

Preparation of $^{198}\text{Au}(\text{I})$ -labelled gold-chloroquine complex $[\text{}^{198}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6$ as a potential antimalarial agent

E.Dadachova

Radiopharmaceuticals R&D, Australian Nuclear Science and Technology Organisation (ANSTO), PMB 1, Menai, NSW 2234 Australia

SUMMARY

$^{198}\text{Au}(\text{I})$ -labelled gold-chloroquine complex $[\text{}^{198}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6$ has been prepared for the enhancement of antimalarial action against chloroquine-resistant strains of *Plasmodium falciparum* of recently reported $[\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6$. $[\text{}^{198}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6$ was synthesized via 3-step route starting with irradiated in the reactor for 2 hours Au foil and purified on Sep-Pak® C18 cartridge. Specific activity of the complex was 1.5 mCi/mg. The possibility of using metal complexes radiolabelled with therapeutic radioisotopes for treatment of chloroquine-resistant malaria is discussed.

Key Words: $[\text{}^{198}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot x\text{H}_2\text{O}$, ^{199}Au , malaria, radiotherapy, radioisotopes, chloroquine-resistance

INTRODUCTION

The speedy spread of resistance in *Plasmodium falciparum*, the parasites responsible for malaria in man, to common antimalarial drugs such as chloroquine (1,2) creates an urgent need for development of alternative compounds capable of curing the resistant strains of malaria. The search for such compounds has lead to investigating the potential of already existing drugs such as cisplatin(II) (3) as well as to designing metal-containing drugs against tropical diseases, eg. platinum group metal complexes with o-vanillin

Present address: E.Dadachova PhD, Radiation Oncology Branch, Building 10, Room B3B69
National Cancer Institute, NIH, Bethesda, MD 20892-1002 USA
Ph: 1-301-402-1383; Fax: 1-301-402-1923; E-Mail: kdx@box-k.nih.gov

thiosemicarbazones (4), transition and platinum group metals with amodiaquine and primaquine (5), ferrocenic compounds mimicking chloroquine (6) and chloroquine complexes with ruthenium, rhodium and gold (7). Practically all of these complexes proved to be relatively active against chloroquine-resistant malaria by *in vitro* or *in vivo* testing, with some, however, exhibiting high toxicity for the host (3-5).

If the above metal complexes-based drugs were labelled with α -, β^+ - or Auger-electron-emitting radioactive isotopes of these metals with activities sufficient for destroying the parasitic cells of *Plasmodium falciparum*, it would allow to administer much lower amounts of the drug to minimize chemical toxicity. Ideally, the curing potency of radiolabelled complexes could be higher than that of unlabelled ones. Also, the radiolabelled complexes in which the radiometal emits imageable γ -rays would be very useful for elucidating the mechanisms of action of these compounds. Here we report the synthesis of $^{198}\text{Au}(\text{I})$ -labelled gold-chloroquine complex [$^{198}\text{Au}(\text{PPh}_3)(\text{CQ})\text{PF}_6$ (7b) and method of its purification.

EXPERIMENTAL

Gold foil, $\text{H}[\text{AuCl}_4]\cdot 3\text{H}_2\text{O}$, KPF_6 , triphenylphosphine PPh_3 and chloroquine diphosphate (CQDP) were purchased from Aldrich. Fresh solvents not requiring purification were used in all experiments. Free chloroquine base (CQ) was obtained according to (7a). To synthesize $^{198}\text{Au}(\text{PPh}_3)(\text{CQ})\text{PF}_6$ we had to modify the procedures described in (8) and (7b) for milligram scale instead of gram amounts as well as to shorten the synthesis time and eliminate some steps which would hinder the synthesis of radiolabelled complex. All synthetic and purification procedures were initially performed with "cold" Au. NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. Elemental analyses were performed by use of the Carlo Erba 1106 Elemental Analyser at the Microanalytical Unit of Australian National University. Silica gel and C18 thin layer chromatography (TLC) plates with UV=254 nm fluorescent indicator were obtained from Aldrich. The plates were developed with the variety of solvents and solvents mixtures: n-hexane, saline, acetone, acetonitrile, methanol : 0.1 M HCl = 10:1, methanol : 1 M NaOH = 25:1, ethanol, hexane : ethanol = 10:1, diethylether : n-hexane : triethylamine = 7:2:1.

Synthesis of AuClPPH_3

The AuClPPH_3 precursor was synthesised as described in (8) starting with solution of 11.91 mg (0.035 mmole) $\text{H}[\text{AuCl}_4]$ in 1.5 mL 1:1 acetone : ethanol mixture to which the solution of 15.72 mg (0.06 mmole) PPh_3 in 1.5 mL of chloroform was added dropwise under stirring. The addition of PPh_3 was stopped when yellow colour of $\text{H}[\text{AuCl}_4]$ disappeared, and the white precipitate of PPh_3 oxide formed.

After filtration through the Centrex® centrifugal filter Unit (Aldrich), the solution was evaporated to dryness under N_2 flow and the product dried *in vacuo*.

