed with significant cytotoxic and anticancer properties. During the past decades, various gold(III) complexes of sufficient stability in the physiological environment have been synthesized and evaluated for *in vitro* anticancer properties. Some of these gold(III) complexes turned out to exhibit relevant cytotoxic effects *in vitro* and were the subject of further biochemical and pharmacological investigations (Casini et al., 2006; Coronnello et al., 2005; Giovagnini et al., 2005; Kostova, 2006; Marcon et al., 2002, 2003; Messori et al., 2000, 2001; Ronconi et al., 2005, 2006; Sundriyal et al., 2006). Our previous findings showed that the gold(III) *meso*-tetraarylporphyrin 1a (Fig. 1A) was stable against demetallation in physiological conditions and exhibited higher cytotoxicity than cisplatin (Fig. 1B) against a panel of human cancer cell lines, indicated that gold(III) porphyrin 1a is a promising candidate for anticancer drug development (Che et al., 2003). In this study we intend to investigate the cellular pharmacological properties of gold(III) porphyrin 1a as a potential anticancer drug lead.

In the present study, we found that the cytotoxicity of gold (III) porphyrin 1a exhibited consistent cytotoxicity and serum noneffective, and the cytotoxic effect of gold(III) porphyrin 1a was not linked to its photosensitizing activity. In addition, an *in vivo* binding assay showed that gold(III) porphyrin 1a acted differently from cisplatin *in vivo*. The *in vitro* DNA binding assay confirmed that gold(III) porphyrin 1a interacted with DNA noncovalently. Flow cytometric study indicated that gold (III) porphyrin 1a induced cell growth inhibition, partly through induction of cell cycle arrest at G_0–G_1 phase and apoptosis in SUNE1 cells. To gain further evidence that gold(III) porphyrin 1a abrogates the cell cycle and induces apoptosis, we investigated the expression of p53 following gold(III) porphyrin 1a treatment. Our results indicated that gold(III) porphyrin 1a induced apoptosis in SUNE1 cells by up-regulation of p53. The present study demonstrated that gold(III) porphyrin 1a is a potential anticancer drug lead.

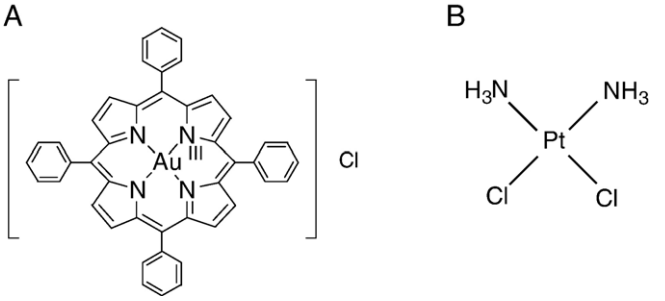

Fig. 1. Schematic draw of chemical structures of gold(III) porphyrin 1a and cisplatin.

2. Materials and methods

2.1. Materials and reagents

Gold(III) porphyrin 1a was synthesized and purified as described previously (Che et al., 2003). Cisplatin was purchased from Sigma-Aldrich Chemical Co. Hypericin was purchased from Invitrogen. All other chemicals, unless otherwise stated, were purchased from Sigma-Aldrich Chemical Co. and Amersham Biosciences. Gold(III) porphyrin 1a, cisplatin and hypericin were dissolved in dimethyl sulfoxide (DMSO) as stock solutions.

2.2. Cell lines and cell culture

Human nasopharyngeal carcinoma (NPC) cell line (SUNE1) was generously provided by Prof. S. W. Tsao (Department of Anatomy, The University of Hong Kong). The cell line was cultured in RPMI 1640 medium with 2.0 g/l sodium bicarbonate plus 10% fetal bovine serum, 2 mmol/l L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin, and maintained in a humidified incubator with an atmosphere of 95% air and 5% CO_2 at 37 °C.

2.3. Cytotoxicity determination

Cell viability was measured by naphthol blue black (NBB) staining assay (Lau et al., 2004). SUNE1 cells were plated in 96-well plates at 2×10^4/well in complete media and incubated overnight. The medium was then changed and the cells were treated with various amounts of gold(III) porphyrin 1a and cisplatin for 24 h. At the end of the experiment, the media were removed and the cells were fixed in 10% formalin for 5–10 min and stained with NBB solution (0.05% NBB in 9% acetic acid with 0.1 M sodium acetate) for 30 min at room temperature. The wells were washed three times with H_2O to remove the free dye. The attached dyes were eluted with 150 μl of 50 mM NaOH. The optical densities were measured at 595 nm using a Model E1 310 Autoplate reader.

2.4. Phototoxicity assay

The cells were preincubated with gold(III) porphyrin 1a or hypericin respectively for 24 h in subdued light conditions (<1 μW/cm^2) and subsequently irradiated in RPMI 1640 medium by placing the samples on a plastic diffuser sheet 5 cm above a 500 W quartz–iodine lamp (Yow et al., 2000). The wavelength of the quartz–iodine lamp was between 190 nm to 1100 nm, which covered the major absorption peaks of gold(III) porphyrin 1a (407 nm) and hypericin (545 and 595 nm). At the surface of the diffuser, the uniform total intensity was measured to be 4.5 mW/cm^2 using a power meter (Bioblock Scientific, Illkirch Cedex, France) (Yow et al., 2000). Cytotoxicity was analyzed 1 h post-irradiation by NBB staining.

