Size effect of gold nanoparticles on various trace elements levels in different tissues of rats

Mohamed Anwar K. Abdelhalim1*, N. J. Siddiqi2, A. S. Alhomida2 and M. S. AlAyed1

1Department of Physics and Astronomy, College of Science, King Saud University, Saudi Arabia.
2Department of Biochemistry, College of Science, King Saud University, Saudi Arabia.

Accepted 16 September, 2011

Despite the many benefits of nanotechnology, some studies have indicated that nanoparticles (NPs) may cause adverse effects, which may be attributed to size, shape, particles number, surface area and unique properties. Very little information exists on gold nanoparticles (GNPs) toxicity in vivo and other biochemical effects. The aim of the present study was to elucidate the size effect of GNPs on various trace elements levels in several tissues of rats. The experimental rats were divided into control and 3 groups (G1A, G2A and G3A; G1: 20 nm; G2: 10 nm; G3: 50 nm; A: infusion of 0.05 ml of GNPs for 3 days). To investigate the size effect of 10, 20 and 50 nm GNPs on Calcium (Ca), Copper (Cu), Iron (Fe) and Zinc (Zn) elements in several tissues of rats, 0.05 ml dose of GNPs was intraperitoneally injected into rats for period of 3 days to identify the toxicity and tissue distribution of various trace elements levels in vivo. All the sizes of GNPs used in this study caused a significant decrease in Ca and Zn concentrations in liver, lung, heart, kidney tissues and blood compared with the control. The 10 and 20 nm GNPs caused a significant increase in Cu concentration in liver, lung, heart, kidney and blood compared with the control. The 50 nm GNPs caused a significant increase in Cu concentration in liver, lung, heart and kidney tissues compared with the control while 50 nm GNPs did not cause significant change in blood Cu concentration compared with the control. The 10 and 20 nm GNPs caused a significant increase in Fe concentration in liver, lung, heart and kidney tissues of rats. The 50 nm GNPs caused a significant increase in Fe concentrations in liver, heart, kidney tissues and blood of rats while it decreased in lung tissue compared with the control. 10 and 20 nm GNPs may be an effective inducer of oxidative stress which was evident by the fact that they caused a decrease in the cellular Ca and Zn concentrations coupled with the increase in the concentrations of Fe and Cu.

Key words: Gold nanoparticles, size, several tissues, trace elements, rats.

INTRODUCTION

Advances in nanotechnology have identified promising candidates for many biological and biomedical applications. Since the properties of NPs differ from that of their bulk materials, they are being increasingly exploited for medical uses and other industrial applications.

In particular, GNPs have been used as Raman sensors (Qian et al., 2008), photocatalysts (Costi et al., 2008), and photo-electrochemical materials for their unique optical properties arising from the surface plasmon oscillation of free electrons (Huang et al., 2007). In addition, GNPs are used as localized photothermal agents mediating tumor cell necrosis from hyperthermia after irradiation with laser light (O’Neal et al., 2004) and have also been useful as biosensors (Nam et al., 2003) and carriers for the delivery of drugs and genes (Gibson et al., 2007).

Despite the huge potential benefit of GNPs in the field of biomedical and industrial applications, very little

*Corresponding author. E-mail: abdelhalimmak@yahoo.com or mabdulhleem@ksu.edu.sa.
information exists on their in vivo toxicity and other biochemical effects. Although some scientists (Shukla et al., 2005) consider NPs as nontoxic, other studies reporting the toxic effects of NPs (Chithrani, and Chan, 2007; Pan et al., 2007). The present study was carried out to study the effect of intraperitoneal administration of GNPs for period of 3 days on the levels of different trace elements in several tissues in rats.

MATERIALS AND METHODS

Animals

Healthy, male Wistar-Kyoto rats were obtained from the Laboratory Animal Center (College of Pharmacy, King Saud University). 8–12 weeks old (approximately 250 g body weight) were housed in pairs in humidity and temperature-controlled ventilated cages on a 12 h day/night cycle. A rodent diet and water were provided. In this study, fifty rats were individually caged, and divided into control group (NG; n = 8), group 1 (G1A: infusion of GNPs of size 20 nm for 3 days; n = 6), group 2 (G2A: infusion of GNPs of size 10 nm for 3 days; n = 6) and group 3 (G3A: infusion of GNPs of size 50 nm for 3 days; n = 6). All experiments were conducted in accordance with the guidelines approved by King Saud University Local Animal Care and Use Committee.

