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Mechanisms of cytotoxicity and antitumor activity of gold(I) phosphine complexes: the possible role of mitochondria

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Contents

Abstract

Well known for their clinical anti-arthritic properties, gold-based drugs have also attracted interest as potential antitumor agents with gold(I) phosphine derivatives being among the most active in vivo. Auranofin, a linear tetraacetylthioglucose gold(I) phosphine complex, increased the life span of mice inoculated with P388 leukaemia, inhibited DNA polymerases and was preferentially cytotoxic to cells with altered mitochondria. Triethylphosphine gold(I) chloride inhibited tumor colony formation in vitro, reacted with DNA, and inhibited oxidative phosphorylation, ATP production and the viability of isolated rat hepatocytes. Bis[1,2-bis(diphenylphosphino)ethane]gold(I) chloride ($[Au(dppe)_2]Cl$) had reproducible and significant antitumor activity in a number of murine tumor models in vivo. $[Au(dppe)_2]Cl$ also inhibited tumor colony formation in vitro, formed DNA strand beaks, induced DNA-protein cross links and had antimitochondrial effects on P388 leukemia cells and isolated hepatocytes. Tetrahedral Au(I)complexes of bidentate pyridyl phosphines have shown promising in vitro and in vivo antitumor properties that are determined by their drug lipophilicity. Although the exact intracellular targets responsible for their antitumor activity are unclear, gold(I) phosphines are directly cytotoxic and many appear to have antimitochondrial activity. Optimization of their hydrophilic–lipophilic balance may be key to improving their selectivity for tumor mitochondria versus oxidative phosphorylation pathways of normal cells.

Keywords: Antitumor agents; Cytotoxicity; Gold(I) phosphines; Mitochondria; Mode of action; Selective toxicity

1. Introduction

For many decades, gold derivatives have been used clinically in the treatment of rheumatoid arthritis. Although very well known for this clinical application, gold derivatives have also shown promise as antitumor agents. Among the many gold complexes that have been investigated for potential antitumor properties, gold(I) phosphine derivatives have shown the most reproducible and significant activity in murine tumor models in vivo. Initial interest had focused on the 2-co-ordinate linear gold phosphine complexes, auranofin and triethylphosphine gold(I) chloride (Fig. 1). More recently, the

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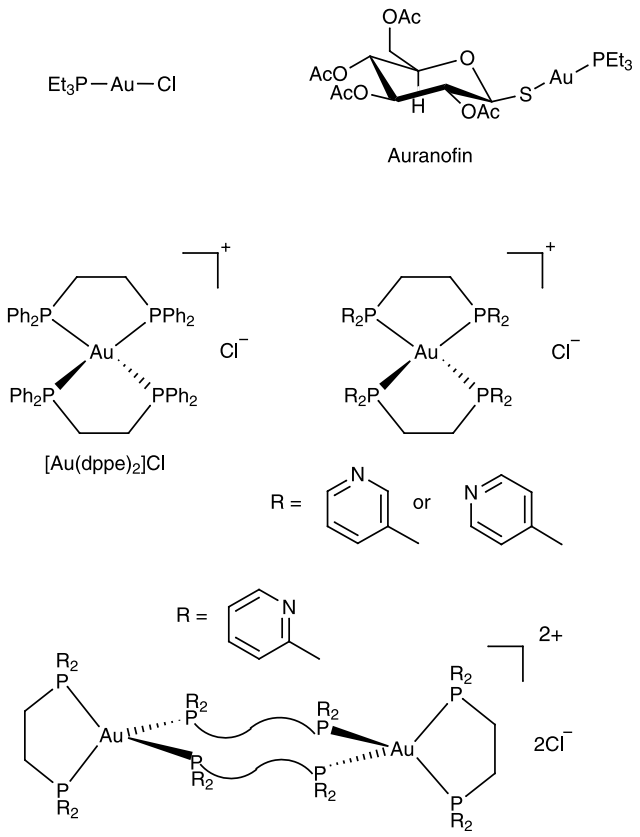

Fig. 1. Chemical structures of antitumor gold(I) phosphine complexes.

cationic tetrahedral gold(I) phosphine complexes, bis[1,2-bis(diphenylphosphino)ethane]gold(I) chloride and bis[1,2-bis(di-*n*-pyridylphosphino)ethane]gold(I) chloride complexes (Fig. 1), have shown a greater degree and wider spectrum of activity in murine tumor models in vivo. The structure–activity relationships that determine the antitumor properties of these gold(I) phosphines have been reviewed previously [1,2]. Progress in this field, and the design of metal–phosphine complexes as antitumor drugs, depends on an understanding of the mechanism of action. Since the linear two co-ordinate and tetrahedral gold(I)phosphine complexes have very different co-ordination chemistry it is particularly important to identify whether they act by a similar mechanism. In this article, current understanding of the mechanism of antitumor and cytotoxic activities is reviewed, in relation to their effects on mitochondria and potential as selective antitumor agents.