2.5. Time course of treatment at the dose of IC_{50}

SUNE1 cells were plated in 6-well plates at 1×10^5/well in complete media and incubated overnight. The medium was then

changed and the cells were treated with IC_{50} dose of gold(III) porphyrin 1a and cisplatin respectively for 24 h. At the end of each treatment, cells were trypsinized and resuspended with phosphate buffer solution (PBS), then mixed with an equal volume of 0.4% trypan blue dye solution for 5 min. Stained (dead) and unstained (live) cells were counted by using a hemocytometer, and the percentage of dead cells/total cells was determined by scoring an average of over 300 cells, twice per plate.

2.6. Serum dependent cell viability

SUNE1 cells were plated in 96-well plates at 2×10^4/well in serum free media and incubated overnight. The medium was then changed and the cells were treated with a set dose of gold (III) porphyrin 1a (1 μM) and cisplatin (30 μM) in different concentrations of fetal bovine serum (FBS) for 24 h. The correspondent controls were incubated in the media with the same amount of FBS but without drug treatment. At the end of the experiment, cell viability was measured by NBB staining assay as described above.

2.7. In vivo DNA binding analysis

Cells were planted in 6-well plates at 4×10^5/well in complete media, incubated overnight, and treated with various concentrations of gold(III) porphyrin 1a or cisplatin and ethidium bromide for the indicated time or left untreated as control. At the end of the experiment, cells were harvested and lysed with DNA isolation lysing buffer (10 mM Tris–HCl, 0.1 M EDTA, 0.5% (*w/v*) sodium dodecyl sulfate (SDS), pH 8.0). The whole cell lysate was subjected to 1% agarose gel electrophoresis. The gel was run at 100 V for 4 h, with thermostating at 4 °C. When the electrophoresis was completed, the gel was stained with ethidium bromide and visualized with UV light.

2.8. DNA binding assays by spectrophotometry

Native DNA (isolated from SUNE1 cells with Qiagen Kit according to the manufacturer's instructions, Qiagen, Hilden, Germany) was titrated with gold(III) porphyrin 1a in 5 mM Tris–HCl buffer (pH 7.3) containing 150 mM NaCl. Solutions of gold(III) porphyrin 1a and cisplatin was prepared by dilution of a stock solution in DMSO with buffer to a final concentration of 0.05 mM and 6.66 mM respectively. Equilibrium binding experiments were performed on a Cary 3E UV–Visible Spectrophotometer equipped with a thermostatted cell holder according to a published protocol (Jenkins, 1997). Briefly, 10 μl of gold(III) porphyrin 1a solution (1.0 mM) was added to 190 μl prewarmed Tris–HCl buffer, and titrated at 25 °C in a 1-cm quartz cuvet with 0.2–2.0 μl aliquots of 2–3 μM isolated DNA stock solutions. After each addition, solutions were allowed to equilibrate for 40 min. Spectral changes were recorded at 409 nm for gold(III) porphyrin 1a. The effects of detergent (Tween 20 and SDS) on DNA binding were also studied by adding detergents into Tris–HCl buffer to reaction mixtures.

2.9. X-ray crystal determination

Crystals of gold(III) porphyrin 1a were obtained by a slow diffusion of *n*-pentane into a solution of gold(III) porphyrin 1a in chloroform. A purple crystal having dimensions $0.4\times0.1\times0.1$ mm mounted on a glass fiber was used for data collection at 28 °C on a MAR diffractometer with a 300 mm image plate detector using graphite monochromatized Mo–K_α radiation ($\lambda=0.71073$ Å). Data collection was made with 3° oscillation step of φ, 300 s exposure time and scanner distance at 120 mm. 48 images were collected. The images were interpreted and intensities integrated by using program DENZO (Otwinowski and Minor, 1997). The structure was solved by direct methods employing SHELXS-97 program (Sheldrick, 1997). Au, Cl and many non-H atoms were located according to the direct methods. The positions of the other non-hydrogen atoms were found

