Absorption optical density as a diagnostic tool for indicating the toxicity of gold nanoparticles

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Nanotechnology has recently emerged as a promising approach for treatment and diagnosis of a variety of diseases. The aim of this study was to evaluate the blood absorption spectra to assess bioaccumulation and toxic effects of 100 µl of 10 and 50 nm gold nanoparticles (GNPs) upon intraperitoneal administration in rats for periods of three and seven days. Healthy 30 male Wistar-Kyoto rats were individually caged and divided into control group (NG: n = 10), group 1 (A: infusion of 10 nm GNPs for three days; n = 5; B: infusion of 10 nm GNPs for seven days; n = 5) and group 2 (A: infusion of 50 nm GNPs for three days; n = 5; B: infusion of 50 nm GNPs for seven days; n = 5). Dose of 100 µl of 10 and 50 nm GNPs were administered intraperitoneally to the animals. The blood absorbance peaks for G1A, G1B, G2A and G2B significantly decreased compared with the control. The blood absorbance peaks for G1A significantly decreased compared with G1B; and G2A significantly decreased compared with G2B. This implies that G1A is highly reactive than G1B, and G2A is highly reactive than G2B. A significant decrease (p<0.05) in all blood absorbance peaks observed by the administration of 10 and 50 nm GNPs for periods of three and seven days compared with the control. For the same GNP sizes, the accumulation of GNPs in the blood after the administration of GNPs for three days was greater than that for seven days. In addition to non-significant blood absorbance peak differences were observed with the different GNP sizes for the same period of administration of GNPs. This study suggests that the 10 nm GNPs are mostly uptaken and accumulated by tissues which support the toxic effects of the smaller GNPs by tissues. However, the 50 nm GNPs are and highly accumulated in blood, suggesting the toxic effects of the larger GNPs by blood. Thus, the absorption optical density can be considered as a diagnostic tool indicating the bioaccumulation and toxicity effects of GNPs in the tissues of rats.

Key words: Gold nanoparticles, UV-visible spectroscopy, toxicity, histology.

INTRODUCTION

There has recently been a great deal of interest in the scientific community concerning the application of nanotechnology in medicine. One particular exciting field of research involves the use of GNPs in the detection and treatment of cancer cells. Current methods of cancer diagnosis and treatment are costly and can be very harmful to the body.

GNPs, however, offer an inexpensive route to targeting only cancerous cells, leaving healthy cells untouched (Caruthers et al., 2007). GNPs of small sizes could get close to a biological target. Metallic nanoparticles (NPs) can be made to resonantly respond to a time-varying magnetic field with the transfer of energy to the particles...
bioaccumulation of the smaller GNPs by tissues of rats absorption spectra as an important diagnostic tool for Shim and Gupta, 2007). It has been reported that GNPS is particle size-dependent organ distributed in vivo (Kamat, 2002; Shipway et al., 2000; Kuwahara et al., 2001; Mirkin et al., 1996; Huber et al., 2004). The orally administrated GNPs appeared in various tissues in mice and the amount of absorption and distribution in the body inversely correlated with the size of the particles (Lasagna-Reeves et al., 2010). NPs of small sizes induce physical and chemical properties that are very different from those of the same material in the bulk form, indicating a large surface to volume ratio, enhanced or hindered particle aggregation (depending on the type of surface modification), enhanced photoemission, high electrical and heat conductivity and improved surface catalytic activity (Kogan et al., 2007; El-Sayed et al., 2006; Zharov et al., 2006; Hirsch et al., 2003).

The most widely used method for characterizing the optical properties and electronic structure of NPs are related to the diameter and aspect ratio of metal NPs. Solutions of colloidal GNPs have a distinctive red colour, which arises from their tiny dimensions. At nanometer dimensions, the electron cloud can oscillate on the particle surface and absorb electromagnetic radiation at a particular energy. The changes in the UV-Visible spectra of the resultant colloids were measured to study the size effect of metal nanoparticles on the surface plasmon resonance. It is known that GNPs exhibit a strong surface plasmon band, which have been widely investigated, but studies on the photoluminescence from GNPs are scarce (Shim and Gupta, 2007).

Currently, there is no data available regarding the absorption spectra as an important diagnostic tool for bioaccumulation of the smaller GNPs by tissues of rats in vivo after repeated administration. In an attempt to cover and understand the bioaccumulation, toxicity and their potential therapeutic and diagnostic use, the absorption spectra of 100 µl/day dose (10 and 50 nm GNPs upon intraperitoneal administration in rats for three and seven days) were evaluated.

