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Full Length Research Paper

The period effects of intraperitoneal administration of different gold nanoparticle sizes on kidney tissue of rat *in vivo* using fluorescence measurements

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Despite the many benefits of nanotechnology, some studies indicate that certain nanoparticles may cause adverse effects because of their small size and unique properties. The aim of the present study was to elucidate the period effects of intraperitoneal administration of different gold GNP sizes on the rat kidney tissue *in vivo* using fluorescence spectroscopy. The experimental rats were divided into control and six groups (G1A, G1B, G2A, G2B, G3A and G3B; G1: 20 nm; G2: 10 nm; G3: 50 nm; A: infusion of GNPs for 3 days; B: infusion of GNPs for 7 days). To investigate the period effects of GNPs 10, 20 and 50 nm on the kidney tissue of rats, 50 µl dose of GNPs (of concentration 0.1% Au) were intraperitonealy injected into rats for periods of 3 and 7 days to identify the toxicity and tissue distribution of GNPs *in vivo* using fluorescence measurements. GNPs of sizes 10 and 20 nm show spherical morphology with good particle size distribution dispersed in the solution while GNPs of size 50 nm have no spherical shape, but they have hexagonal shape. At the infusion period of 3 days, the fluorescence intensity of the 1st peaks increased for G1A and decreased for G2A and G3A compared with the control while the fluorescence intensity of the 2nd peaks decreased for G1A, G2A and G3A compared with the control. At the infusion period of 7 days, the fluorescence intensity of the 1st and 2nd peaks increased for G1B and G2B and sharply decreased for G3B compared with the control. At the infusion periods of 3 and 7 days, the fluorescence intensity of the 1st peaks increased for G1A, G1B and G2B and decreased for G2A, G3A and G3B compared with the control while the fluorescence intensity of the 2nd peaks decreased for G1A, G1B, G2A, G2B and G3A compared with the control. Fluorescence intensity of GNPs varied with the GNP size. The decrease in fluorescence intensity may be attributed to size, shape, number of GNPs, quenching of 50 nm GNPs, surface area of GNPs and slow clearance for GNPs of 10 and 20 nm through urine and bile from the kidney. Moreover, decreasing size may lead to an exponential increase in surface area relative to volume, thus making the GNPs surface more reactive on itself (aggregation) and to its surrounding environment (biological components). Size and shape of GNPs may be related to their useful characters and also plays a key role in toxicity. The alterations of accumulation in the kidney tissue, depending on GNP size, which may be mediated by dynamic protein binding and exchange. A better understanding of these mechanisms will improve drug delivery and period and dose estimation used in the risk assessment.

Key words: Gold nanoparticles, sizes, period effects, kidney tissue, fluorescence spectroscopy.

INTRODUCTION

Fluorescence has many practical applications, including mineralogy, gemology, chemical sensors, fluorescent labeling, dyes and biological detectors (Joseph et al., 2008). Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength. In most cases, emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation (Evans and Xie, 2008). However, when the absorbed electromagnetic radiation is intense, it is possible for one electron to absorb two photons; this two-photon absorption can lead to emission of radiation having a shorter wavelength than the absorbed radiation.

Size, surface area and number of particles appear to

play important roles in facilitating nano-sized particle-related toxicity. It has been proposed that the size of particles plays a key role in their adhesion to and interaction with the biological cells (Foster et al., 1997). The origin of the unique optical properties of GNPs is a phenomenon known as surface plasmon resonance (SPR). When an electromagnetic radiation of a wave-length much smaller than the diameter of the GNPs hits the particles, it induces coherent, resonant oscillations of the metal electrons across the NPs. These oscillations are known as the SPR, which lie within the visible frequencies and result in strong optical absorbance and scattering properties of the GNPs (Schmid, 1992; Jain et al., 2007). This property allows the use of GNPs for many applications, e.g., they can be used as Raman sensors (Tian et al., 2002), photocatalysts (Kamat, 2002), and photoelectrochemical materials (Shipway et al., 2000; Kuwahara et al., 2001). In the bioscience and medical fields, GNPs are used as immunostaining marker particles for electron microscopy, and as chromophores for immunoreactions and nucleic acid hybridization (Mirkin et al., 1996; Huber et al., 2004).