2. Auranofin

Auranofin, the orally administered gold-based anti-arthritic agent, emerged as a clinically useful therapeutic drug for the treatment of rheumatoid arthritis in the late 1970s [3]. Auranofin is a monomeric linear Au(I) complex stabilized by triethyl phosphine and tetracetylthioglucose ligands. Within the body, auranofin is known to undergo ligand dissociation reactions through displacement of both ligands to generate various different Au-based metabolites, including $[Au(CN)_2]^-$ [4]. Although the exact mechanism of anti-arthritic action is unclear, auranofin has been documented to inhibit many aspects of immune cell function, including antibody-dependent complement lysis, release of lysosomal enzymes, antibody-dependent cellular cytotoxicity, superoxide production, lymphocyte responsiveness, cutaneous migration, chemotaxis and phagocytosis (reviewed in [3]). Taken together, this evidence suggests that the anti-arthritic action of auranofin may be based upon its immunosuppressive effects.

In the early 1980s, auranofin was also found to be effective at increasing the life span of mice inoculated with P388 lymphocytic leukemia [5,6]. At the optimal dose, auranofin produced an increase in life span of mice of 59% compared with 117% with cisplatin. Of several tumor models and administration schedules investigated, auranofin showed activity only against P388 leukemia and when the drug was given by intraperitoneal injection. Although demonstrating definite activity, auranofin's antitumor effects were limited compared with conventional chemotherapeutic agents against murine tumor models in vivo.

To investigate possible mechanisms of action, studies of auranofin were carried out on cultured tumor cells in vitro [5]. Under culture conditions, auranofin inhibited the ability of tumor cells to grow, exclude vital dyes and form tumor colonies. Cell cycle analysis by flow cytometry revealed extensive accumulation of debris in the lower channels of DNA histograms but showed no change in the distribution of cells between the G1, S and G2-M phases. Equal toxicity occurred to cells in logarithmic growth compared with cells in the plateau phase of growth. As measured by the incorporation of tritiated precursers of DNA, RNA and protein, auranofin inhibited DNA, RNA and protein synthesis non-selectively and only at drug concentrations that were also associated with altered viability of cells. Morphological changes were documented, such as surface membrane changes, cell rounding and detachment from the culture plate, in cells exposed to auranofin for only 2 h. Taken together, these studies indicated that auranofin may have a direct, rapid onset and potent action against tumor cells that was not mediated through the inhibition of macromolecule synthesis or alteration of the cell cycle.

Further evidence for a direct mechanism of action of auranofin against tumor cells has come from studies of a series of gold(I) analogues of auranofin [7]. A number of analogues, particularly gold(I) phosphine complexes, exhibited potent in vitro cytotoxicity and in vivo antitumor activity. Although a high level of cytotoxicity in vitro was not always predictive of a high degree of

antitumor activity in vivo, a low level of cytotoxicity in vitro was highly predictive of marginal or no activity in vivo. A correlation was obtained between the in vitro cytotoxic potency of the compounds and their maximally tolerated doses in vivo. Compounds with potent cytotoxicity in vitro were more likely to be antitumor active and tolerated at only low doses in vivo. A direct action against target cells, therefore, may be the basis for the antitumor activity and host toxicity of auranofin in vivo.

To investigate the cellular association, distribution and efflux of the drug, studies were carried out with auranofin radiolabeled on the triethylphosphine (^{3}H), gold (^{195}Au) and tetracetylthioglucose (^{14}C) ligands [5,8]. Gold and triethylphosphine moieties, but not the tetracetylthioglucose ligand, were associated with cells and distributed to cellular membranes, cytoplasm and nuclear fractions. Cellular association of gold was linearly related to auranofin concentration and showed no evidence of saturation. The association of gold with cells was also time-dependent, temperature-dependant and inhibited by extracellular serum proteins and prior treatment of cells with the sulfhydryl alkylating reagent *N*-ethylmaleimide. Auranofin-derived gold effluxed from cells in a manner that depended on temperature and extracellular serum proteins, during incubation in fresh medium. At the IC_{50}, auranofin exposed cells contained between 4 and 9×10^{-16} moles of gold per cell and over 100-fold higher concentrations compared with the extracellular fluid. These findings suggest that the mechanism of auranofin association with cells may occur by cell-surface membrane thiols displacing the thioglucose ligand from the drug allowing gold–triethylphosphine or other forms of gold to shuttle between membrane, cytosolic and nuclear sulfhydryl groups. Since extracellular thiols compete with the cell for auranofin-derived gold, and reduce its uptake and cytotoxicity, the cellular association of gold appears to be directly related to the cytotoxic effects of auranofin.