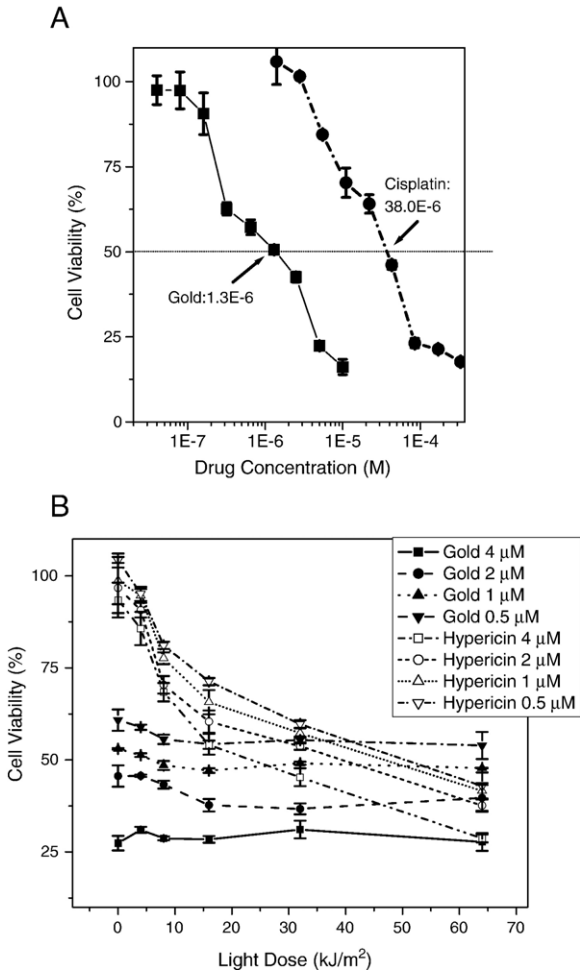

Fig. 2. Cytotoxicity of gold(III) porphyrin 1a on SUNE1 cells. (A) SUNE1 cells were plated in 96-well plates at 2×10^4 cells/well and incubated overnight. On the following day the cells were treated with different doses of gold(III) porphyrin 1a and cisplatin respectively. After treatment for 24 h, the number of viable cells was measured by NBB staining assay. (B) SUNE1 cells were treated with different doses of gold(III) porphyrin 1a and hypericin for 24 h respectively, and then irradiated with different dose of light. The number of viable cells was measured by NBB staining assay. Data are mean values from three independent experiments.

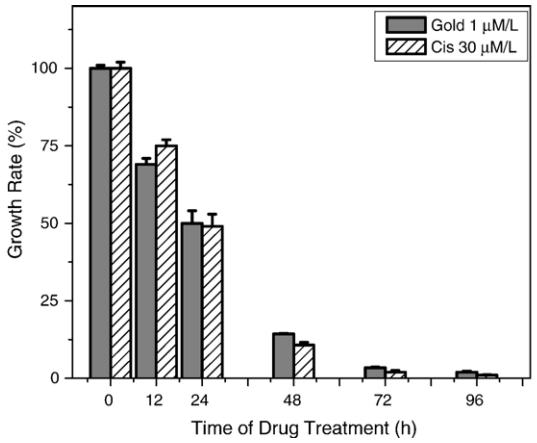

Fig. 3. Time course treatment of SUNE1 cells with IC_{50} dose of gold(III) porphyrin 1a or cisplatin. SUNE1 cells were treated with 1 μM of gold(III) porphyrin 1a or 30 μM of cisplatin for various times as indicated. At the end of the experiment live and dead cells were distinguished by trypan blue staining and the number of live cells was counted. Results from three independent experiments were quantitated and presented as a chart.

after successful refinement by the full-matrix least-squares using program SHELXL-97.

2.10. Flow cytometric analysis of cell cycle

Cell cycle distribution was determined by using flow cytometry as described (Wang et al., 2006). Cells were cultivated for 24 h in serum free medium and then either left untreated or treated with 4 μM of gold(III) porphyrin 1a or 30 μM of cisplatin for 24 h respectively. The second set of experiment with 4 μM of gold(III) porphyrin 1a for 8, 15 and 24 h was also studied. At the end of each experiment, cells were harvested, resuspended in PBS solution, stained by propidium iodide, and analyzed with a FACStar Plus flow cytometer. For each sample, 1×10^6 cells were analyzed, providing a solid statistical basis for the determination of the percentage of cells in each cell cycle phase using the WinMDI 2.8 software program.

2.11. Western blot analysis

The cells were lysed and protein extraction was performed. The samples were separated on 12% SDS gel and electrophoretically transferred to polyvinylidene difluoride membranes (PVDF). The membranes were blotted with 5% skim milk, washed, and probed with primary antibodies against p53 (Santa Cruz Biotechnology) and β-actin (Sigma-Aldrich) respectively. After washing, the membranes were incubated with corresponding secondary antibodies and visualized by enhanced chemiluminescence (ECL) in accordance with the manufacturer's recommendations (Amersham).

2.12. Statistical analysis

Statistical analysis was performed by using two-tailed Student's *t*-test. $P<0.05$ (labeled with * in figures) was considered significant. Data were expressed as the mean±S.D. of triplicate samples, and reproducibility was confirmed in three separate experiments respectively.

3. Results

3.1. Cytotoxicity of gold(III) porphyrin 1a is higher than cisplatin

The cytotoxicity of gold(III) porphyrin 1a was previously evaluated with a panel of human carcinoma cells lines by MTT assay (Che et al., 2003). In the present study, we re-evaluated it by NBB assay in order to achieve results directly relevant to the actual cell number in SUNE1 cells instead of mitochondrial enzyme activity in MTT assay (Mosmann, 1983). For the purpose of comparison, the cytotoxicity of cisplatin was re-evaluated

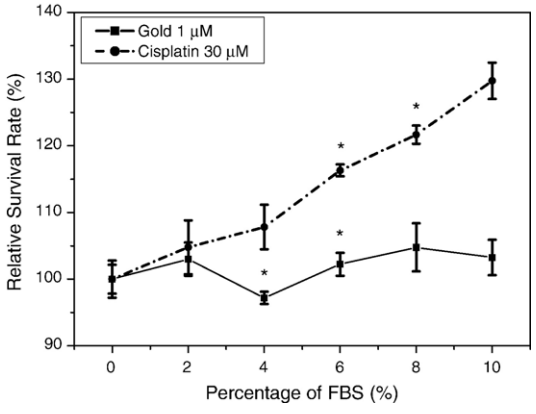

Fig. 4. The effect of FBS on cytotoxicities of gold(III) porphyrin 1a and cisplatin on SUNE1 cells. The effects of FBS on cytotoxicities of gold(III) porphyrin 1a and cisplatin on SUNE1 cells. Cells were treated with 1 μM of gold(III) porphyrin 1a or 30 μM of cisplatin in the media containing various percentages of FBS for 24 h. The amount of survival cells was determined by NBB assay. Relative survival rate under different percentage of FBS was presented as mean± S.D. from three independent experiments (*, $P<0.05$ compared with control).

