Toxicokinetics of zinc oxide nanoparticles in rats

HE Chung¹, J Yu¹, M Back¹, JA Lee¹, MS Kim², SH Kim², EH Maeng², JK Lee³, J Jeong³ and SJ Choi¹

¹Department of Food Science and Technology, Seoul Women’s University, 621 Hwarang-ro, Nowon-gu, Seoul 139-774, Republic of Korea
²Korea Testing & Research Institute, Health Care Research Lab, 7-6 Gomak-ri, Wolgot-myeon, Gyeonggi-do, 415-817, Republic of Korea
³Toxicological Research Division, National Institute of Food and Drug Safety Evaluation, KFDA, Osong-eup, Chungchungbuk-do 363-700, Republic of Korea

E-mail: sjchoi@swu.ac.kr

Abstract. Zinc oxide (ZnO) nanoparticle have been extensively applied to diverse industrial fields because they possess UV light absorption, catalytic, semi-conducting, and magnetic characteristics as well as antimicrobial property. However, up to date, toxicological effects of ZnO nanoparticles in animal models have not been completely determined. Moreover, little information is available about kinetic behaviors of ZnO nanoparticles in vivo, which will be crucial to predict their potential chronic toxicity after long-term exposure. The aim of this study was, therefore, to evaluate the pharmacokinetics and toxicokinetics of ZnO nanoparticles after single-dose and repeated dose 90-day oral administration in male and female rats, respectively. The blood samples were collected following administration of three different doses (125, 250, and 500 mg/kg) and ZnO concentration was assessed by measuring zinc level with inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The result showed that the plasma ZnO concentration significantly increased in a dose-dependent manner, but decreased within 24 h after single-dose oral administration up to 500 mg/kg, without any significant difference between gender. However, when repeated dose 90-day oral toxicity study was performed, the elevated plasma concentrations did not return to normal control levels in all the cases, indicating their toxicity potential. These findings suggest that repeated oral exposure to ZnO nanoparticles up to the dose of 125 mg/kg could accumulate in the systemic circulation, thereby implying that the NOAEL values could be less than 125 mg/kg via oral intake.

1. Introduction
Zinc is an essential trace element in human body and ZnO has been used as a source of zinc in food industry. Since nano-sized particles have high reactivity related to large surface area compared to bulk-sized ones, ZnO nanoparticles have been extensively applied to many industrial domains such as alloys, ceramics, paints, rubber as well as biological fields including medicine, personal care products, sunscreens, and food additives [1-4]. The wide range application of ZnO nanoparticles is attributed to their unique properties such as UV light absorption, antimicrobial, catalytic, semi-conducting and magnetic characteristics [5-7]. Along with rapid development of nanotechnology in human-related products, the potential toxicity of nanomaterials in human health has been raised concern. Some studies were conducted to evaluate the toxicity of ZnO nanoparticles in cell lines [8-10] and in animal.
models [11-12]. However, there is little information about their chronic or sub-chronic toxicity after the long-term exposure. In this study, we investigated the pharmacokinetics and toxicokinetics of ZnO nanoparticles after single-dose and repeated dose oral administration for 90 days to male and female rats, respectively, which will provide critical information about their potential toxicological effects after long-time exposure.

2. Experimental methods

2.1. Preparation of nanoparticle suspension

20 nm-sized ZnO nanoparticles were purchased from Sumitomo Osaka (Tokyo, Japan). For oral administration of ZnO nanoparticles, the nanoparticles were suspended in pH 7.2 20 mM HEPES buffer containing 1% sodium citrate, and then dispersed by vortexing for one minute. The final pH of the suspension was adjusted to 7.3, and 20% of the ZnO nanoparticle suspension were used as a stock solution. The suspension was vortexed for 10 seconds just before administration, and then diluted with distilled water.

2.2. Characterization of nanoparticles

Particle size was determined by transmission electron microscope (TEM: JEM-1010, JEOL, Tokyo, Japan). The surface charge (zeta potential) of thus prepared ZnO nanoparticles was determined using a zeta potentiometer (Zetasizer Nano ZS system, Malvern Instruments, Worcestershire, UK).

2.3. Animals

Five-week-old male and female Sprague-Dawley rats weighing 120-140 g were purchased from G-bio (Seoul, Republic of Korea). Animals were acclimatized as follow for 7 days before receiving experimental treatment; they were housed in plastic laboratory animal cages in a ventilated room. The room was maintained at 20°C±2°C and 60%±10% relative humidity on a 12-hour light/dark cycle. Water and commercial laboratory complete food for rats were available ad libitum. All animal experiments were performed in compliance with the animal and ethics review committee of the Korea Testing & Research Institute.