Numerous studies have showed that the exposures to nanoscale particles produce greater inflammatory and cytotoxic effects when compared to exposures to larger sized particles at equivalent mass concentration (Wang et al., 2007). It is considered that NPs can be more reactive with the biological components and have adverse effects due to large surface area and much particle number (Nel et al., 2006).

Toxicity has been thought to originate from nano-material size and surface area, composition, and shape. Size plays a role in how the body responds to, distributes, and eliminates materials (Kamat, 2002; Huber et al., 2004). Particle size can also affect the mode of endocytosis, cellular uptake, and the efficiency of particle processing in the endocytic pathway (Liu, 2006; Baptista et al., 2008).

GNPs can be used in various biomedical applications, however, very little is known about their size-dependent *in vivo* kinetics. Here we focus our attention on the aspects related to fluorescence spectroscopy for different GNP sizes dissolved in aqueous solution. Moreover, the particles size and shape were monitored by the transmission electron microscopy (TEM). Thus, the aim of the present study was to elucidate the period effects of intraperitoneal administration of different GNP sizes on the rat kidney tissue *in vivo* using fluorescence spectroscopy.

MATERIALS AND METHODS

Gold nanoparticles (GNPs)

Different GNP sizes of 10, 20 and 50 nm were purchased (Product MKN-Au-010, MKN-Au-020 and MKN-Au-050 in aqueous solution of concentration 0.01% of gold, Canada) and used in this study. The mean size and morphology with good particle size distribution for these GNPs were calculated from the images taken by the (TEM) in addition to the assessment of the high electron densities of GNPs as well as the homogeneity of the particles shape and size.

Animals

Healthy, male Wistar-Kyoto rats obtained from the Laboratory Animal Center (College of Pharmacy, King Saud University). 8 to 10 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. To study the infusion period effects of GNP size, fifty rats were individually caged, and divided into control group (NG: n = 8), group 1 (A: infusion of GNPs of size 20 nm for 3 days; n = 6 and B: infusion of GNPs of size 20 nm for 7 days; n = 6), group 2 (A: infusion of GNPs of size 10 nm for 3 days; n = 6 and B: infusion of GNPs of size 10 nm for 7 days; n = 6) and group 3 (A: infusion of GNPs of size 50 nm for 3 days; n = 6 and B: 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. The 10, 20 and 50 nm GNPs in aqueous solutions intraperitonealy administered to the animals. The rats were anesthetized by inhalation of 5% isoflurane until muscular tonus relaxed. Kidney tissues were collected from each rat.

Digestion of rat tissue samples

Kidney rat tissue samples were wet digested with nitric acid and converted into acidic digest solutions for the analysis by fluorescence spectroscopy. 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 HNO_3 were added. The closed reaction vessel was heated in 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 later fluorescence spectroscopy analysis.

Fluorescence spectroscopy

Fluorescence characterization of different GNP sizes 10, 20 and 50 nm GNPs) was performed using FluoroMax-2 (JOBIAN YVON-SPEX, Instruments S.A., Inc., France). Fluorescence measure-ments were made over the wavelength range of 250 to 700 nm. The fluorescence measurements were made using 1 cm path length quartz cuvettes which were cleaned before each use by sonicating them for 5 min in deionized water and then rinsing with deionized water.

RESULTS AND DISCUSSION

Size and morphology of different GNPs size

The GNPs sizes 10 and 20 nm show spherical morphology with good particle size distribution dispersed in the solution. The mean sizes for these GNPs were calculated from the images taken by the TEM. Mean size was 9.45 ± 1.33 nm for GNPs of size 10 nm and 20.18 ± 1.80 nm for GNPs of size 20 nm while GNPs of 50 nm

