Effect of gold nanoparticles on the respiratory activity of peritoneal macrophages

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Abstract

This work was undertaken to investigate the effect of antigen-conjugated gold nanoparticles on the functional activity of cells of the reticuloendothelial system. Experiments using rat and mouse peritoneal cells assessed the dynamics of the total respiratory activity of macrophages and the activity of mitochondrial dehydrogenases on exposure to colloidal gold conjugates of high- and low-molecular-weight antigens. Both gold nanoparticles and their antigen conjugates stimulated the respiratory activity of the macrophages and the activity of macrophage mitochondrial enzymes. This stimulation may be an essential factor determining the adjuvant properties of colloidal gold, found by us in prior work.

Keywords: Colloidal gold nanoparticles; Respiratory activity; Macrophages; Mitochondrial enzymes; MTT test

Abbreviations: CG, colloidal gold; DMSO, dimethylsulfoxide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide

Introduction

Over the past few years, much attention has been given to corpuscular carriers as delivery vehicles for drugs, genetic material, and various antigens. Specifically, work in this direction is aimed at improving the effectiveness of drugs and at lowering their toxicity during treatment of humans and animals. Use has been made of such carriers as metallic and paramagnetic nanoparticles, dendrimers, liposomes, fullerenes, carbon nanotubes, polymer (polymethylmetacrylate, polyalkylcyanocrylate) particles, and other materials [1]. However, the role of the corpuscular particle itself as a structure recognized by an organism's reticuloendothelial system and utilized by it is often overlooked.

This is demonstrated by the injection of metallic gold into laboratory animals, which may cause inflammatory response, accumulation of gold in the reticular cells of lymphoid tissue, and activation of cell-mediated and humoral immunity [2, 3]. Nevertheless, there has been interest in colloidal gold (CG) as a vector for direct delivery of drugs to the inflammation foci and affected organs in the macroorganism [4–6] and, because of its antitumor activity, as a therapeutic means in its own right [7]. Of interest in this context is the influence of nanoparticle size and shape (and the size and nature of nanoparticle-adsorbed molecules) on the ability of conjugates to penetrate into cells [8–10] and on cytotoxicity [11].

Paciotti et al. [4] examined the possibility of using CG for malignant-tumor therapy. They applied a tumor necrosis factor to the surface of colloidal particles and injected the resultant conjugate into tumor-affected mice. They found that the conjugate was less toxic and more effective than the native factor, although the gold-nanoparticle-conjugated factor had been administered at a lower dose.

Gold nanoparticles are also being actively used in DNA vaccination [12, 13] and in the *in vivo* preparation of antibodies specific to complete antigens and various haptens [14–17]. As an antigen carrier, CG enhances the phagocytic activity of macrophages and influences lymphocyte functioning; this fact possibly determines the immunomodulating effect of CG. The most interesting aspect of manifestation of immunogenic properties by a hapten after immobilization on CG is that gold nanoparticles serve as both a carrier and an adjuvant; i.e., they somehow present the hapten to T cells. Several authors reported on responses of Kupffer cells [18] and peritoneal macrophages [19, 20] during interaction with gold nanoparticles. Vallhov et al. [21] discussed the influence of dendritic cells on the formation of an immune response upon administration of an antigen conjugated to gold nanoparticles. Those authors also noted that the use of nanoparticles in medical practice requires making sure that their surface is free from lipopolysaccharides. However, all these results do not provide a final answer on the mechanism underlying the immunomodulating properties of gold nanoparticles in interaction with cells of the reticuloendothelial system.

Here we examine the interaction of CG with phagocyting cells by estimating changes in the activity of cellular respiration and in the activity of mitochondrial dehydrogenases of rat and mouse peritoneal macrophages during macrophage interaction with CG conjugates of high- and low-molecular-weight antigens.

