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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

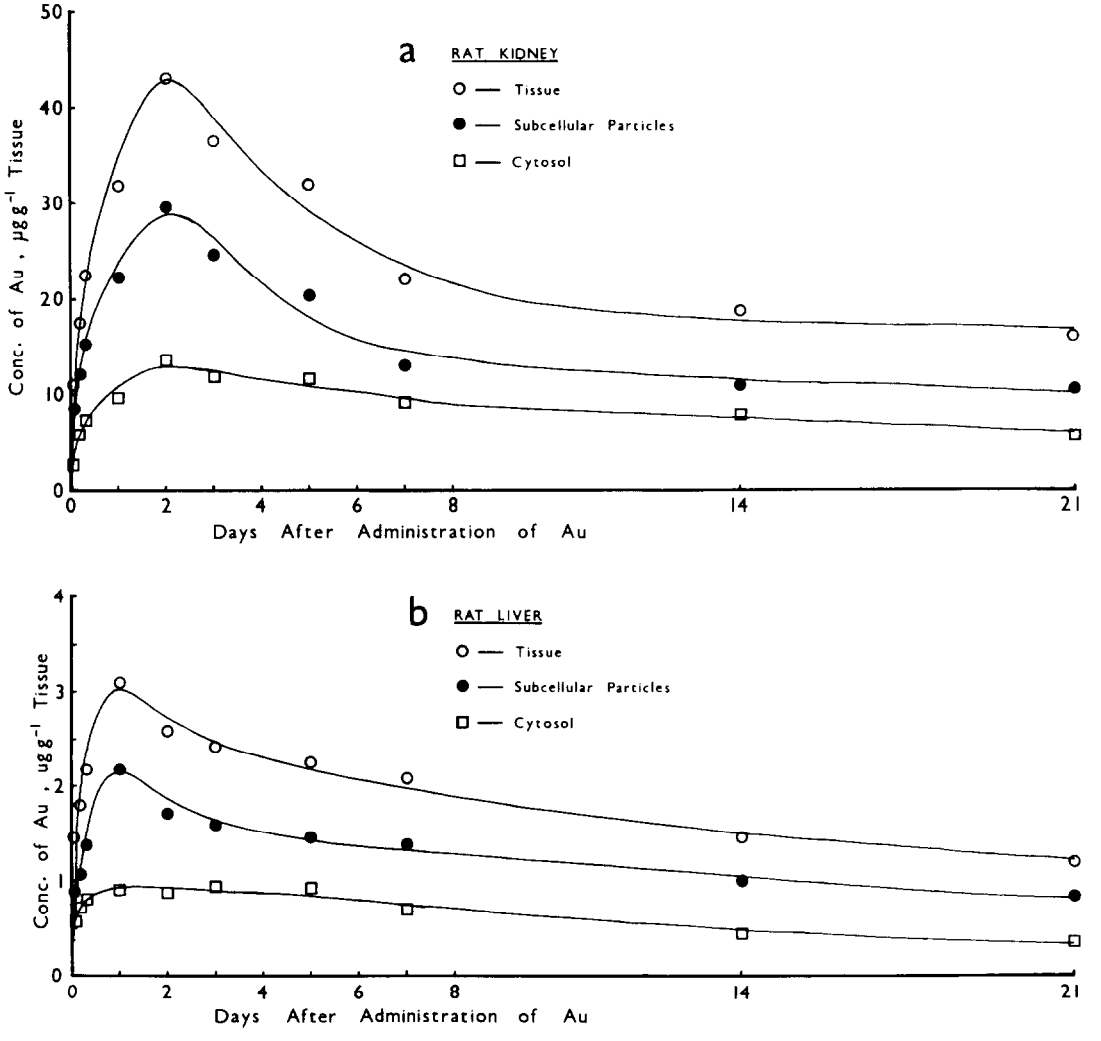

Fig. 1. Time course of Au uptake into the rat kidney (panel a) and liver (panel b) tissues, subcellular particles and the cytosol.

