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THE BINDING OF GOLD TO CYTOSOLIC PROTEINS OF THE RAT LIVER AND KIDNEY TISSUES: METALLOTHIONEINS

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Abstract—Male Wistar rats were given a single s.c. injection of 2.5 mg gold per kg body wt and the binding of gold to the cytosolic proteins was investigated over a period of 21 days. In both the organs, gold was bound to two groups of high and one group of low molecular weight cytosolic proteins. Gold was present in the metallothionein fractions (the low mol. wt Au-binding proteins) within 0.5 hr after exposure to gold. The binding of gold to the cytosolic proteins in the kidney was significantly higher, up to 17 times that in the liver. The incorporation of gold into the metallothionein fraction was complete within 24 hr in the liver but continued to increase over a period of four days in the kidneys. Up to 51 per cent of the cytosolic gold in the kidney was bound to the metallothionein fractions. It is suggested that the 'metallothioneins' may play an important role in the sequestration and the intracellular localization of gold, particularly in the kidneys.

Gold salts, particularly sodium aurothiomalate, have been used successfully for many years in the treatment of rheumatoid arthritis, but little is known about the mode of therapeutic action and the mechanism of the chronic toxicity of the drug. Current theories of the mode of action of gold postulate an effect on subcellular organelles and in particular on the lysosomal bodies [1–5]. Our previous investigations demonstrated a steady accumulation of gold in subcellular organelles of the liver, kidney and the spleen tissues in rhesus monkeys [6]. However, a significant amount of intracellular gold was also present in the cytosol and hence it is desirable to attempt to define the nature of the binding proteins in the cytosol, particularly in view of the contribution that low molecular weight metal-binding proteins (metallothioneins) make to the sequestration of metals such as Zn^{2+}, Cu^{2+}, Cd^{2+} and Hg^{2+} [7–9].

Recent investigations have demonstrated that Au(III) binds to low molecular weight cytosolic proteins and stimulates the biosynthesis of the metalloprotein in the liver [10] and kidney [11]. Furthermore, it has been shown [5, 12] that Au(I) may also bind to the metallothioneins. In the present experiments, the incorporation of Au(I) by liver and kidney tissues, subcellular particles and cytosolic proteins of rats given single doses of sodium aurothiomalate has been investigated.

Since only supportive evidence has been provided for the actual identity of the low molecular weight gold-binding proteins, the term 'metallothionein', in inverted commas, is used to denote the gold-binding proteins.

METHODS

Eleven groups of three male Wistar rats (weighing approx. 250 g) received a single s.c. injection containing 2.5 mg Au/kg body wt as Myocrisin (May & Baker Ltd., Dagenham, U.K.) in isotonic saline. Two more groups of three rats were used as controls and received a single injection of isotonic saline. The animals were exsanguinated in groups of three at intervals during the following three weeks. The liver and kidneys were obtained and either fractionated immediately or frozen and analysed later.

Equal weights of the liver and kidney tissues within each group were pooled, minced and washed in ice-cold 0.25 M sucrose solution. The tissues were then homogenized at 4° in 25% (w/v) 0.25 M sucrose solution using a Sorvall Omni-Mixer Homogeniser. The homogenates were centrifuged at 10,000 *g* (av.) for 30 min in a Sorvall RC-2B refrigerated (4°) centrifuge. The supernatant obtained was centrifuged again at 100,000 *g* (av.) for 1 hr in a Beckman L-4 ultra-centrifuge using a Beckman 60Ti fixed angle rotor.

A portion (5 ml) of the clear supernatant fraction (the cytosol) was applied to a column (2.5 × 75 cm) of Sephadex G-75 (Pharmacia Fine Chemicals AB, Uppsala, Sweden), equilibrated and eluted with 0.1 M ammonium formate solution containing 8 mM Tris–HCl, pH 8.0. Fractions of 5 ml were collected with an ISCO (Instrumentation Specialties Co. Inc.) Refrigerated Fraction Collector. The protein content of the eluates was determined by monitoring the absorbance at 254 and 280 nm with an ISCO Dual Beam Optical Unit Type 4, Channel Alternator Model 1130 and Absorbance Monitor Model UA-4. To determine the relative molecular weights of the cytosolic proteins, the Sephadex column was calibrated with blue dextran, bovine serum albumin, cytochrome *c* (obtained from horse heart muscle), Cd-thionein (rat kidney) and potassium dichromate.

The eluates were analysed for Au by graphite furnace atomic absorption spectrometry with an Instrumentation Laboratory Model 351 Spectrophotometer and Model 555 CTF Flameless Atomiser. Samples of serum, liver and kidney tissues, the homogenates, subcellular particles and the cytosol were also analysed for Au. The average recovery of Au from the samples was 99.1 ± 1.5 per cent. The

average recovery from the Sephadex column was 100.8 ± 4.8 per cent.

Portions (10 ml) of the metallothionein fractions from the liver and kidney were heated to determine the heat stability of the low molecular weight proteins in a water bath at 75° for 5 min. The samples were chilled immediately, then centrifuged and the supernatant analysed for Au. This experiment was carried out for samples obtained at 0.5 hr, 24 hr and 21 days after the administration of Au.

The fractionation of the tissues and the subsequent analysis of the cytosolic proteins was performed in duplicate (or triplicate) and the values expressed in Results are an average of the two.

