Modulation of the Antitumor and Biochemical Properties of Bis(diphenylphosphine)ethane with Metals

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Abstract

Bis(diphenylphosphine)ethane (DPPE) and its bis[chlorogold(I)] [DPPE(AuCl)_2], and bis[chlororhodogold(III)] [DPPE[PtCl(PhCl)]_2], complexes have in vitro antitumor activity. To determine if interaction with metals in situ can play a role in the antitumor activity of DPPE, we have studied the effects of DPPE, DPPE(AuCl)_2, DPPE(CuCl)_2 and mixtures of DPPE with metal salts on in vitro and in vivo biological systems. The in vitro cytotoxic potencies of the two DPPE-gold complexes were approximately 10-fold greater than that of DPPE. In addition, the cytotoxic potency of DPPE was increased when incubated with cells in the presence of Au(III) and Cu(II) salts, whereas Mg(II), Zn(II), Mn(II), Fe(II), Co(II), and Cd(II) had no effect. The effects of DPPE, DPPE(AuCl)_2 and mixtures of DPPE and metal salts on the activity of a model enzyme system, DNA polymerase α, were measured. While DPPE did not inhibit the activity of DNA polymerase α, the DPPE(AuCl)_2 complex and mixtures of DPPE and Cu(II) salts inhibited the enzyme’s activity. Consistent with the effects observed in vitro, coadministration of Cu(II) or Au(III) increased the in vivo potency of DPPE in mice bearing i.p. P388 leukemia. Fifteen other DPPE analogues were evaluated for in vivo antitumor activity and for the effect of Cu(II) on their in vitro cytotoxic potency; there was a relationship between the ability of Cu(II) to potentiate the cytotoxic activities of DPPE analogues and their having in vivo antitumor activity.

Introduction

A number of complexes containing gold in the +1 or +3 oxidation state have been reported to be cytotoxic in vitro and to display antitumor activity in vivo rodent tumor models (1–4). In addition, structure-activity relationships have been defined for these activities relative to the ligands coordinated to the gold in these complexes (4).

The cytotoxic action of the antiarthritic gold complex, auranofin [1-[thio-β-D-glucopyranose 2,3,4,6-tetraacetato-S(triethylphosphine)gold(I)]; Ridaura] has been shown to correlate with cellular concentration of gold(I) (1). The cellular uptake and distribution of gold from auranofin and certain other gold complexes has been shown to result from a sequential ligand exchange process (5, 6). An important component of this process is the nature of the coordinating ligands in the gold complexes. These modulate complex stability, subcellular distribution and potential interaction of the gold with various cellular components. In addition, some of these gold complexes contain ligands such as phosphines that may be oxidized by peroxidases and other oxidative reactions which may generate cytotoxic free radicals.

Recently we reported that complexes related to DPPE are active in a spectrum of transplantable tumor models (7). DPPE, administered i.p. on days 1–5 at its MTD of 40 μmol/kg, reproducibly gives 100% ILS in mice bearing i.p. P388 leukemia. Coordination of chlorogold(I) moieties to each phosphine in DPPE gave a complex which had similar antitumor activity (i.e., similar maximum ILS) but which was considerably more potent (i.e., lower MTD) than DPPE. The MTD for the gold(I) complex was 7 μmol/kg; thus, the activity of the gold complex cannot be ascribed solely to the DPPE. Structure-activity studies have revealed that among DPPE analogues, replacement of phenyl groups with ethyl or benzyl groups resulted in inactivity for both ligands and gold complexes. Analogues in which the ethane bridge of DPPE was varied between 1 and 6 carbons, unsaturated or substituted revealed that activity was maximal with ethane or cis-ethylene. In addition, compounds of these classes with significant levels of antitumor activity against P388 were also active in i.p. L1210 leukemia, i.p. B16 melanoma, i.p. M5076 reticulum cell sarcoma, and s.c. mammary adenocarcinoma 16/c. This spectrum of in vivo antitumor activity suggests that these compounds may represent an important novel class of antitumor agents.