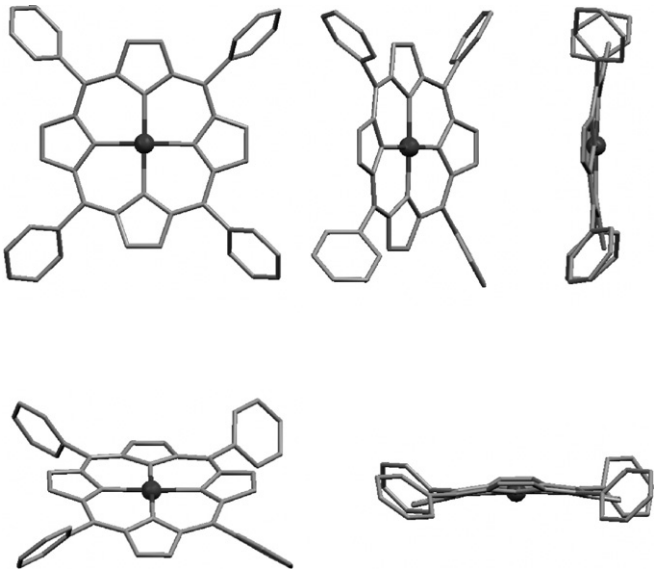

Fig. 5. Different perspective views of X-ray structure of gold(III) porphyrin 1a.

under the same conditions. The IC_{50} of gold(III) porphyrin 1a was about 1.3 μM and cisplatin was about 38.0 μM, clearly indicating that gold(III) porphyrin 1a exhibited higher cytotoxicity than cisplatin under the same conditions (Fig. 2A). We then investigated the photocytotoxicity of gold(III) porphyrin 1a. Fig. 2B showed that cytotoxicity of gold(III) porphyrin 1a did not change under exposure to different dose of light, while the cytotoxicity of hypericin was enhanced with increasing dose of light exposure. This result suggested that the cytotoxic effect of gold(III) porphyrin 1a was not linked to its photosensitizing activity. We choose 1.0 μM of gold(III) porphyrin 1a and 30.0 μM of cisplatin as IC_{50} value for further study.

3.2. Gold(III) porphyrin 1a exhibit consistent cytotoxicity with the dose of IC_{50}

The cell proliferation assay in the time course treatment with the dose of IC_{50} of gold(III) porphyrin 1a and cisplatin respectively was compared based on the cell-counting technique. Fig. 3 showed that SUNE1 cells almost died out with IC_{50} treatment of gold(III) porphyrin 1a after 96 h. This proved that the cytotoxicity of gold(III) porphyrin 1a was high, and the performance of gold complex followed consistent cytotoxicity.

3.3. The effects of serum on the cytotoxicity of gold(III) porphyrin 1a and cisplatin

Since previous studies found that the effects of some metal drugs were interfered by interacting with serum proteins (Aubry et al., 1995; Litterst and Schweitzer, 1988), we used serum dependent test to check whether proteins in the serum could affect the cytotoxicity of gold(III) porphyrin 1a. We used the dose of IC_{50} of gold porphyrin 1a and cisplatin to treat SUNE1

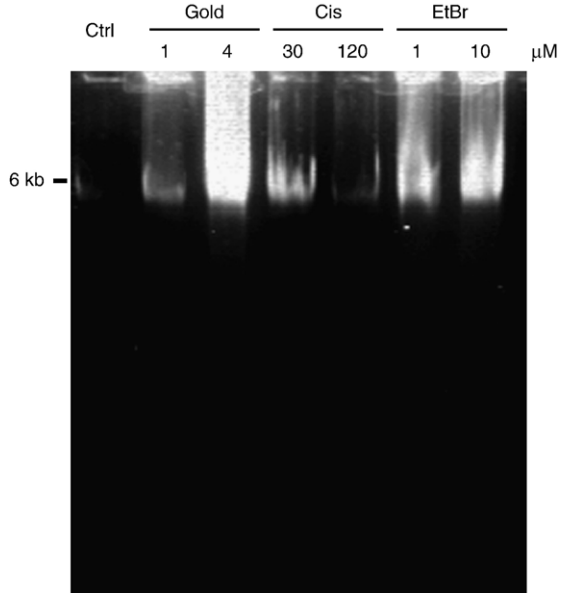

Fig. 6. *In vivo* studies of DNA-gold(III) porphyrin 1a interactions. The amount of DNA in the lower molecular weight region changed as the dose of complexes increased during the treatment. This result is representative of three independent experiments.

