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Studies on the effect of gold nanoparticles on oxidative stress and antioxidants defense indices in various rat tissues

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Gold nanoparticles (AuNPs) have a wide range of applications in various fields. It is therefore essential to study their interaction with the biological system. In the present study, the effect of AuNP was studied on oxidative stress and antioxidants in various tissues of rats. In this study, liver was the organ most sensitive to the effects of AuNP. Liver of AuNP treated rats showed a significant increase in lipid peroxidation, reduced glutathione and ascorbic acid when compared with the control group. This was followed by kidneys which showed significant increase in reduced glutathione and ascorbic acid when compared to the control group. Spleen showed a significant increase in the ascorbic acid concentration when compared with that of control rats. Heart tissue was most resistant to the effects of AuNP and showed no significant change in any of the oxidative stress and antioxidant indices studied. These results suggest that at low concentration, the properties of AuNP could be exploited for use in nanoimmunology, nanomedicine, nanobiotechnology and various other fields.

Key words: Gold nanoparticles (AuNPs), oxidative stress, antioxidants, rat tissues, antioxidant enzymes.

INTRODUCTION

Nanoparticles have a wide range of applications due to their unique properties. Gold nanoparticles (AuNPs) can easily enter cells (Connor et al., 2005) and the demonstration that amine and thiol groups bind strongly to AuNP has enabled their surface modification with amino acids and proteins for biomedical applications (Dani et al., 2008; Shukla et al., 2005; Xu and Han, 2004). AuNP are used in biosensors where they markedly enhance

sensitivity and specificity of detection, because of their unique physical, chemical, mechanical, magnetic and optical properties (Zhang et al., 2009). Gold nanoparticles are also potential candidates for use in food industry due to their antimicrobial properties. Beside their useful effects, the interaction of nanomaterials with the biological system may also cause undesirable effects. There are conflicting reports about oxidative stress and cytotoxicity of gold nanoparticles (Jia et al., 2009; Renault et al., 2008; Tedesco et al., 2008; Tedesco et al., 2010a,b; Cho et al., 2009; Murphy et al., 2008). Crucial variables seem to be physical dimensions, surface chemistry, shape, method of synthesis, concentration and time of exposure (Tedesco et al., 2010a,b). In the present study, rats were injected with gold nanoparticles (20 nm)

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for three days and their effects on oxidative stress and antioxidant defense indices were investigated in different tissues.

MATERIALS AND METHODS

Animals

Male Wister rats weighing 150 to 200 g were obtained from the animal house of Pharmacy College of King Saud University, Riyadh. After one week of acclimatization period, the rats were injected with 20 µg/kg body weight of 20 nm AuNP for three days through the intraperitoneal route (ip). This route of administration of AuNPs was chosen, because it has been reported to be more toxic than other routes even in small doses (Sabella et al., 2011).

Rats were killed by carbon dioxide asphyxiation 24 h after the last dose of AuNP injection. Ethical animal care guidelines were followed.

Chemicals

All the chemicals used were purchased from Sigma Chemical Company, St Louis, MO, USA. Double distilled water was used throughout the study.

AuNPs

AuNPs of 20 nm purchased (Product MKN-Au-020) in aqueous solution of 0.01% concentration of gold; Canada were used in this study.

Preparation of sample

The rats were killed by carbon dioxide asphyxiation 24 h after the last dose of AuNP injection. The tissues were dissected out, washed in ice cold saline and homogenized in saline (10% weight/volume) at 4°C. The homogenates were centrifuged at 3000 rpm for 10 min in a cooling centrifuge. This supernatant was divided into two parts, namely, A and B. Fraction A was used for immediate determination of lipid peroxidation, reduced glutathione and ascorbic acid. Fraction A was further centrifuged at 6000 rpm for 10 min in a cooling centrifuge. The supernatant constituted fraction B. This was used for estimation of catalase activity.

Lipid peroxidation

Lipid peroxidation was determined by the method of Utley et al. (1967). One milliliter of homogenate was incubated in metabolic shaker at 37°C for 1 h. 1.5 ml of 20% trichloroacetic acid (TCA) was added to it and centrifuged at 600 g for 10 min. To 1 ml of supernatant was added fresh 1 ml of thiobarbituric-acid (TBA, 0.67%). The reaction was kept in water bath for 10 min. On cooling, absorbance was read at 535 using a reagent blank.

Values were expressed as nmoles of thiobarbituric acid reactive substances (TBARS) formed hour⁻¹mg protein⁻¹.

