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Nano-based antileishmanial agents: A toxicological study on nanoparticles for future treatment of cutaneous leishmaniasis

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ABSTRACT

Cutaneous leishmaniasis (CL) is endemic in the tropical and subtropical countries. Antileishmanial drugs that are traditionally used for treatment of CL are mainly toxic, ineffective for some parasite isolates, and mostly expensive. Previous studies showed that some metal and metal oxide nanoparticles have antimicrobial activity. Moreover, the use of nanoparticles together with ultra violet (UV) and infra red (IR) light increases toxic effects of nanoparticles by generation of reactive oxygen species (ROSs) and heat, respectively. There is little information on antileishmanial activity of nanoparticles, alone or together with UV/IR. Thus, the purpose of this research was to study antileishmanial effects of some nanoparticles including silver nanoparticles (Ag NPs), gold nanoparticles (Au NPs), titanium dioxide nanoparticles (TiO_2 NPs), zinc oxide nanoparticles (ZnO NPs), and magnesium oxide nanoparticles (MgO NPs) on *Leishmania major* parasites under UV, IR, and dark conditions. After 24 h exposure to nanoparticles, different biological parameters such as cell viability, proliferation, infectivity, and infection index were investigated under UV/IR/dark conditions. In this study, the highest antileishmanial activity was seen for Ag NPs, followed by Au NPs, TiO_2 NPs, ZnO NPs, and MgO NPs. Both UV and IR light increased antileishmanial properties of all nanoparticles. In spite of antileishmanial activity of nanoparticles under UV, IR, and dark conditions, these nanoparticles had high cytotoxicity on macrophages, which must be considered in future studies. The authors declare that the use of nanoparticles for treatment of CL may have both positive and negative consequences.

1. Introduction

Leishmaniasis is endemic in near 100 countries worldwide, and is a health problem in most of the developing countries. The annual worldwide prevalence of leishmaniasis is approximately 12 million, and 350 million people are at risk. It is estimated that there are 500,000 new cases of visceral leishmaniasis and 1.5 million new cases of cutaneous leishmaniasis (CL) each year. Furthermore, global warming and the increasing of leishmanial vectors lead to high incidence of both forms of this disease. Although pentavalent antimonial is the gold standard for treatment of leishmaniasis, it has toxicity to liver and kidney. Despite its toxicity, the use of traditional antileishmanial drugs is mostly limited to undeveloped and developing countries, because these drugs are much expensive. On the other hand, the ineffectiveness of antileishmanial drugs against some species of *Leishmania* parasites have also been reported (Croft and Coombs, 2003; Natera et al., 2007). Because of these limitations, new drugs and protocols must be researched for leishmaniasis.

Currently, with a rapid growth of nanotechnology, different nanoparticles have been presented for medical science applications. Nanomaterials have unique chemical and physical properties, and will be used in treatment of different severe or chronic diseases in the future (Angeli et al., 2008). To date, it has been shown that some metal and metal oxide nanoparticles have antimicrobial activities (Elechiguerra et al., 2005). It has long been demonstrated that silver ions, silver nanoparticles (Ag NPs), and nanosilver-containing complexes have antimicrobial behavior with high ability to inactivate bacteria and viruses (Shrivastava et al., 2007). Also, some reports indicate that gold nanoparticles (Au NPs), titanium dioxide nanoparticles (TiO_2 NPs), zinc oxide nanoparticles (ZnO NPs), magnesium oxide nanoparticles (MgO NPs), etc. have antibacterial properties (AshaRani et al., 2009; Chen et al., 2011; Li et al., 2010; Xia et al., 2006; Zheng et al., 2008). Recently, even though the mechanisms of antimicrobial effect of nanoparticles are not well known, but catalytic oxidation, binding to protein and cell components, and ion release are some proposed

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mechanisms. Particularly, some nanoparticles are able to produce reactive oxygen species (ROSs) under ultra violet (UV) light, and can kill microorganisms (Lodge and Descoteaux, 2006; Misawa and Takahashi, 2011; Murray, 1981). Macrophages produce high level of ROS in order to kill microbial agents such as viruses, fungi, and parasites (Lodge and Descoteaux, 2006). Moreover, ROS production is inhibited by *Leishmania* parasites through enzymatic pathways, and leads to *Leishmania* survival inside macrophages as the host cells (Mehta and Shaha, 2006). ROS-inducing nanoparticles may overcome the inhibition of ROS production in macrophages by *Leishmania* isolates.

On the other hand, some nanoparticles have photo thermal effect after exposure to near infra red (NIR) light. These nanoparticles absorb NIR energy, and alter it to heat. Then, temperature is increased and cells will be damaged (Chatterjee et al., 2011). It is stated that *Leishmania* parasites are sensitive to heat, and heat therapy has been used as a new procedure. Researchers at the Uniformed Services University of the Health Sciences and in Walter Reed Army Medical Center (WRAMC) showed that heat therapy by Thermo Med™ device was as effective on CL as 10 days of intravenous administration of sodium stibogluconate (Pentostam) (Aronson et al., 2010). Also, Junaid (1986) showed treatment of cutaneous leishmaniasis with infrared heat. They indicated that the lesions can be removed in approximately 5–6 weeks by this device (Junaid, 1986). Interestingly, this procedure has been used together with nanoparticles for cancer cell killing by NIR hyperthermia (Chatterjee et al., 2011), and so may be used for treatment of leishmaniasis. It must be mentioned that although nanoparticles have antimicrobial effects, they have some toxicities on different human cells, and their cytotoxicity should be considered as new antimicrobial agents. To date, there are little data on antileishmanial activity of nanoparticles, alone or under irradiation. Therefore, the aim of this study was to evaluate antileishmanial effects of different nanoparticles on *Leishmania major* parasites. In this study, various biological parameters such as cell viability, proliferation, infectivity, and infection index were measured under UV light, infra red (IR) light, and at darkness.

