*Biochemical Pharmacology*, Vol. 34, No. 18, pp. 3243–3250, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00


# EFFECTS OF COORDINATED GOLD COMPOUNDS ON *IN VITRO* AND *IN SITU* DNA REPLICATION

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(*Received* 11 *November* 1984; *accepted* 4 *February* 1985)

**Abstract**—Auranofin, a coordinated gold compound, inhibits *in vitro* DNA synthesis and displays *in vivo* antitumor activity. To understand the mechanisms of inhibition of DNA replication, we have examined the effects of auranofin and other gold complexes on the activities of purified cellular and herpesvirus-induced DNA polymerases, and on *in situ* DNA replication in permeabilized S phase KB cells. Evaluation of the data suggests the following conclusions. (1) The gold compounds varied in their abilities to inhibit DNA polymerase activities. DNA polymerase $\alpha$ was more sensitive to inhibition by gold compounds than DNA polymerase $\beta$; (2) Inhibition of purified DNA polymerases by gold (I) compounds was noncompetitive with both DNA template and triphosphate substrates. Inhibition by SKF 101675, a gold (III) complex was competitive with DNA. (3) None of the gold compounds tested preferentially inhibited herpesvirus-induced DNA polymerases. (4) The gold complexes that inhibited *in vitro* DNA replication also inhibited *in situ* DNA synthesis. However, the potency and order of potency of the compounds varied between the *in vitro* and *in situ* systems. (5) Auranofin and other gold compounds inhibited the clonogenic capacity of KB cells in a concentration-dependent manner. The $IC_{50}$ values measured in the clonogenic assay were significantly lower than those obtained from the *in vitro* and *in situ* DNA replication assays.

Auranofin, an orally absorbed, coordinated gold compound with antiarthritic activity [1], has been reported to be cytotoxic *in vitro* and to display antitumor activity against P388 lymphocytic leukemia *in vivo* [2–9]. As a result of these activities and those of platinum complexes [6, 7], we are studying a variety of gold compounds for potential antineoplastic activities and are attempting to determine the mechanisms of cytotoxicity.

Gold compounds have been shown to inhibit DNA, RNA and protein syntheses at approximately equivalent concentrations. Furthermore, this cytotoxicity correlates with the amount of gold taken up by cells [8, 9]. Gold (III) compounds and some gold (I) compounds have also been shown to interact with DNA [10, 11].

To understand the mechanisms of inhibition of DNA synthesis and structure–activity relationships associated with this activity, we have investigated the effects of auranofin and other gold compounds on the *in vitro* activity of DNA polymerases $\alpha$ and $\beta$ from human leukemia cells and *in situ* DNA replication in permeabilized, S phase synchronized KB cells. We have also studied the effects of gold complexes on DNA polymerases induced by HSV-1‖ and HSV-2 and EBV.

## MATERIALS AND METHODS

*Reagents.* [$^3$H]dTTP (10–20 Ci/mmole), [$^3$H]-dATP (10–20 Ci/mmole), and [$^3$H]dGTP (10–20 Ci/mmole) were obtained from Dupont New England Nuclear. Calf thymus DNA was converted to the activated form by treatment with DNase I (Worthington Chemicals) and used as the template for purified DNA polymerases [12]. The gold complexes used in our studies were synthesized by Smith Kline & French Laboratories. Cisdiaminedichloroplatinum (cisplatinum) was provided by Bristol Laboratories (Syracuse, NY). The compounds tested and their corresponding SKF identification numbers are listed in Table 2. All other chemicals were purchased from the Sigma Chemical Co.

*Isolation of cellular and viral DNA polymerases.* DNA polymerases $\alpha$ and $\beta$ were isolated from leukocytes of a patient with acute myelogenous leukemia [13]. EBV-DNA polymerase was purified from P3HR-1, a virus producing cell line derived from a patient with Burkitt's lymphoma [14]. HSV-1 and HSV-2 DNA polymerases were isolated from Vero cells infected with HSV-1 strain Cl 101 and HSV-2 strain 333 respectively.