Reduced glutathione

Reduced glutathione was estimated by the method of Beutler et al. (1963). 0.4 ml of homogenate was mixed with 3.6 ml of double

distilled water and treated with 0.6 ml of precipitation reagent (containing 1.67 g of glacial metaphosphoric acid, 0.2 g of ethylenediaminetetraacetic acid (EDTA) and 30.0 g of NaCl and made up to 100 ml with double distilled water). The reaction mixture was centrifuged at 600 g for 10 min. To 0.3 ml of supernatant was added 2.00 ml of Na₂HPO₄ (0.3 M), 0.25 ml of 5,5'-dithio-bis-2-nitrobenzoic acid (0.4% in 1% sodium citrate) and volume made up to 3.0 ml with double distilled water. Absorbance was read at 412 nm against blank. The amount of ascorbic acid was calculated from the standard graph. Values were expressed as µg of reduced glutathione µg protein⁻¹.

Ascorbic acid

Ascorbic acid was determined by the method of Jagota and Dani (1982). 0.2 ml of homogenate was treated with 0.8 ml of 10% TCA. After vigorous shaking, tubes were kept in ice cold bath for 5 min and centrifuged at 1200 g for 5 min. 0.2 to 0.5 ml of the supernatant were diluted to 2 ml with distilled water and 0.2 ml of Folin reagent (0.2 M) was added in it. After 10 min, the absorbance was read at 760 nm against a reagent blank. The amount of ascorbic acid was calculated from the standard graph. Values were expressed as µg of ascorbic acid µg protein⁻¹.

Catalase

Catalase activity was estimated in the whole homogenate by the method of Aebie (1984). The reaction mixture in a total volume of 3 ml contained 1.5 ml 0.2 M sodium phosphate buffer pH 7.2, 1.2 ml of H₂O₂ and suitably diluted enzyme. The reaction was started by adding H₂O₂ and reading the change in absorbance at 240 nm for 2 min. Values were expressed as millimoles of H₂O₂ consumed min⁻¹ mg protein⁻¹.

Protein

The protein content in the sample was measured by the modified method of Markwell et al. (1978) using bovine serum albumin as the standard. The amount of protein was calculated from the standard graph.

Statistical analysis

Each sample was run in duplicate. Values were expressed as mean ± standard deviation (SD) tissue, for n = 5 to 6 rats. Values between groups were compared using Dunnet's comparison tests. Values were considered significant if P < 0.05. Statistical analysis was performed by means of In-Stat package for personal computers version 5 (GraphPad Software, Inc., San Diego, USA).

RESULTS AND DISCUSSION

Nanotechnology involves the study of the control of matter on atomic and molecular scales. Nanotechnology is being applied in diverse fields, including extensions of conventional device physics, new approaches based upon molecular self-assembly, the development of novel materials with dimensions on the nanoscale, and even the direct control of matter on the atomic scale. The

Figure 1. The effect of injected AuNPs on lipid peroxidation in different tissues of rats. Values are expressed as $\pm$ SD nmols of thiobarbituric-acid reactive substances formed/h/mg protein. * $P < 0.05$; NS: Non significant.

application of nanotechnology in biology (nanobiotechnology) encompasses development of nanomaterials for delivering and monitoring biologically active molecules, disease staging, therapeutical planning, surgical guidance, neuro-electronic interfaces, and electronic biosensors (Huang et al., 2010). Nanoparticles possess better tissue penetration and higher biological potency than coarse (2.5 to 10 μ m) and fine (<2.5 μ m) particles due to their small sizes and large reactive surfaces (Huang et al., 2010). However, it is also essential to understand the adverse effects of nanoparticles on the biological system. Oxidative stress is a causative factor for many diseases and underlying pathologies. In this study, AuNP were found to induce significant oxidative stress in the liver of rats. Oxidative stress plays important roles in cellular signaling, inflammatory, and genotoxic and proliferative responses (Schins, 2003; Knaapen et al., 2004; van Maanen et al., 1999; Driscoll et al., 1997; Zhong et al., 1997; Leanderson and Tagesson, 1992; Borm et al., 2004).

Figure 1 shows the effect of injected AuNP on lipid peroxidation in different tissues in rats. The size and dose of the nanoparticles used caused a significant increase in lipid peroxidation of 95% ($P < 0.05$) in liver when compared with the liver of the control group of rats. In the other organs studied, namely, lungs, kidneys, spleen and heart, there was no significant change in lipid peroxidation when compared with the control group of rats ($P > 0.05$). Nanoparticles have the potential to

interact with the biological system and cause undesirable effects. One of these damaging effects could be the disturbance in the natural balance between oxidative stress and antioxidant defense indices which in turn can lead to various pathologies. Oxidative stress has been identified as a likely mechanism of nanoparticle toxicity (Li et al., 2008). Since liver is an important organ for storage of iron, it may be susceptible to lipid peroxidation than other tissues. AuNPs are taken up by the Kupffer cells of the liver and their bioaccumulation is regulated by the reticuloendothelial system.

