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Original article

Anti-inflammatory, analgesic and anti-tumor properties of gold nanoparticles

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ABSTRACT

Background: Gold nanoparticles (GNPs) are regarded as potential platforms for drug delivery. However, their interaction with live organisms must be understood prior to their utilization as drug carriers. The present study reports the anti-inflammatory, analgesic and anti-tumor effects of GNPs. The biodistribution of GNPs and their effect on various tissues have also been studied.
Methods: GNPs were synthesized through an environmentally friendly route and characterized with TEM and UV–vis. After HT-29 cells had been exposed to GNPs, apoptosis was assessed with Annexin V and propidium iodide staining and caspase-3 activity determined with a confocal laser scanning microscope. GNPs were administrated to male and female Swiss mice for posterior assessment of their anti-inflammatory and analgesic properties. The biodistribution of GNPs and their impact on tissues were studied with UV–vis and histopathological analysis, respectively.
Results: Cell apoptosis was observed in a dose-dependent manner for GNPs concentrations ranging from 40 μg/mL to 80 μg/mL ($p < 0.05$). The best anti-inflammatory activity was observed at the dose of 1500 μg/kg, which caused a reduction of 49.3% in leukocyte migration. GNPs showed peripheral analgesia at the dose of 1500 μg/kg and have been found in liver, spleen, kidney and lungs. Histopathological examination revealed extravasation of red blood cells in lungs.
Conclusion: The study draws attention to gold nanoparticles as a resource for technological innovation in the anti-inflammatory, analgesic and anti-tumor fields. GNPs have biological effects that deserve investigation to assess their full interaction with organic systems.

Background

Metal nanoparticles have been employed in biomedicine since the 1970s thanks to the conjugation of gold nanoparticles (GNP) with antibodies, opening the era of immune gold labeling [1]. Nanotechnology has since then furthered its boundaries for potential early detection, diagnosis, and targeted treatment of diseases [2,3]. However, for safe use of this relative new technology, it is imperative that its biological and toxicological effects on living systems be understood prior to its dissemination. Due to their minute dimensions and high surface-to-volume ratio, nanomaterials may enter the circulatory and lymphatic systems producing irreversible injuries through exacerbate oxidative stress [4]. As an example, silver nanoparticles induced oxidative stress and genotoxicity in cultured cells and animal tissues [5]. On the other hand, studies have shown that nanoparticles may be used as carriers of anti-inflammatory [6] and immunosuppressive [7]

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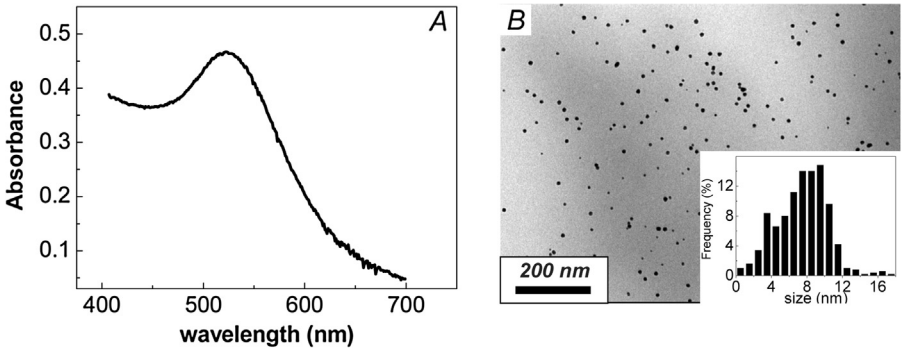

Fig. 1. (A) UV–vis spectrum of the colloidal GNPs, (B) TEM image of the GNPs. Inset: size distribution of the GNPs.

drugs with mild acute citoxicity [8]. Gold or silver nanoparticles conjugated with heparin exhibited anti-inflammatory properties without any significant effect on systemic hemostasis [9]. Chen et al. [10] reported a reduction in TNFα and IL-6 mRNA levels when 21-nm gold nanoparticles were injected into mice, a result attributed to fat loss and inhibition of inflammatory effects. Administration of nanoparticles of MnO_2 caused accumulation of Mn in brain, spinal cord and muscle tissues of rats [11], leading to an impact on pain sensation. Finally, nanoparticles have also presented anti-tumor properties, which is quite interesting from the viewpoint of formulating new drugs against cancer [12].

