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Review

Gold complexes as prospective metal-based anticancer drugs

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Summary. Medical and therapeutic value of gold has been recognized thousands of years ago, but its rational use in medicine has not begun until the early 1920s. Cisplatin is one of the first metal-containing compounds with anti-cancer activity discovered in the 1960s. Despite the fact that cisplatin treatment is efficient for several types of solid tumors, its effectiveness is limited by toxic side effects and tumor resistance that often leads to the occurrence of secondary malignancies. Since gold(III) is isoelectronic with platinum(II) and tetracoordinate gold(III) complexes have the same square-planar geometries as cisplatin, the anticancer activity of gold(III) compounds has been investigated. Previous studies suggested that, in contrast to cisplatin, gold complexes target proteins but not DNA. Recently, we have investigated gold(III) dithiocarbamates for their anticancer activity and showed that their primary target is the proteasome. Treatment of human breast tumor-bearing nude mice with a gold(III) dithiocarbamate complex resulted in significant inhibition of tumor growth, associated with proteasome inhibition and massive apoptosis induction *in vivo*. Better understanding of physiological processing of gold compounds will provide a rational basis for their further development into novel anticancer drugs.

Key words: Metal-based drugs, Gold complexes, Anti-cancer drugs

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Introduction

Medical and therapeutic value of gold has been recognized thousands of years ago, but its rational use in medicine has not begun until the early 1920s. It was initiated with the finding of the bacteriologist Robert Koch that $K[Au(CN)_2]$ could kill the bacteria that cause tuberculosis. However, because of the serious side effects associated with $K[Au(CN)_2]$ observed, the treatment of pulmonary tuberculosis was switched to the less toxic Au(I) thiolate complexes. French physician Jacque Forestier then introduced these complexes for treatment of rheumatoid arthritis, a condition which he believed was related to tuberculosis (Forestier, 1935). For many decades, Au(I) thiolate complexes were considered as a drug of choice for rheumatoid arthritis treatments in many countries. The toxic side effects observed with these compounds prompted the search for new, less toxic gold complexes. Since coordination of gold(I) with phosphine ligands was shown to stabilize its 1+ oxidation state, many new gold(I) phosphine complexes have been investigated. Among them, triethylphosphine compounds were shown to have optimal pharmacological activity in models of rheumatoid arthritis, and as a result, a new drug, auranofin (Fig. 1), for treatment of rheumatoid arthritis was developed. After auranofin's introduction in 1985, no other gold drug has been clinically approved for either rheumatoid arthritis or any other disease. Unfortunately, not all the patients benefit from auranofin treatment and the toxicity problem has still remained. For that reason, gold(I) drugs are currently used more as a last-resort treatment for severe cases of rheumatoid arthritis (Tiekink, 2003).

However, the known immunosuppressive and anti-inflammatory actions of anti-cancer drugs have established, at least in principle, a connection between anti-arthritic and anti-cancer therapies (Ward, 1988). Also, most of the major classes of pharmaceutical agents

contain metal compounds that are used for treatment of different types of disorders, and new areas of application are rapidly emerging (Sadler and Guo, 1998). Since cancer incidence and its mortality are rising worldwide, it is not surprising that anti-cancer therapy is one area explored for the use of metal-based drugs. After the successes achieved with cisplatin (Fig. 1) in cancer chemotherapy, other metal complexes have been widely studied as anti-cancer agents. The most extensively investigated metal complexes are those containing gold, primarily because gold is one of the oldest metals used in medicine (Kostova, 2006).

Gold(I) complexes and cancer

In the past years, a number of studies have shown that auranofin indeed presented an in vitro activity similar to that of cisplatin (Simon et al., 1979; Mirabelli et al., 1986; Ni Dhubhghail and Sadler, 1993). Encouraged by these data, a series of gold(I) coordination complexes, including auranofin analogs, were evaluated for *in vitro* cytotoxic potency against both B16 melanoma cells and P388 leukemia cells and *in vivo* anti-tumor activity against P388 leukemia in mice (Mirabelli et al., 1986). Auranofin and a number of its analogs showed potent cytotoxic activity against both cell lines *in vitro* and anti-tumor activity against leukemia in vivo, and among them, phosphine-coordinated gold(I) thiosugar complexes appeared to be the most potent. However, these complexes were found to be completely inactive against solid tumors (Fricker et al., 2003). The main observations from these experiments were that (i) lack of potency *in vitro* correlates well with lack of anti-tumor activity; (ii) potent cytotoxicity in vitro is not necessarily predictive of anti-tumor activity in vivo; and (iii) in vivo anti-tumor activity is generally optimized by ligation of Au(I) with a substituted phosphine and a thiosugar.

More promising indications were achieved with a series of digold phosphine complexes, such as gold(I) 1,2-bis(diphenylphosphine)ethane (DPPE), which were shown to confer *in vitro* cytotoxic activity especially in some cisplatin-resistant cell lines (Sadler and Sue, 1994). Mechanistic studies suggested that, in contrast to cisplatin, DNA was not the primary target for these gold(I) complexes and that their cytotoxicity was mediated by their ability to alter mitochondrial function and inhibit protein synthesis by causing DNA-protein cross-links (Kostova, 2006). Although these compounds had marked cytotoxic and anti-tumor activity against P388 leukemia, they had limited activity against solid tumor models. These compounds were not entered for clinical trials, due to problems associated with cardiotoxicity highlighted during pre-clinical toxicology studies (Fricker, 1999).