The DNA polymerases used in these experiments were purified by successive chromatography using DEAE-cellulose, phosphocellulose, and single-

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‖ Abbreviations: HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; EBV, Epstein–Barr virus; DEAE, diethylaminoethyl; NEM, *N*-ethylmaleimide; DTT, dithiothreitol; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; MEM, Eagle's Minimum Essential Medium; NBCS, newborn calf serum; PBS, phosphate-buffered saline; PEP, phosphoenolpyruvate; and TCA, trichloroacetic acid.

strand DNA-cellulose columns according to procedures described before [15]. In brief, approximately $10^9$ cells were mixed with 5 ml of homogenization buffer [0.5% (v/v) Triton X-100 in 0.8 M KCl] and disrupted in a chilled Dounce homogenizer. An equal volume of buffer A consisting of 20 mM potassium phosphate (pH 7.0), 0.5 mM EDTA and 1 mM dithiothreitol (DTT) and 50% glycerol (v/v) was added and mixed well. Nucleic acids from the cells were removed by passing the extract through a DEAE-cellulose column equilibrated with the same buffer. Proteins were eluted with buffer A containing 0.3 M KCl. Salt was removed by dialysis and the extract was applied to a phosphocellulose column equilibrated with buffer B consisting of 50 mM Tris–HCl (pH 8.0), 0.5 mM EDTA, 1 mM DTT and 30% glycerol (v/v). DNA polymerases were fractionated on this column with a linear KCl gradient. DNA polymerase $\alpha$ and $\beta$ eluted at 0.1 M KCl and 0.35 M KCl respectively. DNA polymerase $\alpha$ and $\beta$ eluted at 0.1 M and 0.21 M KCl, respectively, on a single-strand DNA-cellulose column. HSV-1 and 2 DNA polymerases eluted at 0.1 M and 0.25 M KCl on both phosphocellulose and single-strand DNA-cellulose columns.

The purified enzymes were free of cross-contamination and resemble respective viral and cellular DNA polymerases in that they displayed normal preferences for primer-templates and sensitivities to NEM, aphidicolin, and mono and divalent cations [16, 17].

*Purified DNA polymerase assays.* To study inhibition of purified polymerases by gold compounds, DTT was omitted from the reaction mixture. The enzymes were also dialyzed against buffer B consisting of 50 mM Tris–HCl (pH 8.0), 0.5 mM EDTA and 50% glycerol (v/v) to remove any DTT. DNA polymerase $\alpha$ activity was assayed in a 50-$\mu$l reaction mixture containing 50 mM Tris–HCl (pH 8.0), 1 mM DTT, 8 mM $MgCl_2$, 100 $\mu$M each of dCTP, dGTP and dTTP, and 20 $\mu$M [$^3$H]dATP (1500 cpm/pmole), 10 $\mu$g activated calf thymus DNA, 10–20 $\mu$g BSA, 5–10% glycerol (v/v) and enzyme. Incubations were performed at 37° for 30 min. Acid-insoluble radioactivity was collected onto nitrocellulose filters (Millipore, 0.45 $\mu$m). These filters were washed six times with 5% (w/v) TCA containing 2 mM sodium pyrophosphate, once with 70% ethanol, and dried. After drying, the filters were counted in a liquid scintillation counter. All determinations were performed in duplicate.

DNA polymerase $\beta$ activity was assayed under similar conditions except that pH 9.0 Tris–HCl buffer, a 50 $\mu$M concentration of non-radioactive triphosphates, 40 $\mu$M [$^3$H]dATP and 40 mM KCl were used.

The reaction mixture for assaying HSV-1 DNA polymerase activity contained 50 mM Tris–HCl (pH 8.3), 2 mM DTT, 4 mM $MgCl_2$, 10 $\mu$M each of dATP, dCTP and dTTP, 0.5 to 1.0 $\mu$M [$^3$H]dGTP (4300 cpm/pmole), 5 $\mu$g activated calf thymus DNA, 50 mM ammonium sulfate, 10 $\mu$g BSA, 5–10% glycerol (v/v) and enzyme. Other conditions were similar to those described for measuring DNA polymerase $\alpha$ activity. EBV DNA polymerase activity was assayed under similar conditions for DNA polymerase $\alpha$ except that 4 mM $MgCl_2$, 10 $\mu$M [$^3$H]dGTP and 100 mM KCl were used.

*DNA synthesis in KB cells.* KB cells, a human epidermoid carcinoma cell line, were obtained from the American Type Culture Collection (ATCC CCl-17). *In situ* DNA replication was examined in permeabilized, S phase synchronized, KB cells by modifying a procedure established by Miller *et al.* [18] for studying DNA synthesis. Optimal DNA synthesis was obtained by incubating $6.25 \times 10^4$ cells per tube in a reaction mixture containing 35 mM HEPES (pH 7.4), 50 mM sucrose, 20 mM PEP, 0.25 mM dGTP, dATP, dCTP, and 0.32 mM [methyl-$^3$H]TTP, 0.25 mM dADP, dCDP, dGDP, 2 mM ATP, 5 mM potassium phosphate (pH 7.4) and 5 mM $MgCl_2$ for 4 min at 37° in a shaking water bath. Total assay volume was 100 $\mu$l. The reaction was stopped by placing tubes on ice and adding 100 $\mu$l yeast RNA and 10% (w/v) ice-cold TCA.