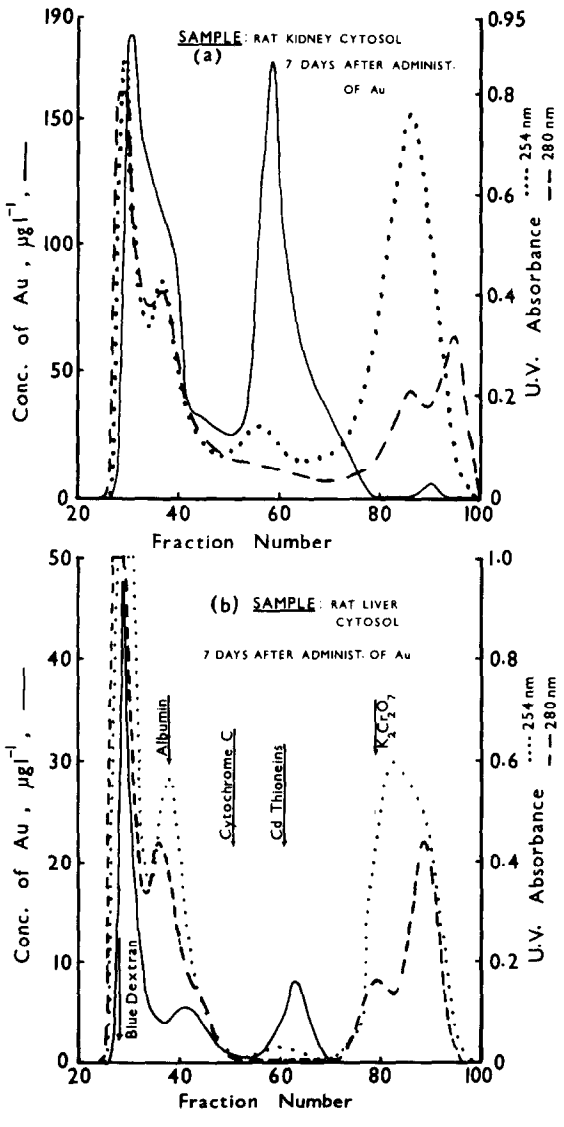

Fig. 2. Binding of Au to the rat kidney (panel a) and liver (panel b) cytosolic proteins, chromatographed on a Sephadex G-75 column, equilibrated and eluted with 0.1 M ammonium formate buffer at pH 8.0. The H.M.W. proteins were eluted between fraction Nos. 25 and 45 and the 'metallothioneins' between fraction Nos. 55 and 65.

kidney and liver cytosol were found to be heat stable at 75°. The recovery of Au from the metallothionein fractions after heat treatment is shown in Table 1. An average of 96.0 ± 3.7 per cent of the L.M.W. protein-bound Au remained in the soluble fraction after the heating. The supernatants of the heat treated L.M.W. Au-binding proteins from the liver and the kidney both approached an absorption maximum at 225 nm.

In the kidney, Au was present in the metallothionein fractions (L.M.W. gold-binding proteins) within 0.5 hr after the administration of Na-aurothiomalate. The binding of Au to H.M.W. proteins was rapid, achieving maximum incorporation within 24 hr (Fig. 3a). Uptake of Au into the metallothionein fractions was not complete until 4 days after the administration of Au. In the liver, as in the kidney, Au was present in the metallothionein fractions of the cytosol within 0.5 hr after exposure to Na-aurothiomalate. However, the incorporation of Au into the metalloprotein(s) was complete within 7 hr and into the H.M.W. proteins in about 24 hr (Fig. 3b). The binding of Au to the 'metallothionein(s)' in the kidney was significantly higher, increasing to up to 38 times (at 4 days) those in the liver. Between days 3 and 4 about 50 per cent of the rat kidney cytosolic Au was bound to the 'metallothionein(s)'.

Up to 44 per cent of the 'metallothionein'-bound Au in the kidney was retained at the end of the experimental period (Fig. 3b). About 96 per cent of the rat liver 'metallothionein'-bound Au was retained at 21 days after the exposure to Na-aurothiomalate.

DISCUSSION

The present experiments have shown that a significant proportion (an average of 35 per cent) of the intracellular gold in the rat kidney and liver was bound to the cytosolic proteins (Figs. 1a and b). Separation of the cytosolic proteins on a Sephadex G-75 column revealed that gold was bound to two groups of H.M.W. proteins and to a group of L.M.W. proteins (Figs. 2a and b). Some of the physical and chemical properties of the L.M.W. Au-binding protein(s) studied (heat stability, relative molecular weight, absorption at 225 nm, and the

