Potential anti-inflammatory properties of biologically-synthesized nanoparticles of gold or silver

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ABSTRACT

We observed that cartilage tissue and chondroitin sulfate (CS) can generate nanoparticles of gold (AuNP) or silver (AgNP) from ionic solutions of Au or Ag. These observations prompted us to hypothesize that the anti-inflammatory effects observed during aurotherapy of rheumatoid arthritis might be due to the *in vivo* generation of AuNPs. To test this hypothesis we synthesized AuNP or AgNP using cartilage tissue or CS, and evaluated them in an embryonic zebrafish inflammation model assay and in a natural killer (NK) cell activity assay. Results from the zebrafish assays indicate little toxicity from exposure to the AuNP or AgNP preparations; however, AgNPs proved to be cytotoxic towards the NK cells. The presence of AuNP or AgNPs decreased the magnitude of the inflammatory response in zebrafish and the cytotoxic activity of NK cells towards cancer cells.

Keywords: cartilage, chondroitin sulfate, gold, silver, inflammation

1 INTRODUCTION

Ionic gold (Au) compounds have been used for many years in the treatment of rheumatoid arthritis (aurotherapy).[1] Despite their long history, the basis for their therapeutic, immune-modulating properties has never been established definitively. We have observed that cartilage tissue and chondroitin sulfate (CS), a major biochemical component of cartilage tissue, can generate AuNP or AgNP when incubated with ionic solutions of Au or Ag.[2] Published reports regarding the pharmacology of Au compounds[3] and our preliminary observations prompted us to hypothesize that during aurotherapy with Au compounds, ionic Au could be coordinated to the CS molecules ubiquitously present in the cartilage tissue of the joints and be reduced into metallic NP of Au(0). The *in vivo* transformation of ionic Au into AuNP may be responsible for its immune-modulating properties. To test this hypothesis, we synthesized AuNP or AgNP using cartilage tissue or CS and evaluated the preparations in an embryonic zebrafish inflammation model assay and in a natural killer (NK) cell cancer-cytotoxicity model assay.

2. EXPERIMENTAL

2.1 Materials

Cartilage powder and chondroitin sulfate (type A, sodium salt from bovine trachea, approx 70% and balanced with CS type C) were purchased from Sigma-Aldrich (St. Louis, MO). $AgNO_3$ and $HAuCl_4.3H_2O$ and all other chemicals were purchased from Fisher Scientific (Suwanee, GA).

2.2 Synthesis of nanoparticles

Our synthesis methods are compatible with the concepts of green nanoscience, in that no solvent or toxic reducing agents are used in the procedures. Varying amounts of cartilage powder (between 200 and 600 mg) or aqueous solutions containing CSA (final concentration between 6 and 30 mg/mL) were mixed with aqueous solutions containing $AgNO_3$ or $HAuCl_4.3H_2O$ (final concentrations between 1 and 2 mM). These were left at 37°C (AuNP) or at room temperature (RT) exposed to ambient light (AgNP). The mixtures were filtered through a 0.2μM filter and filtrate aliquots were characterized and tested.

2.3 Atomic absorption spectroscopy (AAS)

AAS measurements were performed using a PerkinElmer AAnalyst 300 spectrometer (PerkinElmer Instruments LLC, Shelton, CT) equipped with a PerkinElmer Au or Ag cathode lamp to estimate the total amount of Au or Ag present in the sample.

2.4 Gel permeation chromatography (GPC)

GPC analyses were performed on a Varian Prostar chromatography system (Walnut Creek, CA) using a Waters Ultrahydrogel 500 column (Milford, WA). Analyses were performed at room temperature with a mobile phase consisting of 50 mM Na-acetate:methanol (9:1) at a flow rate of 0.75 mL/min and monitored using a photodiode array detector. The sample injection volume was 20 µL. For samples containing AuNP, the area under the curve (AUC) of any peaks observed at 540nm were integrated and taken as a relative measurement of the amount of AuNP present.

2.5 NK cell studies

These experiments were performed as described elsewhere.[4] Briefly, NK cells, freshly isolated from human blood samples, were preincubated with the AuNP or AgNPs and the appropriate control samples for 24h. Following the removal of the NPs or the control samples, the NK cells were mixed with cancer cells and the cytotoxic capacity of the NK cells was evaluated using a ^{51}Cr release assay.

2.6 Embryonic zebrafish assays

Two day old transgenic zebrafish with GFP-labeled neutrophils [7] were caudal fin amputated to induce a localized inflammatory response and immediately co-exposed to 100µl of 10µM test solution in water and 5ug/ml neutral red (a macrophage stain; macrophage data not reported). These embryos were then incubated at 28 °C for 6 hours, after which their tails were mounted in 1% carboxy-methyl-cellulose and photographed using an inverted fluorescent light microscope (Axiovert 200M, Zeiss). The number of neutrophils at the injury site was quantified using Image-Pro Plus (Version 5.1.0.20 for Windows 2000) and graphed using Sigma Plot 2001 (SPSS Inc, Chicago, IL).