To identify possible intracellular targets responsible for the cytotoxic and antitumor activity of auranofin, studies have been carried out of its interaction with DNA [9]. Drug-interactions with pBR322 DNA and calf thymus DNA were determined by measuring DNA electrophoretic mobility and fluorescence induced by ethidium bromide intercalation. Unlike a number of other gold and platinum complexes, auranofin showed a lack of reactivity with DNA in vitro, suggesting that its antitumor activity is not the result of a direct interaction with DNA.

To understand the mechanism of auranofin-induced inhibition of DNA synthesis, investigations were carried out on purified and cellular DNA polymerases [10]. Auranofin and other gold complexes inhibited DNA replication but only at high concentrations compared with those that inhibited tumor colony formation. Inhibition of DNA replication may have occurred via an interaction between auranofin and sulfhydryl groups on DNA polymerases since the effect was observed only in the absence of the sulfhydryl binding reagent dithiothreitol. Although auranofin clearly inhibited DNA replicative enzymes, the high drug concentration required for the effect suggested that DNA polymerases might not be a critical factor for the cytotoxic or antitumor actions of auranofin.

Some forms of acquired resistance to cisplatin have been associated with alterations in mitochondrial structure, mitochondrial dysfunction and hypersensitivity to antimitochondrial cytotoxins [11]. Dong and coworkers carried out studies on the effects of auranofin on the growth of two different cell culture models of cisplatin-resistance associated with altered mitochondria [12]. The cisplatin-resistant cells were hypersensitive to auranofin, and a number of other antimitochondrial agents, compared with cisplatin-sensitive cells with normal mitochondria. These results suggest that auranofin may have an antimitochondrial action that could possibly underlie the cytotoxic and antitumor activity of the drug.

Taken together, the various results discussed about indicate that auranofin has antitumor activity in murine tumor models in vivo and cytotoxic activity against cultured tumor cells in vitro. Accumulation of cellular debris in lower channels of flow cytometry DNA histograms, cell rounding, cell detachment and membrane changes were suggestive of the cells undergoing apoptosis, during exposure to auranofin, although this form of cell death was not well appreciated at the time of these experiments in the early 1980s. Cellular association and internalization of auranofin-derived gold appears to occur via interactions with membrane, cytosolic and nuclear sulfhydryl groups, and is directly related to its cytotoxicity. While the exact intracellular targets responsible for the cytotoxic and antitumor activity of auranofin are unclear, evidence exists in mitochondria, rather than DNA and DNA polymerases, being critical intracellular targets for the drug. Overall, the antitumor action of auranofin may occur via a direct cytotoxic action that possibly involves the induction of apoptotic cell death, targets on the mitochondria, and interactions between auranofin-derived gold and cellular sulfhydryl groups.

3. Triethylphosphine gold(I) chloride

Et_3PAuCl has shown potent cytotoxic activity in vitro but less antitumor activity in vivo compared with the closely related tetracetylthioglucose derivative, auranofin [7]. In this way, replacement of the thiosugar moiety of auranofin with chloride appears to reduce the selectivity of the metal complex for antitumor activity

relative to toxicity, although Et_3PAuCl remains highly cytotoxic in vitro.

To understand the mechanistic basis of the cytotoxic action of Et_3PAuCl, the ability of the complex to interact with DNA has been investigated [9]. Unlike auranofin, Et_3PAuCl appeared to interact with DNA under certain conditions (pH 9.5, borate buffer) as evidenced by induced changes in electrophoretic mobility of pBR322 DNA and ethidium bromide fluorescence of intercalated calf thymus DNA. Binding of Et_3PAuCl to DNA was completely inhibited by co-addition of thioglucose at a 1:1 molar ratio and also by NaCl when added in excess. Other halogen-substituted gold complexes also bound to DNA perhaps due to the greater liability of gold–halogen bonds compared with gold–phosphine or gold–sulfur bonds. Although Et_3PAuCl appeared to interact with DNA, this compound did not produce DNA inter-strand cross-links and DNA breakage [13] and the DNA-reactivity of Et_3PAuCl was significantly less that that of cisplatin. Et_3PAuCl also inhibited the activity of purified cellular and viral DNA polymerases and slowed cellular DNA replication in situ but high concentrations of triethylphosphine gold(I) chloride were required for these effects compared with those that inhibited the formation of tumor colonies in vitro [10].