Materials and methods

A protein complex produced from *Salmonella typhimurium* by dimethylsulfoxide (DMSO) extraction was used as a complete antigen. The DMSO antigen was obtained as follows. A 24-h microbial suspension (the cells had been grown overnight in a nutrient broth on a thermostated shaker [180 rev min^{-1}] at 37°C) was centrifuged five to six times at 1500 g for 15 min in a phosphate-buffered physiological saline (pH 7.3). The resultant bacterial mass was dispersed in acetone (1 volume of bacteria to 2 volumes of acetone) and was placed in a thermostat operating at 37°C for 1.5 h, with occasional shaking. After that, the cells were harvested by centrifugation (2500 g for 15 min) and the acetone was decanted. The bacterial mass was again dispersed in acetone, and the procedure was repeated twice. The acetone-treated cells were dispersed in a DMSO solution at 6 ml of solution per 1 g of bacteria and were shaken at 37°C for 40 min. Then, the cells were separated by centrifugation at 2500 g for 15 min. The extract was dialyzed against 0.01 M carbonate–bicarbonate buffer (pH 6.9) for 2 days with five changes of the buffer. The resulting antigen was conjugated to CG (15 nm mean particle diameter), synthesized according to Frens [22]. Briefly, CG particles were prepared by the reduction of tetrachloroauric acid with sodium citrate. A 242.5-ml portion of 0.01% aqueous tetrachloroauric acid was heated on a magnetic stirrer in an Erlenmeyer flask fitted with a water-cooled reflux tube. This was followed by the addition of 7.5 ml of 1% aqueous sodium citrate to the flask. The mean particle size was controlled by the spectrophotometric calibration described by Khlebtsov et al. [23] (A_{518} = 1.1).

For comparison purposes, our experiments also used the antiparasitic drug ivermectin conjugated to CG, as a low-molecular-weight antigen (hapten).

Rat peritoneal macrophages were isolated conventionally [24]. After isolation, the resultant cell suspension was transferred (at 100 µl per tube [$4x10^6$ cells per sample]) to microtubes containing:

1. 200 µl of nutrient medium and 100 µl of CG (15 nm particle size);
2. 200 µl of nutrient medium and 100 µl of ivermectin–CG conjugate (100 µg ivermectin concentration, 15 nm particle size);
3. 200 µl of nutrient medium and 100 µl of ivermectin (100 µg concentration) dissolved in diethylacetamide;
4. 200 µl of nutrient medium and 100 µl of diethylacetamide;
5. 100 µl of nutrient medium.

Cell incubation with the preparations was done at 37°C for 48 h.

The same procedure was followed in an *in vitro* experiment to study the effect of CG conjugates of the other antigen, DMSO, on the peritoneal macrophages.

In the *in vivo* experiments, nine mice were divided into three groups (three animals per group). The group 1 mice were injected with the CG–antigen complex. The group 2 mice received the antigen (at the same concentration as used for the group 1 mice) diluted with distilled water. The group 3 mice served as controls. The antigen was administered intraperitoneally at 100 µg (500 µl CG–antigen conjugate) per animal, and the mice had been weighed before injection (20 g mouse weight). A week later, the mice in all the groups were immunized again with the same antigen used at the same dose, and after an additional 6 days, peritoneal macrophages were isolated.

Peritoneal cells were diluted to $4x10^9$ cells L^{-1} with the culture medium by using an Arcus hematological analyzer (Diatron, Austria). A count was made of the total number of leukocytes present in a sample. Respiratory activity was measured conventionally [25] (the MTT test). Dehydrogenase activity was determined by reduction of the MTT salt to the water-insoluble formazan [26, 27]. Reduced formazan was measured with a Power Wave microplate spectrophotometer (Bio-Tek Instruments, USA).

Results and discussion

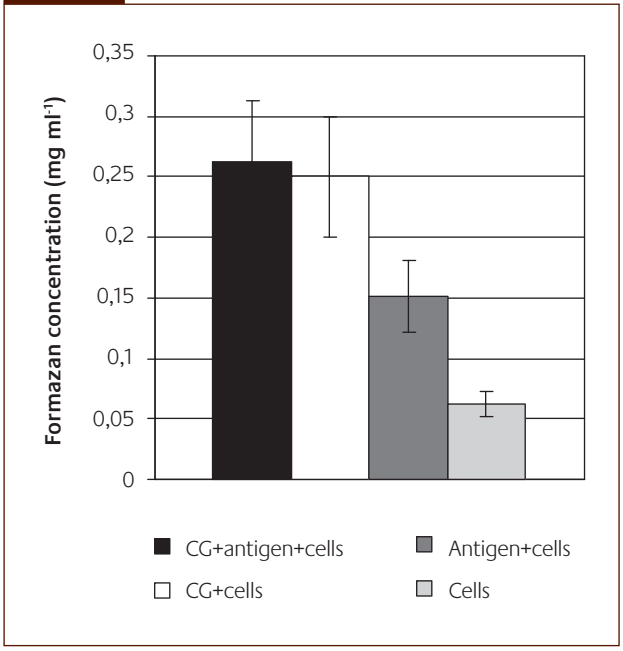
Firstly we evaluated the effect of CG conjugates of the high-molecular-weight antigen on the *in vitro* respiratory activity of rat peritoneal cells. Formazan was most reduced when the cells were cultured with the DMSO-antigen–CG conjugate and with CG itself (Fig 1). In the cells cultured with and without (control) the antigen alone, the amounts of reduced formazan were much smaller.

