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An investigation into the interactions of gold nanoparticles and anti-arthritic drugs with macrophages, and their reactivity towards thioredoxin reductase

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ABSTRACT

Gold(I) complexes are an important tool in the arsenal of established approaches for treating rheumatoid arthritis (RA), while some recent studies have suggested that gold nanoparticles (Au NPs) may also be therapeutically efficacious. These observations prompted the current biological studies involving gold(I) anti-RA agents and Au NPs, which are aimed towards improving our knowledge of how they work. The cytotoxicity of auranofin, aurothiomalate, aurothiosulfate and Au NPs towards RAW264.7 macrophages was evaluated using the MTT assay, with the former compound proving to be the most toxic. The extent of cellular uptake of the various gold agents was determined using graphite furnace atomic absorption spectrometry, while their distribution within macrophages was examined using microprobe synchrotron radiation X-ray fluorescence spectroscopy. The latter technique showed accumulation of gold in discrete regions of the cell, and co-localisation with sulfur in the case of cells treated with aurothiomalate or auranofin. Electrospray ionization mass spectrometry was used to characterize thioredoxin reductase (TrxR) in which the penultimate selenocysteine residue was replaced by cysteine. Mass spectra of solutions of TrxR and aurothiomalate, aurothiosulfate or auranofin showed complexes containing bare gold atoms bound to the protein, or protein adducts containing gold atoms retaining some of their initial ligands. These results support TrxR being an important target of gold(I) drugs used to treat RA, while the finding that Au NPs are incorporated into macrophages, but elicit little toxicity, indicates further exploration of their potential for treatment of RA is warranted.

1. Introduction

Chrysotherapy is the use of gold compounds for medical applications, foremost of which is the treatment of rheumatoid arthritis (RA). The use of gold compounds to treat RA dates back to pioneering studies by Forestier in the 1920s and 1930s [1–3]. Subsequently, for over 50 years, chrysotherapy became a mainstay of treatment for RA, with sodium aurothiomalate (Myochrisine), gold thioglucose (Solganol), sodium bis(thiosulfato)gold, and auranofin (Ridaura) among the most used therapeutic agents [4,5]. Each of these compounds contains gold in the +1 oxidation state (Fig. 1). Although their use has waned recently, chrysotherapy still remains an important chemotherapeutic approach to the treatment of RA.

Both aurothio-malate and -glucose are polymeric compounds containing Au(I) ions bound to two sulfur atoms. In contrast, sodium bis(thiosulfato)gold and auranofin are monomeric complexes in which the Au(I) center is bound to two sulfur atoms or a sulfur and a phosphorus, respectively. Auranofin entered clinical trials in the 1980s with much promise owing to its ability to be given to patients by oral administration. Nevertheless, the polymeric compounds are more effective treatments for RA than auranofin, although their use is associated with greater toxicity and unfavorable side effects [6].

Despite their long history of clinical use, the precise details of the mechanism(s) of action of chrysotherapeutic agents are not known. This is due in part to the complexity of the autoimmune response that is at the center of RA, and the wide range of biological targets *in vivo*

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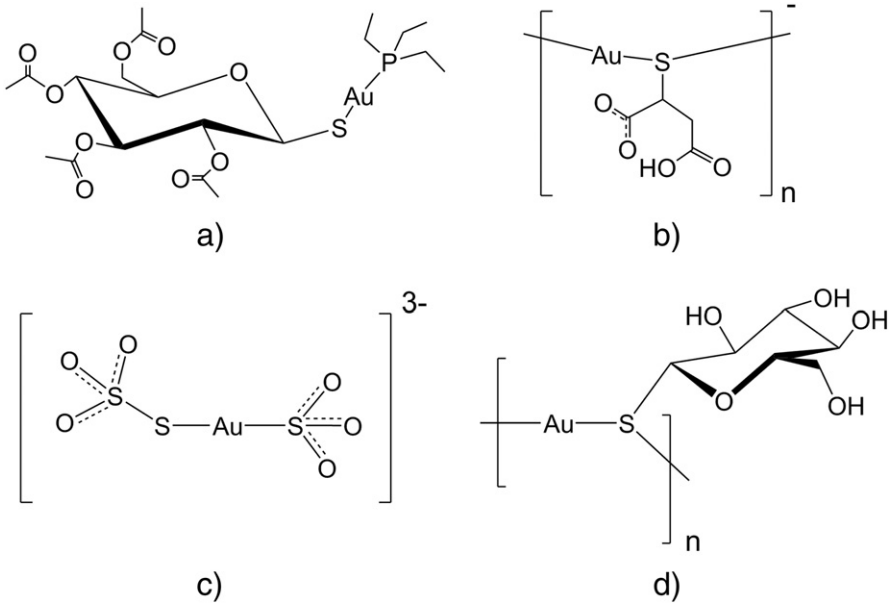

Fig. 1. Structures of clinically used gold drugs: a) auranofin, b) (sodium) aurothiomalate, c) (sodium) aurothiosulfate, d) aurothioglucose.