To investigate the mechanism of acute cytotoxicity of Et_3PAuCl, studies were carried out to determine the biochemical and morphological changes induced in freshly isolated rat hepatocytes [14]. Et_3PAuCl caused rapid loss of cell viability that was apparent as early as 60 min and that was complete after only 180 min. Et_3PAuCl increased the formation of malonaldehyde, indicating a possible role for lipid peroxidation. Although the antioxidants *N*,*N*′ diphenyl-*p*-phenylenediamine and promethazine completely blocked the formation of malonaldehyde, the time-course of cell death and other biochemical changes, such as depletion of glutathione, were unaltered. Lipid peroxidation, therefore, was probably not the main mechanism of cytotoxicity of Et_3PAuCl in freshly isolated rat hepatocytes.

Other changes occurred within a few minutes of exposure to Et_3PAuCl in isolated hepatocytes [14]. Flocculent electron-dense precipitations appear in the mitochondria of hepatocytes as early as 5 min after exposure to Et_3PAuCl. Cellular NADPH and NADH levels were decreased within 10 min of drug exposure. Cellular ATP levels and oxygen consumption were also decreased in a concentration-dependent manner soon after exposure of hepatocytes to Et_3PAuCl. Since mitochondria were the first organelle to show changes and decreases in ATP levels and oxygen consumption preceded the onset of cell death, the mitochondria and oxidative phosphorylation pathways may be the initial targets for Et_3PAuCl in freshly isolated rat hepatocytes.

Further evidence for the involvement of mitochondria in the cytotoxicity of Et_3PAuCl was provided by studies of isolated rat hepatocyte mitochondria [15]. Et_3PAuCl inhibited various different states of mitochondrial respiration and caused the dissipation of the mitochondrial-membrane hydrogen ion gradient. Differential inhibition of uncoupled mitochondrial respiration supported by pyruvate–malate compared with ascorbate–tetramethyl-*p*-phenylenediamine provided evidence for the inhibition of site I or II of the electron transport chain by Et_3PAuCl. Dithiothreitol reversed some of the effects of Et_3PAuCl on isolated mitochondria and partially protected cells against Et_3PAuCl-induced depletion of ATP and loss of cell viability. Together, these findings suggested that Et_3PAuCl may act directly against mitochondria, as an electron transport chain site I or II inhibitor and through dithiothreitol-inhibitable interactions with mitochondrial thiols.

To define the molecular mechanisms of Et_3PAuCl-induced dissipation of the mitochondrial membrance potential, further studies were carried using isolated rat hepatocyte mitochondria [16]. Et_3PAuCl reduced the potential difference across the inner mitochondrial membrane and caused release of sequestered calcium from mitochondria. Addition of dithiothreitol caused mitochondria to re-sequester the calcium released in response to Et_3PAuCl and partially restored the mitochondrial membrane potential. Dibucaine, a non-specific inhibitor of phospholipases, also partially inhibited Et_3PAuCl-induced collapse of the mitochondrial membrane potential. Et_3PAuCl also induced mitochondrial swelling and increased permeability of inner mitochondrial membrane as determined by oxalacetate uptake.

Taken together, the results of the various studies of Et_3PAuCl discussed above suggest that mitochondria are the primary target for its cytotoxic effects against isolated rat hepatocytes, rather than DNA or DNA polymerases. The effects of Et_3PAuCl on mitochondria are complex and involve inhibition of the electron transport chain, depletion of ATP, inhibition of oxidative phosphorylation, increase inner membrane permeability, mitochondrial swelling, release of calcium and dissipation of the inner membrane potential difference. Cytotoxicity to isolated rat hepatocytes may be initiated by interactions between Et_3PAuCl and mitochondrial thiols, inhibition of the electron transport chain or permeability changes in the inner mitochondrial membrane.