Acid-insoluble radioactivity was measured as described for the purified DNA polymerases. All determinations were performed in triplicate.

*Cell culture techniques.* KB cells were grown in monolayer in MEM containing 10% NBCS, 0.1% gentamicin in a 5% $CO_2$ humidified incubator at 37°. These cells were determined to be mycoplasma-free by the mycotrim-TC mycoplasma test (Hana Media, Inc.).

*Cell synchronization.* KB cells to be synchronized were grown in roller bottles in MEM with 10% NBCS and 20 mM HEPES in a 37° warm room. Cultures were synchronized in S phase of the cell cycle by double thymidine treatment [19]. Efficiency of synchronization was assessed by autoradiography. Greater than 98% of the cells were in S phase as determined by this method.

*Permeabilization.* KB cells were scraped from roller bottles 2 hr after release from thymidine block, collected, and washed twice with PBS. They were diluted to the desired concentration in PBS containing 150 mM sucrose, 80 mM KCl, 35 mM HEPES (pH 7.4), 5 mM potassium phosphate (pH 7.4), 5 mM $MgCl_2$ and 0.5 mM $CaCl_2$. Synchronized cells were then permeabilized by fast freezing in a 95% ethanol and dry ice bath and slow thawing at 25°. Permeabilization was assessed by cellular uptake of trypan blue. Permeabilized cells were stored in a −20° freezer and thawed just prior to use in the *in situ* DNA replication assays.

*Clonogenic assay.* Asynchronous KB cells were harvested and 5000 cells were plated in sterile 60 mm × 15 mm petri dishes. Cells were allowed to attach to the plate surface overnight. Cells were treated for 2 hr with gold compounds in MEM/10% NBCS. At the end of 2 hr, the medium was aspirated, plates were washed once with 5 ml of PBS, and fresh medium was added. Plates were incubated for 5–6 days at 37° in a $CO_2$ incubator. Viability was measured by the ability of a KB cell to form a colony greater than 50 cells. Colonies were fixed and stained with 0.5% crystal violet in 95% ethanol. Plates were washed, dried and counted with a Biotran III Automatic Count Totalizer (New Brunswick Scientific Co., Edison, NJ). Means and S.D. of triplicate samples were determined for each drug concentration. The data were then analyzed by plotting the

log of the survival fraction versus the drug concentration.

## RESULTS

*Effect of DTT on the inhibition of DNA polymerase activities.* DTT is required for optimal DNA polymerase $\alpha$ activity. However, in preliminary experiments in which DTT was included, the gold compounds tested were minimally active. If DTT was omitted from the reaction mixture, a significant increase in inhibition of enzyme activity was observed (Fig. 1). Similar differences in the extent of inhibition were also observed for other gold complexes (data not shown). Consequently, the effects of DTT on the activities of the various DNA polymerases were evaluated. In the absence of DTT, DNA polymerase $\alpha$ activity was 78% of the activity in the presence of an optimal concentration of DTT. No qualitative changes in activity were observed. None of the activities of the other DNA polymerases were affected by the deletion of DTT. We, therefore, performed all subsequent studies in the absence of DTT. To further examine the effects of thiols on the inhibition of DNA replication by gold complexes we have compared the effects of DTT, glutathione and cysteine. Results shown in Table 1 indicate that all three thiol agents used in this study have similar effects in reducing the inhibitory effect of gold complexes on DNA replication.

*Inhibition of purified cellular and viral DNA polymerases.* A number of gold (I) and gold (III) complexes, shown in Tables 2 and 3, inhibit cellular and viral DNA polymerases *in vitro*. Purified DNA polymerase $\alpha$ was more sensitive to inhibition by gold compounds than DNA polymerase $\beta$. For example, 20 $\mu$M SKF 101510 inhibited 86% of DNA polymerase $\alpha$ activity, while only 5% of DNA polymerase $\beta$ activity was inhibited by the same amount of drug.