Gold(III) complexes and cancer

Primarily because of the high reactivity of gold(III) complexes, they have not been as thoroughly investigated as gold(I) complexes. Having a high redox potential and relatively poor stability, the use of gold(III) complexes as anticancer drugs was questioned under physiological conditions (Ronconi et al., 2005). Given that the mammalian environment is generally reducing, compounds with gold(III) were expected to be reduced in vivo to gold(I) and metallic gold, which makes them less effective as drugs (Ronconi et al., 2006). In recent years, new gold(III) compounds with much higher stability have been synthesized using better ligands that usually have nitrogen atoms as donor groups (Messori et al., 2003).

The interest for metal complexes was markedly increased after platinum(II) complexes exhibited promising results against selected types of cancers. Since gold(III) is isoelectronic (d^8) with platinum(II) and tetracoordinate gold(III) complexes have the same square-planar geometries as cisplatin (Ronconi et al., 2006), the anticancer activity of gold(III) compounds has been investigated.

It should be noted that at the beginning of exploration of metal complexes as potential anti-cancer drugs, it was believed that the more the metal complexes are chemically and structurally related to cisplatin, the more their mechanism of action will resemble that of cisplatin (Calamai et al., 1998). That was mostly due to a tendency to believe that the activity of metal compounds is determined solely by the presence of the metal itself. It has been known that metal centers are very important for the biological activity of various metal-containing proteins and enzymes, and that metals are often responsible for the activity of organic drugs. The classic example is cisplatin, which exerts its anti-tumor activity

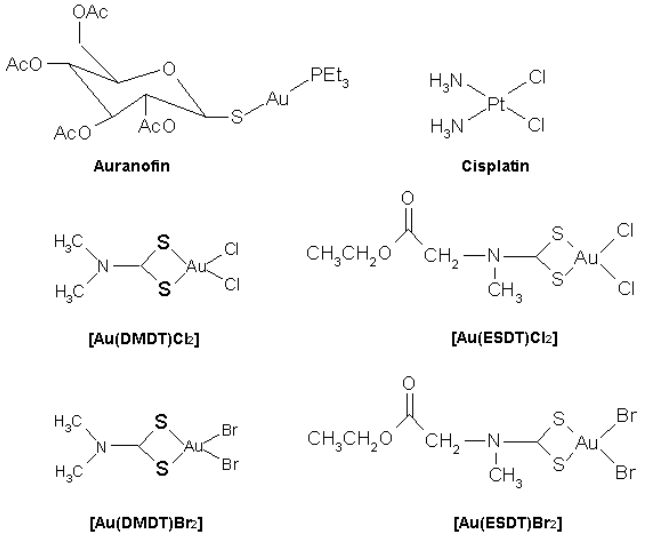

Fig. 1. Chemical structures of cisplatin, auranofin and gold(III) dithiocarbamates.

by interacting with DNA (Zhu et al., 2005), forming a unique lesion that has not been mimicked by any other organic drug. However, the metals that exist in different oxidation states, such as gold, have rich coordination chemistry (Sadler, 1982), and therefore, even subtle changes in the structure of these metal complexes can result in dramatic changes in their physicochemical and thus biological properties. Another important consideration is that the precise molecular structure of many metal-based drugs is not completely established, thus their mechanisms of action are often not well understood. Sometimes, even when a compound is well characterized, it is still difficult to understand the molecular mechanism underlying its biological effects. For instance, gold(III) compounds were synthesized in such a way as to reproduce the main features of cisplatin, and were later found not to have the same molecular target (see below). Additionally, other factors such as kinetic lability, hydro- and lipophilicity, redox behavior, and electric charge may also affect their mechanisms of action. Therefore, the activity of a metal complex depends not only on the metal itself, but also on its oxidation state, number and types of ligands bound, and the coordination geometry of the complex. Since many metal-based drugs act as pro-drugs that undergo ligand substitution and redox reactions before they reach their targets, it is important to recognize these processes and learn how to control them.

The first promising result was obtained with a class of gold(III) complexes with 2-[(dimethylamino) methyl]phenyl] (damp) ligand, synthesized in such a way to stabilize gold in its 3+ oxidation state. These complexes exhibited cytotoxic effects against several human cancer cell lines, comparable to, or greater than cisplatin. Moreover, these complexes retained cytotoxic activity even against cisplatin-resistant cell lines (Fricker, 1999).

Dinger and Henderson synthesized cycloaurated N,N-dimethylbenzylamine complexes with either diphenylurea (Dinger and Henderson, 1998a) or salicylate and thiosalicylate (Dinger and Henderson, 1998b) as the dianionic ligands. All these complexes demonstrated cytotoxic activity *in vitro*, with the methoxy substituted N,N-dimethylbenzylamine cyclometallated gold(III) thiosalicylate complex being the most potent. However, these compounds were evaluated only against the P388 leukemia cell line, and therefore their potential activity against human tumors requires further investigation.

Recently *in vitro* activity of a series of gold(III) complexes, $[Au(en)_2]Cl_3$, $[Au(dien)Cl]Cl_2$, $[Au(cyclam)](ClO_4)Cl_2$, $[Au(terpy)Cl]Cl_2$, and $[Au(phen)Cl_2]Cl$, against the A2780 ovarian cancer cell line and a cisplatin-resistant variant were described (Messori et al., 2000; Marcon et al., 2002). The relative order of cytoxicity was: Au(terpy) >> Au(phen) > Au(en), Au(dien) >> Au(cyclam). Interestingly the three most active compounds retained activity against the cisplatin-resistant cell line (Messori et al., 2000; Marcon et al., 2002).

