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In Vitro Distribution of Gold in Serum Proteins after Incubation of Sodium Aurothiomalate and Auranofin with Human Blood and its Pharmacological Significance

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Abstract This study presents a comparative drug–protein, in vitro, binding profile of sodium aurothiomalate and auranofin. It was found that about 40% of total protein-bound gold is attached to albumin after incubation of aurothiomalate with whole blood for 24 h and about 29% of it was with α_1-globulin and the least amount was found with γ-globulin (6.1%). On the other hand, approximately 84% of the protein-bound auranofin gold attached to globulins of which 51% was found with β-globulin band. It was almost equally distributed among albumin, α_2-globulin and γ-globulin, and showed least affinity for α_1-globulin. The gold analyses were performed by standardless instrumental neutron activation method duly validated by use of an established atomic absorption method. The results of this study explain to some extent the difference in, in vivo, pharmacokinetics and pharmacodynamics of the two drugs.

Keywords Chrysotherapy · Myocricin® · Auranofin · Electrophoresis · Serum proteins · Neutron activation analysis

Introduction

Gold compounds have been in use for treatment of rheumatoid arthritis for about 70 years but several questions relating to efficacy and toxicity still remain unanswered. The two

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main types of gold compounds available in the market are: (1) injectable preparations and (2) an oral preparation. The injectable preparations include sodium aurothiomalate and the only oral preparation available is 5,(1-thio-ß-D-glucopyranose-2,3,4,6-tetraacetato-S)-(triethylphosphine) gold(I) commonly known as auranofin. These are two different chemical entities shown to have different in vivo chemistry and pharmacokinetics [1]. With a weekly 50-mg injection of sodium aurothiomalate, the peak serum gold concentration goes to approximately 7 $\mu g\ mL^{-1}$ 6–8 h after the injection and declines to approximately 3 $\mu g\ mL^{-1}$ within 1 week. The initial serum half life is 5.5 days [2, 3]. Most of the intramuscularly injected gold (>95%) is protein bound, which has been reported to be mainly bound to serum albumin [2, 4]. After oral administration of auranofin the serum gold concentrations reach a peak value between 0.2 and 1 $\mu g\ mL^{-1}$ depending on the dose. The plasma half life has been reported as 16–25 days [2, 5, 6] which is 3–5 times longer than that of sodium aurothiomalate, which is most probably because of drug–protein interactions.

Drug–protein interactions provide highly useful information for interpretation of various pharmacokinetic parameters including volume of distribution, elimination rate constant, and clearance. Reactions of metal-based drugs with proteins are even more important with reference to their mode of action and toxicity as such drugs are able to form coordinate covalent bonds with them. Interaction of aurothiomalate, in vivo, with plasma/serum proteins has been studied in animals [7, 8] and, in vitro, with humans [9–11]. The previous in vitro studies suggested that most of the protein-bound gold is held by albumin [7, 9, 11–14] and a small fraction is bound to immunoglobulins [14–16] and low-molecular-weight substances [13, 14]. In humans, the in vivo study of gold–protein distribution after administration of auranofin has been reported as: 81.8% to albumin, 4.8% to α_1-globulin, 6.9% to α_2-globulin, and 6.5% to β and γ globulins at blood gold level of 1.5 $\mu g\ mL^{-1}$. In this study, the protein bands separated by electrophoresis were cut, eluted, and then analyzed by neutron activation analysis (NAA) [17]. A comparative study of binding of gold to serum proteins of patients treated with sodium aurothiomalate, gold keratinate, and triethylphosphine gold has also been reported [18], which showed different distribution patterns. The aurothiomalate gold was 94% bound to albumin and only 6% to globulin. Whereas in case of phosphine gold the corresponding figures were 70% and 30%, respectively. The keratinate gold did not bind completely with serum proteins, in vitro, even after 24-h incubation. The affinity of keratinate gold to globulin was found to be high (20%). It appears that the affinity of gold to globulins changes with the nature of the drugs. This was evident in the in vivo study [1] where the level of gold in erythrocyte membrane was initially very high and decreased rapidly afterwards in case of aurothiomalate and auranofin produced a constant high levels up to 36 weeks. In order to understand the different behavior of sodium aurothiomalate and auranofin a study was warranted to compare drug–protein binding profiles of the two drugs which is the subject of the present work. This information is expected to help in designing more effective and less toxic gold drugs.

Materials and Methods

The drugs used were sodium aurothiomalate (Alfa Aesar) and auranofin (gifted by GlaxoSmithKline, USA). All reagents used were of analytical grade. High purity de-ionized water was used in the preparation of all solutions.

Serum Preparation

The drug (10 mg) was vortex-mixed with an aliquot of fresh blood (10 mL) from a healthy human and incubated at 37°C for 24 h. After this serum was separated by centrifugation at 3,000×*g*.

Electrophoresis

Barbital buffer solution (pH 8.6; 0.05 M) was prepared by dissolving barbitone (1.84 g) and barbitone sodium (10.3 g) in water (1 L). A staining solution was prepared by dissolving Ponceau S (0.5 g) in of 5% trichloroacetic acid (100 mL).The destaining solution used was 5% acetic acid.

Separation of serum proteins was carried out on Interlab Genio Electrophoresis 18602571 apparatus using cellulose acetate strips (60×76 mm) supported on Mylor® and the barbital buffer. The sample (30 μL) was run at 150 V for 15 min. The separated bands were stained by immersing the strip in the staining solution for 5 min. The strips were then washed thrice with the destaining solution to obtain a white background. Water was removed by dipping the strips in methanol for 60 s followed by treatment with clearing solution (Cellogel, Code 06A06-S1). The strips were then air-dried for 2–3 min followed drying at 70°C in an oven for 20–30 min. The five protein bands were visible on clear background.

