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Short Communication

Transient increase in IL-1β, IL-6 and TNF-α gene expression in rat liver exposed to gold nanoparticles

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ABSTRACT. Most studies have used *in vitro* systems to test inflammatory responses of nanoparticles; these may not reflect the real biological response of body organs. In fact, certain nanoparticles have provoked opposite effects under *in vitro* and *in vivo* conditions. Current understanding of the biocompatibility of gold nanoparticles is controversial. We studied the acute (1 day) and sub-chronic (5 days) effects of gold nanoparticles (10 and 50 nm in diameter) on expression of interleukin-1 beta (IL-1β), IL-6 and tumor necrosis factor alpha (TNF-α) in rat liver. Real-time PCR analysis showed that gold nanoparticles of both sizes significantly increased cytokine gene expression on day 1; this had subsided by day 5. The 50-nm gold nanoparticle produced more severe inflammation than the smaller gold nanoparticle. These findings indicate a possible biocompatibility of medium-sized gold nanoparticles, as they caused only a transient

increase in proinflammatory cytokines, followed by normalization during sub-chronic repeated exposure.

Key words: Gold nanoparticles; Inflammation; *In vivo* toxicity; Rats; Cytokines

INTRODUCTION

Several investigators have studied the inflammatory properties of different nanomaterials. *In vitro* studies have shown upregulated expression and release of proinflammatory cytokines, including interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α), by zinc oxide (Heng et al., 2011) and silver (Martínez-Gutierrez et al., 2012) nanoparticles. Despite an inability to induce the production of reactive oxygen species, $ZnCl_2$ stimulated TNF-α production, which was synergistically enhanced by 14-nm carbon black (Wilson et al., 2007). Auger et al. (2006) observed that nanoparticle-induced oxidative stress and proinflammatory cytokine expression are restricted to small nanoparticles (<40 nm). Their findings suggest that airway epithelial cells exposed to particles augment the local inflammatory response in the lung but cannot alone initiate a systemic inflammatory response. Ultrafine particles of two very different materials (titanium dioxide and carbon black) induce inflammation and epithelial damage to a greater extent compared to their fine counterparts, where the effect of ultrafine carbon black is greater than that of ultrafine titanium dioxide, suggesting that there are differences in the likely harmfulness of different types of ultrafine particles (Renwick et al., 2004). Gojova et al. (2007) showed that inflammation in human aortic endothelial cells following acute exposure to metal oxide nanoparticles depends on particle composition. However, Y_2O_3 and ZnO nanoparticles elicit a pronounced inflammatory response while the later nanoparticles are cytotoxic and lead to considerable cell death at higher concentration (Gojova et al., 2007). Rothen-Rutishauser et al. (2007) studied the potential of different particle types (different sizes and materials) to induce a cellular response and observed a 2- to 3-fold increase in TNF-α in the supernatants after applying polystyrene fine particles and gold nanoparticles, but not with polystyrene and titanium dioxide nanoparticles.

In the emerging field of nanomedicine, gold nanoparticles (GNPs) possess promising therapeutic possibilities due to their unique properties such as biocompatibility, high surface reactivity and resistance to oxidation. The promise of GNPs for so many different biological applications has led to a strong interest in studying their potential to cause deleterious effects in biological systems, and how these effects could be mitigated. The systematic toxicity of the intermediate size (18-37 nm) citrate-capped GNPs has been linked to major organ damage in the liver, spleen, and lungs of mice, but the same nanoparticles have been found to be nontoxic *in vitro* using HeLa cell lines (Chen et al., 2009). This discrepancy between *in vitro* and *in vivo* results points towards the notion that simple *in vitro* experiments may not lead to good predictions regarding *in vivo* results. To date, only limited nanoparticle structures and compositions have been tested, most of them using *in vitro* systems. There is a real need to investigate the *in vivo* effects of nanomaterials before any potential therapeutic applications (Fischer and Chan, 2007). In this investigation, we studied the time-course of the effects of 10- and 50-nm GNPs on the expression of the proinflammatory cytokines IL-1β, IL-6 and TNF-α in the liver of rats.

MATERIAL AND METHODS

Animals and treatment groups

Adult male Wistar-Kyoto rats, weighing 230 ± 20 g, were obtained from the Laboratory Animal Centre, College of Pharmacy, King Saud University, Riyadh. The animals were housed in humidity- and temperature-controlled ventilated cages on a 12-h light/dark cycle, with free access to standard laboratory food and tap water. The animals were randomly divided into 5 groups of 5 animals each. One group served as control and received vehicle only. Two groups were treated with GNPs (10 nm in diameter) for 1 and 5 days. The remaining two groups received GNPs (50 nm in diameter) for 1 and 5 days.