Gold nanoparticles administration

Dose of 50 μl of 10, 20 and 50 nm GNPs in aqueous solutions were intraperitoneally administrated to the animals for a period of 3 days. The rats were anesthetized by inhalation of 5% isoflurane until muscular tonus relaxed. Blood and several tissues (liver, heart, lung and kidney) were collected from each rat.

Digestion of rat tissues

Several rat tissues were wet digested with nitric acid and converted into acidic digest solutions for analysis by atomic absorption spectroscopy (AAS). The tissue was freeze dried in order to minimize loss of analytes and to facilitate subsequent sample preparation steps, and then homogenized to a fine powder by ball-milling in plastic containers. Approximately 0.20 to 0.25 g of powdered tissue was weighed into a Teflon reaction vessel and 3 ml of HNO₃ were added. The closed reaction vessel was heated in a 130°C oven until digestion was completed. Samples were then diluted to a final volume of 20 ml with quartz distilled water and stored in 1 oz. polyethylene bottles for the analysis by AAS.

Atomic absorption spectroscopy measurements

AAS determines the presence and concentration of trace elements (Ca, Fe, Cu and Zn) levels in different tissues of rats. The trace elements absorbed ultraviolet (UV) light when they were excited by heat. The AAS instrument looks for a particular metal by focusing a beam of UV light at a specific wavelength through a flame and into a detector. The sample of interest was aspirated into the flame. If that metal is present in the sample, it will absorb some of the light, thus reducing its intensity. The instrument measures the change in intensity. A computer data system converted the change in intensity into an absorbance. As concentration goes up, absorbance also goes up. A calibration curve was constructed by running standards of various concentrations (10, 15 and 20 PPM) on the AAS and observing the corresponding absorbance. A calibration curve was made and then samples were tested and measured against this curve. AAS measurements were carried out at the Research Center for Girls, King Saud University. Ca, Fe, Cu and Zn were measured using a Specter AA-220 series double-beam digital atomic absorption spectrophotometer. The concentration of trace elements in each tissue sample was calculated by comparing the absorbance produced by the sample with that produced by a series of standards as follows:

\[
\text{Concentration of trace element in sample} = \frac{[(\text{Absorbance of Sample/ Absorbance of Standard}) \times (\text{Conc. of Standard})] }{100}
\]

RESULTS AND DISCUSSION

Figures 1, 2, 3 and 4 show the effect of intraperitoneal administration of different GNPs sizes on Ca, Zn, Cu and Fe concentrations on various tissues in rats, respectively. Results show that all the sizes of GNPs used in this study caused a significant decrease in Ca and Zn concentrations in liver, lung, heart, kidney tissues and blood compared with the control (Figures 1 and 2). The 10 and 20 nm GNPs caused a significant increase in Cu concentrations in liver, lung, heart, kidney and blood compared with the control (Figures 3). The 50 nm GNPs caused a significant increase in Cu concentrations in liver, lung, heart and kidney tissues compared with the control while the 50 nm GNPs did not cause significant change in blood Cu concentration compared with the control (Figure 3).

The 10 and 20 nm GNPs caused a significant increase in Fe concentration in liver, lung, heart and kidney tissues of rats (Figure 4). The 50 nm GNPs caused a significant increase in Fe concentrations in liver, heart, kidney tissues and blood of rats while it decreased in lung tissue compared with the control (Figure 4).

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 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).

In the present study all the sizes of GNPs used caused a significant decrease in Ca and Zn concentrations in liver, lung, heart, kidney tissues and blood compared with the control. Calcium is the single most abundant element in the body. The human body contains about 1 kg of calcium most of which is sequestered in the bone. However a small amount of calcium is present in the extracellular and intracellular fluids. Intracellular calcium concentration is generally several orders of magnitude lower than extracellular calcium concentration due to the
Figure 1. The effect of intraperitoneal administration of different sizes of GNPs on Ca concentration in various tissues of rats.

Figure 2. The effect of intraperitoneal administration of different sizes of GNPs on Zn concentration in various tissues of rats.
Figure 3. The effect of intraperitoneal administration of different sizes of GNPs on Cu concentration in various tissues of rats.

Figure 4. The effect of intraperitoneal administration of different sizes of GNPs on Fe concentration in various tissues of rats.
activity of specific membrane protein pumps, although the concentrations inside specific intracellular compartments can themselves differ quite markedly (Baird, 2011).

