In Vitro and in Vivo Antitumor Properties of Tetrakis((trishydroxymethyl)phosphine)gold(I) Chloride

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Abstract: A novel hydrophilic gold compound, tetrakis((trishydroxymethyl)phosphine)gold(I) chloride **1**, has been investigated for its antitumor properties. In vitro studies demonstrate that **1** is active against HCT-15, AGS, PC-3, and LNCaP tumor cells. Cell cycle analysis of the HCT-15 cells by flow cytometry revealed elongation of the G1 phase of the cell cycle leading to growth inhibition. Administration of **1** to Balb/C mice inoculated with syngenic meth/A cells demonstrated statistically significant dose-dependent survival time.

Gold-based therapeutic agents have been used for over 50 years in the treatment of rheumatoid arthritis with nearly 70% success rate.[1,2] The FDA-approved gold-based drug auranofin comprises a linear arrangement of phosphine (PEt_3) and thiolate ligands bound to the gold(I) center.[3] Several phosphine-ligated gold(I) complexes[4−7] including auranofin[8] and various gold(III) complexes[9−14] have been found to be active in suppressing growth of cultured tumor cells such as HCT-15 and malignant meth/A cells in vitro. Administration of specific gold agents has resulted in prolonged life with limited side effects in tumor-bearing mice. Therefore, there is a considerable interest in developing gold-based drugs focused on anticancer use.[5,6] In general, the higher drug tolerance profiles of gold-based drugs compared to the acute toxic effects of cisplatin have provided attractive prospects for future applications of gold-based drugs in chemotherapy. However, clinical trials in humans of anticancer applications of gold compounds are still limited.[15,16]

The pharmacokinetic studies on gold-based agents to date reflect that a majority of these compounds are hydrophobic.[7−14] The utility of hydrophilic gold compounds for therapeutic applications in the treatment of cancer is less explored in part because of the lack of well-defined gold agents with hydrophilic characteristics. As part of our ongoing research efforts on the design and development of metal- and radiometal-based cancer diagnostic and therapeutic agents,[17−20] we report herein the in vitro cytotoxic properties of tetrakis((trishydroxymethyl)phosphine)gold(I) chloride ($[Au(P(CH_2OH)_3)_4]Cl$, **1**) against prostatic, gastrointestinal, and colonic carcinoma tumor cells. We also present our findings that **1** elongates the G1 phase of the cell cycle and exhibits significant in vivo cytotoxic properties.

Chemistry. The literature procedure[18] has been modified as shown in Scheme 1 to afford multigram-scale synthesis and easy purification of **1**. To a vigorously stirring solution of trishydroxymethylphosphine (4.10 g, 33.04 mmol) in 30 mL of deoxygenated water, a solution of triphenylphosphinegold(I) chloride (4.00 g, 8.08 mmol) in dichloromethane (50 mL) was added dropwise, and the stirring was continued for 1 h after addition. Then the aqueous layer was separated and washed with dichloromethane (2 × 25 mL) and the solvent was evaporated under reduced pressure to yield the crude product as a white, pasty mass. The crude product was dissolved in 15 mL of methanol, layered with 1 mL of diethyl ether, and cooled to −15 °C for 3 days to afford pure **1** as a white crystalline solid. The mother liquor was concentrated and the process was repeated to get second batch of crystals of **1**. The combined yield was 4.12 g (70%). The formation and purity of **1** were confirmed by NMR spectroscopy and elemental analysis.

Results and Discussion. Cell inhibition properties of **1** were studied using LNCaP, PC-3 cells derived from prostate cancer, HCT-15 cells derived from human colon carcinoma, and AGS cells derived from human gastric carcinoma. The cytotoxic properties of **1** on the cell lines were evaluated by cell count analysis, and the results are summarized in Table 1.

The cell inhibition data (Table 1) clearly indicate that the new gold agent **1** inhibited the growth of different types of tumor cells. The PC-3 cell line required much higher concentrations of **1** for inhibition (see Supporting Information for further details). A 50% cell growth inhibition of the PC-3 cell line occurred with the gold agent concentration around 26 nM. This compound demonstrated excellent cytotoxicity against androgen-dependent LNCaP cells, HCT-15, and AGS and even against the androgen-independent PC-3 prostate tumor cells. Indeed, the cell growth inhibition property of **1** for the androgen-independent PC-3 cells is significant because cytotoxic effects on hormone refractory cancers are rarely observed among metal-based cytotoxic agents.

The results of cell cycle analysis on synchronized HCT-15 cells by flow cytometry are summarized in Table 2. The data demonstrate that the percent of cells in the G1 phase was significantly higher for cell samples that were incubated with gold agent **1** than for the control set. This result suggests that **1** may induce cell growth inhibition via elongation of the G1 phase in the cell cycle.