4. Bis[1,2-bis(diphenylphosphino)ethane]gold(I) chloride

Unlike the neutral, two-co-ordinate linear gold complexes discussed above, bis[1,2-bis(diphenylphosphino)ethane]gold(I) chloride is a cationic, tetrahedral four-coordinate gold(I) phosphine complex with che-

lated diphosphine ligands. The complex is more stable with respect to ligand exchange reactions than the linear complexes, and an important difference is that [Au(dppe)$_2$]Cl is much less reactive towards thiols [1]. [Au(dppe)$_2$]Cl has shown reproducible and significant in vivo antitumor activity in a range of murine models including P388 leukemia, M5078 reticulum cell sarcoma, B16 melanoma, mammary adenocarcinoma 16/C, intraperitoneal transplanted tumors and subcutaneous transplanted tumors [17]. Against a subline of P388 leukemia resistant to cisplatin, [Au(dppe)$_2$]Cl produced increases in life span that were similar to that produced by the gold complex in the cisplatin-sensitive parental line. Combination of [Au(dppe)$_2$]Cl with cisplatin provided greater activity compared with either agent given alone in P388 leukemia [17]. In these ways, [Au(dppe)$_2$]Cl had a broader range of activity in murine tumor models in vivo compared with auranofin, and exhibited activity in both solid tumor and leukemia murine models.

[Au(dppe)$_2$]Cl inhibited the formation of tumor colonies from B16 melanoma and P388 leukemia cells in vitro, but unlike auranofin, this activity was not associated with acute loss of cell viability as indicated by cellular trypan blue exclusion [17]. In contrast to auranofin, inhibition of tumor colony formation by [Au(dppe)$_2$]Cl was associated with preferential inhibition of protein synthesis compared with DNA and RNA synthesis, and the production of DNA protein cross-links and DNA strand breaks. The in vitro activity of [Au(dppe)$_2$]Cl was less inhibited by the presence of serum proteins in the tissue culture medium, and the compound was more chemically stable in the presence of serum proteins, thiols and disulfides, compared with auranofin. These findings provided evidence for a mechanism of antitumor activity for [Au(dppe)$_2$]Cl that differed from that for auranofin.

To investigate the mechanism of action of [Au(dppe)$_2$]Cl, a subclone of murine P388 leukemia resistant to the bis(diphenylphosphino)ethane ligand was developed by serial tumor transplantation in bis(diphenylphosphino)ethane-treated mice [18]. The P388-bis(diphenylphosphino)ethane resistant subclone was cross-resistant in vitro and in vivo to [Au(dppe)$_2$]Cl but not to auranofin. Compared with the P388-parental line, the P388-bis(diphenylphosphino)ethane resistant subclone was cross-resistant with several mitochondrial uncouplers, accumulated less rhodamine and bis(diphenylphosphino)ethane and had lower basal levels of ATP and mitochondrial respiration. [Au(dppe)$_2$]Cl depleted cells of ATP and inhibited mitochondrial respiration more extensively in sensitive P388 cells compared with the bis(diphenylphosphino)ethane-resistant cells. These results suggested that alterations in mitochondria could be the basis for the cross-resistance of bis(diphenylphosphino)ethane-resistance P388 cells to [Au(dppe)$_2$]Cl. Cross-resistance between [Au(dppe)$_2$]Cl and uncouplers of mitochondrial oxidative phosphorylation has also been demonstrated in human ovarian carcinoma cell lines [12]. Mitochondria, therefore, may be one of the cellular targets involved in the antitumor and cytotoxic activity [Au(dppe)$_2$]Cl.

To determine whether [Au(dppe)$_2$]Cl-induced cytotoxicity was related to changes in mitochondria, studies were carried out of induced toxicity and biochemical alterations in intact isolated rat hepatocytes. Within 30 min of exposure, [Au(dppe)$_2$]Cl had been taken up by isolated hepatocytes and distributed to mitochondria, nucleus, cytoplasm and cellular membranes. Within the same period of time, isolated rat hepatocytes formed blebs on the plasma membrane, lost ATP, showed increased oxygen consumption and developed morphological changes in mitochondria as detected by electron microscopy. Induced increased hepatocyte oxygen consumption suggested that [Au(dppe)$_2$]Cl may act as an uncoupler of oxidative phosphorylation. Following the onset of these various biochemical and morphological changes, isolated rat hepatocytes lost cellular viability, as indicated by the release of lactate dehydrogenase. These result suggested that mitochondria were a possible target organelle for [Au(dppe)$_2$]Cl cytotoxicity in intact isolated rat hepatocytes.

The in vivo significance of [Au(dppe)$_2$]Cl-induced cytotoxicity to liver cells was evaluated in male beagle dogs during the development of the compound as an antitumor agent [19]. [Au(dppe)$_2$]Cl was highly hepatotoxic in the dog in vivo as well as being cytotoxic to suspensions of isolated canine hepatocytes. Serum asparate aminotransferase, alanine aminotransferase and alkaline phosphatase were markedly elevated 48 h after a single intravenous dose of [Au(dppe)$_2$]Cl. Livers showed multiple areas of focal necrosis throughout all zones of the organ after treatment with [Au(dppe)$_2$]Cl. Cytotoxicity to hepatocytes, therefore, produced hepatotoxicity in vivo that may have limited the therapeutic application of [Au(dppe)$_2$]Cl as an antitumor agent.