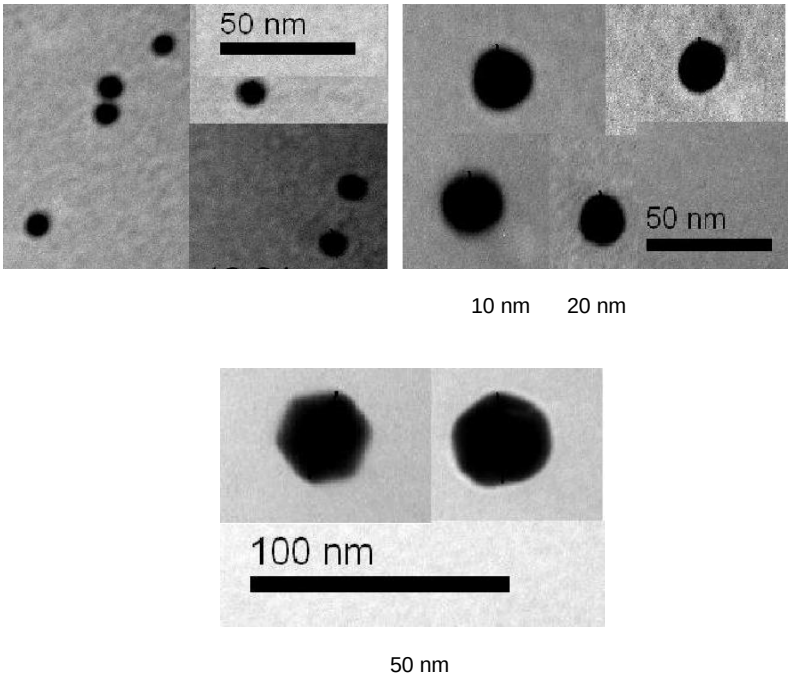

Figure 1. TEM images for different GNP samples

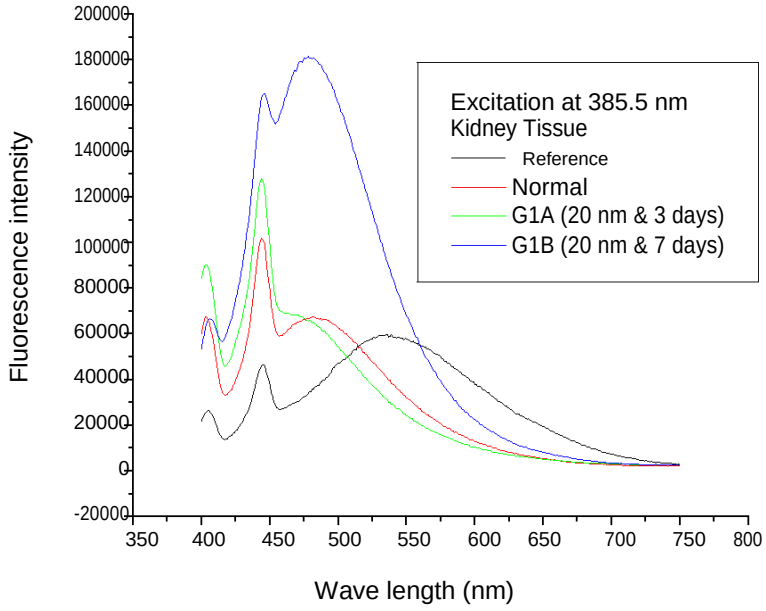

Figure 2. The emission fluorescence peak intensity after infusion periods of 3 and 7 days for GNP of size 20 nm.

have no spherical shape, but they have hexagonal shape as shown in Figure 1. The high electron densities of GNPs as well as the homogeneity of the particles shape and size make them highly conspicuous under the TEM.

The maximum absorption surface plasmon band (SPB) of GNPs only was shifted from 517 to 532 nm when the GNPs size changed from 10 to 50 nm which were attributed to the surface plasmon oscillation of free electrons.

The data of Figures 2 and 3 represent the relation between fluorescence intensity and wavelengths for GNPs of sizes 20 nm and 10 nm and infusion periods of3 and 7 days. The results show that fluorescence intensity