Table 2 shows DNA polymerase $\alpha$ and HSV-1 DNA polymerase to be equally sensitive to inhibition by gold, while HSV-2 and EBV DNA polymerases

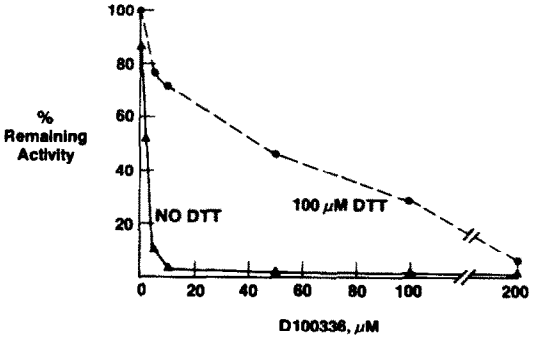



Fig. 1. Inhibition of DNA polymerase $\alpha$ activity by SKF 100336 in the presence and absence of DTT. The enzyme activity was measured by [$^3$H]dAMP incorporation into activated DNA template. One hundred percent of DNA polymerase activity represented 12.9 to 13.8 pmoles [$^3$H]-dAMP incorporation under optimal conditions for enzyme activity except that DTT was omitted from the reaction mixture. Key: (▲—▲) no DTT and (●—●) 100 $\mu$M DTT in the reaction mixture.

Table 1. Effects of thiol agents on inhibition of DNA polymerase $\alpha$ activity* by gold compound

| | % Inhibition by D100336 | |
|---|---|---|
| Addition† | 2 $\mu$M | 20 $\mu$M |
| None | 89 | 95 |
| DTT | 0 | 25 |
| Glutathione | 0 | 22 |
| Cysteine | 39 | 86 |

* One hundred percent activity represents 11.5 pmoles of [$^3$H]dAMP incorporation into activated DNA template.
† A 100 $\mu$M concentration of thiol agents was used.

were much less sensitive. None of the complexes tested to date preferentially inhibit viral polymerase. The effect of cisplatinum on DNA polymerase activity was compared with that of gold complexes. Cisplatinum was less potent than the gold complexes. For example, 50 $\mu$M cisplatinum inhibited the DNA polymerase $\alpha$ activity by only 16% (data not shown); a 5 $\mu$M concentration of the gold complex SKF 100336, in contrast, inhibited 95% of the DNA polymerase $\alpha$ activity.

*Mode of inhibition by gold complexes.* To examine the mechanism by which gold complexes inhibit *in vitro* DNA synthesis, we determined the extent of their inhibition as a function of increasing concentration of both activated DNA template and triphosphate substrate. The Lineweaver–Burk plots for inhibition of polymerase $\alpha$ activity by SKF 100336, a gold (I) compound, indicate that inhibition was non-competitive with both DNA template (Fig. 2) and substrate (data not shown). A similar mode of inhibition was also observed for other gold (I) complexes. The $K_i$ values for those compounds are shown in Table 4. The results illustrate that auranofin, SKF 39162, was not as potent an inhibitor of DNA polymerase $\alpha$ activity as the other compounds tested.

Gold complex interactions with DNA polymerase $\beta$ were also investigated. This inhibition was also non-competitive with DNA template and substrate, although $K_i$ values were generally higher than those measured for DNA polymerase $\alpha$ (data not shown).

The mode of inhibition by 101675, a gold (III) complex, was different from that of gold (I) complexes in that it was competitive with the DNA template (Fig. 3). This is consistent with reports from this laboratory showing that gold (III) compounds bind to isolated PBR322 DNA and calf thymus DNA [11].

*Inhibition of* in situ *DNA replication.* Figure 4 shows the inhibition of *in situ* DNA replication by gold complexes. All of those compounds that inhibited purified DNA polymerases also inhibited *in situ* DNA replication. Table 5 compares the effects of gold compounds on the activities of purified DNA polymerase $\alpha$ and *in situ* DNA replication. The potency of the gold complexes varied between the two systems, with *in situ* inhibition requiring up to 20-fold greater concentration of gold compounds than *in vitro*.