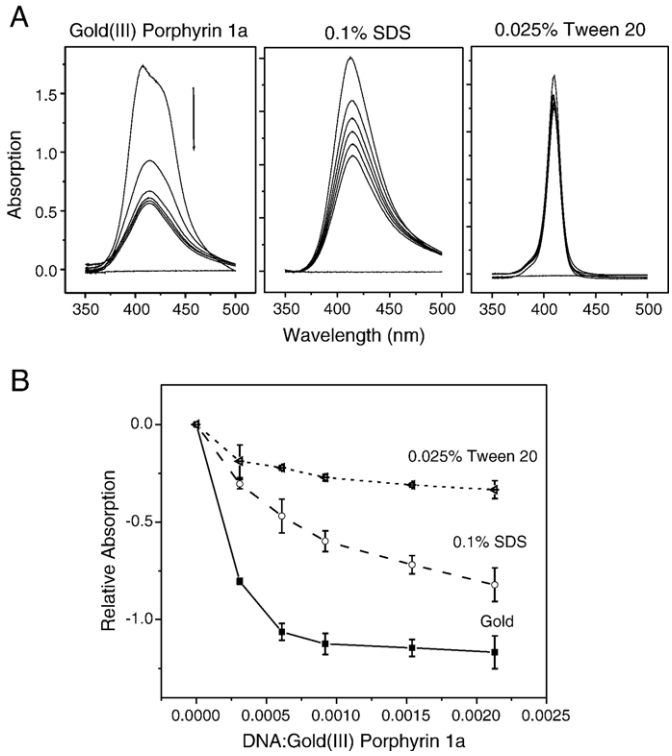

Fig. 7. Spectrophotometric binding assay of gold(III) porphyrin 1a to isolated SUNE1 DNA. (A) Titration curve of DNA-gold(III) porphyrin 1a interaction in the presence or absent of detergents. Absorption spectra of gold(III) porphyrin 1a (0.05 mM) in the absence (top) and presence of DNA (DNA:Gold = 3.1E-4, 6.1E-4, 9.2E-4, 1.5E-3, 2.1E-3 respectively) in 5 mM Tris–HCl buffer pH 7.3. Arrow shows that the absorbance changes upon increasing DNA concentration. (B) Detergents reduced DNA-gold(III) porphyrin 1a interaction. The binding of gold(III) porphyrin 1a to DNA decreased after detergent was added into the reaction mixture. Data are mean values from three independent experiments.

cell in media with 0% to 10% of FBS respectively for 24 h. Interestingly, we found that the cytotoxicity of cisplatin was significantly reduced by the increasing contents of FBS in the media, while cytotoxicity of gold(III) porphyrin 1a was less effected (Fig. 4). Molecular structure of gold(III) porphyrin established by X-ray crystallography showed that the gold ion was located in the center of the porphyrin ring (Fig. 5). All the respective angles between the *trans* pyrrolic nitrogen atoms (i.e., N–Au–N = 177.3°) were close to linearity, and all the Au–N distances are found identical (i.e., 2.03 Å). The robust square-planar geometry of the gold(III) porphyrin may hinder the demetallation or binding events induced by serum protein. Thus, its cytotoxicity was maintained with increasing concentration of FBS. These results suggested that the proteins in the serum could not bind gold(III) porphyrin 1a, but interacted with cisplatin. The difference in plasma protein binding may have profound effects on both the disposition and activity of drugs, especially drugs like the anticancer platinum derivatives such as cisplatin, which could covalently bind to serum proteins (Gamelin et al., 1998; Timerbaev et al., 2006). Our data suggested that gold(III) porphyrin 1a would have fewer problems in this aspect as an anticancer drug. This may also be the reason that the cytotoxicity of gold(III) porphyrin 1a is higher than that of cisplatin.

3.4. Gold(III) porphyrin 1a binds DNA in vivo

Previous findings indicated that most drugs interacted with DNA, for example, cisplatin caused inter- and intra-strand DNA cross-link (Eastman, 1990; Howe-Grant et al., 1976; Poirier et al., 1982) and ethidium bromide caused DNA breakage (Begusova et al., 2000; Huang et al., 1983). We therefore investigated whether gold(III) porphyrin 1a could also bind to DNA. Our data showed that the amount of DNA in the lower molecular region increased with gold(III) porphyrin 1a treatment, which was similar to ethidium bromide treatment. By contrast, cisplatin decreased the amount of low molecular DNA due to its cross-linkage action (Cohen et al., 1979; Lippard, 1982; Lippard and Hoeschele, 1979). With the increasing dose of gold(III) porphyrin 1a, the amount of small molecular weight DNA increased, suggesting that gold(III) porphyrin 1a causes