GNPs and dosage

GNPs with diameters of 10 nm (MKN-Au-010 with a concentration 0.01% Au) and 50 nm (MKN-Au-050 with a concentration 0.01% Au) were purchased from MK Impex Corp., Ontario, Canada. Doses of 50 µL 10 and 50 nm GNPs in aqueous solution were administered to animals via intraperitoneal injection daily for 1 or 5 days. The rats were sacrificed 24 h after the last injection of GNPs. The specimens of liver were isolated and immediately immersed in RNA Later solution (Qiagen) and stored at 4°C until RNA extraction. All experiments were conducted in accordance with guidelines approved by the local Animal Care and Use Committee.

Real-time RT-PCR

Expressions of mRNAs for the proinflammatory cytokines, IL-1β, IL-6 and TNF-α, were quantified by real-time RT-PCR. Total RNA was isolated from liver tissues (approximately 30 mg) using the RNAEasy kit (Qiagen, Germany), according to the manufacturer protocol. The extracted RNA was dissolved in 30 µL nuclease-free distilled water and stored at -20°C. The concentration and purity of RNA were determined by Nanodrop Spectrophotometer (Thermo Scientific, USA). Real-time PCR was performed using 2 µL template in a 20-µL reaction containing 0.25 µM of each primer and 12.5 µL Sybr Green Real-time PCR MasterMix (Applied Biosystems, USA). Each run consisted of 50°C for 2 min and 95°C for 10 min followed by 45 cycles of 95°C for 15 s, 60°C for 20 s, and 72°C for 60 s in a real-time qPCR machine (Stratagene, Agilent Biosciences, USA). GAPDH was used as a housekeeping gene for normalizing the expression data (Ronis et al., 2005). The primer sequences are given in Table 1.

Table 1. Primers used for real-time PCR amplifications.

Primer name	Sequence	bp
IL-1β-FW	CAC CTT CTT TTC CTT CAT CTT TG	23
IL-1β-RW	GTC GTT GCT TGT CTC TCC TTG TA	23
IL-6-FW	TGA TGG ATG CTT CCA AAC TG	20
IL-6-RW	GAG CAT TGG AAG TTG GGG TA	20
TNF-α-FW	ACT GAA CTT CGG GGT GAT TG	20
TNF-α-RW	GCT TGG TGG TTT GCT ACG AC	20
GAPDH-FW	GTA TTG GGC GCC TGG TCA CC	20
GAPDH-RW	CGC TCC TGG AAG ATG GTG ATG G	22

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by the Dunnett multiple comparison test using the SPSS statistical package. $P < 0.05$ was considered to be statistically significant.

RESULTS

There were significant and dose-dependent increases in IL-1β mRNA expression in liver on day 1 post-dosing of 10 nm (10.143-fold) and 50 nm GNPs (14.805-fold), while expression was significantly reduced on day 5 (ANOVA, $F = 5.489$, $P = 0.004$) (Figure 1). The expression of IL-6 in liver was also dose-dependently increased by 10 nm (2.456-fold) and 50 nm (8.256-fold) GNPs after 1 day, but this increase was only significant when using 50 nm GNPs (ANOVA, $F = 3.177$, $P = 0.039$). IL-6 gene expression was normalized on day 5. TNF-α mRNA expression in liver was significantly increased on day 1 by 10 nm (6.337-fold) and 50 nm (22.650-fold) GNPs, which was significantly reduced on day 5 (ANOVA, $F = 3.096$, $P = 0.040$) (Figure 1). The real-time RT-PCR amplification plots are shown in Figure 2.

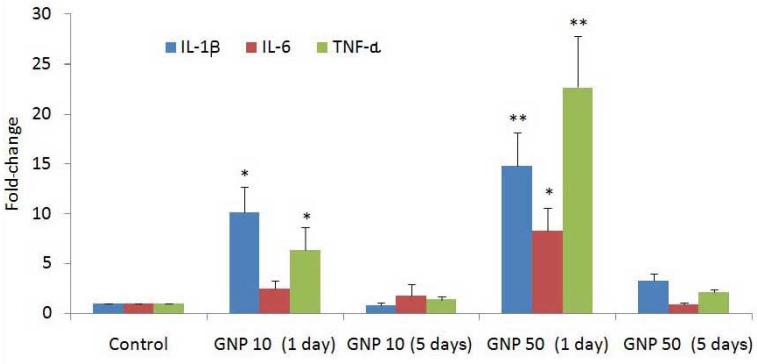

Figure 1. Time-course effects of gold nanoparticles (GNPs) of 10 and 50 nm in diameter on proinflammatory cytokine gene expression in rat liver. *$P < 0.05$ and **$P < 0.01$ versus control group using the Dunnett test.