*Cytotoxic effect of gold complexes on cultured KB*

Table 2. Effects of gold complexes on the activities* of DNA polymerase α and DNA polymerases of three human herpesviruses

| Compound SKF number† | | Conc (μM) | Percent inhibition | | | |
|---|---|---|---|---|---|---|
| | | | DPα | HSV-1 DP | HSV-2 DP | EBV-DP |
| 100336 | Au(I) | 5 | 93 | 87 | 10 | 3 |
| 85626 | Au(I) | 5 | 88 | 87 | 34 | 12 |
| 36914 | Au(I) | 5 | 85 | 81 | 25 | 5 |
| 101510 | Au(I) | 5 | 81 | 87 | 10 | 3 |
| 39162 | Au(I) | 5 | 3 | 3 | 0 | 0 |
| | | 50 | 62 | 43 | 0 | 0 |
| 60646 | Au(I) | 5 | 51 | 35 | 22 | 0 |
| 80544 | Au(I) | 5 | 28 | 20 | 0 | 0 |
| 40117 | Au(I) | 5 | 26 | 17 | 15 | 3 |
| 39807 | Au(I) | 5 | 14 | 24 | 0 | 0 |
| 41170 | Au(I) | 5 | 13 | 10 | 10 | 5 |
| 60817 | Au(I) | 5 | 12 | 32 | 0 | 0 |
| 58889 | Au(I) | 5 | 0 | 0 | 0 | 0 |
| 89754 | Au(I) | 5 | 11 | 5 | 3 | 0 |
| 80019 | Au(I) | 5 | 7 | 4 | 0 | 0 |
| 101675 | Au(III) | 5 | 35 | 38 | 5 | 0 |
| | | 50 | 89 | 97 | 23 | 23 |

* One hundred percent activity represents 12 to 13.2 pmoles of [$^3$H]dAMP incorporation into activated DNA template.

† SKF compound numbers and corresponding chemical names: 100336, 2,3,4,6-tetra-*O*-acetyl-2-β-D-glucopyranosyl bis [(triethylphosphine)-aurio] sulfonium nitrate; 85626, 2,3,4,6-tetra-*O*-acetyl-1-β-D-glucopyranosyl bis [(triethylphosphine)-aurio] sulfonium chloride; 36914, chloro(triethylphosphine)gold; 101510, tris[(triethylphosphine)-aurio] sufonium nitrate; 39162, ((1-thio-β-D-glucopyranosato)(triethylphosphine gold)2,3,4,6-tetra-acetate); 60646, bromo-(triethylphosphine)gold; 80544, bis-(triethylphosphine)gold (1-chloride); 40117, *S*-(triethyl-phosphine)gold-thio-malic acid; 39807, *S*-(triethylphosphine)gold-2-mercaptoethanol; 41170, tris(dimethylaminophosphine)gold chloride; 60817, cyano(triethylphosphine)gold; 58889, di-μ-(diethylphosphinoethyl)mercaptodigold (I); 89754, *S*-(diethylethoxyphosphine)gold, 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glycopyranoside; 80019, (triethylphosphine)methyl gold; and 101675, (2-hydroxyethylpyridine)trichloro gold (III).

*cells.* Auranofin and other coordinated gold compounds have been reported to be cytotoxic against neoplastic cells *in vitro* [2–4]. We examined the cytotoxic effects of those gold compounds that were inhibitory to *in vitro* and *in situ* DNA replication on KB cells, an epidermoid cancer cell line. Figure 5 shows the effect of auranofin, SKF 39162, on the clonogenic capacity of KB cells after a 2-hr treatment with increasing concentrations of the drug. The survival curves of auranofin and the other gold complexes tested (data not shown) were both monophasic and exponential, implying that all cells were equally sensitive to the effects of the complexes. Table 6 compares the $IC_{50}$ values obtained from the KB clonogenic assay to those measured with B16 melanoma cells.

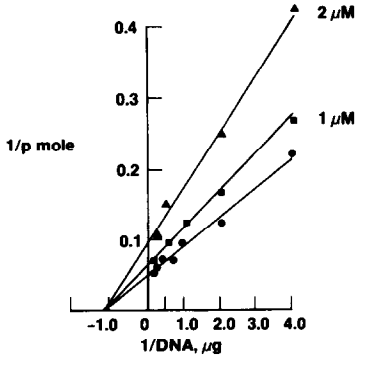



Fig. 2. Effect of SKF 100336 on DNA synthesis catalyzed by DNA polymerase α with different concentrations of activated DNA template. Key: (●—●) no inhibitor; (■—■) 1 μM inhibitor and (▲—▲) 2 μM inhibitor.

