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# TOXICITY OF ZINC OXIDE NANOPARTICLES IN RATS TREATED BY TWO DIFFERENT ROUTES: SINGLE INTRAVENOUS INJECTION AND SINGLE ORAL ADMINISTRATION

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**Toxicokinetics of zinc oxide nanoparticles (ZnONP) was studied in rats via a single intravenous (iv) injection and a single oral administration (3 mg/kg or 30 mg/kg), respectively. Blood concentrations of zinc (Zn) were monitored for 7 d and tissue distribution were determined in liver, kidneys, lung, spleen, thymus, brain, and testes. To ascertain the excretion of ZnONP, Zn levels in urine and feces were measured for 7 d. ZnONP were not readily absorbed from the gastrointestinal tract (GIT) after oral administration and were excreted mostly in feces. When the nanoparticles were injected iv to rats at a dose of 30 mg/kg, peak concentration appeared at 5 min but returned to normal range by d 2 (48 h after injection). ZnONP were distributed mainly to liver, kidneys, lung, and spleen, but not to thymus, brain, and testes. The distribution level was significantly decreased to normal by d 7. Feces excretion levels after iv injection supported biliary excretion of ZnONP. In rats injected iv with 30 mg/kg, mitotic figures in hepatocytes were significantly increased and multifocal acute injuries with dark brown pigment were noted in lungs, while no significant damage was observed in rats treated orally with the same dosage.**

Zinc (Zn) is one of the most widely used micronutrients (Boreiko, 2010). Zinc oxide (ZnO) accounts for the largest industrial use of Zn compounds. Zn is used primarily in the rubber industry, as a heat conductor, white pigment, and to absorb ultraviolet (UV) light. Zinc oxide nanoparticles (ZnONP) have also been used in a variety of products including semiconductors, catalysts, and paints. Further, these particles are increasingly found in consumer products, such as sunscreen, because of the strong UV absorption properties of ZnO (Osmond and McCallet al., 2010; Ludi and Niederberger, 2013; Ma et al., 2013). Products containing ZnONP may release nanoparticles (NP) or Zn ions, which may be translocated from the environment into the human blood circulation and accumulate in organs, potentially leading to toxicity (Hackenberg and Kleinsasser, 2012; Li et al., 2012; Nohynek and Defour, 2012; Baky et al., 2013; Sahu et al., 2014).

Many toxicity studies on ZnONP have been published; more than 350 publications were identified on PubMed as of July 2014. Among these, several in vitro studies suggested that ZnONP induce genotoxicity in primary human epidermal keratinocytes (Sharma et al., 2011a; Demir et al., 2014), produce cytotoxicity and enhance inflammatory cytokines levels in murine macrophages (Roy et al., 2011), induce oxidative stress and genotoxicity in human liver cells (HepG2) (Sharma et al., 2011b), and generate reactive oxygen species (ROS) to damage human bronchial epithelial cells (Heng

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et al., 2010). In vivo studies explored various routes of exposure of ZnONP. Toxicities including oxidative stress were observed after bronchoalveolar lavage fluid was collected in ZnONP-treated rats (Ho et al., 2011). Repeated application of ZnONP to rat skin with 3 different doses (75, 180, or 360 mg/kg) for 28 d decreased dermal collagen content (Surekha et al., 2012). Oral administration of ZnONP also produced toxicity in some animal models. ZnONP induced oxidative stress, DNA damage, and apoptosis in mouse liver after subacute oral exposure to 300 mg/kg for 14 d (Sharma et al., 2012). When rats were treated with a single oral administration of 5, 50, 300, 1000, or 2000 mg/kg, inverse dose-dependent increase was noted in aspartate aminotransferase and alanine aminotransferase (Pasupuleti et al., 2012).

The transport or absorption mechanism of ZnONP in the gastrointestinal tract (GIT) following oral administration is still unclear. Further, the toxicity mechanisms underlying ZnONP are still unclear; the active form producing toxicity may be $Zn^{2+}$ released from ZnONP, given the correlation between ZnONP toxicity and dissolved Zn (Song et al., 2010; Reed et al., 2012). In these studies, $Zn^{2+}$ concentration significantly correlated with cell death and elevated levels of lactate dehydrogenase (LDH) activity, which was induced by supernatants of ZnONP suspensions.

In addition to toxicity tests, a few ZnONP toxicokinetic studies investigated the absorption rate, tissue distribution, and clearance of NP in an attempt to correlate levels of Zn or ZnONP and the observed toxicity in target organs. In a study to compare the absorption rate of NP and micro-sized particles after oral administration, Zn levels in serum of mice receiving the nano-sized particles were higher compared to the micro-sized treated group (Li et al., 2012). It seemed to be easier for the smaller particles to penetrate the membrane barrier of the GIT. Li et al. (2012) also compared tissue distribution; Zn levels in liver, spleen, and lung were significantly higher in mice treated with nano-sized particles than micro-sized particles. In another toxicokinetic study, Baek et al. (2012) suggested that ZnONP were not readily absorbed into the bloodstream via GIT after a single oral dose, and were mainly excreted via feces. In the study, rats were treated with a single dose of 50, 300, or 2000 mg/kg and data indicated that the distribution levels in treated groups were not higher than expected compared to nontreated control because of a low absorption rate. Another study examined the effects of particle size and surface charge on the pharmacokinetics, tissue distribution, and excretion of ZnONP after a single oral dose in rats (Paek et al., 2013). Surface charge, rather than particle size, was the critical modulator of the pharmacokinetic behavior of ZnONP. ZnONP were found in feces and levels in blood were low compared to the administered doses, suggesting that absorption of ZnONP through the GIT was poor.