2.4. Dosing and sample collection

Three groups of male and female rats (n=9 per group) were daily administered via oral gavage for 90 days with a dose of 125, 250, 500 mg/kg of ZnO nanoparticles. And an additional group of 3 rats received an equivalent volume of citrate/HEPES buffer as controls for all the experiments. Changes in body weight and behaviours including food and water intake, as well as possible appearance of symptoms in the rats were carefully recorded every day after administration of the ZnO nanoparticles. Blood samples were collected via the tail vein at several time points (0, 0.5, 1, 2, 4, 6, 10, 24 hours) on 0, 28, and 90 days after oral administration to evaluate the plasma ZnO nanoparticle concentration. The blood samples were centrifuged at 12,000 rpm for 3 minutes at 4°C to obtain the plasma. Several toxicokinetic parameters such as maximum concentration (C_max), time to maximum concentration (T_max), area under the plasma concentration-time curve (AUC), half-life (T1/2), and mean residence time (MRT), were estimated using the Kinetica program (version 4.4, Thermo Electron Corporation, Waltham, MA).

2.5. Sample preparation for ICP-AES analysis

The plasma samples were pre-digested in 5 ml of ultrapure nitric acid, heated at about 180°C, and then, 0.5 mL of H2O2 was added. Each sample mixture was heated until completely digested. The remaining solution was then removed by heating until the solutions were colorless and clear.

2.6. ICP-AES analysis

Quantitative analysis was carried out using external five-point calibration with internal standard correction spiking experiments. Stock solutions were diluted with 20% ultrapure nitric acid to designated concentrations. Analysis of standards and biological samples was undertaken using
inductively coupled plasma-atomic emission spectroscopy (ICP-AES: Jobin Y von Horiba, JY 2000 Ultrace). The instrument operating conditions were as follows: RF power and nebulizer gas flow were set at 1000 W and 0.02 mL per minute of argon, respectively.

2.7. Statistical analysis
Statistical analysis were performed using SAS software (Tukey’s test, version 11.0, Cary, NC) for unpaired data and p values of less than 0.05 were considered significant.

3. Results and discussion

3.1. Characterization of ZnO nanoparticles
Particle size and surface charge of ZnO nanoparticles were measured by TEM and zeta potentiometer, respectively, and presented in Table 1. The ZnO nanoparticles were capped with citrate molecules, widely used as capping agents for inorganic nanoparticles, giving rise to negatively charged surface property. Citrate was also used as a dispersing agent for oral administration. The result showed that the average size of ZnO nanoparticles was determined to be 20±9 nm with surface charge of -28.1 mV.

| ZnO dispersed in citrate/HEPES | Measured particle size (nm) | Zeta potential (mV) |
|-------------------------------|-----------------------------|---------------------|
|                               | 20 ± 9                      | -28.1               |

Table 1. Particle size and surface charge of ZnO nanoparticles

Figure 1. TEM image (A) and size distribution (B) of ZnO nanoparticles dispersed in citrate/HEPES buffer. Average particle size and size distribution were measured by randomly selecting 500 particles from transmission electron micrographs

3.2. Effects of ZnO nanoparticles on body weight changes and symptoms
During oral administration of the ZnO nanoparticles for 90 days, changes in body weight, mortality, behaviors, and appearance of symptoms were observed and recorded daily. No mortality was observed in all the ZnO nanoparticles-treated groups up to the dose of 500 mg/kg. Any statistically significant difference in body weight was not found between the controls and the groups administered ZnO nanoparticles as shown in Fig. 2. However, piloerection and alopecia was observed after 8-day and 13-day oral administration, respectively, both in male and female rats, and these symptoms were continued in all the experimental groups during all the periods of treatment. Salivation was also
observed after 14-day repeated administration in male and female rats when they were treated with 500 mg/kg, and this was found in all treated groups by the end of the experiment. Abnormal symptoms such as soft stool, wound, diarrhea, staining around mouth, spoiled fur, and opacity of eyeball were observed in some groups. It seems that the present nanoparticles up to the dose of 500 mg/kg did not cause acute oral toxicity, but can induce some toxicological effects.

Figure 2. Body weight gain in male and female rats treated with ZnO nanoparticles. The data show no significant difference from the control group (p < 0.05).