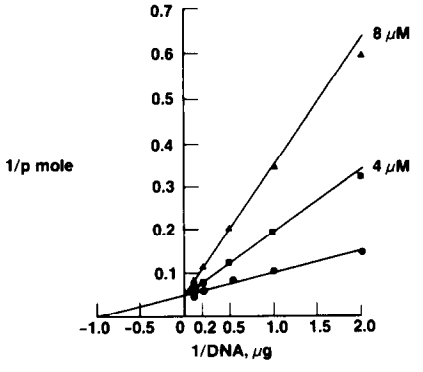
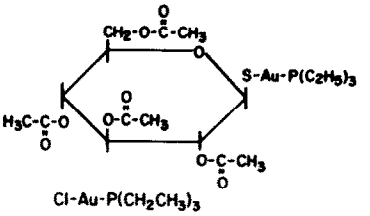
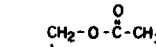
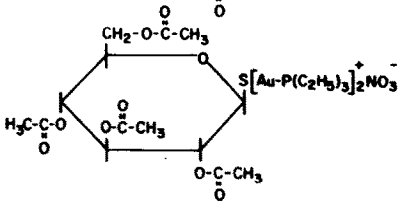
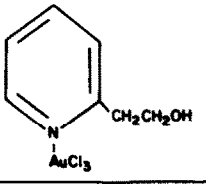



Fig. 3. Effect of SKF 101675 on DNA synthesis catalyzed by DNA polymerase α with increasing concentrations of activated DNA template. Key: (●—●) no inhibitor; (■—■) 4 μM inhibitor; and (▲—▲) 8 μM inhibitor.

Table 3. Structure chart

| SKF # | CHEMICAL NAME | OXIDATION STATE | STRUCTURE |
|---|---|---|---|
| 39162 | ((1-thio-β-D-glucopyranosato) (triethylphosphine gold) 2,3,4,6-tetra-acetate) | Au(I) |  |
| 36914 | Chloro (triethylphosphine) gold | Au(I) |  |
| 85626 | 2,3,4,6-tetra-O-acetyl-1-β-D-glucopyranosyl bis [(triethylphosphine)-aurio] sulphonium chloride | Au(I) |  |
| 100336 | 2,3,4,6-tetra-O-acetyl-2-β-D-glucopyranosyl bis [(triethylphosphine)-aurio] sulfonium nitrate | Au(I) |  |
| 101675 | (2-hydroxyethylpyridine) trichlorogold | Au(III) |  |

## DISCUSSION

In a previous study, Simon *et al.* [3] reported that auranofin preferentially inhibits DNA synthesis in Hela cells as measured by inhibition of macromolecular incorporation of [$^3$H]thymidine. To understand the mechanisms of inhibition of DNA replication, we studied the interactions of fifteen gold compounds with purified DNA polymerases. Our data show that gold complexes varied significantly from each other in their abilities to inihibit *in vitro* replication. This variation in ability to inhibit polymerase activity probably reflects the differences in chemical structure between each of the compounds, since changes in the ligand and the stereochemistry of those ligands coordinated with the gold nucleus have been reported to alter biological activities [6, 20].

Table 4. Kinetic analysis of inhibition by gold complexes of DNA polymerase α activity

| Compound | $K_i$ (μM) |
|---|---|
| 36914 | 0.9 |
| 85626 | 1.5 |
| 100336 | 1.6 |
| 101510 | 1.8 |
| 39162 | 27.8 |

DNA polymerase α and HSV-1 DNA polymerase activities were more sensitive to inhibition by gold compounds than HSV-2 and EBV DNA polymerases. For example, 5 μM SKF 100336 inhibited DNA polymerase α by 93% and HSV-1-induced DNA polymerase by 87%, while HSV-2 and EBV DNA polymerases were inhibited by 10 and 3% respectively. DNA polymerase α was generally twenty times more sensitive to inhibition by gold compounds than DNA polymerase β. In addition, significant inhibition of all polymerases was observed only in the absence of DTT. It is likely that the sulfhydryl groups of the thiol interact with gold compounds interfering with their association with the DNA replication complex. These data and the report that DNA polymerase α is selectively inhibited by sulfhydryl reagents [21] suggest that gold I complexes that inhibited DNA polymerases do so by interacting with the enzymes at sulfhydryl groups on the polymerases. This manner of inhibition has also been proposed for a number of other enzymes [20, 22].