This result is significant because disruption or malfunction of cell cycle control within the G1 phase has been recognized as the most important biochemical phenomenon for tumor progression and tumorigenesis.[21−23] The ability of certain small molecules to control cell cycle

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Scheme 1. Synthesis of **1**

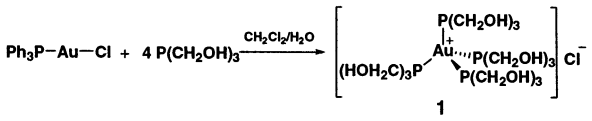

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Table 1. Cell Growth Inhibition Data with Varying Concentrations of **1**

concn of **1** (nM)	cell growth inhibition[a] (%)			
	LNCaP	AGS	HCT-15[b]	PC-3[c]
13.72	83.9 ± 6.7	59.5 ± 8.1	87.5 ± 3.7	27
2.74	59.2 ± 2.7	17.4 ± 8.6	58.3 ± 7.6	
0.54	47.4 ± 11.6	10.2 ± 6.2	22.5 ± 12.6	

[a] Tabulated percentages of cell inhibition of various cancerous cell lines after exposing cell cultures to a 40 μL solution of **1** in methanol and cultured in 5% CO_2 incubator for 7 days. % growth inhibition = [(mean control cell count − mean experimental cell count)/(mean control cell count)] × 100. [b] Incubation time of 4 days. [c] Calculated value (see Supporting Information for further details).

Table 2. Percentage of HCT-15 Cells in a Particular Cell Cycle Phase Incubated with Different Concentrations of **1**

cell cycle phase	% HCT-15 cells		
	control	with [**1**] = 27.4 nM	with [**1**] = 54.8 nM
G1	4	22	53
S	89	70	36
G2 + M	7	9	12

machinery within the G1 phase has provided exciting new opportunities with hopes of developing new types of drugs efficacious against refractory cancers (e.g., hormone refractory prostate cancer).

Phosphorus NMR spectroscopy is a versatile tool for the investigation of in vitro stability profiles of phosphorus-containing pharmaceuticals.[24,25] The stability of gold compound **1** in both cell culture medium and human serum was studied using ^{31}P NMR spectroscopy for a period of 7 days. ^{31}P NMR spectroscopic analysis of aliquots of **1** in cell culture media and human serum indicated no detectable decomposition over a period of 7 days, suggesting a long half-life for this new chemotherapeutic agent in biologically relevant media (see Supporting Information for further details).

We have also examined the in vivo antitumor activity of **1**, using mice administered with syngenic meth/A cells (derived from sarcoma) ip. In in vivo experiments of tumor-bearing mice, the survival was observed for 4−6 weeks until the number of survivors became stable. Wilcoxon's test was used for statistical analysis. The survival curves in Figure 1 show survival patterns in tumor-bearing mice after treatment with **1** in various doses. A statistically significant increase in survival time was unequivocally established in tumor-bearing mice treated with **1** sc at a dose range of 25 mg kg^{-1} dose^{-1} (total dose of 75 mg kg^{-1}) to 125 mg kg^{-1} dose^{-1} (total dose of 375 mg kg^{-1}). Acute toxicity in mice was not found when **1** was administered in the range 10−125 mg kg^{-1} for three times.

At the current time, to the best of our knowledge, there are no chemotherapeutic agents available that can effectively control the growth and metastasis of human androgen independent prostate cancer cells. In this context, new agents that decrease the rate of proliferation either directly or by increasing the rate of programmed cell death, allowing for improved treatment of androgen-independent disease, are clearly needed. Our investigation on gold agent **1** has demonstrated its excellent efficacy in inhibiting the growth of HCT-15 cells, AGS cells, LNCaP, and PC-3 cells. The remarkable efficacy of **1** in suppressing the growth of various tumor cells is of particular significance for its potential use in the treatment of a wide range of cancers. It is also important to note that **1** has demonstrated unique selectivity in elongating (or arresting) the G1 phase of the cell cycle. With this cell cycle specificity, **1** can be potentially used in combination with other chemotherapeutic agents for a greater combined efficacy. Therefore, the potential clinical applications of the new chemotherapeutic agent **1** in the treatment of various cancers are promising. Further studies on the in vivo activity of **1**, its pharmacokinetics, and its schedule dependency in animals are currently underway.

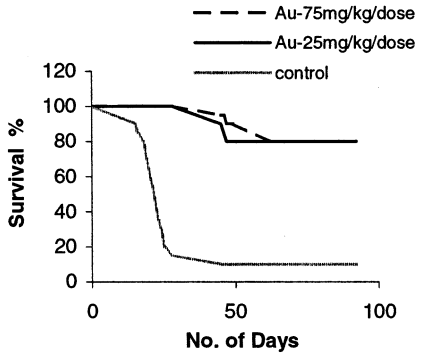

Figure 1. Percentage survival curve of cancerous mice injected sc with three doses of **1** on the first, third, and fifth day of tumor inoculation. Each group consisted of 20 mice.

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Supporting Information Available: Characterization details of compound **1**, experimental details on cytotoxicity assay, stability study, PC-3 cell line inhibition data, flow cytometric analysis, and in vivo study. This material is available free of charge via the Internet at http://pubs.acs.org.

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