Table 1. Recovery of Au from metallothionein fractions after heating

Time after exposure to Au	Source of metallothionein fraction	Weight of Au(ng) Before heating	Weight of Au(ng) After heating	Recovery of Au (%)
0.5 hr	Liver	74.0	67.0	90.5
	Kidney	193.0	186.0	96.4
24 hr	Liver	80.2	78.0	97.2
	Kidney	1150.0	1070.0	93.3
21 days	Liver	59.6	58.3	97.8
	Kidney	945.0	951.0	100.8

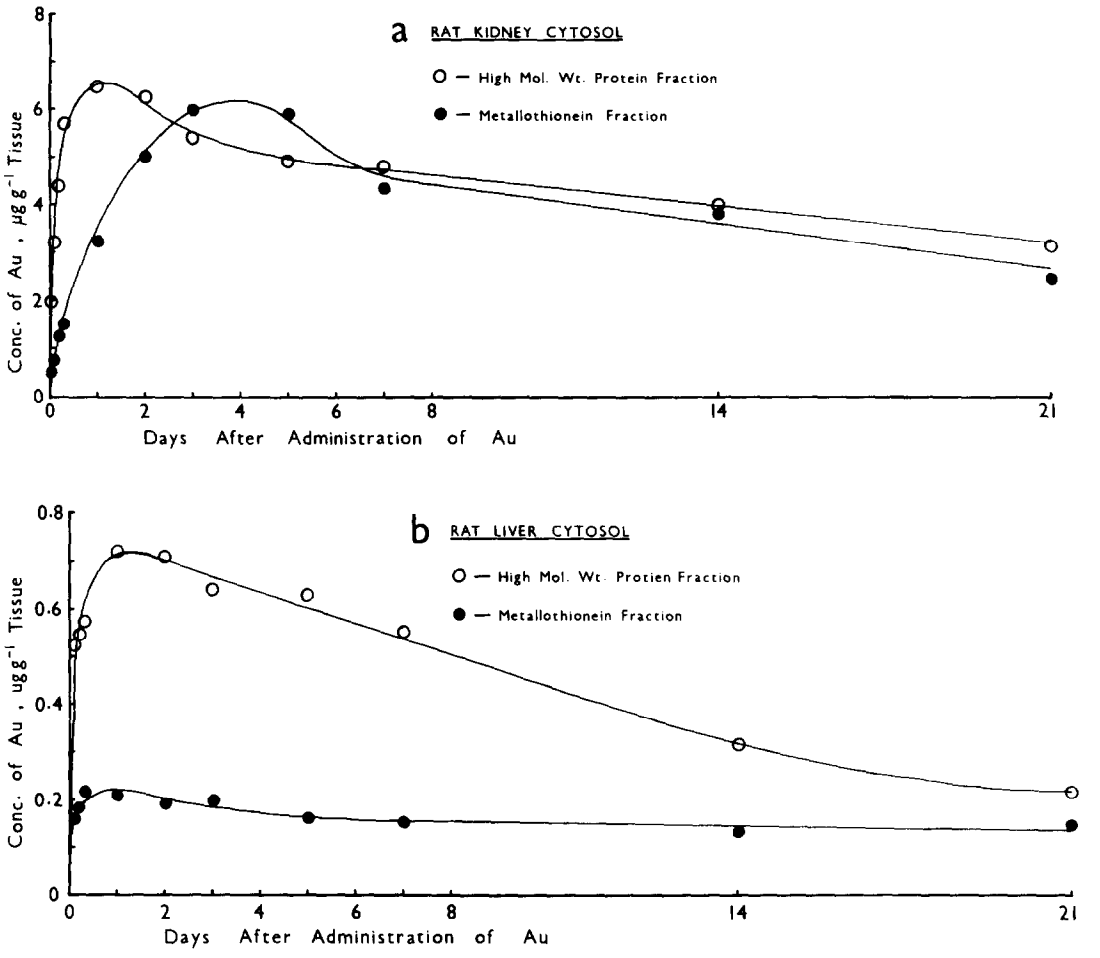

Fig. 3. Time course of Au incorporation into the H.M.W. protein fractions and the metallothionein fractions in the rat kidney (panel a) and the liver (panel b) cytosol.

ability to incorporate Au, Cd, Cu and Zn*) show close similarity to the characteristic properties of metallothioneins [13, 14]. It has been established [15] that the L.M.W. Au-binding protein present in the cytosol of rat liver and kidney tissues following administration of Na-aurothiomalate is in fact metallothionein.