MATERIALS AND METHODS

GNPs size

This study was conducted in College of Science, King Saud University, Saudi Arabia in 2011. The 10 and 50 nm GNPs (M K Impex Corp; Divn MK nano, CANADA) was dissolved in aqueous solution of 0.01% concentration. The mean sizes and morphology were calculated from the images taken by the transmission electron microscope (TEM) in addition to assess the high electron densities of GNPs as well as the homogeneity of the particles shape and size. 30 healthy male Wistar-Kyoto rats were obtained from the Laboratory Animal Center (College of Pharmacy, King Saud University). Eight to 12 weeks old rats (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, 30 rats were individually caged and divided into the control group (NG: n = 10), group 1 (A: infusion of 10 nm GNPs for three days; n = 5; B: infusion of 10 nm GNPs for seven days; n = 5) and group 2 (A: infusion of 50 nm GNPs for three days; n = 5; B: infusion of 50 nm GNPs for seven days; n = 5). Dose of 100 µl of 10 and 50 nm GNPs dissolved in aqueous solution were administered intraperitoneal to the animals. The rats were anesthetized by inhalation of 5% isoflurane until muscular tonus was relaxed. All the experiments were conducted in accordance with the guidelines approved by King Saud University Local Animal Care and Use Committee.

UV-visible spectroscopy (absorption spectra measurement)

The blood absorption spectra of rats infused with 10 and 50 nm GNPs for three and seven days were measured by UV-VIS double beam spectrophotometer (type UV-1601 PC, Shimadzu, Japan; H14 granting UV) through shortwave NIR with optical resolution of 0.4 nm. The absorbance measurements were made over the wavelength range of 200 to 800 nm at room temperature using 1 cm path length quartz cuvettes. The cuvettes were cleaned before each use by sonicating them for 5 min in deionized water and then rinsing with deionized water. The pH values for the different GNP samples were constant during the measurements.

Statistical analysis

To assess the significance differences between the control group and the other two groups (G1: 10 nm and G2: 50 nm; A: infusion of 100 µl GNPs for 3 days; B: infusion of 100 µl GNPs for 7 days), the statistical analysis was performed using one-way analysis of variance (ANOVA) for repeated measurements with significance assessed at 5% confidence level.

RESULTS AND DISCUSSION

UV-visible spectroscopy

Figures 1 to 5 show five peaks for the blood which zcharacterizes the hemoglobin (Hb) macromolecule. The 1st peak observed at 280 nm corresponds to the aromatic amino acids, the 2nd peak observed at 340 nm corresponds to globin-heme interaction, the 3rd peak observed at 420 nm corresponds to the heme, the 4th peak observed at 540 nm corresponds to the heme-heme interaction and the 5th peak observed at 578 nm corresponds to the hemoglobin oxygen affinity. These reviews (Dacie and Lewis, 1991; Khalifa, 1992) are in support of our results.

After the administration of 10 nm GNPs for three and seven days, blood absorbance peaks for G1A and G1B were significantly decreased and shifted towards the UV wavelength compared with the control as shown in Figures 1 and 2 indicating that the decrease in absorbance may be attributed to changes in aliphatic and aromatic amino acids, globin-heme and heme-heme interactions.
Figure 1. UV-visible absorption spectra for the blood of rats after administration of 10 nm GNPs for three and seven days.

Figure 2. UV-visible absorption spectra for the blood of rats after administration of 50 nm GNPs for three and seven days.
Figure 3. UV-visible absorption spectra for the blood of rats after administration of 10 and 50 nm GNPs for three days.

Figure 4. UV-visible absorption spectra for the blood of rats after administration of 10 and 50 nm GNPs for seven days.
interaction bands associated with the accumulation of GNPs in the blood. The blood absorbance peaks for G1A significantly decreased compared with G1B as shown in Figure 1. The blood absorbance peaks for significantly decreased compared with G2B shown in Figure 2. This implies that G1A is highly reactive than G1B, and G2A is highly reactive than G2B. These results demonstrate that for the same GNPs size, the accumulation of GNPs in the blood after the administration of GNPs for three days was greater than that for seven days; for the same GNPs size, the decrease in the blood absorbance peaks were highly administration of GNPs period dependent rather than GNPs size. Moreover, the administration of 10 and 50 nm GNPs induced significant decrease in blood absorbance peaks.

The conformational substrates of Hb molecule appeared from its normal absorption spectrum, indicating the stabilization optical density of Hb as a macro-molecule. Thus, any change in the characteristic absorption spectrum of Hb reflects the changes in the spin state of iron heme. Absorbance of this spin state band gives a clear report about heme-heme interaction and consequently its affinity to O\(_2\) and its delivery to tissue (Dacie and Lewis, 1991; Khalifa, 1992). Proteins are dynamic systems and their motions are essential to their function. A decrease in the absorbance at 280 nm, as an indication of this abnormal motion, reflects its deviation from a normal structure and function, depending on the degree of globin unfolding and random motion of the Hb molecule under the different degrees of oxidative stress. Elevation in the half soret band width and shifting towards shorter wavelength indicate the stretching of iron and nitrogen bonds in prophyrin ring and the imbalance between protein and heme in the Hb molecule (Khalifa, 1992). Figure 3 shows the UV-visible absorption spectra for the blood of rats after administration of 10 and 50 nm GNPs for three days. Figure 4 shows UV-visible absorption spectra for the blood of rats after administration of 10 and 50 nm GNPs for seven days. A significant decrease (p<0.05) in all blood absorbance peaks was observed by the administration of 10 and 50 nm GNPs for periods of three or seven days compared with the control as shown in Figures 3 and 4. Figure 3 indicates that very low non significance differences were observed for G1A and G2A, while Figure 4 indicates that very low non significance differences were observed for G2A and G2B. These results demonstrate a significant decrease in blood absorbance peaks with the administration of 10 and 50 nm GNPs in addition to no significant differences for the accumulation of GNPs in the blood after the administration of 10 and 50 nm GNPs for three or seven days.

