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Original Article

In vivo Genotoxicity of Silver Nanoparticles after 90-day Silver Nanoparticle Inhalation Exposure

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Objectives: The antimicrobial activity of silver nanoparticles has resulted in their widespread use in many consumer products. Yet, despite their many advantages, it is also important to determine whether silver nanoparticles may represent a hazard to the environment and human health.
Methods: Thus, to evaluate the genotoxic potential of silver nanoparticles, *in vivo* genotoxicity testing (OECD 474, *in vivo* micronuclei test) was conducted after exposing male and female Sprague-Dawley rats to silver nanoparticles by inhalation for 90 days according to OECD test guideline 413 (Subchronic Inhalation Toxicity: 90 Day Study) with a good laboratory practice system. The rats were exposed to silver nanoparticles (18 nm diameter) at concentrations of 0.7×10^6 particles/cm^3 (low dose), 1.4×10^6 particles/cm^3 (middle dose), and 2.9×10^6 particles/cm^3 (high dose) for 6 hr/day in an inhalation chamber for 90 days. The rats were killed 24 hr after the last administration, then the femurs were removed and the bone marrow collected and evaluated for micronucleus induction.
Results: There were no statistically significant differences in the micronucleated polychromatic erythrocytes or in the ratio of polychromatic erythrocytes among the total erythrocytes after silver nanoparticle exposure when compared with the control.
Conclusion: The present results suggest that exposure to silver nanoparticles by inhalation for 90 days does not induce genetic toxicity in male and female rat bone marrow *in vivo*.

Key Words: Silver nanoparticles, Genotoxicity, OECD test guidelines, *In vivo* micronuclei test, Good laboratory practice, Inhalation toxicity

Introduction

The antimicrobial activity of silver nanoparticles has resulted in their widespread use in many consumer products, such as disinfectants, deodorants, antimicrobial sprays and powders, bedding, washers, water purification, toothpaste, shampoo and rinses, nipples and nursing bottles, fabrics, deodorants, filters, kitchen utensils, toys, and humidifiers. Yet, while the population exposed to silver nanoparticles continues to increase with ever-new applications, silver nanoparticles remain a controversial research area with respect to their toxicity to biological systems. The toxicity of silver nanoparticles has been studied extensively. The acute inhalation toxicity, LC50, of silver nanoparticles is suggested to be higher than 3.1×10^6 particles/cm^3 (750 mg/m^3) [1]. A toxicity study that exposed rats to twenty-eight days of silver nanoparticle inhalation did not show any significant toxicity up to (1.32×10^6 particles/cm^3, 61 μg/m^3) [2]. In contrast, a study on the oral toxicity of silver

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nanoparticles that exposed rats to silver nanoparticles for 28 days indicated that some significant dose-dependent changes were found in the alkaline phsophatase and cholesterol values in both the male or female rats, which seemed to indicate that exposure to over more than 300 mg/kg of silver nanoparticles may result in slight liver damage [3]. Consistent with these findings, silver nanoparticles were found to be toxic to the liver in both male and female rats. A NOAEL (no observable adverse effect level) of 30 mg/kg and LOAEL (lowest observable adverse effect level) of 125 mg/kg are suggested based on the 90-day the subchronic oral toxicity study in reference [4]. Target organs for silver nanoparticles in another 90-day subchronic inhalation toxicity study were considered to be the lungs and liver in male and female rats [5]. Lung function changes were observed when animals were subchronically exposed to silver nanoparticles over a period of 90 days [6]. A no observable adverse effect level of 100 μg/m^3 is suggested from the experiment in reference [5]. Many researchers have studied the genotoxicity of silver nanoparticles. However, the majority of studies could not reflect the practical genotoxicity effect because they performed experiments with *in vitro* systems on microorganisms and cell lines. In contrast, we conducted a study for an *in vivo* system with rats. Notably, we carried out the genotoxicity studies for animals subchronically exposed *via* oral and inhalation routes. The genotoxicity of silver nanoparticles after 28 days of oral administration was negative for the *in vivo* micronucleus test [3]. Accordingly, we have investigated the genotoxicity of silver nanoparticles after subchronic inhalation exposure.