A number of other gold(III) complexes have been synthesized and their cytotoxic activities have been evaluated. Calamai et al. tested a group of square planar gold(III) complexes containing at least two gold-chloride bonds in cis-position (Calamai et al., 1998). Bruni et al. synthesized four gold(III) complexes: trichloro(2-pyridylmethanol) gold(III) $[AuCl_3(Hpm)]$, dichloro(N-ethylsalicylaldiminato) gold(III) $[AuCl_2(esal)]$, trichlorodiethyl-endiamine gold(III) $[AuCl(dien)]Cl_2$, and trichlorobisethylendiamine gold(III) $[Au(en)_2]Cl_3$ (Bruni et al., 1999). All of these complexes showed significant cytotoxic effects against the A2780 human ovarian cancer cell line, comparable to or even greater than cisplatin, and they were able to overcome resistance to cisplatin to a large extent (Bruni et al., 1999).

Previous studies on potential molecular targets for gold complexes

It is a well-known fact that metals easily lose electrons and form positively charged ions, and therefore have a tendency to bind to and interact with biological molecules. Cisplatin was one of the first metal complexes used for cancer treatment with its anti-cancer activity discovered in the 1960s (Alderden et al., 2006). It is responsible for the cure of more than 90% of testicular cancer cases and plays a vital role in the treatment of cancers such as ovarian, head and neck cancer, bladder cancer, cervical cancer, melanoma, and lymphomas (Wong and Giandomenico, 1999). Cisplatin exerts its anti-cancer effect by interacting with DNA and forming adducts which interfere with transcription and DNA replication, thereby triggering programmed cell death or apoptosis (Eckhardt, 2002). The effectiveness of cisplatin treatment is limited by the phenomenon of tumor resistance, and since it is a non-specific, it results in significant toxicity, including nephrotoxicity, neurotoxicity, and myelotoxicity (Criado et al., 2003).

Unlike cisplatin, the main biological targets for gold compounds are still unknown. Considering that some gold drugs have already been in use and that many gold compounds have been examined for their potential use in medicine, it is surprising that their mechanisms of action are still not well understood. However, it has been established that gold(I) drugs are in fact pro-drugs since, upon administration to the patients, they get rapidly metabolized generating the pharmacologically active species (Tiekink, 2003). It has been shown that within 20 minutes upon auranofin administration, gold(I) primarily exists as a protein-bound in the serum, and its reactions with the albumin are well described (Shaw, 1999). Recently, Rigobello et al. observed that auranofin and other gold(I) compounds, in presence of calcium ions, are able to induce mithocondrial swelling, membrane potential decrease and stimulation of respiration dependent on permeability transition of mithocondrial membrane (Rigobello et al., 2004). The role of mitochondria in the mechanism of cytotoxicity and

antitumor activity exerted by gold derivatives has been reviewed recently (McKeage et al., 2002).

To obtain compounds with superior chemotherapeutic index in terms of increased bioavailability, higher cytotoxicity, and lower side effects than cisplatin, Ronconi et al. synthesized new gold(III) dithiocarbamate derivatives (Ronconi et al., 2005). The choice of dithiocarbamate ligands was not accidental; they were being evaluated for their efficacy as inhibitors of cisplatin-induced nephrotoxicity without decreasing its antitumor activity (Bodenner et al., 1986; Borch et al., 1988; Huang et al., 1995). The synthesized gold complexes were tested for their in vitro cytotoxic activity toward a panel of human tumor cell lines. Remarkably, most of them, in particular gold(III) derivatives of N,N-dimethyldithiocarbamate and ethylsarcosinedithiocarbamate, [Au(DMDT)Cl_2], [Au(DMDT)Br_2], [Au(ESDT)Cl_2] and [Au(ESDT)Br_2] (Fig. 1), were shown to be 1- to 4-fold more cytotoxic than cisplatin and to overcome to a large extent both intrinsic and acquired resistance to cisplatin.

From the few data available and by comparison with platinum(II) complexes, it might be hypothesized that the biological action of gold(III) complexes and, specifically, their anti-tumor activity is possibly mediated by direct interaction with DNA. The probable binding mode of gold(III) to DNA has been modeled by thorough crystallographic and spectroscopic investigations of gold(III) complexes with nucleosides and nucleotides (Novelli et al., 1999). In addition, a number of studies based on different physicochemical techniques suggest that probable binding sites for gold(III) are N(1)/N(7) atoms of adenosine, N(7) or C(6)O of guanosine, N(3) of cytidine, and N(3) of thymidine, which are analogous to the possible binding sites for the isoelectronic platinum(II) ion (Crooke and Mirabelli, 1983). However, recent studies showed that the *in vitro* interactions of some gold(III) complexes with calf thymus DNA are weak, whereas significant binding to model proteins takes place. This implies that their mechanism of action might be substantially different from that of the clinically established Pt(II) compounds and from Pt(II) complexes with the same ligands (Marzano et al., 2004).

This is supported by the recent studies of Fricker et al. who demonstrated that a gold(III)-damp complex showed a clear preference for S-donor ligands such as glutathione and cysteine, with only limited reactivity against nucleosides and their bases (Fricker et al., 2003). Therefore, a new mechanism was proposed that proteins containing exposed cysteine residues might be proper targets for that class of gold(III) complexes. Gold(III) complexes were also shown to interact with bovine serum albumin (He and Carter, 1992), making very stable adducts that, once formed, were destroyed only by the addition of strong ligands for gold(III) such as cyanide (Marcon et al., 2003). Based on these findings, it has been proposed that selective modification of surface protein residues by gold(III) compounds could be the molecular basis for their biological effects. This has prompted a new search for gold-protein interactions in an attempt to identify possible targets responsible for the biological effects of gold compounds. The key proteins that are modified by gold(III) complexes and responsible for triggering apoptosis have yet to be identified.