Systemic calcium homeostasis is critical to the survival of multicellular organisms, and complex, inter-dependent regulatory systems have evolved to maintain \( \text{Ca}^{2+} \) in the extracellular fluid within a narrow range (1.1–1.4 mM \( \text{Ca}^{2+} \) for humans) (Hurwitz, 1996). \( \text{Ca} \) is a ubiquitous cation, yet it is highly regulated. Maintaining near constancy of the extracellular ionized calcium concentration is critical because of calcium’s numerous intra- and extracellular roles in vital bodily processes, such as neuromuscular activity, clotting of the blood and skeletal integrity.

This tight regulation of the serum calcium concentration is achieved through a delicate interplay between PTH, calcitonin and vitamin D acting on their target tissues, especially kidney, bone and intestine. Any dysregulation of calcium regulation is associated with pathological changes (Chattopadhyay and Brown, 2006). Therefore decrease of calcium levels in the various tissues and blood of rats compared with the control would lead to impairment of the essential functions like blood clotting, muscle contraction, etc. In this study GNPs also caused a decrease in calcium concentration in the kidney tissue compared with the control which could in turn affect calcium homeostasis in other tissues. This is due to the fact that the kidney tissue plays key roles in \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \) metabolism by adjusting the tubular reabsorption of these divalent cations from the glomerular filtrate (Chattopadhyay and Brown, 2006).

Zinc is an element present in many enzymes that are involved in different cellular metabolism. Though zinc is redox inert it functions as an antioxidant in the biological system. Therefore a decrease in the zinc concentration would result in an impairment of antioxidant system resulting in an increased susceptibility to oxidative stress. This is further supported by an increase in cellular concentration of iron and copper which participate in oxidative stress.

Several studies have shown that Zn reduced oxidative damage and the risk of cardiovascular disease. Scientists have suggested that because Zn supplementation both reduced the formation of atheromas and lowered lipid peroxidation it may have antioxidant activity. Since Zn is not redox active, it may not act directly as a scavenging antioxidant but instead may act as an indirect antioxidant by competing with pro-oxidant metals such as Fe and Cu for strategic binding sites (Watt et al., 2006; Beattie and Kwun, 2004; Ren et al., 2005).

Cu may increase superoxide dismutase (SOD) expression, thereby reducing the interaction of nitric oxide (NO) with superoxide, and hence potentiating NO-mediated pathways that may protect against atherosclerosis (Alissa et al., 2004).

Fe may participate in diverse pathological processes by catalyzing the formation of reactive oxygen free radicals. The oxidation of LDL and lipid is believed to be one of the crucial events leading to plaque formation in vasculature. It has been hypothesized that iron-mediated oxidation is involved in this process.

Several epidemiological studies have shown that the level of body Fe stores is positively correlated with the incidence of coronary heart disease in human populations. However 50 nm GNPs caused no significant change in blood Cu concentration. The 50 nm GNPs on the other hand caused a decrease in Fe concentration in blood the reason for which is not known. This may be due to the fact that 50 nm GNPs is not an effective inducer of oxidative stress when compared to the smaller sizes of GNPs.

**Conclusions**

The aim of the present study was to elucidate the size effect of GNPs on the various trace elements levels in several tissues of rat. In this study, fifty rats were used and divided into NG, G1A, G2A and G3A. The results of this study can be summarized as follows:

1. All the sizes of GNPs used in this study caused a significant decrease in Ca and Zn concentrations in liver, lung, heart, kidney tissues and blood compared with the control.
2. The 10 and 20 nm GNPs caused a significant increase in Cu concentration in liver, lung, heart and kidney blood compared with the control. The 50 nm GNPs caused a significant increase in Cu concentration in liver, lung, heart and kidney tissues compared with the control while 50 nm GNPs did not cause significant change in blood Cu concentration compared with the control.
3. The 10 and 20 nm GNPs caused a significant increase in Fe concentration in liver, lung, heart and kidney tissues of rats. The 50 nm GNPs caused a significant increase in Fe concentrations in liver, heart, kidney tissues and blood of rats while it decreased in lung tissue compared with the control.
4. The 10 and 20 nm GNPs may be an effective inducer of oxidative stress which was evident by the fact that they caused a decrease in the cellular Ca and Zn concentrations coupled with the increase in the concentrations of Fe and Cu.

This study suggests that extensive further studies in several tissues of rats are needed taken into consideration the role of exposure duration and dose effects in vivo applications.

**ACKNOWLEDGEMENTS**

The authors are very grateful to National Plan of Science and Technology (NPST). This research was financially supported by the National Science and Technology Innovation Plan (NSTIP), Research No. 08-ADV206-02.
REFERENCES