Inasmuch as DPPE is an effective chelator (8), we reasoned that in situ chelation of a metal or metals by DPPE might result in complexes which are responsible for the biological activities of this compound; indeed, structure-activity studies suggest that the chelating potential of analogues of DPPE may be related to their in vivo potency (7). Furthermore, among the various metal complexes of DPPE which have been evaluated, Au(I), Au(III), and Cu(I) complexes were active against P388 (>50% ILS) whereas Pt(II), Ag(I), Ni(II), and Pd(II) complexes had marginal activity (30–40% ILS). The studies described in this paper were designed to better probe the relevance of biologically available metals with DPPE in situ and the antitumor properties of DPPE and DPPE-gold complexes. These studies include evaluations of the effects of various metal salts on the cytotoxic potency of DPPE analogues and on their in vitro antitumor activity and of the effects of DPPE in the presence of various metals on DNA polymerase α, a model enzyme system. These studies demonstrate that Cu(II) salts potentiate the in vitro cytotoxic and in vivo toxic activity of DPPE and that copper may play a role in the pharmacological activity of DPPE.

Materials and Methods

Materials. DPPE and analogues of this ligand were obtained from Streum Chemicals Co. (Newburyport, MA), metal salts from Fisher Scientific Co. (Pittsburgh, PA), and gold complexes from Smith Kline and French Laboratories (Philadelphia, PA).

Cell Culture Techniques. B16 melanoma cells (highly metastatic subline, F10) and KB cells were maintained as monolayer cultures in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, antibiotics, and 2 mM L-glutamine. The cells were maintained at 37°C in a humidified atmosphere of 5% CO2 in air.

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1 Predoctoral trainee supported by the University of Pennsylvania, Department of Pharmacology. To whom requests for reprints should be sent, at Smith Kline and French Laboratories, L-511, P.O. Box 7929, Philadelphia, PA 19101.
2 The abbreviations used are: DPPE, bis(diphenylphosphine)ethane; CDDP, cis-diaminedichloroplatinum(II); ILS, increase in life span; MTD, maximum tolerated dose; 2,9-DMP, 2,9-dimethyl-1,10-phenanthroline; mAMSA, 4’-(9-acridinylamino)methanesulfon-m-aniside.

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in minimal essential media (Grand Island Biological Co., Grand Island, NY) supplemented with 10% fetal calf serum (heat inactivated at 56°C for 30 min) and penicillin (100 units/ml) and streptomycin (100 μg/ml) in a 5% CO2 humidified incubator at 37°C.

Monolayer Clonogenic Assay. Asynchronous populations of cells were harvested and replated at 5000 cells/plate in sterile 60- x 15-cm Petri plates. Plates were incubated overnight to allow attachment of cells to the plate surface. Cells were treated with compound and/or metal salts for 2 h in culture medium under sterile conditions followed by aspiration of medium. Plates were washed with 5 ml of phosphate-buffered saline, followed by addition of 5 ml of fresh medium. Plates were incubated for 5 days at 37°C in a CO2 incubator. Viability was measured by the ability of a cell to form a colony of greater than 50 cells. Colonies were fixed with 0.5% crystal violet in 95% ethanol. Plates were dried and counted with a Biotron III automatic counter.

In Situ DNA Polymerase Assay. KB cells were synchronized in S phase of the cell cycle by double thymidine block (10). Synchronized cells were then permeabilized by fast freezing in 95% ethanol/dry ice bath and slow thawing at 25°C. Optimal DNA synthesis was obtained by incubating 0.625 × 10⁶ cells/tube with 35 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (pH 7.4), 50 mM sucrose, 20 mM phosphoenolpyruvate, 0.25 mM dGTP, dATP, dCTP, 0.32 mM [methyl-³H]dTTP (15 Ci/mmol), 0.25 mM dADP, dCDP, dGDP, 2 mM ATP, 0.1 mM GTP, CTP, UTP, 0.75 mM CaCl₂, 80 mM KCl, 5 mM potassium phosphate (pH 7.4), and 5 mM MgCl₂ for 4 min at 37°C in a shaking water bath. Total assay volume was 100 μl. Inhibition by DPPE and DPPE(Au₂Cl₂) was measured in this system. Reactions were stopped and quantitated as described below.