Ubiquitin-proteasome pathway and cancer

Recent developments in cancer therapy have focused on targeting the ubiquitin-proteasome system in tumors. As aberrant proteasome-dependent proteolysis seems to be associated with the pathophysiology of some malignancies, there has been a great deal of interest in the possibility that proteasome inhibitors might prove useful as a novel class of anti-cancer drugs. The ubiquitin-proteasome pathway (Fig. 2) plays a major role in the degradation of oxidatively damaged and mutated proteins as well as proteins involved in cell cycle progression, proliferation and apoptosis (Nalepa et al., 2006). The 26S proteasome (Fig. 2) is a large multi-subunit protease complex that is local-ized in the nucleus and cytosol and selectively degrades intracellular proteins. The 26S proteasome identifies and therefore degrades the proteins that have been tagged by a chain of ubiquitin molecules. Ubiquitin is a highly conserved 76-amino acid protein that can be covalently ligated at a lysine residue of the target protein by a multi-enzymatic system consisting of Ub-activating (E1), Ub-conjugating (E2), and the Ub-ligating (E3) enzymes (Fig. 2). An ubiquitinated protein is escorted to the 26S proteasome, the multi-component enzymatic complex, where it undergoes final degradation and the ubiquitin is released and recycled (Ciechanover, 2006). The 20S proteasome, the proteolytic core of 26S proteasome complex, contains multiple peptidase activi-ties (chymotrypsin-like, trypsin-like and peptidylglutamyl peptide hydrolyzing-like/PGPH) and functions as a catalytic machine (Seemuller et al., 1995).

The ubiquitin-proteasome pathway is essential for many fundamental cellular processes, including the cell cycle, apoptosis, angiogenesis and differentiation (Orlowski and Dees, 2002a). The proteasome contributes to the pathological state of several human diseases including cancer, in which some regulatory proteins are either stabilized due to decreased degradation or lost due to accelerated degradation (Ciechanover, 1998). The possibility of targeting the ubiquitin-proteasome pathway therapeutically has met great skepticism, since this pathway plays an important role in normal cellular homeostasis, as well. However, with the demonstration that proteasome inhibitors were well tolerated and had activity in models of human malignancies in vivo (Orlowski et al., 1998), the proteasome inhibitor Velcade/ PS-341 was introduced into Phase I safety trials (Adams et al., 1999). The data from the Velcade trials showed acceptable toxicity with significant clinical benefit (Orlowski et al., 2002b). Furthermore, the fact

that actively proliferating cancer cells are more sensitive to apoptosis-inducing stimuli, including proteasome inhibitors, makes proteasome inhibitors even more attractive (Dou and Nam, 2000; Almond and Cohen, 2002; Adams, 2003).

The proteasome is a primary target of Gold(III)-dithiocarbamate complexes in human breast cancer cells

In our previous work, we investigated the molecular mechanism responsible for gold(III) dithiocarbamate-mediated tumor cell-killing activity (Milacic et al., 2006). We first showed that selected gold(III) dithiocarbamate complex, compound 2 or Au(DMDT)Br_2 (Fig. 1), potently inhibited proliferation of different breast cancer cell lines, including pre-malignant MCF10K.cl2, malignant MCF10dcis.com, estrogen receptor α-positive MCF7, and estrogen receptor α-negative MDA-MB-231. Also, compound 2 was much more potent than cisplatin under our experimental conditions. Compound 2, at 5 μM, inhibited 85% of MDA-MB-231 cell proliferation, compared to less than 20% inhibition by 5 μM of cisplatin. Even when 50 μM of cisplatin was used, only ~ 40% inhibition was observed. Moreover, compound 2 at 5 μM for 2 h induced apoptotic morphological changes in MDA-MB-231 cells, while cisplatin at 50 μM for 48 h did not induce such changes (Milacic et al., 2006). Our data support the argument that the mechanisms of action of platinum(II) and gold(III) complexes are different.

Since we have previously shown that copper was capable of irreversible inhibition of the proteasome in time- and concentration-dependent manners under in vitro conditions (Daniel et al., 2004, 2005), we hypothesized that gold and copper might use the same mechanism against cancer cells. We found that four tested gold(III) dithiocarbamates inhibited the proteasomal chymotrypsin-like activity in MDA-MB-231 whole cell extract in a concentration-dependent manner. To provide direct evidence for proteasome inhibition by gold compounds, we performed a cell-free proteasome activity assay using a purified 20S proteasome and compound 2. We discovered that compound 2 significantly inhibited all three activities of the purified 20S proteasome, especially its chymotrypsin-like activity (Milacic et al., 2006). This finding is particularly important since it has been reported that inhibition of the proteasomal chymotrypsin-like activity is associated with growth arrest and/or apoptosis induction in cancer cells (Lopes et al., 1997; An et al., 1998).