3. RESULTS

3.1 AAS

AAS analyses of the filtrate samples indicate that between 60 and 100% or between 40 and 75% of the initial amount of Au remained for AuNP synthesized using CSA or cartilage tissue respectively. For the synthesis of AgNP these numbers were between 85 and 100% or between 25 and 45% for synthesis using CSA or cartilage tissue respectively.

3.2 GPC

Figure 1 presents typical GPC profiles obtained for samples containing AuNP synthesized with cartilage tissue. The figure shows the absorbance profiles obtained at 210 and 540nm.

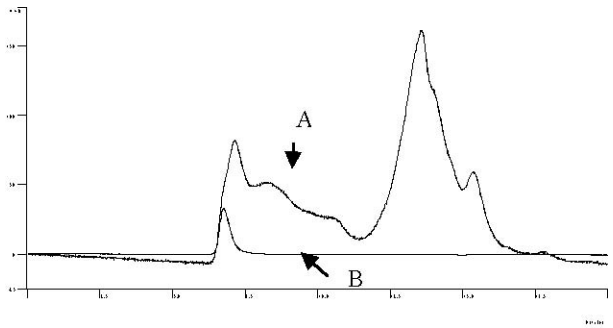

Figure 1: GPC profiles at 210 (A) and 540nm (B) of samples containing NP of Au synthesized using cartilage tissue.

3.3 NK cell studies

AuNP synthesized using cartilage tissue did not significantly alter the viability of the NK cells, nor their cytotoxic capabilities (results not shown).

AgNP synthesized with either cartilage tissue or CSA significantly decreased (sometimes up to 100%) the viability of the NK cells, leading to a decreased cytotoxic capacity of these cells (results not shown).

AuNP synthesized using CSA did not alter the viability of the NK cells but did have a concentration-dependent effect on the cytotoxicity of the NK cells. Figure 2 represents the relationship between % inhibition of the cytotoxic capacity of NK cells and the relative amount of AuNP present. The relative amount of AuNP was calculated by dividing the AUC of the GPC profile at 540nm of the sample by the total amount of Au present obtained from the AAS analysis of the sample.

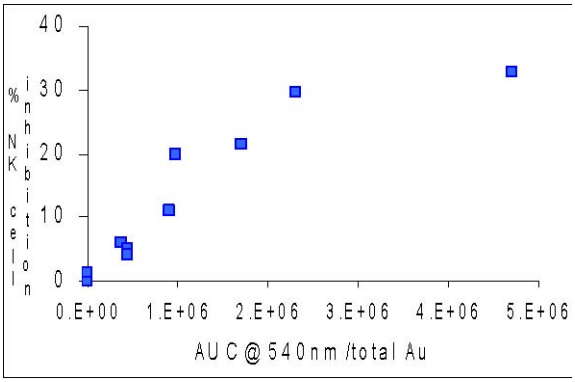

Figure 2: Percentage inhibition of NK cells cytotoxic capacity as a function of the relative amount of AuNP.

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3.4 Embryonic zebrafish assays

Two known anti-inflammatory compounds, dexamethasone and scopoletin, were used to validate the zebrafish inflammation assay for detection of compounds with anti-inflammatory properties. A reduction in neutrophil numbers was detected for both compounds compared to DMSO controls (Figure 3). AuNP and AgNP produced in cartilage-rich (Figure 4) and CS-rich medium (Figure 5) caused a reduction in neutrophil numbers at the injury site in subsequent embryonic zebrafish assays. The NPs shown in Figures 4 and 5 did not produce significant toxicity in a zebrafish dose-response assay conducted prior to the inflammation assays.

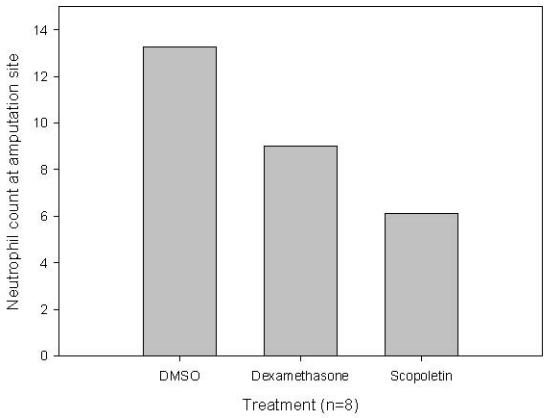

Figure 3: Zebrafish inflammation assay validation. Two known anti-inflamatory drugs, dexamethasone and scopoletin, reduced the neutrophil count at the site of injury compared to DMSO alone.