Figure 5 shows UV-visible absorption spectra for the blood of rats after the administration of 10 and 50 nm GNPs for three and seven days. A significant decrease (p<0.05) in all blood absorbance peaks was observed by the administration of 10 and 50 nm GNPs for periods of three or seven days compared with the control, in addition to non-significant blood absorbance peak differences were observed with the different GNP sizes for the same period of administration of GNPs.
This study demonstrates that the absorption spectra of GNPs are highly GNPs size dependent rather than administration period and accompanied with the high accumulation of 50 nm GNPs in the blood after three days.

The marked decrease in absorbance may be attributed to the high accumulation of GNPs in the blood, suggesting that the toxic effects of the larger GNPs may be induced in the blood. However, the toxic effects of the smaller GNPs may be induced in the tissues.

It has been reported that increased uptake into certain tissues may lead to accumulation, where they may interfere with critical biological functions. The rate of exocytosis of GNPs was size dependent with more accumulation of larger GNPs in the cell. Absorbed NPs within the systemic circulation can be excreted through various routes. A possible elimination route for NPs could be renal and biliary clearance. Renal clearance of solid nano-sized materials was affected by particle size and surface charge (Ramanujam et al., 1999; Liu et al., 2007).

This study demonstrates that the physical and chemical properties as well as the applications of NPs are highly GNPs administration period dependent rather than particle size, number of NPs and shape dependent. The 10 nm GNPs may be taken up faster and more intensively than the other sizes by macrophages of the liver and disappear thereafter.

NPs for therapy need to have a long retention time for targeting and therapy. However, a long retention time can evoke the toxic effects in vivo. Thus, the clearance rate and route of nanomaterials is an important issue (Ramanujam et al., 1999; Kehoe et al., 1988).

Abdelhalim and Jarrar (2011) reported that hepatocytes exhibited cloudy swelling with pale cytoplasm and poorly delineated and displaced nuclei in all GNPs treated rats. This ballooning degeneration was more prominent with 100 µl dose than 50 µl and with 10 nm size particles than 50 nm larger ones (Abdelhalim and Jarrar, 2011). This swelling may occur as a result of disturbances of membranes function that lead to massive influx of water and Na+ due to GNPs effects. Cellular swelling might be accompanied by leakage of lysosomal hydrolytic enzymes that lead to cytoplasmic degeneration and macromolecular crowding (Lasagna-Reeves et al., 2010; Abdelhalim and Jarrar, 2011; Cho et al., 2009).

A previous study demonstrated that in all organs studied, there was a significant increase in gold levels after treatment, which was proportional to the dose administered. However, the levels of gold in blood did not increase in proportion to the dose, indicating that GNPs are mostly uptaken and accumulated by tissues (Cho et al., 2009). The accumulation of 12.5 nm GNPs in kidney could be explained by the bigger size of the particle with respect to the glomerular pores that measure 5.5 nm. So, it is unlikely that NPs can pass through the glomerular filtration due to its size and negative electrostatic potential (Longmire et al., 2008). In the spleen and liver, the bioaccumulation of GNPs may be regulated by the reticulo-endothelial system, which is part of the immune system involved in the uptake and metabolism of exogenous molecules and particles in these tissues. In addition, it is also known that NPs are taken up by kupffer cells in the liver and by macrophages in other places, regardless of the particle size (Sadauskas et al., 2007).

Conclusions

The blood absorbance peaks for G1A, G1B, G2A and G2B significantly decreased compared with the control. G1A is highly reactive than G1B, and G2A is highly reactive than G2B. A significant decrease in all blood absorbance peaks were observed by the administration of 10 and 50 nm GNPs for periods of three or seven days compared with the control. For the same GNPs size, the accumulation of GNPs in blood after the administration of GNPs for three days was greater than that for seven days. In addition to non-significant blood absorbance peak, differences were observed with the different GNPs sizes for the same period of administration of GNPs. This study demonstrates that the blood absorption spectra for the same size of GNPs are highly administration period dependent rather than particle size dependent. The 10 nm GNPs is mostly up taken and accumulated by tissues which support the toxic effects of the smaller GNPs by tissues. However, the 50 nm GNPs were mostly and highly accumulated in the blood, suggesting the toxic effects of the larger GNPs by blood. Thus, the absorption optical density can be considered as a diagnostic tool for investigating the bioaccumulation and toxicity effects of GNPs in the tissues of rats. Further additional histological experiments are required to document bioaccumulation and toxic effects of the smaller GNPs by tissues.

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