In an attempt to further define the mitochondrial effects of [Au(dppe)$_2$]Cl, studies were carried using isolated rat hepatocyte mitochondria [20]. [Au(dppe)$_2$]Cl induced a rapid dissipation of the inner mitochondrial membrane potential, efflux of calcium, increased mitochondrial respiration, mitochondrial swelling and increased permeability of the inner membrane to cations and protons in isolated mitochondria. Structure–activity relationship comparisons suggested that these mitochondrial effects were determined by the uptake of [Au(dppe)$_2$]Cl into mitochondria, which in turn was possibly related to the cationic–lipophilic nature of the molecule. The mechanism of uncoupling of oxidative phosphorylation, therefore, appeared to be related to the ability of [Au(dppe)$_2$]Cl to increase the permeability of the inner mitochondrial membranes.

The various studies discussed above suggest that the antitumor activity of $[Au(dppe)_2]Cl$ may be related to the formation of DNA strand-breaks, formation of DNA-protein cross-links, inhibition of protein synthesis or altered mitochondrial function and ATP synthesis. The hepatotoxicity of the compound appeared more clearly related to $[Au(dppe)_2]Cl$-induced cytotoxicity in isolated hepatocytes arising from increased permeability of mitochondrial membranes and uncoupling of oxidative phosphorylation.

5. Bis[1,2-bis(di-*n*-pyridylphosphino)ethane]gold(I) chloride complexes

To determine the effect of introducing aqueous solubility, variations of the phenyl substituents of $[Au(dppe)_2]Cl$ were examined by replacement with pyridyl groups to decrease the lipophilicity of the diphosphine ligand [21]. The 2-pyridyl complex exhibited similar activity against intraperitoneal P388 leukemia compared with $[Au(dppe)_2]Cl$. The 2-pyridyl complex was also active against the B16 melanoma in mice. The 4-pyridyl complex was inactive in all tumor models but toxic to mice. Some pyridyl tetrahedral bis(diphosphino) gold(I) complexes, therefore, have significant in vivo antitumor activity comparable to bis[1,2-bis((diphenylphosphino)ethane]gold(I) chloride.

To determine the potential relationship of their structure and solution properties to the antitumor activity of the gold(I) complexes of bidentate pyridyl phosphines, the 1:2 adducts of Au(I) with 1,2-bis(di-*n*-pyridylphosphino)ethane (dnpype) were characterized for $n = 2,3$ and 4 by NMR and crystallographic studies [22]. The 3- and 4-pyridyl complexes exist as simple monomeric cations in the solid state and solution and have a much higher water solubility than the 2-pyridyl complex which crystallizes in the solid state as a dimer (Fig. 1) and exists in solution as an equilibrium mixture of monomeric, dimeric and tetrameric clusters. The position of the N-atom in the pyridyl ring influences the interaction with solvent providing a series of compounds which are structurally similar to $[Au(dppe)_2]^+$, and in which the hydrophilic character covers a very large range. Based on their solution chemistry, it was thought that tetrahedral bis(dipyridylphosphino) gold(I) complexes could act like lipophilic cations that are taken up by mitochondria.

To determine the physicochemical basis for the cellular uptake and cytotoxicity of $[Au(dppe)_2]Cl$ and a series of gold(I) complexes of bidentate pyridylphosphines, studies were carried out in CH-1 human ovarian carcinoma cells [23]. Drug lipophilicity, as measured by high performance liquid chromatography or *n*-octanol–water partition, showed a non-linear relationship with the growth inhibition of CH-1 cells, with a fall and then a rise in IC_{50} values with increasing lipophilicity (see Fig. 2). Initial rate of drug uptake, as measured by inductively coupled plasma mass spectrometry, also showed a complementary nonlinear dependence on drug lipophilicity. Correction for drug binding to serum proteins, in the culture medium, converted the nonlinear dependence of activity and uptake on lipophilicity to linear correlations. Changes on free drug concentration under culture conditions, therefore, explained most of the nonlinearity of the relationships with drug lipophilicity.

Antitumor activity in vivo of $[Au(dppe)_2]Cl$ and tetrahedral bis(dipyridylphosphino) gold(I) complexes also showed a dependence on lipophilicity in subcutaneous colon 38 tumors [23]. The most lipophilic and hydrophilic compounds had no significant tumor growth delay. However, a compound of intermediate lipophilicity showed significant antitumor activity, less dose-limiting toxicity and higher gold concentration in plasma and tumors compared with more lipophilic or hydrophilic compounds. Lipophilicity of tetrahedral bis(dipyridylphosphino) gold(I) complexes, therefore, plays a key role in determining their cellular uptake, cytotoxicity, host toxicity and in vivo antitumor activity. The possible role of mitochondria in the cytotoxic and antitumor properties of tetrahedral pyridyl bis(diphosphino) gold(I) complexes remains unclear.