2. Materials and methods

2.1. Materials

In this research, analytical grade of materials were used. All nanoparticles including Ag NPs, Au NPs, TiO_2 NPs, ZnO NPs, and MgO NPs were obtained from Lolitech Company, Germany. $RPMI_{1640}$, fetal calf serum (FCS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), Giemsa, and methanol were purchased from Sigma–Aldrich Chemical Co, (St Louis, MO). Also, Hanks' balanced salt solution (HBSS) was sourced from Gibco, Invitrogen, UK. Standard isolate of *L. major* promastigotes (MRHO/IR/75/ER) was provided from Center for Research and Training in Skin Disease and Leprosy, Tehran University of Medical Sciences, Tehran, Iran.

2.2. Preparation and characterization of nanoparticles

Different concentrations of nanoparticles (200, 20, and 2 μg/mL) were prepared in $RPMI_{1640}$ supplemented with 10% FCS, mixed for 5 min, sonicated for 30 min, and stored at 5 °C. The size distribution of nanoparticles was investigated by dynamic light scattering (DLS) (Malvern Instruments, Italy). Briefly, nanoparticle suspensions at concentration of 20 μg/mL were prepared in $RPMI_{1640}$, and then 50 μL of each nanoparticle suspension was placed in Zetasizer Nanoinstrument. DLS analysis was carried out at 25 °C, and the size range of nanoparticles was recorded after triplicate independent tests. To investigate structure and shape of nanoparticles, scanning electron microscopy (SEM) (Hitachi S-2400) was used. Briefly, nanoparticle samples were dried on a copper grid, coated with a thin layer of gold by sputtering, and then examined at 15 kV.

2.3. Cells and cell culture

L. major promastigotes (MRHO/IR/75/ER) were cultured at 25 °C for 3 days in $RPMI_{1640}$ medium with L-glutamine supplemented with 10% FCS and antibiotics. Each week, 3×10^6 promastigotes were transferred to 4 mL of $RPMI_{1640}$ enriched with 10% FCS and incubated at 25 °C.

Peritoneal macrophages of Balb/c mice (approximately 18–20 g) were prepared aseptically, and rinsed with HBSS. Then, 5×10^5 - cells were added to $RPMI_{1640}$ medium enriched with 10% FCS in 96-well plates, and incubated at 37 °C for 24 h for adhesion of macrophages to the wells. After 24 h, the non-adherent cells were removed by washing with RPMI medium.

Mice were maintained at standard conditions, according to guidelines of the NIH (Guide for the Care and Use of Laboratory Animals, 1996), and all experiments related to mice were in compliance with the ethics committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2.4. Cytotoxicity evaluation

Firstly, both promastigotes and macrophages were treated separately with different concentrations of nanoparticles for 24 h at 25 °C and 37 °C, respectively. After addition of nanoparticles to cells, UV light (200–300 nm, 8 watt, Ultra-Lum Inc., USA) and IR light (800–1400 nm, 8 watt, Tungsram-Schreder, Hungary) were used for 1 h in separate microplates, while the other was held in the dark. Then, the cytotoxicity was quantified by MTT assay, and formazan formation was observed using light microscopy. After 24 h exposure, cells were washed three times with HBSS, and then 100 μL of $RPMI_{1640}$ and 25 μL of MTT at concentration of 5 mg/mL were added to each well. After 5 h incubation, 50 μL of 70% isopropanol was added, and optical densities (OD) of each well was read at 490 nm using a microplate reader (Novin Gostar, Iran). Finally, cell viability was measured. In the control groups, promastigotes and macrophages were not exposed to any nanoparticles, UV, or IR light. Also, in a separate test, promastigotes and macrophages were exposed to UV and IR light alone, and the effects of irradiation were evaluated. On the other hand, for observational study of formazan formation, parasites and macrophages were spread on microscopic slides after incubation, fixed with methanol, stained with Giemsa, and examined with a light microscopy (magnification 400×).

2.5. Proliferation evaluation test

For this assay, 100 μL of *L. major* promastigotes was added separately to 100 μL of different concentrations of nanoparticles, and were then incubated at 25 °C for 24 h. Like cytotoxicity evaluation test, UV and IR light were used for 1 h after addition of nanoparticles, and the other microplate was held in the dark. Parasites were counted by Neubauer hemacytometer slide (Marienfeld, Germany) after 24 h incubation. In the control groups, promastigotes were not treated with nanoparticles or any irradiation. Also, promastigotes were exposed to UV and IR light alone, and the effects of irradiation were evaluated.

2.6. Infectivity evaluation test

Firstly, *L. major* parasites were incubated with different concentrations of nanoparticles at 25 °C for 24 h. Similar to cytotoxicity