In other experiments AuClPPh_3 was synthesized starting with elemental Au. Typically, 6.00 mg (0.03 mmole) of gold foil was dissolved in 1 mL of aqua regia. The solution was brought to near dryness using a hot plate. Next 1 mL of concentrated HCl was added and again heated to near dryness. Finally, $\text{H[AuCl}_4]$ was taken up in 1.5 mL 1:1 acetone : ethanol mixture and the synthesis proceeded as above.

Synthesis of $[\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot 2\text{H}_2\text{O}$

The synthetic steps were carried out according to (7b). Use of Schlenk glassware was omitted. AuClPPh_3 (0.03 mmole, 14.84 mg) was dissolved in 3 mL acetonitrile followed by addition of KPF_6 (0.06 mmole, 11.00 mg) and refluxing for 30 min. Chloroquine free base was added (0.06 mmole, 19.00 mg), and the mixture was stirred and refluxed for 48 hours. The reaction flask was flushed with N_2 following addition of each reagent. On completion of the synthesis the mixture was cooled to room temperature and filtered through the Centrex® centrifugal filter.

To purify $[\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot 2\text{H}_2\text{O}$ we substituted overnight recrystallisation suggested in (7b) for solid phase extraction (SPE) which is more convenient for synthesis of radiolabelled compounds and as well as less time consuming. The volume of the solvent was reduced under N_2 to near dryness and the mixture was redissolved in 1 mL acetonitrile. It was loaded with a syringe onto Sep-Pak® C18 cartridge (Waters) previously activated with the sequence of 1 mL methanol and 1 mL acetonitrile. The eluant was collected (fraction 1). The column was eluted with 2 1-mL aliquotes of n-hexane (fractions 2 and 3) followed by final elution with 1 mL acetone (fraction 4).

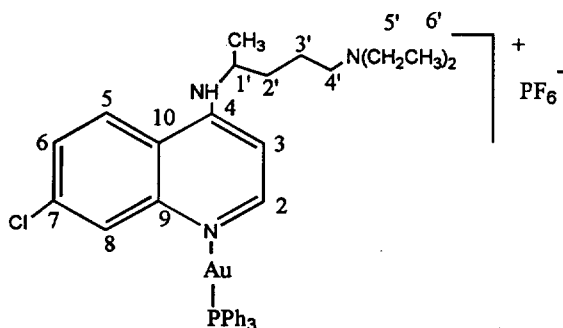


Fig.1 $[\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6$ complex (redrawn from Navarro M., Pérez H., Sánchez-Delgado R.A. - J. Med. Chem. 40: 1937 (1997))

All fractions were collected, the solvents evaporated to dryness on rotational concentrator, the solid residues weighted and subjected to elemental analysis. No solid was found in fraction 1, fraction 2 contained the dark yellow-coloured product $[\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot x\text{H}_2\text{O}$ (calculated, %: C – 46.5, H – 4.8, N – 4.57; found, %: C – 45.55, H – 4.68, N – 4.88); fraction 3 – white CQ (calculated, %: C – 67.59, H – 8.19, N – 13.14; found, %: C – 65.36, H – 7.61, N – 9.41); fraction 4 – dark yellow mixture of 83% $[\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot x\text{H}_2\text{O}$ + 17% CQ (found, %: C – 51.10, H – 4.93, N – 5.07). 60% of $[\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot x\text{H}_2\text{O}$ calculated yield was recovered in fraction 2, and 14% – in fraction 4.

The product $[\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot x\text{H}_2\text{O}$ was analysed by ^1H NMR ($(\text{CD}_3)_2\text{CO}$): ppm 8.55 (d, $J=6$ Hz, H2), 6.70 (d, $J=6$ Hz, H3), 8.33 (d, $J=9.00$ Hz, H5), 7.47 (dd, $^3J=9$ Hz, $^4J=2$ Hz, H6), 8.12 (d, $J=2$ Hz, H8), 3.81 (br, H1'), 1.33 (d, $J=6.5$ Hz, H1''), 1.61 (m, H2', H3'), 2.60 (m, H4', H5'), 0.98 (t, $J=7$ Hz, H6'), PPh₃ 7.66 (m, 15H).

Synthesis of $[\text{}^{198}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot x\text{H}_2\text{O}$

6.00 mg of gold foil was irradiated for 2 hours in HIFAR (High Flux Australian Reactor) in neutron flux of $3.5 \times 10^{13} \text{ n/cm}^2 \cdot \text{s}$. The activity of ^{198}Au at EOB was 55.4 mCi (9.24 mCi/mg). The target was digested in 1 mL aqua regia, The solution was brought to near dryness using a hot plate. Next 1 mL of concentrated HCl was added and again heated to near dryness. Finally, $\text{H}[\text{}^{198}\text{AuCl}_4]$ was taken up in 1.5 mL 1:1 acetone : ethanol mixture and $^{198}\text{AuClPPh}_3$ was synthesized as described above. It was used without drying *in vacuo* for the synthesis of $[\text{}^{198}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot x\text{H}_2\text{O}$. The $[\text{}^{198}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot x\text{H}_2\text{O}$ product was purified on Sep-Pak® C18 cartridge and kept in the dessicator at 4°C until all ^{198}Au decayed followed by elemental analysis.