Purified DNA Polymerase α Assay. DNA polymerase α was isolated from leukocytes of a patient with acute myelogenous leukemia and assayed as described previously by Allaudeen et al. (11). Briefly, DNA polymerase α activity was assayed in a 50-μl reaction mixture containing 50 mM tris-HCl (pH 8.0), 8 mM MgCl₂, 0.1 mM dCTP, dGTP, dATP, 0.02 mM [³H]dTTP, 10 μg activated calf thymus DNA, 10–20 μg bovine serum albumin, 5–10% glycerol, enzyme, and either DPPE, DPPE(Au₂Cl₂), CuCl₂, or mixtures of DPPE and CuCl₂. Incubation was for 30 min at 37°C in a shaking water bath. The reaction was stopped by placing tubes on ice and adding 100 μl yeast RNA (100 μg/ml) and ice-cold trichloroacetic acid to 10%. Acid insoluble radioactivity was collected on 0.45-μm nitrocellulose filters (Millipore, Bedford, MA). The filters were washed several times with 5% trichloroacetic acid, once with 70% ethanol, and dried. After drying, the filters were counted in a liquid scintillation counter.

Evaluation in i.p. P388 Leukemia. P388 leukemia cells (10⁶), maintained by serial transplantation in syngeneic DBA/2 mice, were inoculated i.p. in C57BL/6 × DBA/2 F1 (hereafter called B6D2F1) mice. Twenty-four h later, if the tumor inoculum proved to be free of bacterial contamination (as determined by 24-h incubation in thioglycollate broth) animals were randomized into groups of 6 and housed in shoebox cages. For administration to animals, DPPE and DPPE gold complexes were initially dissolved in N,N-dimethylacetamide. An equal volume of Cremophor (polyethoxylated castor oil; Sigma, St. Louis, MO) was added, followed by water or an aqueous solution of the desired metal salt. The final volume percentage of the organic vehicles was less than 10%. Formulations were prepared immediately prior to injection. Compounds were administered i.p. on days 1 through 5 (i.e., treatment was initiated 24 h after tumor inoculation). Each experiment included 3 groups of 6 animals as untreated controls which survived for a median of 9–11 days and animals treated with a positive control, CDDP (Sigma), at 2 dose levels. In these experiments, CDDP at 2 mg/kg/day produced 125 ± 38% (SD) ILS. In all these experiments, CDDP was active (>50% ILS).

RESULTS

Cytotoxic Potencies of DPPE and DPPE-Gold Complexes. The effect of DPPE (see Fig. 6, compound 1.) on the clonogenic capacity of B16 melanoma cells following a 2-h treatment is shown in Fig. 1A. The survival curve was monoeponential. The concentration of DPPE required to reduce cell survival by 50% was 60 μM. The survival curves resulting from treatment with the chlorogold(I) and chlorogold(III) complexes of DPPE were also monoeponential (Fig. 1, B and C, respectively). Both of the gold complexes were significantly more potent than DPPE with values of 8 and 3.5 μM for the concentration of DPPE required to reduce cell survival by 50% for the gold(I) and gold(III) complex, respectively.

Effects of Metal Salts on the Cytotoxic Potency of DPPE. To determine what effects exogenous metals might have on the biological activities of DPPE a series of experiments was performed in which B16 cells were incubated in the presence of a minimally toxic concentration of DPPE and a variety of transition-metal salts. As shown in Fig. 2, both CuCl₂ and NaAuCl₃...
enhanced the cytotoxicity of DPPE. Whereas 25 μM DPPE produced only a 15% reduction in colony formation, 25 μM DPPE with 10 and 50 μM CuCl₂ (nontoxic concentrations of CuCl₂) reduced colony formation by 65 and 85%, respectively. Similar results were observed when DPPE was incubated with CuSO₄. Neither ZnCl₂, MgCl₂, CoCl₂, MnCl₂, FeCl₂, nor CdCl₂ (at 10, 50, and 100 μM) altered the cytotoxic potency of DPPE (data not shown). NiCl₂ appeared to inhibit the in vitro cytotoxic potency of DPPE. At a concentration of 50 μM of DPPE, which alone produced a 50% reduction in colony formation, admixtures with 1, 10, and 100 μM of NiCl₂ resulted in 13, 7, and 0% reductions in colony formation, respectively.