[7]. For example, auranofin inhibits activation of the transcription protein complex NF-κB [8], and reduces expression of the inflammatory enzyme COX-2 [8,9]. Production of nitric oxide [10] and the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 are also reduced by auranofin treatment [9,11], while aurothiomalate also inhibits production of TNF-α [12].

The Au(I) drugs are metabolized into their pharmacologically active forms after administration to patients. Initially the majority of the gold becomes bound to Cys-34 of serum albumin in processes that result in the loss of most, if not all, of the original ligands [4]. Several metabolites of the gold(I) complexes have been identified, including $[Au(CN)_2]^-$, Au(III) complexes and Au(0) [7]. It has also been shown that cells sequester gold into lysosomal bodies termed aurosomes [13,14]. X-ray absorption near edge spectroscopy (XANES) showed that aurosomal gold in the tissues of rats treated with aurothiomalate or $[AuCl_4]^-$ is present in the +1 oxidation state, while extended X-ray absorption fine structure (EXAFS) spectroscopy showed that the metal was bound to two sulfur atoms [15].

One mechanism by which the gold(I) drugs exert their anti-RA effects is inhibition of hydrolytic enzymes such as β-glucuronidase and elastase [5]. In addition, aurothiomalate and auranofin inhibit cathepsins K and S, which play a role in the progression of RA [16]. Thioredoxin reductase (TrxR) is another enzymatic target for metabolites of the gold drugs. This dimeric protein regulates cellular processes via reduction of thioredoxin (Trx) [17]. Both Trx and TrxR are over-expressed in synovial cells or fluid of RA patients [18,19]. Inhibition of TrxR by gold drugs has attracted interest due to the presence of a selenocysteine residue at its active site, and the greater affinity of Au(I) for selenol groups compared to thiols [20,21]. In addition, while platinum complexes bind to the active site of TrxR [22], some gold drugs inhibit the enzyme more potently [23–26]. Matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) showed that multiple molecules of gold compounds bind to TrxR [27], suggesting the presence of binding sites other than the active site selenocysteine. Several other mechanisms of anti-RA action have been proposed, including inhibition of leukocyte infiltration [28], modulation of the adhesion of polymorphonuclear neutrophils [29] and modification of macrophage activity [30]. It has also been suggested that various metabolites of the Au(I) drugs modulate activity of the immune system by binding directly to T-cell receptors to block antigen signaling [31].

The formation of Au(III) and Au(0) metabolites from the Au(I) drugs suggests the latter undergo redox chemistry *in vivo* [32,33]. It has also been proposed that Au(0) is responsible for the anti-arthritic activity of Au(I) complexes, whilst Au(III) gives rise to the side-effects. Not surprisingly the anti-arthritic activity of gold nanoparticles (Au NPs) have been investigated in an effort to avoid side effects. In one study, rats that had been chemically induced to exhibit symptoms of inflammation were treated with either Au NPs or sodium aurothiomalate [34]. Those treated with Au NPs showed a greater reduction in the severity of symptoms, encouraging further investigations. One such study involved human patients with chronic RA that had proven unresponsive to other treatments. A significant reduction in the severity of symptoms was demonstrated in 9 of 10 patients [35]. Moreover, studies focusing on cell systems provided clues as to how Au NPs may elicit their anti-inflammatory activity. For example, they inhibit production of reactive nitrogen [36] and oxygen [37] species, as well as activation of NF-κB [36]. In addition, Au NPs do not induce secretion of the pro-inflammatory cytokines TNF-α and IL-1β [37].

Here we present the first systematic comparison of the cytotoxicity, uptake and cellular distribution of auranofin, sodium aurothiomalate and Au NPs with RAW264.7 macrophages. Macrophages are of particular interest in the study of RA, since they have been implicated in the pathogenesis of the disease [38–40]. In addition, we used electrospray ionization mass spectrometry (ESI-MS) to compare the binding of the gold drugs and Au NPs to a modified form of TrxR, containing an active site cysteine instead of selenocysteine.

2. Experimental

2.1. Materials

All aqueous solutions were prepared using Milli-Q water (18.2 MΩ, Millipore). Sodium aurothiomalate, aurothioglucose, auranofin, gold(III) chloride (99.9%) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich. Sodium aurothiosulfate was obtained from Alfa Aesar. Fetal bovine serum (FBS, heat-inactivated) was purchased from Bovogen Biologicals. GlutaMAX and Roswell Park Memorial Institute-1640 cell culture medium powder (RPMI-1640) were purchased from Life Technologies.

2.2. Synthesis of gold nanoparticles

A stock solution of Au NPs was synthesized according to Turkevich et al. [41], with some modifications. Water (30 mL) was heated to