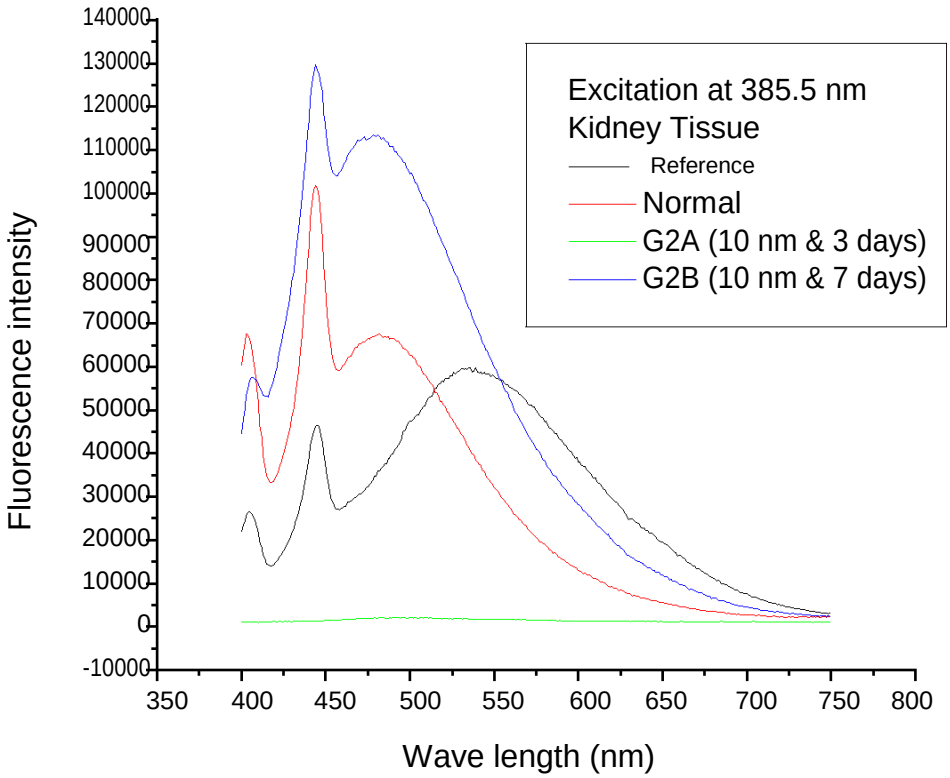

Figure 3. The emission fluorescence peak intensity after infusion periods of 3 and 7 days for GNPs of size 20 nm.

of the 1st peaks increased for G1A, G1B and G2B and decreased for G2A with the increase of the infusion periods of GNPs compared with the control while the fluorescence intensity of 2nd peaks increased for G1B and G2B and decreased for G1A and G2A with the increase of the infusion periods of GNPs compared with the control.

The data of Figure 4 represents the relation between fluorescence intensity and wavelengths for GNPs of size 50 nm and infusion periods of 3 and 7 days. Figure 4 shows that the fluorescence intensity (1st and 2nd peaks) of GNPs was sharply decreased for G3A and G3B compared with the control.

The data of Figure 5 represents the relation between fluorescence intensity and wavelengths for GNPs of sizes 10, 20 and 50 nm at the same infusion period of 3 days. Figure 5 shows the size effect of GNPs for G1A, G2A and G3A at the infusion period of 3 days. The fluorescence intensity of the 1st peaks increased for G1A and decreased for G2A and G3A compared with the control while the fluorescence intensity of the 2nd peaks decreased for G1A, G2A and G3A compared with the control.

This sharp decrease in fluorescence intensity for G3A and G3B may be attributed to the following: (1) the occurrence of a strong quenching of the fluorescence from serum albumins due to the formation of a ground state complex with GNPs (static quenching); (2) electron microscopy revealed that most GNPs of size 50 nm were of hexagonal morphology than spherical morphology (GNPs of sizes 10 and 20 nm); (3) the physical and chemical properties as well as the applications of NPs are controlled and limited by their dimensions and shape; (4) the 50 nm GNPs may be taken up faster and more intensively than the other sizes by macrophages of the kidney and disappeared thereafter.

The data of Figure 6 represents the relation between fluorescence intensity and wavelengths for GNPs of sizes 10, 20 and 50 nm at the infusion period of 7 days. Figure 6 shows the size effect of GNPs at the infusion period of 7 days. The fluorescence intensity of the 1st and 2nd peaks increased for G1B and G2B and sharply decreased for G3B compared with the control.