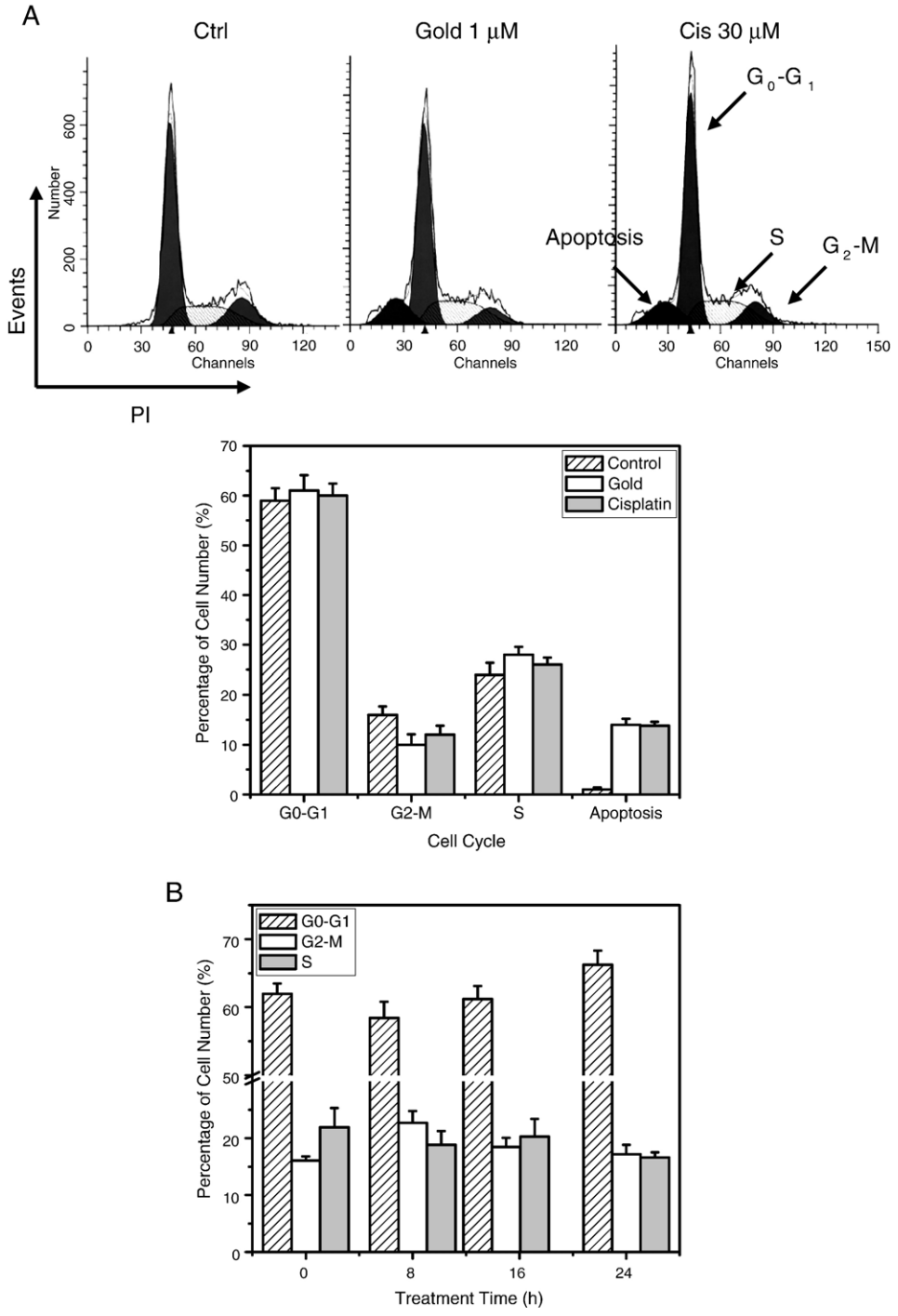

Fig. 8. Flow cytometric analysis on the cell cycle distribution of SUNE1 cells after treatment with gold(III) porphyrin 1a and cisplatin. (A) Flow cytometric analysis on the cell cycle distribution of SUNE1 cells after treatment with gold(III) porphyrin 1a and cisplatin. (B) Time course results of 4 μM of gold(III) porphyrin 1a for 8, 15 and 24 h. Data are mean values from three independent experiments. Columns, means from three separate experiments; bars, S.D.

DNA fragmentation instead of crosslinkage (Fig. 6). To further study the effects of gold(III) porphyrin 1a on DNA molecular, we carried out spectrophotometric titrations of DNA with gold (III) porphyrin 1a to test if this gold containing complex formed DNA adducts similar to those of other metal compounds.

3.5. Gold(III) porphyrin 1a interact with DNA noncovalently

Spectrophotometric titrations of gold(III) porphyrin 1a with naked DNA were carried out to detect and quantify the binding of the gold complex to nucleic acid. The binding of gold(III) porphyrin 1a to DNA was also compared by adding various concentrations of detergents in the reaction mixtures. Spectral changes observed in the range of 350–500 nm suggested a high-affinity association of the gold complex with double-stranded nucleic acids. The reduction of absorption spectra upon the addition of detergents indicated that gold(III) porphyrin 1a did not form a covalent bond with DNA (Fig. 7). This was in contrast to cisplatin, which did form a covalent bond with DNA (Aird et al., 2002; Boudsocq et al., 2005). These results were consistent with previous reports that metalloporphyrins interacted with DNA in a noncovalent fashion (Araki et al., 2000; Dixon et al., 2005; Jin et al., 2006; Kang et al., 2005; Mettath et al., 1999).

3.6. Gold(III) porphyrin 1a induces SUNE1 cell cycle arrest and apoptosis

It has been reported that cisplatin caused cell cycle arrest and inhibition of DNA synthesis in a panel of human cancer cell lines (Orlandi et al., 2001). We investigated the effects of gold (III) porphyrin 1a on cell cycle regulation and apoptosis. In the current experiment, we performed dose dependent cell cycle analysis of gold(III) porphyrin 1a and compared it with cisplatin. Twenty-four hours incubation with 4 μM of gold (III) porphyrin 1a and 30 μM of cisplatin respectively caused SUNE1 cell arrest at G_0–G_1 phase and apoptosis (Fig. 8A). In order to confirm these results, we carried out a time course study on 4 μM gold(III) porphyrin 1a under the same conditions. Our results showed that initially gold complex caused cell arrest at G_2-M phase (at 8 h treatment). The SUNE1 cells then progressed from G_2 to G_0 phase gradually, with a concomitant cell number decrease in the G_2-M and S phase and an increase in the G_0–G_1 phase (Fig. 8B). These results indicated that gold (III) porphyrin 1a can inhibit cell growth, partly through induction of cell cycle arrest and then induce apoptosis in SUNE1 cells.