A comparison of sensitivities of purified cellular and herpesvirus specific DNA polymerases revealed that none of the compounds examined thus far selectively inhibited the viral enzymes. Furthermore, the

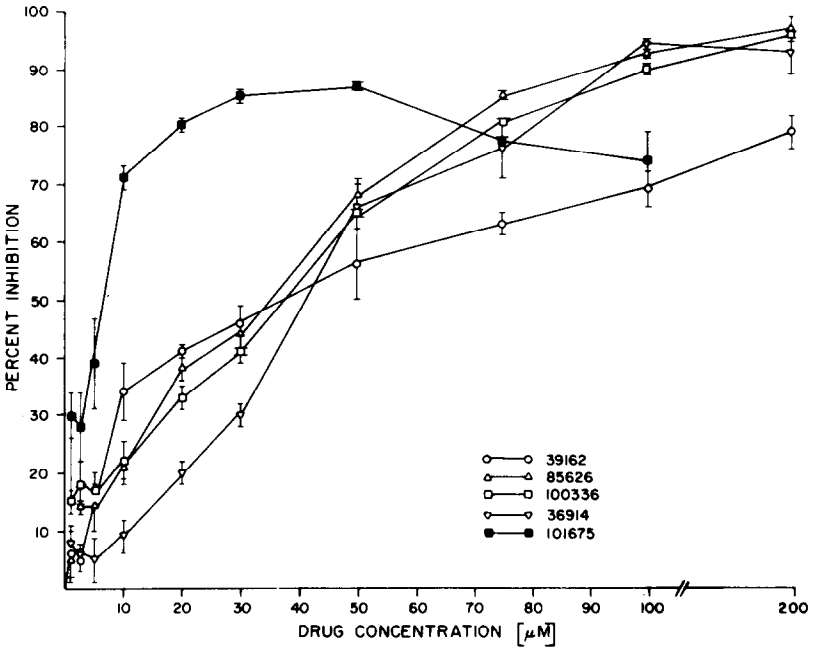



Fig. 4. Inhibition of *in situ* DNA replication by increasing concentrations of gold complexes. Permeabilized cells were incubated with assay reaction mixture described in Materials and Methods; 1.53 to 3.87 pmoles of [$^3$H]dTMP was incorporated into TCA precipitable counts under optimal assay conditions. Inhibition was measured by the decrease in TCA precipitable counts. Points: mean of triplicate samples; bars: S.E.M.

concentration of gold complexes that inhibited HSV-1 replication in Vero cells in culture also proved to be toxic to uninfected cells (data not presented).

Kinetic analysis shows that the inhibition of DNA polymerase activity by gold (I) complexes was non-competitive with DNA as well as the triphosphate substrate. The mode of inhibition of DNA polymerases by a gold (III) complex, SKF 101675, differs from that of gold (I) compounds in that this complex was found to be competitive with the DNA template. This result correlates with the observation that gold (III) complexes, which possess square planar geometry much like cis-diaminedichloroplatinum (II), bind to isolated PBR322 DNA and calf thymus DNA [11, 19]. Some gold (I) compounds, like SKF 36914, also bind to and cause changes in the electrophoretic mobilities of DNA similar to those produced by cis-diaminedichloroplatinum and SKF 101675 [11]. The binding of SKF 36914 to DNA and subsequent DNA mobility changes occurred at pH 9.5, while DNA polymerase assays were performed at pH 8.0. Therefore, pH differences in experimental systems may explain why SKF 36914 inhibition of purified DNA polymerase activity was found to be non-competitive with the DNA template. In addition, gold (I) can also be stabilized by complexation to "soft" or "class b" ligands such as thiolates and phosphines [6, 20]. This high affinity of gold (I) for sulfur may imply a greater attraction of gold (I) complexes to sulfhydryl containing proteins than to DNA.

Gold (III) compounds may also interact with replicative enzymes since they bind to sulfhydryl groups as well as nitrogen bases. They have also been reported to precipitate proteins and to oxidize methionine, cysteine and carboxylate residues [20].

Table 5. Comparison of $IC_{50}$ values of gold compounds on the activity of DNA polymerase $\alpha$ and *in situ* DNA replication

| | $IC_{50}$ ($\mu$M) | |
|---|---|---|
| Compound | DNA polymerase $\alpha$ | *In situ* DNA replication |
| 39162 | 19.2 | 37.5 |
| 36914 | 2.7 | 41.0 |
| 85626 | 2.6 | 35.0 |
| 100336 | 2.5 | 37.5 |
| 101675 | 5.5 | 6.5 |