6. Discussion

Overall, the antitumor properties of the various gold(I) phosphine complexes discussed above appear to be closely associated with their direct cytotoxic effects on tumor cells in vitro. Most of the gold(I) phosphine compounds that have been documented to exhibit antitumor activity in vivo also have been shown to be cytotoxic to tumor cells in vitro. Those lacking in vitro cytotoxicity almost always lack antitumor activity in vivo. Direct cytotoxicity also appears to play a role in the in vivo hepatotoxicity of gold(I) phosphine complexes since compounds that were hepatotoxic in vivo were also toxic to isolated hepatocytes in vitro. In these ways, their therapeutic and toxic activity of gold(I) phosphine derivatives appears to be closely related to their direct cytotoxicity rather than occurring via indirect mechanisms.

Although the exact mechanism of gold(I) phosphine-induced cytotoxicity is unclear, several lines of evidence point to the involvement of mitochondria. Cisplatin-resistant cell-lines with altered mitochondria were hypersensitive to auranofin and $[Au(dppe)_2]Cl$, along with a number of other antimitochondrial agents [12]. Isolated rat hepatocytes exposed to Et_3PAuCl and $[Au(dppe)_2]Cl$ lost cellular viability following the onset of mitochondrial morphological changes, depletion of

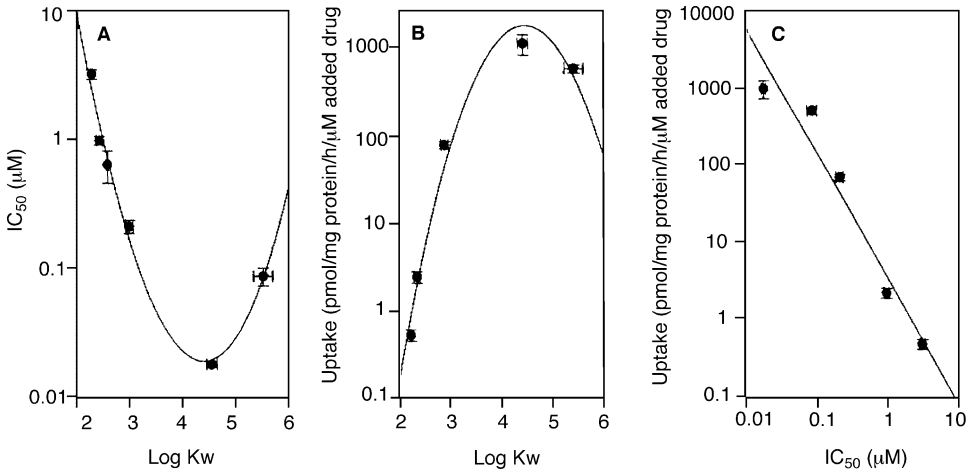

Fig. 2. Relationship between lipophilicity, uptake and growth inhibition of gold phosphine complexes in CH-1 tumor cells in vitro. (A) Growth inhibition versus lipophilicity. (B) Uptake versus lipophilicity. (C) Growth inhibition vs. uptake. The most lipophilic complex is $[Au(dppe)_2]Cl$ (log $kw = 5.42$) and the others are Au(I) complexes of bidentate pyridyl phosphines. Adapted from ref. [23].

ATP and changes in oxygen consumption [14,24]. Isolated mitochondria exposed to Et_3PAuCl and $[Au(dppe)_2]Cl$ showed mitochondrial swelling, dissipation of the mitochondrial membrane potential, efflux of calcium and altered oxygen consumption [15,16,20]. A pair of P388 murine leukemia cell lines that differed in their oxygen consumption, basal levels of ATP, lipophilic cation uptake and sensitivity to mitochondrial uncouplers, also showed differential sensitivity to $[Au(dppe)_2]Cl$. Although some gold(I) phosphine complexes have been documented to interact with DNA or DNA polymerases, these various studies discussed above suggest that mitochondria and pathways of oxidative phosphorylation may be their primary intracellular targets.