The presence of L.M.W. Au-binding protein, 'Au-metallothionein', within 0.5 hr (in both the kidney and the liver cytosol) indicates that, at least initially, Au(I) may incorporate into the presynthesized metallothionein. It was evident, however, that a substantial increase in the cytosolic 'metallothionein'-bound Au(I) in the kidney continued over a four-day period. This was also evident, although to a lesser degree and over a shorter period, in the liver. Recent studies show that Au(III) binds to metallothioneins in the rat liver [10] and kidney [11] cytosol and that the cation induces the synthesis of the L.M.W. Au-binding protein. It would seem likely that the increased uptake of Au(I) into the 'metallothionein' in the kidney cytosol (Fig. 3a) of rats exposed to Na-aurothiomalate, and the similarity in the renal uptake of Au(I) in the present experiments and that of Au(III) recently [11] studied, would suggest that Au(I) may similarly induce biosynthesis of renal 'metallothionein'. Furthermore, the similarity in the binding of Au(I) and Au(III) to the liver 'metallothioneins', and the stimulation of hepatic (and renal) uptake of Cu (by 36 per cent) into the 'metallothionein' by Na-aurothiomalate* suggests that Au(I) may also enhance the biosynthesis of 'metallothioneins' in the liver as well as the kidney.

The lower percentage retention of gold in the kidneys (44 per cent retention), as against the liver (96 per cent retention), at 7 days after the administration of Na-aurothiomalate may be due to the significantly higher concentration of the metal in the kidney cytosol, which may have exceeded the ability of Au(I) to stimulate the biosynthesis of the renal L.M.W. gold-binding protein. This may also explain the time difference between the liver (within 7 hr) and the kidney (about 4 days) for the complete incorporation of Au into the metallothionein fractions (Figs. 3a and b).

Metalloproteins have been classified into two types [16], one which controls metal ion concentration, the sequestering proteins (e.g. metallothioneins), and one which contains metals which control the

* R. P. Sharma, unpublished data.

substrate concentration (e.g. haemoglobin). The sequestering proteins not only control the metal concentration and carry it to the site that requires it, but in addition can act as catalysts in placing the metal ion into its new molecule (e.g. transferrin which transports Fe to a porphyrin ring to make the heme group). The significant binding of Au to 'metallothioneins', particularly in the kidney (up to 51 per cent of cytosolic Au), suggests that these proteins may play an important role in the sequestration and localization of intracellular gold. The transport of Au from other tissues to the kidney, coupled with a high glomerular filtration and cumulative tubular reabsorption by the endocytic epithelial cells [17], together with the incorporation and retention of Au by the 'metallothioneins', may to some extent account for the increasingly higher presence of the metal in the kidney tissues.

Treatment of rheumatoid arthritis with Au salts is limited by the chronic toxic reactions it may provoke in the skin, the liver and in particular the kidneys. In animals, the most extensive damage caused by Au in high dosage is in the proximal tubules [18]. The primary site of injury in man is also the tubules [19–21]. It is possible that protection against the chronic toxicity of Au might be conferred by pretreatment with low doses of Au and/or other metallothionein biosynthesis stimulating agents such as zinc [22]. Recent investigations [9, 23, 24] with Cd^{2+} and Hg^{2+} ions have indicated that predosing with small amounts of the metals stimulates the biosynthesis of the metalloproteins which then take up any additional amount of the toxic metals, thereby reducing the chances of toxicity. Such a mechanism may also explain the lower susceptibility to toxicity of Au in patients receiving small doses of Au salts and also in patients who receive oily suspensions of Au salts rather than the fast absorbing aqueous solutions [25].

The pathogenetic mechanism whereby Au salts may act as a nephrotoxic agent ultimately responsible for an immune complex nephritis is not known. Skrifvars [26] suggests that Au, the primary 'antigen', couples to tissue or serum proteins and the resultant gold–protein conjugate acquires immunogenic properties and provokes an antibody response. Immune complexes then become deposited in the glomeruli and ultimately give rise to membranous glomerulonephritis. The sensitivity of immune systems to exposure to heavy metals [27] warrants further investigations into the role (if any) of 'Au-metallothionein(s)' in the formation of the immune complexes.

The significance of the binding of Au to the 'metallothioneins' with respect to the mode of therapeutic action of the metal is not clear. Further studies with regard to the induction of metallothionein biosynthesis by Au and the presence (or absence) of Au-thionein in the human synovial cells are planned.

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