The u.v. absorption maxima of the supernatants from the heat treated liver and kidney metallothionein fractions (24 hr samples only) was also measured.

RESULTS

Gold uptake by the kidney tissues, subcellular particles and the cytosol (Fig. 1a) reached maximum concentrations at 48 hr. The time course of Au uptake (Fig. 1b) into the liver tissue, subcellular particles and cytosol shows that the maximum concentrations of Au were reached within 24 hr. However, the concentrations of Au in the kidney samples were approximately 14 times those in the liver.

The binding of Au to various cytosolic proteins of the kidney tissues at 7 days is shown in Fig. 2a. Gold was present in basically two groups of proteins; a group of high molecular weight (H.M.W.) proteins eluting near the void volume and a group of low molecular weight (L.M.W.) proteins eluting with a V_R (relative elution volume) close to that of Cd-thioneins (rat kidney). In the liver cytosol at 7 days (Fig. 2b), Au was bound to three groups of proteins; two groups of H.M.W. proteins, one eluting near the void volume and the other eluting with a V_R similar to albumin, and a group of L.M.W. proteins eluting with a V_R similar to that of Cd-thioneins. The binding of Au to the H.M.W. proteins in the kidney cytosol was not resolved to the same extent as for the liver cytosol.

The Au-bound L.M.W. proteins present in the

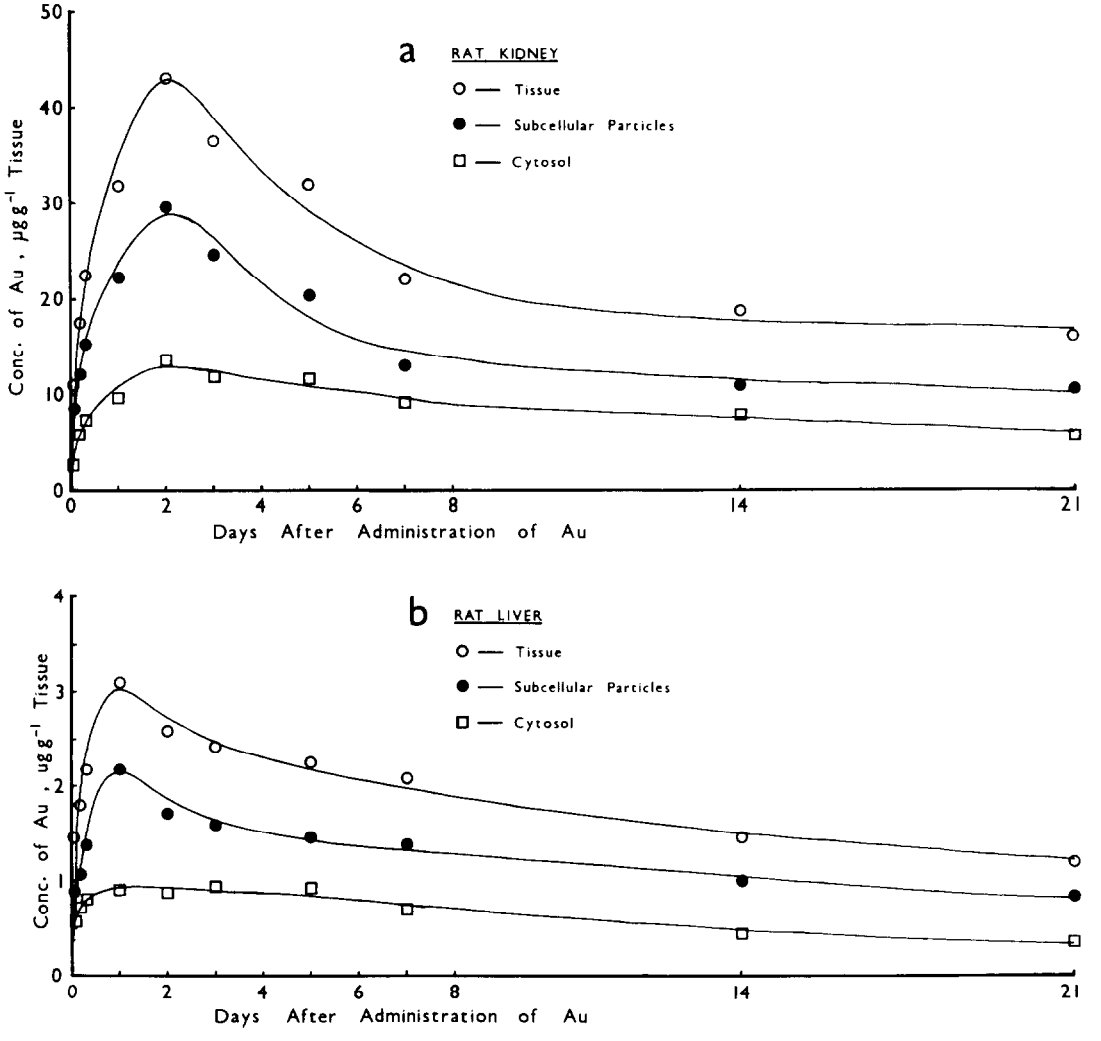

Fig. 1. Time course of Au uptake into the rat kidney (panel a) and liver (panel b) tissues, subcellular particles and the cytosol.