After we demonstrated that compound 2 could inhibit the purified proteasomal chymotrypsin-like activity, we then tested its effect in intact MDA-MB-231 cells and found similar inhibitory effects. Proteasomal inhibition by compound 2 was confirmed by decreased proteasomal activity and increased levels of ubiquitinated proteins and the proteasome target protein p27. Most importantly, inhibition of the proteasome activity and accumulation of p27 were also found in MDA-MB-231 xenografts treated with compound 2 (Milacic et al., 2006). All together, these findings clearly

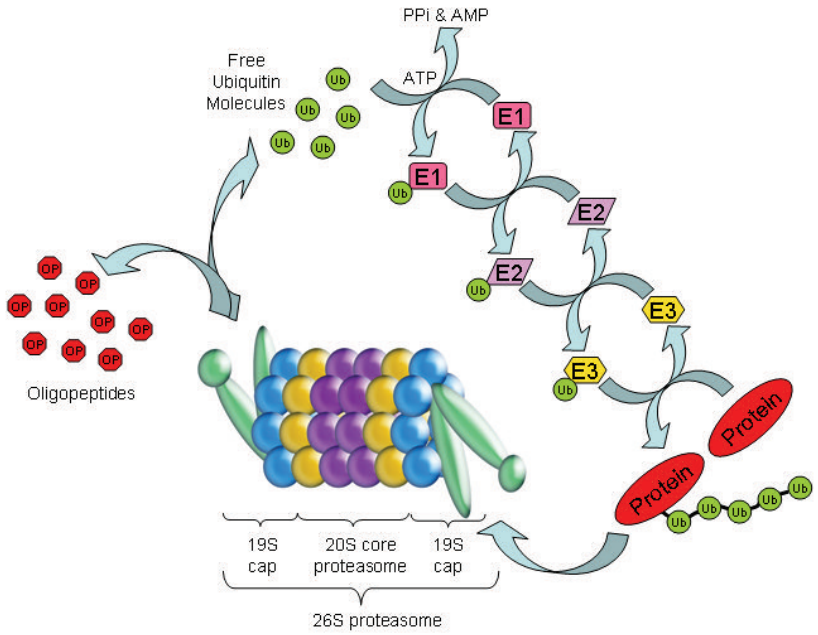

Fig. 2. The ubiquitin-proteasome pathway. A target protein degraded by the ubiquitin-proteasome pathway is first covalently modified by multiple ubiquitin (Ub) molecules in a three-stepped, highly regulated enzymatic process involving an Ub-activating (E1), Ub-conjugating (E2), and the Ub-ligating (E3) enzymes. The ubiquitinated protein is then escorted to the 26S proteasome, recognized by the 19S cap and then degraded by the catalytic 20S core into oligopeptides. The ubiquitin molecules are released and recycled.

indicate that compound 2 can directly target the tumor proteasome *in vivo*.

It has been reported that various proteasome inhibitors potently induce apoptosis (Lopes et al., 1997; An et al., 1998; Dou and Li, 1999; Almond and Cohen, 2002; Adams, 2003). Therefore, we investigated if gold(III) compound 2 behaved similarly. Indeed, we found that inhibition of the proteasomal chymotrypsin-like activity by compound 2 induced apoptosis in cultured MDA-MB-231 breast cancer cells and in the tumors developed from the same breast cancer cell line. Induction of apoptosis by compound 2 in vitro and in vivo has been shown by multiple assays that measure characteristic cellular and biochemical hallmarkers. For instance, apoptotic morphological changes, the presence of apoptotic nuclei, and apoptosis-specific PARP cleavage were observed in cultured MDA-MB-231 cells treated with compound 2. In the treated tumors, apoptosis induction was confirmed by PARP cleavage, TUNEL and H&E staining assays (Milacic et al., 2006).

Our data strongly suggest that the proteasome is the primary target for gold(III) dithiocarbamates and that inhibition of the proteasomal activity by gold(III) dithiocarbamates is associated with apoptosis in cancer cells. We found that the effect of compound 2 could be completely blocked by two different S-donor ligands, 1,4-Dithio-DL-threitol (DTT), and N-Acetyl-L-Cysteine (NAC). DTT at 1 mM entirely reversed inhibition of the purified 20S proteasome by compound 2, and NAC at 200 µM completely blocked proteasome inhibition by compound 2 in intact MDA-MB-231 cells at both early (4 h) and later (24 h) time points. Consistently, apoptotic morphological changes, PARP cleavage, and accumulation of p36/Bax were also completely prevented in the cells co-treated with compound 2 and NAC (Milacic et al., 2006). It should be noted that NAC at lower concentrations was unable to inhibit copper-mediated proteasome inhibition *in vitro* and in tumor cells (Daniel et al., 2004). We are currently investigating whether NAC at higher concentrations could reverse organic copper-induced events.

There are several possible mechanisms that might be responsible for the reversal of compound 2-mediated proteasomal inhibition by NAC and DTT. First, it has been previously reported that some gold(III) complexes could bind some S-donor ligands, such as glutathione and cysteine, and cleave their disulfide bond(s) (Zou et al., 1999), an event which might be responsible for the biological effects of gold(III) complexes (Marcon et al., 2003). Therefore, it is possible that gold(III) dithiocarbamates could bind sulfhydryl groups found in NAC or DTT. NAC or DTT at high concentrations could react with all the compound 2 molecules, thereby preventing binding to and inhibition of the proteasome (Fig. 3). Second, NAC or DTT could reduce gold(III) to gold(I), an ionic state that does not have the affinity to bind the proteasome and inhibit its activity (Fig. 3). Third, it has been reported that gold(III) porphyrin 1a induces intracellular ox*f*Bidation, altering glutathione (GSH) levels in the cell (Wang et al., 2005). GSH is the main antioxidant system in the cell, and its depletion might facilitate accumulation of reactive oxygen species (ROS) in cells treated with anticancer drugs, which in turn increases the drug lethality (Troyano et al., 2001).