Figure 7 shows the size effect of GNPs at the infusion periods of 3 days (G1A, G2A and G3A) and 7 days (G1B, G2B and G3B). The fluorescence intensity of the 1st peaks increased for G1A, G1B and G2B and decreased for G2A, G3A and G3B compared with the control while the fluorescence intensity of the 2nd peaks decreased for G1A, G1B, G2A, G2B and G3A compared with the control. This result indicates that decreasing size may lead to an exponential increase in surface area relative to volume, thus making the GNPs surface more reactive on itself (aggregation) and to its surrounding environment

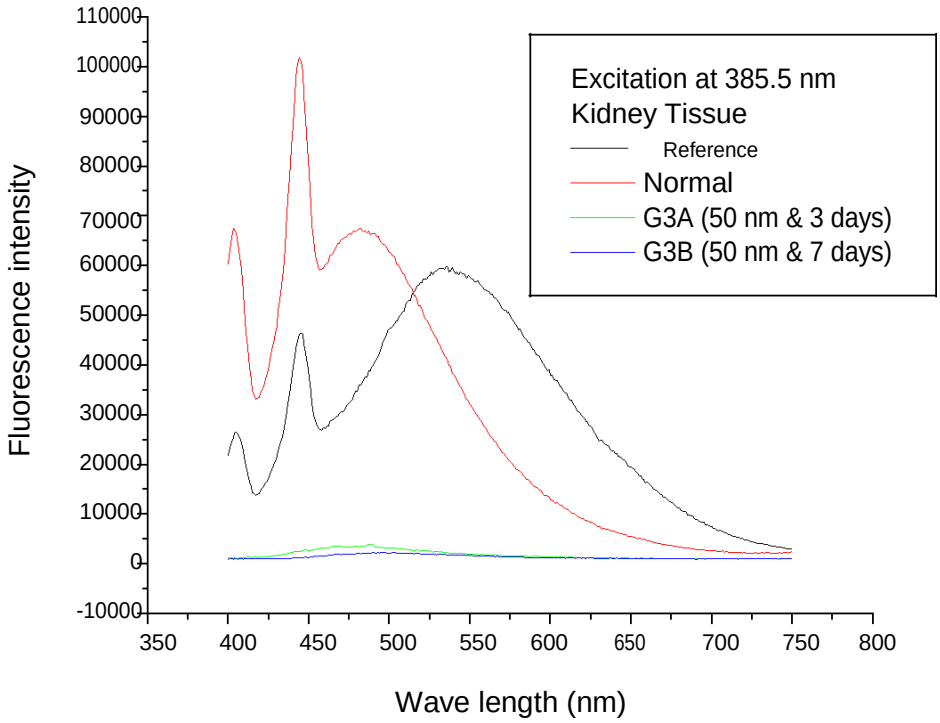

Figure 4. The emission fluorescence peak intensity after infusion periods of 3 and 7 days for GNPs of size 50 nm.

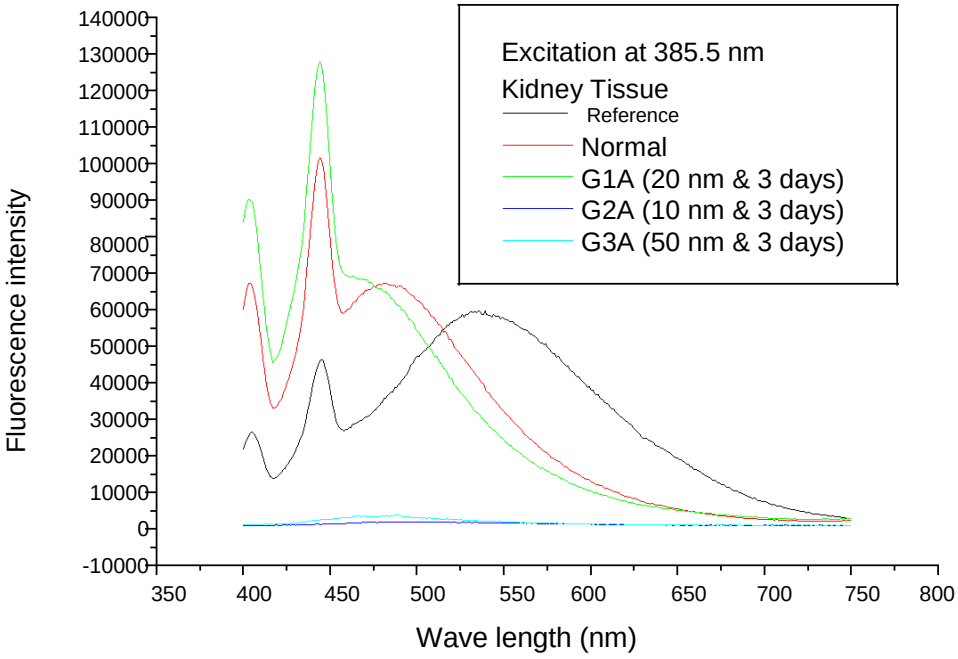

Figure 5. The emission fluorescence peak intensity after infusion period of 3 days for GNP of sizes 10, 20 and 50 nm.