Effects of DPPE, DPPE(Au₂Cl₄) and Metal Salts on DNA Polymerase Activities. Previous studies in our laboratory demonstrated that a variety of gold-containing complexes inhibited DNA polymerases (11). For most of the gold complexes the inhibition was noncompetitive with substrate (DNA) and the inhibition was due to interactions with the enzyme. In addition, DNA polymerase α was the most sensitive of the polymerases tested to the inhibitory action of the gold complexes. As a model enzymatic activity, the effects of DPPE, DPPE(Au₂Cl₄), and DPPE in combination with metal salts were determined. Fig. 3A shows that DPPE had no effect on the activity of DNA polymerase α, whereas DPPE(Au₂Cl₄) produced a 30% inhibition at concentrations as low as 2.5 μM. Similar results were obtained when the effects of these 2 compounds on in situ DNA polymerase activity were measured (Fig. 3B). The concentration of DPPE(Au₂Cl₄) required to achieve maximal inhibition of polymerase activity in situ was 5- to 10-fold higher than that required to achieve maximal inhibition of isolated DNA polymerase α.

The effects of a variety of metal salts (i.e., CuCl₂, CuSO₄, ZnSO₄, NiCl₂, RuCl₃, CdCl₂) on DNA polymerase α activity in the presence or absence of DPPE were studied. None of these metal salts had an inhibitory effect on DNA polymerase α at concentrations from 1-100 μM. An inhibitory effect by DPPE on DNA polymerase α activity was only observed when CuCl₂ or CuSO₄ was added. Fig. 4 shows that although CuCl₂ (1-100 μM) when used alone did not inhibit the activity of DNA polymerase α, it produced a concentration-dependent increase in the inhibitory activity of DPPE.

Effects of Metal Salts on the Antitumor Activity and Toxicity of DPPE. The experiments described above demonstrated that Cu(II), Au(I), and Au(III) can modulate the in vitro biological properties of DPPE both when complexes to DPPE in a defined
structure [in the case of Au(I) and Au(III)] or when added as mixtures of metal salts with DPPE (in the case of CuCl2, CuSO4, and NaAuCl3). We previously reported that complexing of Au(I) and Au(III) to DPPE results in complexes that are 5- to 10-fold more potent in vivo and retain the in vivo antitumor activity of the ligand (7). We next attempted to determine whether exogenous metal salts could modulate the in vivo biological activities of DPPE as predicted by our in vitro findings. Mice bearing P388 leukemia were treated with admixtures of DPPE and metal salts. DPPE produced a dose-dependent ILS of tumor-bearing animals (Table 1). Under the experimental protocol used, the compound produced 100% ILS at a MTD of 25 μmol/kg. DPPE was evaluated in combination with a 2.5- fold higher concentration on a molar basis of transition-metal salts. Coadministration of Co(II), Fe(II), Zn(II), and Mn(II) did not change the antitumor activity or host toxicity of DPPE. Similar to the effect observed on the in vitro cytotoxic activity of DPPE, coadministration of Cu(II) markedly increased the toxicity of DPPE. Although CuCl2 induced an 8- to 16-fold increase in toxicity, it did not affect the level of antitumor activity of the suboptimal doses of DPPE which were tolerated by mice when given in combination. These results and our observation that the oxidized form of DPPE is nontoxic and devoid of antitumor activity (7) suggest that the exogenous CuCl2 may serve to stabilize the biologically active form of DPPE. The ability of exogenous Cu(II) to increase the cytotoxicity of DPPE analogues and the intrinsic in vivo antitumor activity of the latter. The interaction of Ni(II) with DPPE may have eliminated the host toxicity of DPPE as well, although the metal-free ligand (DPPE) had in vivo antitumor activity (3). Compounds related to DPPE and their metal complexes were found to have activity in murine tumor models (7). Although DPPE without increasing the optimal antitumor activity. Coadministration of Ni(II) with DPPE appeared to abolish the antitumor activity of the latter. The interaction of Ni(II) with DPPE may have eliminated the host toxicity of DPPE as well, but NiCl2 alone was toxic at the dose (250 μmol/kg/day) which was given in combination with the toxic dose of DPPE (50 μmol/kg/day).