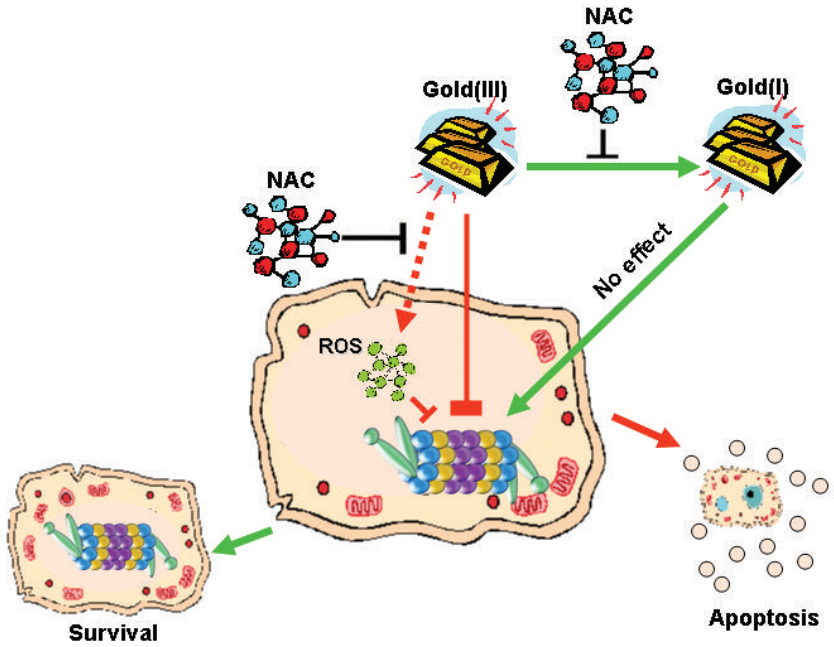

Fig. 3. A schematic representation of possible mechanisms used by NAC to prevent proteasome inhibition and apoptosis induced by gold(III) dithiocarbamates. A gold(III) dithiocarbamate directly binds and inhibits the 26S proteasome. It might also stimulate production of ROS (reactive oxygen species), which then oxidize and inactivate the proteasome. NAC blocks proteasome inhibition either by interacting with gold(III) dithiocarbamate, or by scavenging ROS, or by reducing gold(III) into gold(I).

Compound 2 might stimulate production of ROS, which then oxidize and inactivate the proteasome (Fig. 3). This argument is supported by a report that the proteasome is susceptible to oxidative modification and inactivation upon exposure to free radical generating systems (Szweda et al., 2002). Moreover, we observed that the effect of NAC is much stronger in intact cells compared to cell-free conditions. When intact cells were treated with compound 2, NAC at much lower concentrations could reverse proteasomal inhibition induced by compound 2, arguing that NAC can increase the cellular pool of ROS scavengers. We are currently investigating all these possibilities. A schematic representation of possible interactions between gold(III) compounds and NAC molecules within the cell are shown in Figure 3.

Future directions

Despite the widespread use of metallodrugs, their interactions with cell membranes, proteins, and DNA are still poorly understood. To improve design of metallodrugs, a better knowledge of coordination chemistry under biologically relevant conditions is essential. This includes the understanding of both the thermodynamics (equilibrium constants and structures of products) and kinetics (mechanisms, pathways, ligand-exchange dynamics) of substitution and redox reactions. Since gold has rich coordination chemistry, its biochemistry is potentially very complicated and difficult to understand. Recently, several new gold(III) compounds have been investigated for their anticancer activity. Initial studies using some of them, such as gold(III) dithiocarbamates, were encouraging and indicated that these complexes have a mechanism of action different from that of platinum anti-cancer drugs, and that their primary target is the proteasome. Better understanding of physiological processing of gold compounds will provide a rational basis for their further development into novel anticancer drugs. Future studies should be focused on the selective delivery of gold complexes to cancer cells and to specific tumor targets to increase the effectiveness and better control of the side effects of these compounds.

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References

Adams J. (2003). Potential for proteasome inhibition in the treatment of cancer. Drug Discov. Today 8, 307-315.

Adams J., Palombella V.J., Sausville E.A., Johnson J., Destree A., Lazarus D.D., Maas J., Pien C.S., Prakash S. and Elliott P.J. (1999). Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res. 59, 2615-2622.

Alderden R.A., Hall M.D. and Hambley T.W. (2006). The discovery and development of cisplatin. J. Chem. Edu. 83, 728-734.

Almond J.B. and Cohen G.M. (2002). The proteasome: a novel target for cancer chemotherapy. Leukemia 16, 433-443.

An B., Goldfarb R.H., Siman R. and Dou Q.P. (1998). Novel dipeptidyl proteasome inhibitors overcome Bcl-2 protective function and selectively accumulate the cyclin dependent kinase inhibitor p27 and induce apoptosis in transformed, but not normal, human fibroblasts. Cell Death Differ. 5, 1062-1075.

Bodenner D.L., Dedon P.C., Keng P.C. and Borch R.F. (1986). Effect of diethyldithiocarbamate on cis-diamminedichloroplatinum(II)-induced cytotoxicity, DNA cross-linking, and gamma-glutamyl transpeptidase inhibition. Cancer Res. 46, 2745-2750.

Borch R.F., Dedon P.C., Gringeri A. and Montine T.J. (1988). Inhibition of platinum drug toxicity by diethyldithiocarbamate. In: Platinum and other metal coordination compounds in cancer chemotherapy. Nicolini M. (ed). Martinus Nijhoff. Boston. pp 216-227.