Mitochondria are involved in many of the normal processes occurring in living cells such as ATP synthesis, oxidative phosphorylation, calcium uptake and release, production of NADPH, pH control, synthesis of DNA, the tricarboxylic acid cycle and β-oxidation pathway [25,26]. Known mitochondria toxins act via various and often complex mechanisms involving these various pathways [26,27]. For example, the classic mechanism of mitochondrial poisoning involves the inhibition or uncoupling of oxidative phosphorylation resulting in loss of ATP and cell death, such toxicity induced by cyanine. Other potential mechanisms of antimitochondrial toxicity include the liberation of reactive oxygen species, interference with mitochondrial calcium regulation, induction of mitochondria permeability transition and accumulation of point mutations and deletions in the mitochondrial genome. More recently, mitochondria have been recognized to play a critical role in the regulation of apoptosis induced by many different stimuli [28,29]. Mitochondria integrate death signals through various bcl-2 proteins and sequester cytochrome *c* and other proapoptotic factors in the intermembrane space. Upon release from mitochondria, these pro-apoptotic factors activate caspases and amplify or initiate apoptotic cell death. Located on the outer mitochondrial membranes, the bcl-2 family of proteins regulate apoptosis, via some family members acting as an on–off switch determining cytochrome *c* release while others transduce molecular death signals to the mitochondria. Compounds affecting mitochondria, therefore, could potentially cause cytotoxicity via the induction of apoptosis by stimulating the release of cytochrome *c* for mitochondria and activation of caspases.

Whether any of the above mechanisms of antimitochondrial toxicity could produce selective toxicity in cancer cells relative to normal host cells is an important consideration for the potential of mitochondria as targets for cancer chemotherapy. For decades, solid tumors have been known to display high rates of glucose uptake and glycolysis even under aerobic conditions [30]. More recently, ^{18}F-fluoro-2-deoxyglucose positron emission tomography has consistently demonstrated much higher rates of glucose uptake in tumors in human subjects compared with normal tissues, and molecular studies have shown that oncogenes and tumor suppressor genes up-regulate the expression of glycolytic enzymes in tumors. Since many normal cells are reliant on oxygen consumption and oxidative phosphorylation for ATP production and tumors are primarily reliant on glycolysis for production of ATP, antimitochondrial agents that target oxidative phosphorylation pathways are likely to be limited in their therapeutic application by host toxicity and poor antitumor selectivity. In this respect, phase I clinical trials of the rhodacyanine dye MKT077 have shown that dosing of antimitochondrial agents may be limited by renal toxicity and changes in

skeletal muscle energetics, as indicated by ^{31}P phosphorous magnetic resonance spectroscopy [31].

Early results of other clinical trials [32,33] suggest the feasibility of targeting mitochondrial-based apoptosis regulatory pathways in human cancer therapy without limiting toxicity or significant effects on oxidative phosphorylation pathways. Augmerosen (G3139), an antisense 18-base phosphorothioate oligonucleotide complementary to the first 16 codons of bcl-2 mRNA, hybridizes to the target mRNA causing decreases in mRNA and protein levels of Bcl-2 on the membrane of mitochondria. In clinical trials, augmerosen has been demonstrated to induce decreased bcl-2 protein in tumor samples, biologically relevant plasma concentrations and clinical antitumor responses, at doses associated with no dose-limiting systemic toxicity. These early experiences provide some support for the principle of targeting mitochondria with therapeutic agents to produce selective toxicity and clinically relevant antitumor responses.

In conclusion, auranofin, Et_3PAuCl, $[Au(dppe)_2]Cl$ and tetrahedral bis(dipyridylphosphino) gold(I) complexes have all demonstrated antitumor activity in murine tumor models in vivo. Although the exact cellular targets responsible for their antitumor properties are unclear, they are all directly cytotoxic to tumor cells and appear to have antimitochondrial activity. Cytotoxic effects to isolated hepatocytes appear to be due to the inhibition or uncoupling of oxidative phosphorylation by gold(I) phosphine complexes. The mechanism of their antitumor properties is less clear but could possibly involve interference with oxidative phosphorylation, induction of mitochondrial-dependent apoptosis or other mechanisms. Given the different chemical reactivities of the linear Au(I) phosphine complexes compared with the tetrahedral Au(I) complexes with bidentate phosphines, it is unlikely that these compounds will act by the same mechanism(s). Reactions with sulfhydryl groups are likely to be involved for auranofin and Et_3PAuCl, whereas the lipophilic cationic properties of $[Au(dppe)_2]^+$ and tetrahedral bis(dipyridylphosphino) gold(I) complexes may be important. Improving the tumor selectivity of mitochondrial-targeted gold(I) phosphine complexes, by optimizing their lipophilic–hydrophilic balance and other measures is now critical for their future as potential antitumor drugs.

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