3.7. Gold(III) porphyrin 1a enhances expression of p53

To obtain further evidence that gold(III) porphyrin 1a causes cell death and accumulates apoptotic cells in the sub-G_0 phase, we investigated the expression of the apoptosis-related and cell cycle-controlled protein p53 by Western blot analysis. The level of p53 increased in a time dependent manor with gold(III) porphyrin 1a treatment, indicating that gold(III) porphyrin 1a indeed induced apoptosis in SUNE1 cells, and the cell cycle arrest caused by gold(III) porphyrin 1a was related to p53 elevation (Fig. 9).

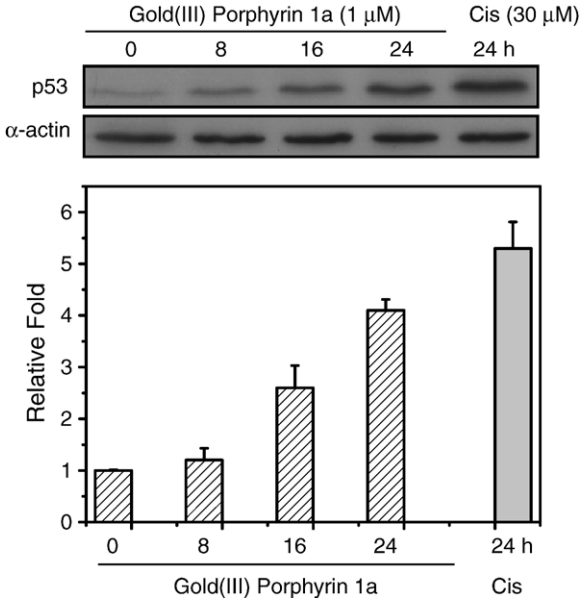

Fig. 9. Western blot analysis of p53 expression under gold(III) porphyrin 1a treatment. The same blot was also re-probed with a monoclonal anti-β-actin antibody to monitor the loading difference. This is representative from three independent experiments. The relevant fold expression of p53 was normalized to β-actin intensity and presented as a chart. Columns, means from three separate experiments; bars, S.D.

4. Dicussion

4.1. Gold(III) porphyrin 1a exerts higher cytotoxicity than cisplatin

Our study indicated that the cytotoxicity of gold(III) porphyrin 1a was higher than that of cisplatin by NBB staining assay in SUNE1 cell lines (Fig. 2A), and previous data also reported that only minimal cross-resistance with cisplatin was detected (Che et al., 2003; Coronnello et al., 2000). Phototoxicity assay further revealed that cytotoxicity of gold(III) porphyrin 1a was not linked to its photosensitizing activity (Fig. 2B). The visible absorption spectrum of gold(III) porphyrin 1a showed that the prominent absorption band was at 407 nm (Supplemental Fig. 1), which was different from hypericin (Supplemental Fig. 1) and most other photosensitizing agents. The major absorption band of previous reported photosensitizing agents belong to the 500 nm to 700 nm region (Hendrickx et al., 2005; Tong et al., 2000).

The impaired cytotoxicity of cisplatin was perhaps partly due to its high rate of non-specific binding to a number of extracellular and intracellular proteins, especially the interaction with blood constituents, such as serum albumin (Cole and Wolf, 1980; Gullo et al., 1980; Morazzoni et al., 1998; Timerbaev et al., 2006). It has been previously reported that following intravenous administration of cisplatin, more than 90% of platinum in the blood was covalently bond to the serum proteins (Bednarski, 1995; Gullo et al., 1980). The cisplatin-protein

interaction certainly has a major impact on drug pharmacology and efficacy, strongly decreasing the effective concentration of the drug that reaches tumor cells. Since albumin plays a central role in the molecular pharmacology of drugs used in cancer chemotherapy and could change the biological clinical effectiveness of certain anticancer drugs, we carried out serum (Fetal Bovine Serum) dependent test to gain insight into gold (III) porphyrin 1a in this respect. Our FBS-dependent cell survival experiment showed that cell survival rate did not change significantly with 1 μM gold(III) porphyrin 1a treatment, but steadily increased with 30 μM cisplatin treatment with the increase of FBS concentrations in cultural media. As revealed by X-ray crystallography, complexation of gold(III) ion with free-based porphyrin resulted in the formation of gold(III) porphyrin complex with nearly perfect square-planar geometry (Fig. 5). The tetradentate porphyrin system provides strong chelating effect and the rigid scaffold which render the resulting complex to have adequate stability against protein-induced demetallation and undesirable reduction to two-coordinate gold (I) by raising kinetic barrier (inner-sphere reorganization energy). This accounts for the stability of gold(III) porphyrin in physiological condition and in turn explain the fact that this compound is more potent than other gold(III) analogues as well as the clinically-used anticancer agent cisplatin. In addition, in call cases, the gold center remains in the +3 oxidation state largely due to the stabilization effects played by the porphyrin ligand. No redox interaction with protein side chains was observed both in our previous study and in others' reports (Che et al., 2003; Messori et al., 2000).