Bruni B., Guerri A., Marcon G., Messori L. and Orioli P. (1999). Structure and cytotoxic properties of some selected gold(III) complexes. Croatica Chemica Acta 72, 221-229.

Calamai P., Carotti S., Guerri A., Mazzei T., Messori L., Mini E., Orioli P. and Speroni G.P. (1998). Cytotoxic effects of gold(III) complexes on established human tumor cell lines sensitive and resistant to cisplatin. Anticancer Drug Res. 13, 67-80.

Ciechanover A. (1998). The ubiquitin-proteasome pathway: on protein death and cell life. EMBO J. 17, 7151-7160.

Ciechanover A. (2006). The ubiquitin proteolytic system: from a vague idea, through basic mechanisms, and onto human diseases and drug targeting. Neurology 66(2 Suppl 1), S7-S19.

Criado J.J., Manzano J.L. and Rodriguez-Fernandez E. (2003). New organotropic compounds. Synthesis, characterization and reactivity of Pt(II) and Au(III) complexes with bile acids: DNA interactions and 'in vitro' anticancer activity. J. Inorg. Biochem. 96, 311-320.

Crooke S.T. and Mirabelli C.K. (1983). Molecular mechanisms of action of auranofin and other gold complexes as related to their biologic activities. Am. J. Med. 75, 109-113.

Daniel K.G., Gupta P., Harbach R.H., Guida W.C. and Dou Q.P. (2004). Organic copper complexes as a new class of proteasome inhibitors and apoptosis inducers in human cancer cells. Biochem. Pharmacol. 67, 1139-1151.

Daniel K.G., Chen D., Orlu S., Cui Q.C., Miller F.R. and Dou Q.P. (2005). Clioquinol and pyrrolidine dithiocarbamate complex with copper to form proteasome inhibitors and apoptosis inducers in human breast cancer cells. Breast Cancer Res. 7, R897-R908.

Dinger M.B. and Henderson W. (1998a). Organogold(III) metallacyclic chemistry. Part 2. Synthesis and characterisation of the first gold(III) ureylene complexes. J. Organomet. Chem. 557, 231-241.

Dinger M.B. and Henderson W. (1998b). Organogold(III) metallacyclic chemistry. Part 4. Synthesis, characterisation, and biological activity of gold(III)-thiosalicylate and -salicylate complexes. J. Organomet Chem. 560, 233-243.

Dou Q.P. and Li B. (1999). Proteasome inhibitors as potential novel anticancer agents. Drug Resist. Update 2, 215-223.

Dou Q.P. and Nam S. (2000). Pharmacological proteasome inhibitors and their therapeutic potential. Exp. Opin. Ther. Patents 10, 1263-1272.

Eckhardt S. (2002). Recent progress in the development of anticancer agents. Curr. Med. Chem. Anticancer Agents 2, 419-439.

Forestier J. (1935). Rheumatoid arthritis and its treatment with gold salts - results of six years' experience. J. Lab. Clin. Med. 20, 827-840.

Fricker S.P. (1999). A screening strategy for metal antitumor agents as exemplified by gold(III) complexes. Metal Based Drugs. 6, 291-300.

Fricker S.P., Skerjl R., Cameron B.R., Mosi R. and Zhu Y. (2003). Recent developments in gold drugs 2003; AnorMED Inc, Langley BC, V2Y 1N5, Canada. GOLD 2003.

He X.M. and Carter D.C. (1992). Atomic structure and chemistry of human serum albumin. Nature 358, 209-215.

Huang H., Zhu L., Reid B.R., Drobny G.P. and Hopkins P.B. (1995). Solution structure of a cisplatin-induced DNA interstrand cross-link. Science 270, 1842-1845.

Kostova I. (2006). Gold coordination complexes as anticancer agents. Anticancer Agents Med. Chem. 6, 19-32.

Lopes U.G., Erhardt P., Yao R. and Cooper G.M. (1997). p53-dependent induction of apoptosis by proteasome inhibitors. J. Biol. Chem. 272, 12893-12896.

Marcon G., Carotti S., Coronnello M, Messori L., Mini E., Orioli P., Mazzei T., Cinellu M.A. and Minghetti G. (2002). Gold(III) complexes with bipyridyl ligands: solution chemistry, cytotoxicity, and DNA binding properties. J. Med. Chem. 45, 1672-1677.

Marcon G., Messori L, Orioli P., Cinellu M.A. and Minghetti G. (2003). Reactions of gold(III) complexes with serum albumin. Eur. J. Biochem. 270, 4655-4661.

Marzano C., Bettio F., Baccichetti F., Trevisan A., Giovagnini L. and Fregona D. (2004). Antitumor activity of a new platinum(II) complex with low nephrotoxicity and genotoxicity. Chem. Biol. Interact. 148, 37-48.

McKeage M.J., Maharaj L. and Berners-Price S.J. (2002). Mechanisms of cytotoxicity and antitumor activity of gold(I) phosphine complexes: the possible role of mitochondria. Coord. Chem. Rev. 232, 127-135.

Messori L., Abbate F., Marcon G., Orioli P., Fontani M., Mini E., Mazzei T., Carotti S., O'Connell T. and Zanello P. (2000). Gold(III) complexes as potential antitumor agents: solution chemistry and cytotoxic properties of some selected gold(III) compounds. J. Med. Chem. 43, 3541-3548.

Messori L., Marcon G. and Orioli P. (2003). Gold (III) compounds as a new family of anticancer drugs. Bioinorg. Chem. Appl 1, 177-187.