To cope with elevated oxidative stress, cells mount protective or injurious responses. For instance, cells activate enzymatic and non-enzymatic antioxidant defense mechanisms like glutathione peroxidases, catalases, superoxide dismutases, etc (Huang et al., 2010). Figure 2 shows the effect of injected AuNPs on reduced glutathione concentration in various tissues in rats. The AuNPs caused a significant increase in reduced glutathione on liver, lungs and kidneys. There was however no significant change in spleen and heart ($P > 0.05$). The increase in reduced glutathione was the highest in kidneys (1130%; $P < 0.001$), followed by lungs (118%; $P < 0.05$) and liver which was 65% ($P < 0.05$) when compared with the control group. The changes in reduced glutathione levels in the other tissues like spleen and heart was not significant ($P > 0.05$). Glutathione is one of the primary cellular antioxidant defenses against oxidative stress. Glutathione is a tripeptide

Figure 2. The effect of injected AuNPs on reduced glutathione concentration in different tissues in rats. Values were expressed as $\pm$ SD μ g of reduced glutathione μ g protein⁻¹. *P < 0.05; ***P < 0.001; NS: Non significant.

(γ -glutamylcysteinylglycine present intracellularly in millimolar concentrations. The cysteine amino acid in glutathione can function as a thiol reducing agent, thus buffering cellular oxidants. Glutathione homeostasis is predominantly regulated by a complex cycle of synthesis and catabolism that occurs in the liver, lung, and kidney. Under physiological conditions, glutathione reductase rapidly reduces any oxidized glutathione (GSSG) to its thiol form (GSH), so that under normal conditions more than 98% of intracellular glutathione is GSH (Deleve and Kaplowitz, 1991). In the present study, nanoparticle treatment caused a significant increase in reduced glutathione which may be due to increase in lipid peroxidation in the liver and a preventive measure in lungs and kidneys. Although there are multiple potential detoxification mechanisms that affect the efficacy of chemotherapeutic drugs and confer drug resistance to targeted cells (Du et al., 2009), GSH has a prominent role in resistance to chemotherapy (Chen et al., 1998). GSH and its associated enzymes play a critical role in the cell susceptibility to the cytotoxic effect of alkylating agents, doxorubicin, cisplatin and nitrosoureas (Tew, 1994). It has been shown that for these drugs, increased cellular GSH levels can confer cells resistance and decreased cellular GSH levels can sensitize cells to the killing effects (Chiba et al., 1996; Yang et al., 2000).

Figure 3 shows the effect of injected AuNP on ascorbic acid content in various rat tissues. Injection of AuNP caused an increase of 142% (P < 0.05) in kidneys, 111% (P < 0.001) in liver and 91% (P < 0.001) in spleen when compared with the control rats. There was however no significant change in the ascorbic acid content in heart

and lungs (P > 0.05). Ascorbic acid is a well known antioxidant. The antioxidant function of vitamin C is to neutralize the free radicals. In liver, the increase in vitamin C may be a response to lipid peroxidation. Alternatively, the higher increase in vitamin C levels in other tissues like lungs, kidneys and spleen might have prevented significant increase in lipid peroxidation in these tissues.

Figure 4 shows the effect of injected AuNPs on catalase activity in various rat tissues. The results show that the AuNPs caused no significant change (P > 0.05) in catalase activity in any of the tissues studied. Catalase is the enzyme responsible for dissipation of hydrogen peroxide formed during oxidative stress. Catalase has been reported to be expressed in relatively low constitutive level in cardiomyocytes (Huang et al., 2010). In the present study, AuNPs injection in rats caused no significant change in the catalase activity in all the tissues studied. Reactive oxygen species (ROS) including H₂O₂ have dual role in the living system. They can oxidize cell components and lead to inactivation of certain enzymes as well as ROS are known to be involved in oxygen sensing and signal transduction as second messengers (Bagnyukova et al., 2005). Since catalase was not significantly altered by nanoparticle treatment, it might be suggested that H₂O₂ might not have been generated in significant amount by the AuNPs to elicit an alteration in the catalase activity.

Therefore, the concentration and/or the type of AuNP in this study may not be toxic to all the organs in rats. These results are in agreement with the study of Shukla et al. (2005) indicating their potential for application in

Figure 3. The effect of injected AuNPs on ascorbic acid concentration in different tissues in rats. Values were expressed as $\pm$ SD μ g of ascorbic acid μ g protein⁻¹. ***P < 0.001; NS: Non significant.

Figure 4. The effect of injected AuNPs on catalase activity in different tissues in rats. Values were expressed as $\pm$ SD millimoles of H₂O₂ consumed min⁻¹ mg protein⁻¹. NS: Non significant.

nanoimmunology, nanomedicine, and nanobiotechnology.

Conclusion

The low doses of AuNPs used in this study caused a significant change in the oxidative stress and antioxidant defense indices only in the liver. Therefore, they offer

potential to be exploited for various *in vivo* uses.

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