Materials and Methods

Generation of silver nanoparticles

The silver nanoparticles were generated as described in previous reports [2,6], and the rats were exposed to the silver nanoparticles in a whole-body-type exposure chamber (1.3 m^3, Dusturbo, Seoul), consisting of a small ceramic heater connected to an AC power (91.6 V) supply and housed within a quartz tube case [7]. The heater dimensions were 50 × 5 × 1.5 mm^3, and a surface temperature of about 1,500°C within a local heating area of 5 × 10 mm^2 could be achieved within about 10 s. For long-term testing, the source material (about 160 mg, Daedeok Science, Daejeon) was positioned at the highest temperature point. The quartz case was 70 mm in diameter and 140 mm long [8]. Clean (dry and filtered) air was used as the carrier gas, and the gas flow was maintained at 30.0 L/min (Re = 572, laminar flow regime) using a mass flow controller (MFC, AERA, FC-7810CD-4V, Japan). In this study, the system produced different concentrations of nanoparticles (high, middle, and low) in three separate chambers. The nanoparticle generator was operated at 30 L/min and this was mixed with the 200 L/min flow rate of the main flow through the high-concentration chamber. Using the MFC for the first particle sampler, a portion of the high nanoparticle concentration was diverted to the middle-concentration chamber and diluted by the MFC flow rate. In the same way, a portion of the middle nanoparticle concentration was also diverted to the low-concentration chamber and diluted by the MFC flow rate. The flow rates for the high, middle, and low doses were 47.02 ± 0.14 lpm, 6.76 ± 0.16 lpm, and 5.42 ± 0.18 lpm (mean ± S.E.), respectively.

Monitoring the inhalation chamber and analysis of silver nanoparticles

In the individual chambers containing the different nanoparticle concentrations, the nanoparticle distribution with respect to size was measured directly using a differential mobility analyzer (nano-DMA, 4220, HCT Co., Ltd. Korea, range 2.5-150 nm) and ultra-condensation particle counter (UCPC, 4312, HCT Co., Ltd. Korea, 3025, 0-108/cm^3 detection range). Nanoparticles from 1.98 to 64.9 nm were measured using sheath air at 5 L/min and polydispersed aerosol air at 1 L/min, with these values being the operational conditions for nano-DMA and UCPC, respectively. The particle numbers per cm^3 in the fresh-air control chamber were measured using a particle sensor (4103, HCT Co., Ltd. Korea) that consisted of channel 1 (below 300 nm) and channel 2 (over 300 nm).

Animals and conditions

Six-week-old male and female, specific-pathogen-free (SPF) Sprague-Dawley rats were purchased from SLC (Tokyo. Japan) and acclimated for 2 wks before starting the experiments. During the acclimation and experimental periods, the rats were housed in polycarbonate cages (5 rats per cage) in a room with controlled temperature (23 ± 2°C) and humidity (55 ± 7%) with a 12-h light/dark cycle. The rats were fed a rodent diet (Harlan Teklab, Plaster International Co., Seoul) and filtered water *ad libitum*. The 8-week-old rats, weighing about 253 g for the males and 162 g for the females, were then divided into 4 groups (10 rats in each group): fresh-air control, low-dose group (target dose, 0.6 × 10^6 particles/cm^3, 1.0 × 10^9 nm^2/cm^2), middle-dose group (target dose, 1.4 × 10^6 particles/cm^3, 2.5 × 10^9 nm^2/cm^2), and high-dose group (target dose, 3.0 × 10^6 particles/cm^3, 5.0 × 10^9 nm^2/cm^2), and exposed to silver nanoparticles for 6 hr/day, 5 days/wk, for 13 weeks [5]. The animals were examined daily on weekdays for any evidence of exposure-related effects, including respiratory, dermal, behavioral, nasal, or genitourinary changes suggestive of irritancy. The body weights were