The aim of the present study is to evaluate the anti-inflammatory, analgesic, and anti-tumor properties of GNPs that were synthesized through a low-toxicity chemical route. We already showed that gold nanoparticles produced via the glycerol route [13] have catalytic [14] properties and can also be combined with antibodies to generate an extremely efficient contrast agent for colorectal cancer cell imaging [15]. The latter study prompted us to understand the effect of GNPs on cancer cell viability and other effects *in vivo*, since the ultimate goal is to formulate, in the future, more efficient drugs that are based on nanoparticles.

Materials and methods

Reagents

The following reagents were purchased as indicated: Dulbecco's modified Eagle's medium (Life Technologies, Grand Island, NY, USA), 10% (v/v) heat-inactivated fetal bovine serum (CULTILAB LTDA/Brazil), trypsin/EDTA (Gibco BRL, Life Technologies, Grand Island, NY, USA), cisplatin (citoplax, 50 mg, Bergamo Taboão da Serra, SP, Brazil). Gold(III) chloride (30% wt in HCl), sodium hydroxide, glycerol, and polyvinylpyrrolidone (PVP, molecular weight = 10,000) were products of Sigma-Aldrich Co.

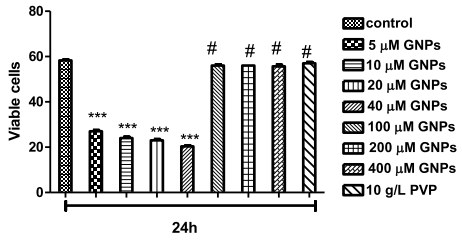

Fig. 2. Half-maximal inhibition of HT-29 growth was observed for concentrations of GNPs ranging from 5 μM to 40 μM. PVP did not alter HT-29 growth. $^{***}p < 0.0001$, $^{\#}p > 0.05$.

Synthesis and characterization of GNPs

100 mg of PVP are mixed with 2 mmol of $HAuCl_4$ and ultrapure water was added to generate a 5-mL solution. In a separate flask, 0.20 mol of glycerol was mixed with 0.20 mol of NaOH and ultrapure water was added to generate a 5-mL solution. The glycerol-NaOH solution was then added to the $HAuCl_4$-PVP to yield 10 mL of GNPs solution. The GNPs colloidal solution had then its pH adjusted to 7 by addition of diluted HCl and was subjected to dialysis for purification. UV–vis absorption spectra of the GNPs were acquired with an Evolution 60S UV–vis spectrophotometer (Thermo Scientific). Transmission electron microscopy (TEM) images were acquired with a Tecnai Spirit BioTwin 12 microscope operating at 120 kV.

Cell culture

A colorectal cancer cell line (HT-29) was purchased from the Culture Collection of the Federal University of Rio de Janeiro (RJCB Collection, Rio de Janeiro, RJ). HT-29 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum. Gold nanoparticles and cisplatin solutions were filtered using a 0.22 μm membrane (EMD Millipore) and stored at −20 °C.

Cell viability

HT-29 cell viability (1×10^5 cells) was determined by Trypan Blue exclusion assay 24 h after inoculation with different concentrations of GNPs (5–400 μM in aqueous suspensions). Briefly, cell aliquots were mixed with 0.5% (w/v) Trypan Blue and incubated at room temperature for 5 min. The number of viable cells was calculated using a hemocytometer. The effect of stabilizing agent of gold nanoparticle (10 g l^{-1} PVP) was also tested.

Annexin V and propidium iodide staining

The apoptotic assay was conducted according Araújo Jr et al. [16]. HT-29 cells were placed in 6-well plates (2×10^5 cells/well) with 2 ml medium/well. After 24 h, different concentrations of gold nanoparticles (10 μM, 20 μM and 40 μM) and cisplatin (50 μM and 100 μM) were added and allowed to react for 24 h and 48 h. In parallel, control cells were maintained in culture medium without gold nanoparticles or cisplatin. The cells were then assayed using the Annexin V-FITC Apoptosis Detection kit I (Biosciences Pharmingen, San Diego, USA). Annexin V-FITC and propidium iodide (PI) were added to the cellular suspension according to the manufacturer's instructions. A total of 1.0×10^6 cells from each sample were then analyzed using a FACS Calibur cytometer (BD Bioscience, Franklin Lakes, NJ, USA) and FlowJo software (BD