The ivermectin–CG interaction led to similar results (Fig 2). When the cells interacted with CG, the concentration of reduced formazan was almost two times higher than the control value. Both ivermectin and dimethylacetamide suppressed cellular respiration. However, CG complexed with ivermectin appreciably decreased the toxic effect.

The effect of CG on the activities of the mitochondrial enzymes succinate dehydrogenase and α-glycerophosphate dehydrogenase is shown in Fig 3. The results for the effects of CG on α-glycerophosphate dehydrogenase are presented in Fig 4.

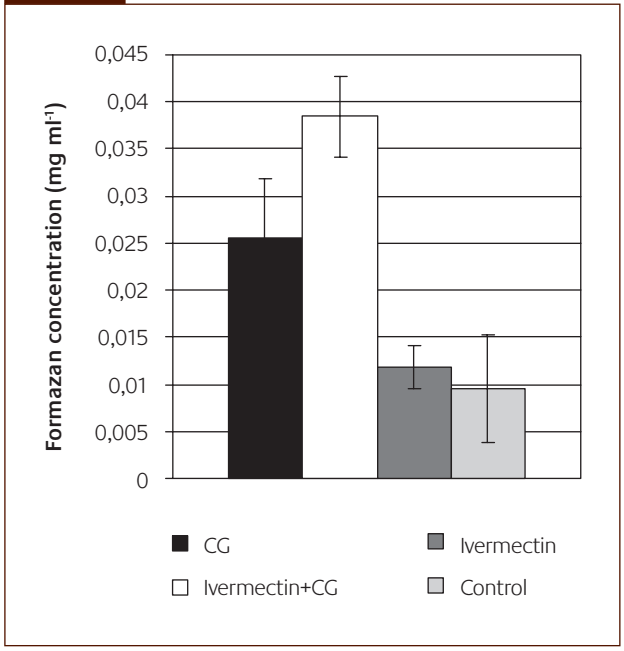
These results show that the ivermectin–CG conjugate enhanced mitochondrial activity. Additionally, CG itself was found to increase cellular mitochondrial activity. These observations, attesting to a CG-induced increase in the respiratory activity of cells of the reticuloendothelial system

Figure 1



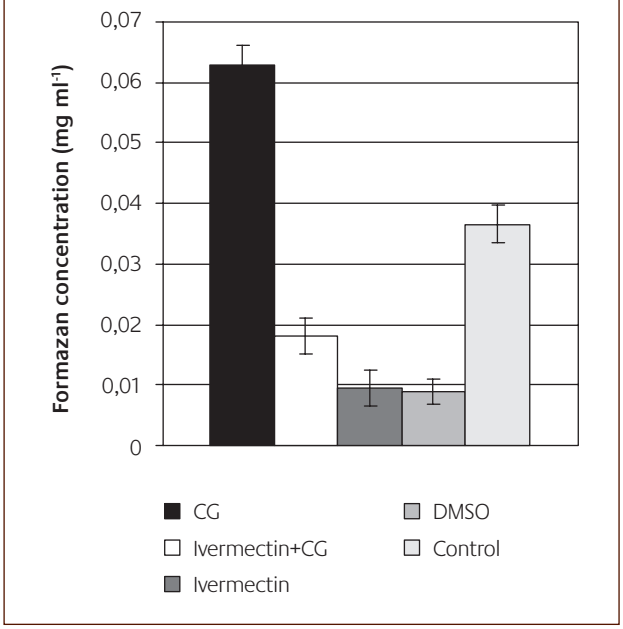
Changes in the concentration of reduced formazan depending on the culture conditions (DMSO antigen) for rat peritoneal macrophages