Milacic V., Chen D., Ronconi L., Landis-Piwowar K.R., Fregona D. and Dou Q.P. (2006). A novel anticancer gold(III) dithiocarbamate compound inhibits the activity of a purified 20S proteasome and 26S proteasome in human breast cancer cell cultures and xenografts. Cancer Res. 66, 10478-10486.

Mirabelli C.K., Johnson R.K., Hill D.T., Faucette L.F., Girard G.R., Kuo G.Y., Sung C.M. and Crooke S.T. (1986). Correlation of the in vitro cytotoxic and in vivo antitumor activities of gold(I) coordination complexes. J. Med. Chem. 29, 218-223.

Nalepa G., Rolfe M. and Harper J.W. (2006). Drug discovery in the ubiquitin-proteasome system. Nat. Rev. Drug Discov. 5, 596-613.

Ni Dhubhghail O.M. and Sadler P.J. (1993). Gold complexes in cancer chemotherapy. In: Metal complexes in cancer chemotherapy. Keppler B.K. (ed). VCH, Weinheim. pp 221-248.

Novelli F., Recine M., Sparatore F. and Juliano C. (1999). Gold complexes as antimicrobial agents. Farmaco. 54, 232-236.

Orlowski R.Z. and Dees E.C. (2002a). The role of the ubiquitination-proteasome pathway in breast cancer: Applying drugs that affect the ubiquitin-proteasome pathway to the therapy of breast cancer. Breast Cancer Res. 5, 1-7.

Orlowski R.Z., Eswara J.R., Lafond-Walker A., Grever M.R., Orlowski M. and Dang C.V. (1998). Tumor growth inhibition induced in a murine model of human Burkitt's lymphoma by a proteasome inhibitor. Cancer Res. 58, 4342-4348.

Orlowski R.Z., Stinchcombe T.E., Mitchell B.S., Shea T.C., Baldwin A.S., Stahl S., Adams J., Esseltine D.L., Elliott P.J., Pien C.S., Guerciolini R., Anderson J.K., Depcik-Smith N.D., Bhagat R., Lehman M.J., Novick S.C., O'Connor O.A. and Soignet SL. (2002b). Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. J. Clin. Oncol. 20, 4420-4427.

Rigobello M.P., Scutari G., Folda A. and Bindoli A. (2004). Mitochondrial thioredoxin reductase inhibition by gold(I) compounds and concurrent stimulation of permeability transition and release of cytochrome c. Biochem. Pharmacol. 67, 689-696.

Ronconi L., Giovagnini L., Marzano C., Bettio F., Graziani R., Pilloni G. and Fregona D. (2005). Gold dithiocarbamate derivatives as potential antineoplastic agents: design, spectroscopic properties and in vitro antitumor activity. Inorg. Chem. 44, 1867-1881.

Ronconi L., Marzano C., Zanello P., Corsini M., Miolo G., Macca C., Trevisan A. and Fregona D. (2006). Gold (III) dithiocarbamate derivatives for the treatment of cancer: solution chemistry, DNA binding, and hemolytic properties. J. Med. Chem. 49, 1648-1657.

Sadler P.J. (1982). The comparative evaluation of the physical and chemical properties of gold compounds. J. Rheumatol. Suppl. 8, 71-78.

Sadler P.J. and Guo Z. (1998). Metal complexes in medicine: Design and mechanism of action. Pure Appl. Chem. 70, 863-871.

Sadler P.J. and Sue R.E. (1994). The chemistry of gold drugs. Metal Based Drugs 1, 107-144.

Seemuller E., Lupas A., Stock D., Lowe J., Huber R. and Baumeister W. (1995). Proteasome from Thermoplasma acidophilum: a threonine protease. Science 268, 579-582.

Simon T.M., Kunishima D.H., Vibert G.J. and Lorber A. (1979). Cellular antiproliferative action exerted by auranofin. J. Rheumatol. Suppl. 5, 91-97.

Shaw C.F. III (1999). The biochemistry of gold. In: Gold: Progress in chemistry, biochemistry and technology. Schmidbaur H. (ed) John Wiley & Sons. New York. pp 260-308.

Szweda P.A., Friguet B. and Szweda L.I. (2002). Proteolysis, free radicals, and aging. Free Radic. Biol. Med. 33, 29-36.

Tiekink E.R. (2003). Gold derivatives for cancer treatment. Bioinorg. Chem. Applns. 53, 1-9.

Troyano A., Fernandez C., Sancho P., de Blas E. and Aller P. (2001). Effect of glutathione depletion on antitumor drug toxicity (apoptosis and necrosis) in U-937 human promonocytic cells. The role of intracellular oxidation. J. Biol. Chem. 276, 47107-47115.

Wang Y., He Q.Y., Sun R.W., Che C.M. and Chiu J.F. (2005). Gold(III) porphyrin 1a induced apoptosis by mitochondrial death pathways related to reactive oxygen species. Cancer Res. 65, 11553-11564.

Ward J.R. (1988). Role of disease-modifying antirheumatic drugs versus cytotoxic agents in the therapy of rheumatoid arthritis. Am. J. Med. 85, 39-44.

Wong E. and Giandomenico C.M. (1999). Current status of platinum-based antitumor drugs. Chem Rev. 99, 2451-2466.

Zhu C., Raber J. and Eriksson L.A. (2005). Hydrolysis process of the second generation platinum-based anticancer drug cis-amminedichlorocyclohexylamineplatinum(II). J. Phys. Chem B. 109, 12195-12205.

Zou J., Guo Z., Parkinson J.A., Chen Y. and Sadler P.J. (1999). Gold(III)-induced oxidation of glycine. Chem. Commun. 1359-1360.

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