4.2. Gold(III) porphyrin 1a binds to DNA noncovalently and differently from cisplatin

The cytotoxicity of cisplatin originates from its binding to DNA in a covalent cross-linking manner. The 1,2-intrastrand *d* (GpG) cross-links represent the major adduct. Binding of cisplatin to DNA causes significant distortion of helical structure and results in inhibition of DNA replication and transcription (Jamieson and Lippard, 1999; Lee et al., 2002). The anticancer activity of cisplatin is also influenced by the efficiency of cisplatin-DNA adduct removed by the repair machinery, with nucleotide excision repair being a major pathway. The clinical success of cisplatin is limited by its significant side effects and acquired or intrinsic resistance. As a result there has been considerable interest in the design of new platinum compounds with improved pharmacological properties and a broader range of anticancer activity. Research is also being conducted into the development of metal complexes that bind to DNA in a manner fundamentally different from cisplatin, in the hope that such an approach can overcome the resistance pathways that have evolved to eliminate the cytotoxicity of the drug.

A previous study (Mirabelli et al., 1986) indicated that the ability of gold complexes bind to DNA and produce gold-DNA adducts is not dependent on the oxidation state of gold in the complex, but is influenced by the nature of the coordinating ligands. By examining the chemical structure of this gold(III) porphyrin 1a (Figs. 1 and 5), we hoped to determine whether it would bind to DNA and, if so, whether it did so in the same fashion as cisplatin. Interestingly, our results showed that gold (III) porphyrin 1a probably causes DNA fragmentation *in vivo*, unlike cisplatin but similar to ethidium bromide. Our DNA titration study indicated that the interaction between gold(III) porphyrin 1a and DNA appeared to be non-covalent and thus reversible. This kind of noncovalent interaction of metalloporphyrins with DNA has been reported by others (Araki et al., 2000; Dixon et al., 2005; Jin et al., 2006; Kang et al., 2005; Mettath et al., 1999).

4.3. SUNE1 cells undergo apoptosis induced by gold(III) porphyrin 1a treatment

Apoptosis is a tightly controlled process in which cell death is executed through the activation of specific signaling pathways. Within cells, there are positive and negative regulatory pathways of apoptosis. There is a balance between these pathways that determines cell fate. In addition, tumor-suppressor genes are able to induce apoptosis. In response to DNA damage, p53 is activated and capable of regulating gene expression. One mechanism of p53-induced apoptosis is the transactivation of several genes that contribute to apoptosis, such as Bax or Fas (Schuler and Green, 2001). Although the exact mechanism by which p53 induces apoptosis is unclear, it is evident that p53 depends on its ability to regulate gene expression.

Based on the flow cytometric data, we found that gold(III) porphyrin 1a induced apoptosis and also caused cell cycle arrest initially at G_2-M phase and then at G_0–G_1 with gold(III) porphyrin 1a treatment. Western blot analysis further demonstrated that the pre-apoptotic proteins p53 is upregulated in gold (III) porphyrin 1a treatment. Our present data suggest that gold porphyrin 1a could induce cancer cell apoptosis by up regulating p53, arresting cell cycle at G_0–G_1, and inhibiting cellular DNA synthesis in SUNE1 cell.

4.4. Gold(III) porphyrin 1a can act as tumor targeting reagent

DNA-alkylating agents, such as cisplatin, are practically nonselective and cannot distinguish cancer cells from normal cells. They thus exhibit severe toxicity to normal tissues. One way to enhance tumor selectivity is to modify the chemical structures of the agents. Porphyrins are known to be rapidly and preferentially taken up by the tumor cells with higher intakes of lipoproteins (Georgiou et al., 1994; Villanueva and Jori, 1993). Rapidly proliferating cells, such as tumor cells, may give more opportunity for the porphyrins to interact with G quadruplexes formed by single-strand overhangs (Rha et al., 2000). Carrano et al. (1977) reported porphyrin accumulation in tumor tissue, and suggested that porphyrin containing compounds can be used for tumor targeting agents. The tumor-selectivity of porphyrin derivatives and their photodynamic action subsequently led to their clinical use as sensitizers for photodynamic therapy (PDT) of various cancers (Ali and van Lier, 1999). Since gold (III) porphyrin 1a is a porphyrin-containing reagent, it is expected to be more tumor selective than other anticancer agents, including cisplatin.

In conclusion, we demonstrated that gold(III) porphyrin 1a executes potent anticancer cytotoxicity in a mode different from cisplatin, and induces apoptosis in SUNE1 cell line. Gold(III) porphyrin 1a exhibits higher cytotoxicity than cisplatin which is not linked to its photosensitizing activity, and its binding to serum proteins is less than cisplatin. The interaction of gold(III) porphyrin 1a and DNA is non-covalent, and with a different consequence. Flow cytometric studies showed that gold(III) porphyrin 1a abrogates G_0–G_1 cell cycle, then undergoes apoptosis. Western blot analysis of the cell cycle-controlled and apoptosis-related proteins p53 further supported this conclusion. Its cytotoxic activity and porphyrin image-containing structure indicate that gold(III) porphyrin 1a offers considerable potential for development as an anticancer drug.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejphar.2006.10.034.

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