Effect of Cu(II) on the Cytotoxicity of DPPE. The ability of exogenous Cu(II) to increase the cytotoxic activity of DPPE was determined. Fig. 5 shows that coinubation of nontoxic concentrations of CuCl2 with analogues of DPPE (Fig. 5, compound 1) in which the ethane bridge was lengthened up to and including (CH2)9, produced increases in the cytotoxic activity of the individual analogues; however, CuCl2 did not increase the activity of the (CH2)3 analogue (Fig. 5, compound 5). In addition, with a 2-carbon bridge, the cytotoxic activity of the analogues with ethane or cis-ethyleno moieties was potentiated by the coadministration of CuCl2, whereas that of the compounds with trans-

diagram

Fig. 5. Effects of CuCl2 on cytotoxic potency of DPPE analogues. Clonogenic assays were performed (as described in "Materials and Methods") in which B16 melanoma cells were incubated for 2 h with the compounds (at concentrations which had previously been shown to inhibit colony formation by 5-10%) in the absence or presence of CuCl2 (10, 50, and 100 μM). The DPPE analogues used in this experiment were bis(diphenylphosphino) compounds in which the ethane bridge was modified; 1, (CH2)3, i.e., DPPE; 2, (CH2)4; 3, (CH2)5; 4, (CH2)3; and 5, (CH2)3. Measurements represent mean of 3 assays; bars, SE.

ethylene or acetylene bridges was not (Fig. 6). A series of other analogues of DPPE was also studied for the ability of CuCl2 to potentiate their in vitro cytotoxic activity. A summary of these data is shown in Fig. 6 in comparison with the corresponding intrinsic in vivo antitumor activity of the analogue as measured in mice bearing i.p. P388 leukemia. These data show that for this series of compounds there is a correlation (15 of 16 analogues) between in vivo antitumor activity and the ability of CuCl2 to enhance in vitro cytotoxicity.

DISCUSSION

Based on a report that auranofin, a complex of gold(I) with triethylphosphine and tetraacetylthioglucose, has antitumor activity (2, 4) our laboratory initiated a program to synthesize and evaluate gold complexes in experimental tumor systems (3). Compounds related to DPPE and their metal complexes were found to have activity in murine tumor models (7). Although the metal-free ligand (DPPE) had in vivo antitumor activity, complexes of Au(I) and Au(III) or Cu(I) with DPPE were 5- to 10-fold more potent in vivo and in vivo. The data presented in this paper demonstrate that the addition of Cu(II) (as well as gold and silver) salt potentiates the biological activity of DPPE (Fig. 2; Table 1). The ability of CuCl2 to enhance the cytotoxicity of DPPE analogues and the intrinsic in vivo antitumor activity of such compounds suggest the possibility that endogenous copper may play a significant role in the pharmacological properties of DPPE (Fig. 6).
A Phosphine Linker Analogs of DPPE

<table>
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<tr>
<th>Compound No</th>
<th>R</th>
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<td>†</td>
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B Replacement of Phosphine(s) with Arsine(s) or Sulfides

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C Substitution of Terminal Phenyl Groups

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Fig. 6. Comparison of modulatory effect of CuCl₂ in vitro cytotoxic activity of a variety of DPPE analogues and their in vivo antitumor activity. A, *, as determined in the colony formation assay using B16 melanoma cells; †, >2-fold increase in the cytotoxic activity of DPPE analogues under conditions in which the analogue was incubated at the concentration required to reduce cell survival by 20% with 10, 50, or 100 μM of CuCl₂; negative, <2-fold increase in activity under conditions described above. Note: for all compounds listed in the table as negative, there was no increase in the cytotoxic potency at any of the concentrations of CuCl₂ used. †, for in vivo evaluation the analogues were evaluated in P388 leukemia (see “Materials and Methods”). A drug was judged as active if it reproducibly produced an ILS of >40% at its MTD and inactive if <40% ILS at its MTD. B, †, one phenyl group per sulfur atom. C, †, †, effect (equal to a 2-fold increase) measured only at 100 μM CuCl₂.

A variety of DPPE-copper(I) complexes have been synthesized and reported previously (7, 12, 13). These include complexes of linear and cubic geometries with copper:DPPE ratios of 2:2, 2:3, and 4:2. The solution chemistry of Cu(II) and DPPE which can include both oxidation/reduction and chelation chemistry are under investigation in our laboratory. UV studies indicate that reaction of CuCl₂ and DPPE can occur rapidly in solution. The results of these studies are consistent with the formulation of spectroscopically silent reaction products which remove 2 equivalents of Cu(II) absorbance from the reaction mixture per equivalent of DPPE. Elucidation of these reaction products should help to define the precise structure(s) responsible for the pharmacological activities of the DPPE-copper mixtures.