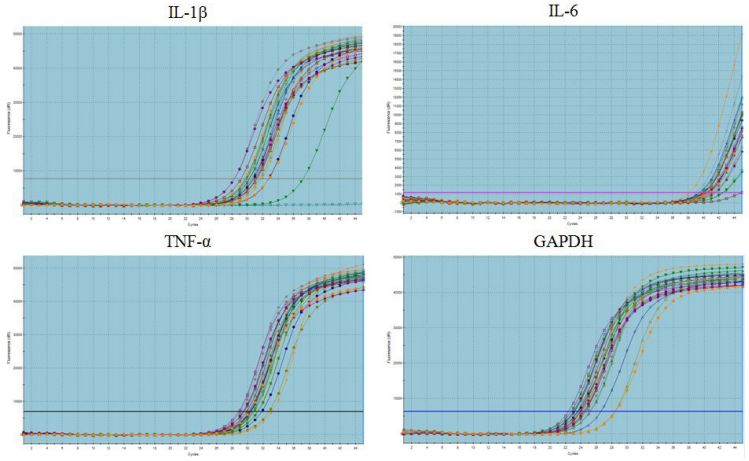

Figure 2. qRT-PCR amplification plots of IL-1β, IL-6, TNF-α, and GAPDH in liver.

DISCUSSION

The administration of GNPs caused significant acute phase induction of proinflammatory cytokines in rat liver (Figure 1). The proinflammatory cascade returned to normal after the subchronic exposure of GNPs (Figure 1). Nishanth et al. (2011) observed significant induction of IL-6 and TNF-α in mouse macrophages by silver nanoparticles followed by aluminum, carbon black and carbon-coated silver nanoparticles; however, no such prominent proinflammatory responses were observed with GNPs, suggesting their biocompatibility. The results of another *in vitro* study on murine macrophages has suggested that 60 nm GNPs are not cytotoxic or do not elicit proinflammatory (IL-6, TNF-α) responses (Zhang et al., 2011). On the other hand, Yen et al. (2009) found that both silver and gold nanoparticles enter the cells but that only the latter (especially those with smaller diameter) upregulate the expression of proinflammatory genes (IL-1, IL-6, TNF-α). They speculated that part of the negatively charged GNPs may adsorb serum protein and enter cells via the more complicated endocytotic pathway, resulting in higher cytotoxicity and immunological response of GNPs than silver nanoparticles (Yen et al., 2009).

A significant inflammatory response has been observed in the release of TNF-α and IL-1β after 24 h of exposure to 15-nm Ag nanoparticles, but there was no detectable level of IL-6 upon exposure to silver nanoparticles (Carlson et al., 2008). The levels of TNF-α and IL-1β in bronchoalveolar lavage fluid at 5 and 24 h were found to be higher in carbon nanotube-exposed animals than in corresponding air-exposed controls (Han et al., 2008). Park and Park (2009) noticed that a single treatment of silica nanoparticles (50 mg/kg, *ip*) activates peritoneal macrophages and increases blood levels of IL-1β and TNF-α; mRNA expressions of inflammation-related genes were also elevated in the cultured peritoneal macrophages harvested from the treated mice. Exposure to anatase TiO_2 nanoparticles has been found to increase significantly oxidative damage expressed as lipid peroxidation and to produce higher inflammation responses in association with the significantly increased TNF-α and IL-1β levels (Wang et al., 2008). A single intratracheal instillation of platinum and silver nanoparticles causes progressive increase in proinflammatory cytokines (IL-1, IL-6, TNF-a) by day 28 post-instillation (Park et al., 2010, 2011). Intragastric administration of TiO_2 nanoparticles has been shown to increase significantly the mRNA expression of several inflammatory cytokines in mouse liver (Cui et al., 2011). The above literature clearly indicates that GNPs are comparatively safer and more biocompatible than other nanomaterials.

Acute chemical intoxication in rats significantly increases the levels of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α, after 24 h, in timely concordance with the inflammatory process present in the liver (Salazar-Montes et al., 2000). At that time, cell proliferation processes occurring in the liver and these cytokines together with other growth factors may be responsible for cell proliferation and liver regeneration followed by decreased expression of these cytokines, indicating that liver damage has been resolved and regeneration has taken place (Salazar-Montes et al., 2000). Higashitsuji et al. (1995) found transient increases in mRNA expression of IL-1β and TNF-α (but not IL-6) during the regeneration phase following partial hepatectomy. Interestingly, induction of the metallothionein and manganese superoxide dismutase by proinflammatory cytokines (TNF-α and IL-6) in the liver exerts an antioxidant effect during acute phase response, thereby preventing tissues from oxidative injury (Sato et al., 1995).

In conclusion, GNPs of both sizes (10 and 50 nm) significantly increased cytokine gene expression on day 1, which subsided on day 5. However, GNP of 50 nm in size produced a stronger induction of proinflammatory cytokines as compared to smaller size GNP. These findings indicate the possible biocompatibility of medium-sized GNPs (10-50 nm), since they caused only a transient increase in proinflammatory cytokines followed by their normalization after the regenerative phase was over. Further studies are warranted on *in vivo* effects of naked and surface-modified GNPs on inflammatory cascade in other organs of rats.

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