Gold Analysis

Neutron Activation The standardless instrumental neutron activation analysis method of quantification, known as k_o-INAA [19, 20], was used as follows.

A 2×5-mm portion of the separated protein band (4×5 mm in case of γ-globulin) was cut, wrapped in a small piece of clean paper, packed inside a polyethylene (PE) rabbit (16 mm diameter, 52 mm length) and irradiated. Irradiations were performed at Pakistan Research Reactor-2 (PARR-2), which is a 30 kW miniaturized neutron source reactor with nominal thermal flux of 1×10^{12} n cm^{-2} s^{-1}. Flux monitors and all samples were irradiated together. The irradiated samples were transferred to PE capsules for gamma spectrometry. Countings were performed by a p-type coaxial HPGe detector (Eurisys Mesures), which has 60% relative efficiency and 1.95 FWHM at 1,332 keV. The detector was connected to an Ortec-570 amplifier and Trump PCI 8k ADC/MCA card with GammaVision-32 ver. 6 software. Full peak efficiency calibration of the detector was carried out at various heights using ^{241}Am, ^{133}Ba, ^{137}Cs, ^{60}Co, and ^{152}Eu point calibration sources. Peak-to-total calibration was performed by ^{203}Hg, ^{65}Zn, ^{198}Au, ^{51}Cr, and ^{137}Cs point sources. All calculations were performed by Microsoft Excel using Solver add-in. In concentration calculations nuclear data were adopted from a recent tabulation by De Corte and Simonits [21] and uncertainties were calculated following the approaches described by Robouch et. al [22]. The results were reported as ng mm^{-2}.

Atomic Absorption The gold stock solution was prepared by dissolving $HAuCl_4.4H_2O$ in distilled water (2.091 gL^{-1}) to obtain 1,000 ppm concentration. From this, 1, 5, 10, 15, 20, and 25-ppb standard solutions were prepared by dilution with distilled water.

Table 1 Gold Concentration in Serum Protein Bands

Protein band	ng mm^{-2} (% of total protein-bound gold)			
	Aur		Atm	
	AAS	NAA	AAS	NAA
Albumin	7.9	8.1 (16.1)	28.9	29.1 (40.0)
α_1-Globulin	1.6	1.9 (3.7)	21.2	21.0 (28.9)
α_2-Globulin	6.7	6.8 (13.7)	9.5	9.9 (13.6)
β-Globulin	25.6	25.5 (51.0)	8.0	8.3 (11.4)
γ-Globulin	7.5	7.7 (15.5)	4.5	4.4 (6.1)

A reported atomic absorption method [23] was used to validate the NAA results. The sample solutions were prepared by placing a 4×8-mm portion of the separated protein band on the cellulose strip in test tubes separately. To each of the sample tube, a suitable quantity of water and concentrated HNO_3 (1 mL) were added and the mixture was boiled to expel NO_2 fumes. Finally the volume was made up to 5 mL with distilled water.

In order to match the matrix with the sample a 4×8-mm piece of blank cellulose acetate strip was added and 1 mL concentrated HNO_3 to each tube while preparing these standards. The mixture was boiled to expel NO_2 fumes, filtered and volume was made up with water to obtain the desired concentration.

The atomic absorption measurements were made according to the reported method [23] by using Shimadzu AA 6300 Spectrophotometer and GFA-EX7i graphite furnace atomizer. The results were reported as ng mm^{-2}.

Results and Discussion

The results are summarized in Table 1. There was a general agreement between the results obtained from NAA and AAS, which validated the new method of sample preparation. The two drugs being chemically different showed up an entirely different binding pattern, in vitro, with the separated serum protein bands on cellulose acetate strips. About 40% of total protein-bound gold was found bound with albumin after incubation of aurothiomalate with whole blood for 24 h and about 29% of it was with α_1-globulin and the least amount was found with γ-globulin (6.1%). On the other hand, approximately 84% of the protein-bound

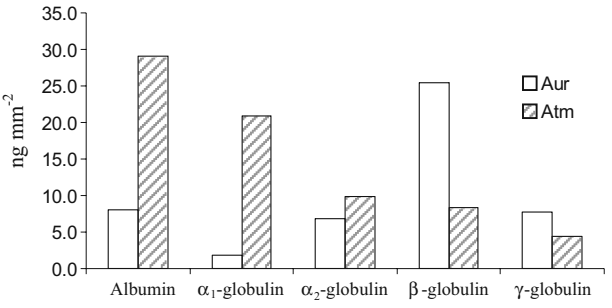

Fig. 1 Comparative gold distribution in serum proteins from auranofin and sodium aurothiomalate

auranofin gold attached to globulins of which 51% was found with β-globulin band. It was almost equally distributed among albumin, α_2-globulin and γ-globulin, and showed least affinity for α_1-globulin. A comparative picture is given in Fig. 1. This different behavior of auranofin may account for its peculiar pharmacokinetics and pharmacodynamics in vivo [1, 24]. Globulins are less soluble in water than albumin thus the auranofin gold is expected to form more lipophilic complexes with them.

The preferential binding of auranofin to globulins is important, in many respects, to understand the mechanism of action of auranofin in the inflammatory process. The globulin levels may become elevated in rheumatoid arthritis. α_1-globulins, especially α_1-antitrypsin, protect tissues from enzymes of inflammatory cells, such as elastase, and their concentrations can rise manyfold upon acute inflammation [25]. α_2-globulins include ceruloplasmin (carrier of copper) whose level is known to be raised in rheumatoids. β-globulins include C-reactive proteins which are also considered important indicators of the disease condition. Based on this study it will be worthwhile to prepare mixed-ligand complexes of triethylphosphine-gold (I) with globulins and study their pharmacokinetics.

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