(biological components). This study suggests that the increase in fluorescence intensity may be attributed to increase in the surface area which is related to number of GNPs and to slow clearance through urine and bile from the kidney. Increased uptake into certain tissues may lead to accumulation, where they may interfere with the

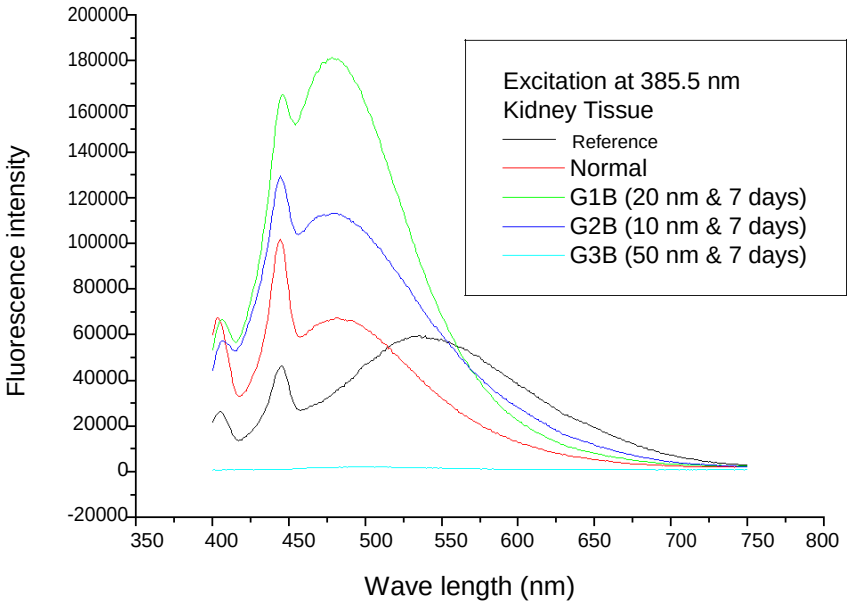

Figure 6. The emission fluorescence peak intensity after infusion period of 7 days for GNP of sizes 10, 20 and 50 nm.

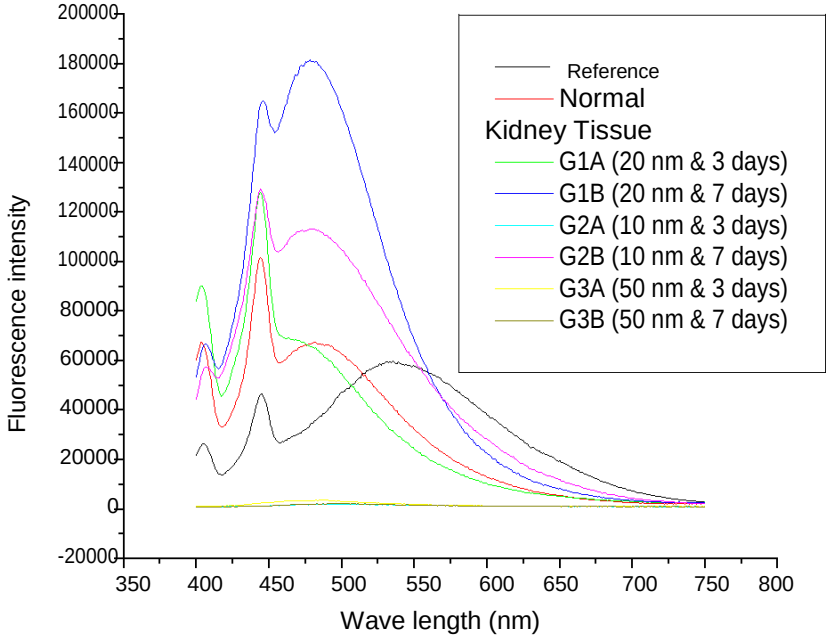

Figure 7. The emission fluorescence peak intensity after infusion periods of 3 and 7 days for GNP of sizes 10, 20 and 50 nm.

critical biological functions (Liu, 2006; Shrestha et al., 2006). The rate of exocytosis of GNPs was size dependent with more accumulation of larger GNPs in the cell (Shrestha et al., 2006).