3.3. Food and water intake in rats

Fig. 3 showed the mean amount of food and water intake in rats administered ZnO nanoparticles. Overall food intake gradually increased both in male and female rats during all the periods of treatment, in comparison with control group. On the other hand, water intake amount significantly increased during the first 1 to 5 weeks of treatment, especially when 500 mg/kg were repeatedly administered. However, increase in food and water intake in a dose-dependent manner was not found. Together with the result on body weight gain (Fig. 2), it is likely that the changes in food and water intake were not resulted from the toxicological effects of ZnO nanoparticles.
Figure 3. Food and water intake in rats during the administration of ZnO nanoparticles. * denote significant differences from the control group (*p < 0.05, **p < 0.01).

3.4. Plasma concentration–time curve
ZnO concentration in plasma after oral administration to male or female rats was evaluated by measuring the zinc level using ICP-AES. The plasma concentration-time curve was highly dependent on the dose administered (Fig. 4). After single-dose administration, elevated zinc levels returned to normal levels with 24 h. However, when 125 mg/kg were administered, there was slight increase in zinc level at 90 day compared to 0 day in male and female rats (Fig. 4A). Meanwhile, both 250 and 500 mg/kg doses resulted in statistically increased plasma zinc level after 28 and 90 day-repeated oral administration (Fig. 4B and 4C). This result suggests that the present nanoparticles could be accumulated in the blood circulation after repeated exposure.

Based on this result, the toxicokinetic parameters of ZnO nanoparticles were presented in Table 2. All parameters increased as the doses administered increased. AUC values, representing total amount of the nanoparticles absorbed into the blood circulation seemed to be slightly higher in female rats than those in male rats, in case of 250 and 500 mg/kg. But different significance was not found between genders. It is worthy to note here that T\textsubscript{1/2} and MRT values, indicators for systemic half-life and mean residence time in the body, respectively, increased gradually as ZnO nanoparticles were repeatedly exposed to rats in all the cases. This is an indication for systemic accumulation of the present nanoparticles. It is strongly probable that 90-day repeated oral administration up to the dose of 125 mg/kg could cause toxicological effects associated with gradually increased ZnO concentration at the systemic level. This may explain some abnormal behaviors or symptoms as observed in section 3.2.
Figure 4. Zinc concentration in plasma after 90 day-repeated oral administration to rats. A, 125 mg/kg; B, 250 mg/kg; C, 500 mg/kg. The data are presented as increased zinc levels after subtraction of the basal zinc level in the control group.

Table 2. Toxicokinetic parameters of 20 nm ZnO nanoparticles in rats.

|               | 125 mg/kg |           | 250 mg/kg |           | 500 mg/kg |           |
|---------------|-----------|-----------|-----------|-----------|-----------|-----------|
|               | Male      | Female    | Male      | Female    | Male      | Female    |
|               | 0         | 28        | 90        | 0         | 28        | 90        |
| $C_{\text{max}}$ | 42.53     | 33.89     | 46.88     | 51.10     | 37.89     | 50.10     |
| $T_{\text{max}}$ | 4         | 4         | 4         | 4         | 2         | 4         |
| AUC           | 249.66    | 296.73    | 528.58    | 469.60    | 216.04    | 438.66    |
| $T_{1/2}$     | 3.69      | 5.06      | 8.81      | 4.63      | 5.01      | 7.12      |
| MRT           | 5.52      | 7.65      | 12.59     | 8.11      | 6.13      | 9.83      |
|               |           |           |           |           |           |           |
4. Conclusion
The present study demonstrated the toxicokinetics of ZnO nanoparticles after single-dose and repeated 90-day oral administration to male and female rats, respectively. The results showed that the present nanoparticles up to the highest dose tested, 500 mg/kg, did not cause mortality and body weight change after 90-day oral exposure. But, some abnormal symptoms such as piloerection and alopecia were observed in the experimental groups. Pharmacokinetic study of ZnO nanoparticles after single-dose oral administration demonstrated that their plasma concentration increased in a dose-dependent manner, but returned to normal levels with 24 h. However, when repeated dose 90-day administration was performed, slightly increased levels of the nanoparticles were found in the groups treated with 125 mg/kg, and moreover, significantly elevated systemic accumulation was evident in rats administered 250 and 500 mg/kg. Significant difference in toxicokinetics between genders was not found. These results suggested that ZnO nanoparticles could be accumulated up to the dose of 125 mg/kg in the systemic circulation in rats. It is, therefore, concluded that the NOAVEL (no observed adverse effect level) values may be less than 125 mg/kg via oral ingestion.

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6. References
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