Figure 3



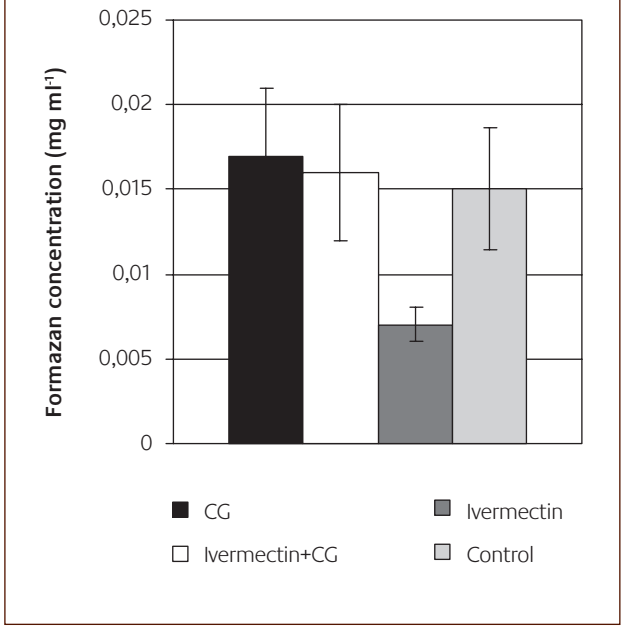
Effect of ivermectin and ivermectin–CG on the activity of succinate dehydrogenase

Figure 2



Results of an MTT test of rat peritoneal macrophages after their incubation with CG–ivermectin

Figure 4



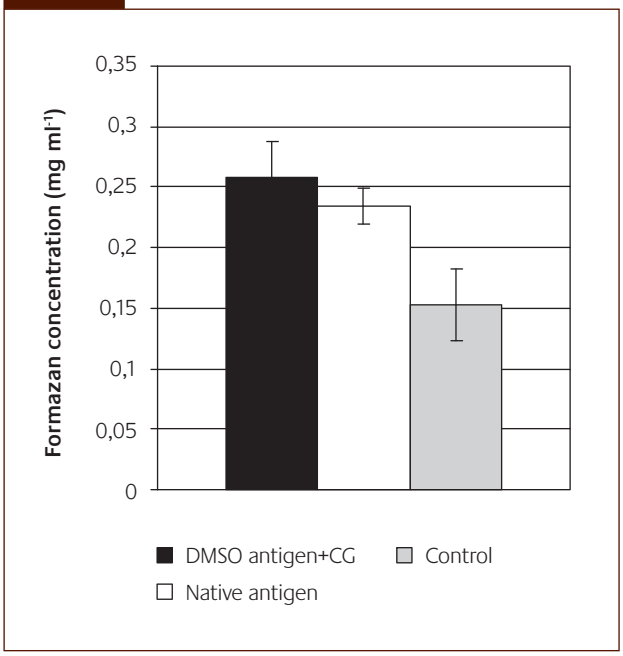
Effect of ivermectin and ivermectin–CG on the activity of α-glycerophosphate dehydrogenase

in our *in vitro* experiments, supplement the existing data on possible mechanisms responsible for the immunomodulating properties of gold nanoparticles.

We next explored the effect of gold nanoconjugates on cells of the reticuloendothelial system of white mice. Injection of the antigen–CG conjugate caused the most macrophage activity (Fig 5). Injection of the antigen alone somewhat decreased the activity. In the control group, the concentration of formazan was the lowest.

Other authors have reported experimental evidence that gold is selectively toxic to the tumor cells of different lines. Patra et al. [28] investigated the interaction of CG with A549 human lung carcinoma cells. They tested two other cell lines, BHK21 (baby hamster kidney) and HepG2 (human hepatocellular liver carcinoma); and showed that gold was toxic only to line A549; and found a relationship between toxicity and CG-particle size (note that the cell viability, after the action of gold nanoparticles, was assessed by an MTT

Figure 5



Results of an MTT test of murine macrophage cells after injection of the antigen and the antigen–CG conjugate

test). Khan et al. [29], working with HeLa cells, found no toxicity of CG.

Our data show that CG is not toxic to the phagocytic peritoneal cells and that it even alleviates the effect of the toxic antigen conjugated to CG. The penetration of gold into and its accumulation in peritoneal cells has, in particular, been confirmed by Shukla et al. [19] and by Pristensky et al [20].

In conclusion, we note that considering the effects of active interaction of CG with cells of the immune system, found by us previously and confirmed in this work, we believe that caution should be exercised when using corpuscular carriers in targeted drug delivery. One has to take into account the possibility that the animal or human body may develop antibodies against the drug administered in this way.

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