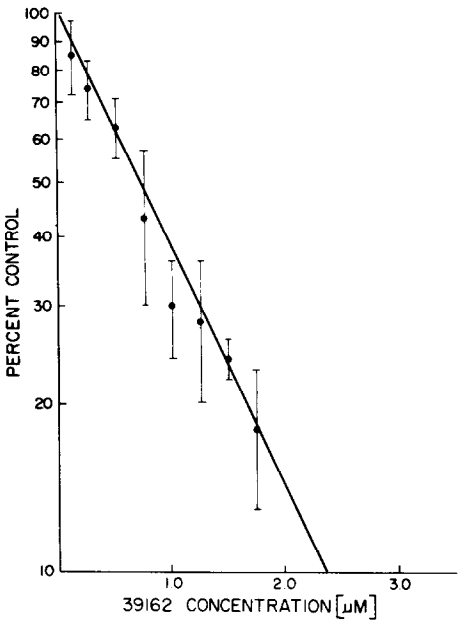



Fig. 5. Cytotoxic effect of auranofin (SKF 39162) on KB cells as measured in the clonogenic assay. Points: mean of triplicate samples; bars: S.D.

We studied the inhibition of DNA replication in permeabilized S phase synchronized KB cells to assess the effects of gold compounds in a physiological milieu with intact DNA replicative enzymes and endogenous template while reducing the potential complicating factors introduced by intact plasma membrane. To avoid artifacts associated with variations in the degree of permeabilization, all experiments were performed with a single batch of permeabilized cells and then repeated with another batch. Batch-to-batch variation in experimental results was less than 10%. The four gold (I) and one gold (III) complexes that inhibited purified DNA polymerase activity also inhibited *in situ* DNA replication. As a rule, more gold compound was required for *in situ* inhibition than *in vitro* inhibition. The order of potency also varied between the *in situ* and *in vitro* systems. Since gold complexes are chemically reactive compounds, they probably undergo extensive intracellular modification through a variety of processes, including disproportionation and ligand exchange. The differences in potency and order of potency between *in situ* and *in vitro* systems may reflect variations in reaction of gold compounds with cellular components such as proteins and low molecular weight thiols [20, 23].

The structure–activity relationships associated with gold (I) inhibition of *in vitro* and *in situ* DNA replication were investigated. In general, gold (I) compounds varied in their abilities to inhibit DNA replication as a consequence of structural differences in the ligands associated with the gold nucleus of the compound. The triethylphosphine gold of SKF 36914 is present in the larger molecule of auranofin, SKF 39162. Auranofin was not as potent an inhibitor of *in vitro* DNA replication as those chloride (SKF 85626) or nitrate (SKF 100336) substituted analogs, or SKF 36914 (chlorotriethylphosphine gold). Those compounds may be better inhibitors of DNA polymerase activity because chloride and nitrate make better leaving groups than the tetraacetylthioglucose moiety of auranofin.

Our data and recent studies from others in our laboratory [9, 24] also suggest that the gold–phosphine moiety is required for *in vitro* and *in situ* inhibition of DNA replication. In general, gold–phosphine complexes are more lipophilic than nonphospine substituted analogs. This increase in lipophilicity may enhance transport of the gold complex through cellular membranes and facilitate association of the complex with the active site of DNA polymerases.

Finally, the abilities of auranofin and other gold compounds to inhibit the clonogenic capacity of cultured KB cells were examined. The capacity of KB cells to form colonies was affected in a concentration-dependent manner. Even though the $IC_{50}$ values were similar to those obtained with another cancer cell line, they were significantly lower than those measured in the *in vitro* and *in situ* DNA replication assays. Recent data in our laboratory show that DNA, RNA and protein syntheses are equally sensitive to inhibition by auranofin [9]. Those data and those presented in this study suggest that the gold compounds studied to date may interact with numerous cellular components other than DNA replicative enzymes. Based on current data, it is not possible to conclude that inhibition of DNA replication is a critical factor in the cytotoxicity of these agents.

Table 6. Cytotoxic effects of gold compounds on two cell lines *in vitro*

| | $IC_{50}$ (μM) | |
|---|---|---|
| | Cell line | |
| Compound | KB | B16* |
| 39162 | 0.7 | 1.0 |
| 36914 | 0.6 | 1.0 |
| 85626 | 0.5 | 0.6 |
| 100336 | 0.5 | 0.4 |

* From Ref. 4.

*Acknowledgements*—We thank Ms. Judy Seaman for her excellent typographical assistance in preparing the manuscript. We also thank Drs. Dave Hill and Blaine Sutton for our supply of gold complexes. We give special thanks to Dr. Christopher Mirabelli for his helpful discussions.

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