A variety of structurally diverse compounds which display clinical and/or experimental model antitumor activity, e.g., bleomycin (14), Adriamycin (15), 1,10-phenanthroline (16), and mAMSA (17, 18) salicylate derivatives (19), thiosemicarbazones (20, 21), and aroylhydrazone analogues of the growth-modulating tripeptide glycine-histidine-lysine (22), are able to form coordination complexes with transition-metal ions. The evidence for the interaction of these compounds with biologically available metal ions suggests that their coordination complexes are in part responsible for their biochemical effects. For example, the effects of 1,10-phenanthroline have been attributed to its ability to combine with zinc and thus inhibit zinc-containing nucleotidyl transferases (23, 24) and DNA synthesis. Alternatively, chelate complexes of 1,10-phenanthroline with the divalent metal ions, copper, cobalt, iron, zinc, and ruthenium have been reported to be cytotoxic in vitro (16). In addition, Mohindru et al. (25) have recently reported that 2,9-dimethyl-1,10-phenanthroline (2,9-DMP) is a potent copper-dependent cytotoxin which is biologically active only as the coordinated metal complex. Similar to our findings with DPPE, other divalent ions, iron and zinc were shown to be ineffective as promoters of the cytotoxic action of 2,9-DMP. The potentiating effect of copper on the cytotoxic activity of 2,9-DMP appears to be related to its ability to increase the cellular uptake...
of the compound (25). The effect of copper on the cellular uptake of DPPE is currently under investigation in our laboratory in an attempt to determine whether (a) the role of copper in the complex is to transport DPPE into the cell, (b) the role of DPPE is to transport copper into the cell, or (c) the DPPE-copper complex is the biologically active molecule. It may be that DPPE, DPPE-metal admixtures, and defined DPPE-gold complexes produce their cytotoxic effects through a common mechanism as a result of an ultimate cellular metabolite common to each.

The effects of metals on the interactions of bleomycin, 1,10-phenanthroline, and mAMSA with DNA have been studied extensively. Each of the drugs, in the presence of the reduced, oxidatively unstable oxidation state of the appropriate metal, binds to and breaks DNA. For example, the available evidence supports the concept that bleomycin exhibits its DNA-cutting activity through the formation of a complex of DNA-bleomycin-chelated Fe(II)-molecular oxygen. Oxidation of the bound Fe(II) ion occurs in this complex with concomitant reduction of oxygen to produce oxygen free radicals (14, 26–28). Several studies have suggested that 1,10-phenanthroline produces DNA degradation via a reaction with copper, oxygen, and a reducing agent (29–31). Hydroxyl radicals are generated in the vicinity of susceptible bonds, resulting in DNA strand breakage (32). Recently, Wong et al. (17, 18) have reported evidence which suggests oxygen free radicals play a role in the DNA breakage induced by mAMSA and Cu(II). While we have found no evidence that DPPE-copper complexes interact with DNA, recent experiments in our laboratory provide evidence that some DPPE-gold complexes can interact with DNA in vitro and produce single-strand DNA breaks in cells as measured by alkaline elution (33). While the locus for the cytotoxic action of DPPE-copper complexes may be similar to that of the other drug-metal complexes discussed above (i.e., DNA), the phosphorous-copper ligating complex unique to DPPE may provide a novel molecular mechanism for the cytotoxic action of DPPE-copper complexes. Clearly, our studies demonstrate the potential of DPPE-copper complexes to inhibit DNA polymerase α, which is required for cellular DNA synthesis (Fig. 5).

As metal ions are intimately involved in many biological systems, the affinity of a drug for metal ions can have pharmacological significance. Metal binding in biological systems is a highly competitive phenomenon and the determination of whether a drug is complexed or uncomplexed under physiological conditions is often difficult (34); nonetheless, a growing number of antineoplastic drugs appear to require a biologically available metal ion for their activity. The role of the interaction of these drugs with metals in conferring selectivity of the drugs for killing tumor cells is unclear. Although a number of hypotheses have been proposed to address this question (35, 36), it is possible that each group of drugs with its specific interactions with certain metals will require a different mechanistic explanation; however, it appears that copper plays a significant role in the biological activities of a number of compounds with cytotoxic and antineoplastic activities. Our results suggest that DPPE may be included in this group of drugs. The modulation of the biological activities of DPPE by copper and the spectrum of in vivo antitumor activity of DPPE suggest that this class of compounds may provide probes for elucidating the role of copper in the tumor cell selectivity of a variety of antineoplastic agents.

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