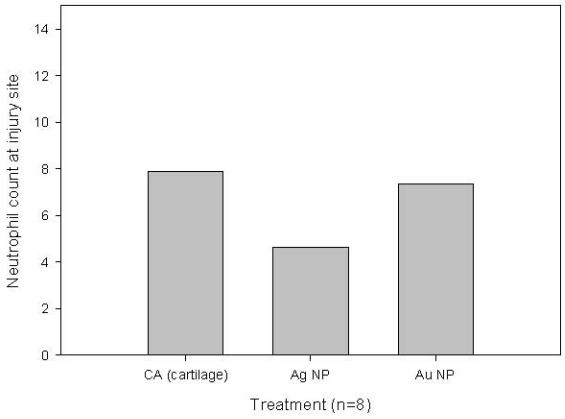

Figure 4: Representative AgNP produced in a cartilage-rich medium caused a reduction in neutrophil count at the injury site.

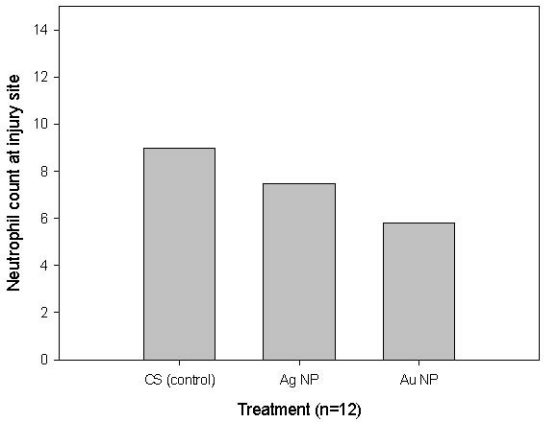

Figure 5: Representative AuNP and AgNP produced in a CS medium caused a slight reduction in neutrophil count at the injury site.

4. DISCUSSION

We were able to produce AuNP and AgNP using biosynthetic "green nanoscience" approaches.[5-7]

Regarding the mechanisms by which AuNP and AgNP are formed under these experimental conditions, we speculate that the highly anionic glycosaminoglycan (GAG) polysaccharides (CS or others) present in cartilage tissue are capable of chelating the Au(III) or Ag(I) cations and that some factor, possibly the reducing ends of the GAG polysaccharides, are capable of reducing these cations to their metallic form.

We have shown that the embryonic zebrafish inflammation assay described here is a relatively rapid, reliable method for identifying potential anti-inflammatory compounds *in vivo*. The power of this model stems from the ability to genetically manipulate embryonic zebrafish to facilitate characterization of NP anti-inflammatory mechanisms of action (e.g., inhibition of neutrophil migration or acceleration of neutrophil apoptosis) in follow-up investigations. Although, the pharmacology of gold compounds and their interaction with various biological molecules (proteins, nucleotides) has been studied extensively, the potential interaction with polyanionic polysaccharides, like the GAGs, and the potential *in vivo* formation of nanosized particles of Au has not been considered.[8] Our observations and preliminary results suggest that the potential formation of nanosized particles of Au in cartilage-rich tissues like the joints should be considered when evaluating the immuno-modulatory properties of Au compounds used in the treatment of rheumatoid arthritis.

REFERENCES

[1] T. Pincus, G. Ferraccioli, T. Sokka, A. Larsen, R. Rau, I. Kushner, F. Wolfe, Rheumatology (Oxford) 41, 1346-56, 2002

[2] K. Vercruysse, A. Williams, D. Ivory, B. Johnson, R. Dixon, M. Tran, J. Moore. Using Chondroitin Sulfate to Synthesize Nanoparticles. In: NSTI Nanotech Symposium. Santa Clara, CA, 2007

[3] I. C. Shaw, Chemical Reviews 99, 2589-600, 1999

[4] M. M. Whalen, B. G. Loganathan, N. Yamashita, T. Saito, Chem Biol Interact 145, 311-319., 2003

[5] S. P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad, M. Sastry, Biotechnol Prog 22, 577-83, 2006

[6] S. L. Harper, J. A. Dahl, B. L. S. Maddux, R. L. Tanguay, J. E. Hutchison, International Journal of Nanotechnology 5, 124-142, 2008

[7] S. A. Renshaw, C. A. Loynes, D. M. I. Trushell, S. Elworthy, P. W. Ingham, M. K. B. Whyte, Blood 108, 3976-3978, 2006

[8] C. F. Shaw, Inorganic Perspectives in Biology and Medicine 2, 287-355, 1979

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