RESULTS AND DISCUSSION

Starting with 6.00 mg (0.03 mmole) of gold, 13.4 mg (0.014 mmole) of pure $[\text{}^{198}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot x\text{H}_2\text{O}$ was obtained after 48 hour synthesis followed by purification on C18 column. The specific activity of the product was 1.5 mCi/mg. $^{198}\text{AuClPPh}_3$ and $[\text{}^{198}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot x\text{H}_2\text{O}$ have the same mobility on TLC plates as "cold" AuClPPh_3 and $[\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot x\text{H}_2\text{O}$, respectively. We were not able to find a suitable TLC system for separating $^{198}\text{AuClPPh}_3$ and $[\text{}^{198}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \cdot x\text{H}_2\text{O}$ and thus estimating the radiochemical purity of the product using silica gel or C18 as the stationary phase. The compounds were either immobile, or decomposed while moving along the TLC plate. For example, $^{198}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6$

$\times 2\text{H}_2\text{O}$ always appeared as two spots on the chromatogram – one with R_f of CQ and another – with R_f of AuClPPh_3 . The same difficulties in developing TLC procedure for another PPh_3 -containing metal complex $\text{ReOCl}_3(\text{PPh}_3)_2$ were reported in (9) and were explained by the reactive nature of such complexes. “Delayed” analysis of radiochemical purity of $[\text{}^{198}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6 \times 2\text{H}_2\text{O}$ product by elemental analysis showed that the product was 98.5% radiochemically pure

^{198}Au ($t_{1/2}=2.7$ d) is an intermediate β -emitter with energies of 960 keV, 99%; ^{199}Au ($t_{1/2}=3.1$ d) is both a β - ($\beta_1=462$ keV, 6% and $\beta_2=296$ keV, 71.6%) and an electron-emitter ($e_1=125$ keV, 6%, $e_2=144$ keV, 16%) which are known to have very high LET; ^{198}Au and ^{199}Au give off imageable γ -rays of 412 (95.5%) and 159 (37%), respectively, suitable for diagnostic experimentation. The decay properties of these isotopes make them favourable for radiotherapy - ^{198}Au colloid has been used for radiation synovectomy and treatment of meningeal leukaemia (10, 11), ^{198}Au gold seeds – in prostate cancer (12) and ^{199}Au - for radioimmunotherapy (13, 14). Both ^{198}Au and ^{199}Au are readily available reactor-produced isotopes obtained by neutron and double neutron absorption on ^{197}Au (100% abundance), respectively.. The large (n, γ) cross sections for ^{197}Au ($\sigma = 98.7$ b) and for ^{198}Au ($\sigma = 26,000$ b) allow high activities of ^{198}Au and ^{199}Au to be produced even in relatively low neutron fluxes – 1 day irradiation in neutron flux of 3.5×10^{13} n/cm 2 s of 10 mg ^{197}Au results in 630 mCi of ^{198}Au , and 3 days – in 140 mCi of ^{199}Au .

It has been recently shown (15) that such blood disease as leukemia may be successfully treated with radionuclide therapy. Malaria is a blood disease caused by *Plasmodium* invasion of host erythrocytes followed by digestion of haemoglobin by the parasite. Chloroquine and other quinoline drugs penetrate the red blood cell where they interfere with the parasite feeding mechanism by inhibiting haemoglobin digestion (16). If the radiation dose sufficient for damaging the parasitic cell is delivered by radiolabelled chloroquine-based drug such as $[\text{}^{198, 199}\text{Au}(\text{PPh}_3)(\text{CQ})]\text{PF}_6$ it will help to destroy the parasite more efficiently thus allowing to decrease the minimum inhibitory concentration (MIC) of the drug.

There is also a potential for use of therapeutic radionuclides in non-chloroquine-based antimalarial drugs. For example, it was shown that well-known anticancer drug cisplatin(II) has an antimalarial potential in chloroquine-resistant strains of *Plasmodium falciparum* (3). This compound exerts its toxicity mainly by damaging and cross-linking parasitic DNA strands. Reactor-produced platinum isotopes $^{195\text{m}}\text{Pt}$ and $^{197\text{m}}\text{Pt}$ emit large number of low-energy electrons many of which have ranges of the order of one cell radius. Labelling of cisplatin(II) with $^{195\text{m}}\text{Pt}$ or $^{197\text{m}}\text{Pt}$ would enhance sterilisation of parasitic cells.

Practically each metal whose complexes or salts were studied as potential antimalarial agents (3-7) has one or several radioisotopes with properties favourable for radiotherapy. *In vitro* and *in vivo* studies of antiplasmodium properties of radiolabelled metal compounds against chloroquine-resistant strains of malaria are needed.

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