It has been published that GNPs of very small sizes impart physical and chemical properties that are very different from those of the same material in the bulk form.

These properties include 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 (Liu, 2006; Shrestha et

al., 2006).

Since this surface area can interact with biological components of cells, nanoparticles can be more reactive in than larger particles. Incidence and severity of inflammatory response was transiently increased with injection of 200 and 100 nm GNPs within 12 h. GNPs were trapped by macrophages in the spleen and kidney and remained there until 4 weeks after the single injection (Cho et al., 2010).

To evaluate the impact of a particle size on tissue distribution, we injected 50 µl different sizes of GNPs. The 50 nm GNPs were taken up faster and more intensively than the other sizes by macrophages of the kidney and disappeared thereafter. This result is related with the inflammatory response of the kidney.

Nanoparticles 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 (Cho et al., 2010; 2007).

Absorbed nanoparticles within the systemic circulation can be excreted through various routes. A possible elimination route for nanoparticles could be renal and biliary clearance. Renal clearance of solid nano-sized materials was affected by particle size and surface charge (Choi et al., 2007; Gupta, 2005).

In our study, we compared fluorescence intensity and distribution of three different GNPs of varying particle size after intravenous injection into rats for periods of 3 and 7 days. It has been demonstrated that fluorescence intensity and kidney tissue distribution was different depending on particle size. The fluorescence intensity for 10 and 20 nm GNPs increased with the increase of the infusion period of GNPs from 3 to 7 days while it decreased for 50 nm GNPs. As particle sizes increased, fluorescence intensity decreased because more particles were trapped by macrophages.

These results could serve as a guideline in the rational design of drug nanocarriers with maximized therapeutic efficacy and predictable *in vivo* properties, in which the control of particle size and shape was of significance.

All injected GNPs showed a propensity to accumulate. The kidney tissue distribution of GNPs is size-dependent, with the smallest particles showing the most widespread organ distribution. Smaller GNPs showed greater cellular accumulation due to the dose-metric treatment. Therefore, GNPs target organs with many phagocytic cells such as kidney, spleen, lung, heart and mesenteric lymph node should be taken into consideration.

Conclusions

The aim of the present study was to investigate the period effects of intraperitoneal administration of different GNP sizes 10, 20 and 50 nm on the kidney tissues of rat *in vivo* using fluorescence measurements. The high electron densities of GNPs as well as the homogeneity of the particles shape and size make them highly conspicuous under the TEM.

The fluorescence intensity of the 1st peaks increased for G1A, G1B and G2B and decreased for G2A with the increase of the infusion periods of GNPs compared with the control while the fluorescence intensity of 2nd peaks increased for G1B and G2B and decreased for G1A and G2A with the increase of the infusion periods of GNPs compared with the control. The fluorescence intensity (1st and 2nd peaks) of GNPs was sharply decreased for G3A and G3B compared with the control. The fluorescence intensity of the 1st peaks increased for G1A and decreased for G2A and G3A compared with the control while the fluorescence intensity of the 2nd peaks decreased for G1A, G2A and G3A compared with the control.

This sharp decrease in fluorescence intensity for G3A and G3B may be attributed to the following: (1) the occurrence of a strong quenching of the fluorescence from serum albumins due to the formation of a ground state complex with GNPs (static quenching); (2) electron microscopy revealed that most GNPs of size 50 nm were of hexagonal morphology than spherical morphology (GNPs of sizes 10 and 20 nm); (3) the physical and chemical properties as well as the applications of nanoparticles are controlled and limited by their dimensions and shape; (4) the 50 nm GNPs may be taken up faster and more intensively than the other sizes by macrophages of the kidney and disappeared thereafter.

The fluorescence intensity of the 1st and 2nd peaks increased for G1B and G2B and sharply decreased for G3B compared with the control. The fluorescence intensity of the 1st peaks increased for G1A, G1B and G2B and decreased for G2A, G3A and G3B compared with the control while the fluorescence intensity of the 2nd peaks decreased for G1A, G1B, G2A, G2B and G3A compared with the control. This study suggests that extensive further studies in several organs of rats are needed for practical *in vivo* applications**.** This study demonstrates that the fluorescence spectra for these GNP sizes were size and shape dependent.

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