Apart from the oral administration, little is known regarding the toxicity and kinetics of ZnONP after intravenous (iv) injection, which is a route of distribution of NP to target organs. For the intrinsic in vivo toxicity of ZnONP, it is essential to deliver NP to target organs. This may be done by iv injection. Exposure through iv injection of ZnONP is infrequent in the workplace or from consumer products. Nevertheless, it is an important route in the aforementioned toxicokinetic studies.

The aim of this study was to investigate differences in toxicity and kinetics, including blood kinetics, tissue distribution, and excretion of ZnONP in rats after treatment by two different routes, a single iv injection and a single oral administration. The toxicities as evidenced by serum biochemistry, hematology, and histopathology of target organs were evaluated based on levels of Zn in the target organs.

## MATERIALS AND METHODS

### ZnONP

ZnONP were purchased from Sigma-Aldrich (St. Louis, MO). The NP provided by the manufacturer were dispersed in deionized water and coated with 3-aminopropyl triethoxysilane to confer a positive surface charge.

The pH of ZnONP suspensions in deionized water was consistently 7 and density was 1.7 ± 0.1 g/ml at room temperature (25°C). According to the information provided by the manufacturer, the average particle size determined by transmission electronic microscopy (TEM) was less than 35 nm. In this study, the suspension for treatment to animal was prepared in 5% glucose solution, which is a non-ionic isotonic solution. The size distribution of ZnONP in suspension was measured using a submicrometer particle size analyzer (Nicomp, Port Richey, FL) and was confirmed to be within the designated range (Figures 1 and 2). Images of NP in the vehicle were also obtained by TEM (JEM-2010, JEOL, Tokyo, Japan) (Figure 3A). Zeta potential of ZnONP suspended in 5% glucose solution was 20–35 mV.

### Animals and Treatment

Seven-week-old Sprague-Dawley male rats (body weight 200–225 g) were purchased from Orient Bio (Gyeonggi-do, Korea). Rats were acclimatized in the lab for 1 wk before initiation of the experiment. Diet and water were provided ad libitum. The animal facility was maintained at 23 ± 3°C with a relative humidity of at 55 ± 15%, air circulation 12 times/h, and a 12-h light/dark cycle (light from 8:00 to 20:00) with a light intensity of 150–300 lux.

The high dose of 30 mg/kg in this study was chosen because rats were dead after a single iv injection of 100 mg/kg in preliminary dose-finding study and the low dose 3 mg/kg was selected as one-tenth of the high dose of 30 mg/kg. The final concentration

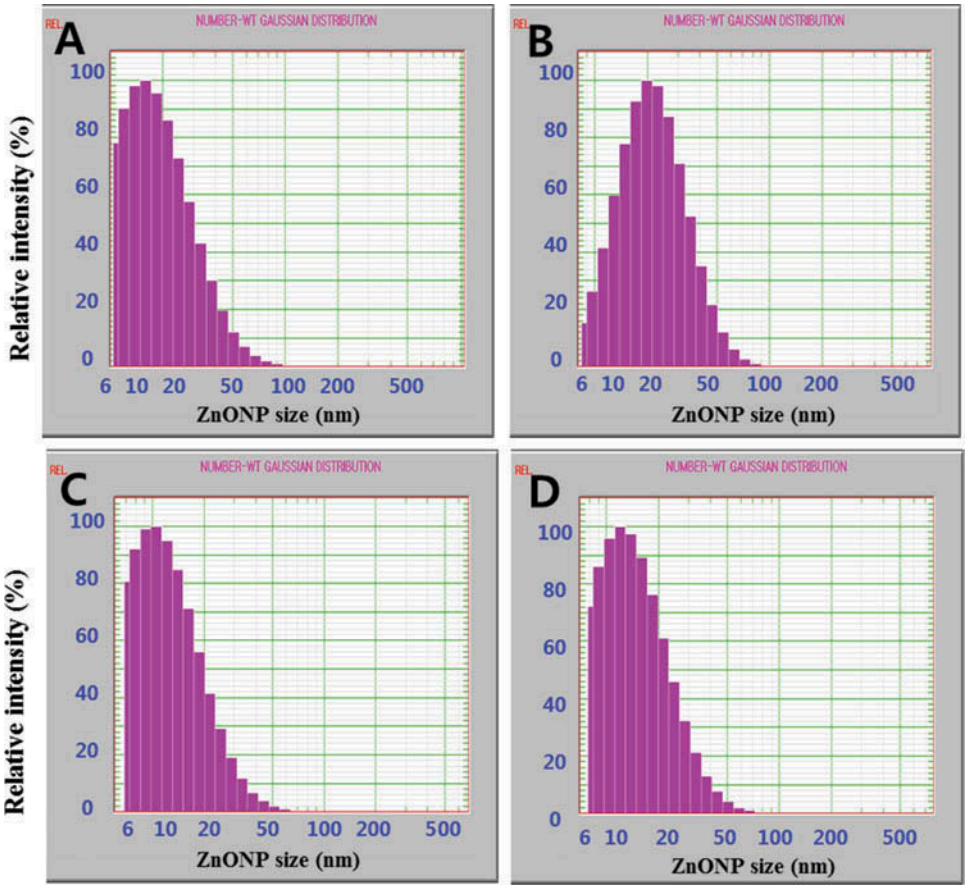


**FIGURE 1.** Size distribution of ZnONP in isotonic 5% glucose solution. Size distribution of 100–15,000 ppm ZnONP in 5% glucose solution was measured at 24 h after preparation: (A) 100 ppm, (B) 1000 ppm, (C) 10,000 ppm, and (D) 15,000 ppm. Average size of diameter was less than 35 nm, which is the estimated size provided by the manufacturer. The highest concentration of 15,000 ppm was based on the dosage of 30 mg/2 ml/kg for intravenous injection.

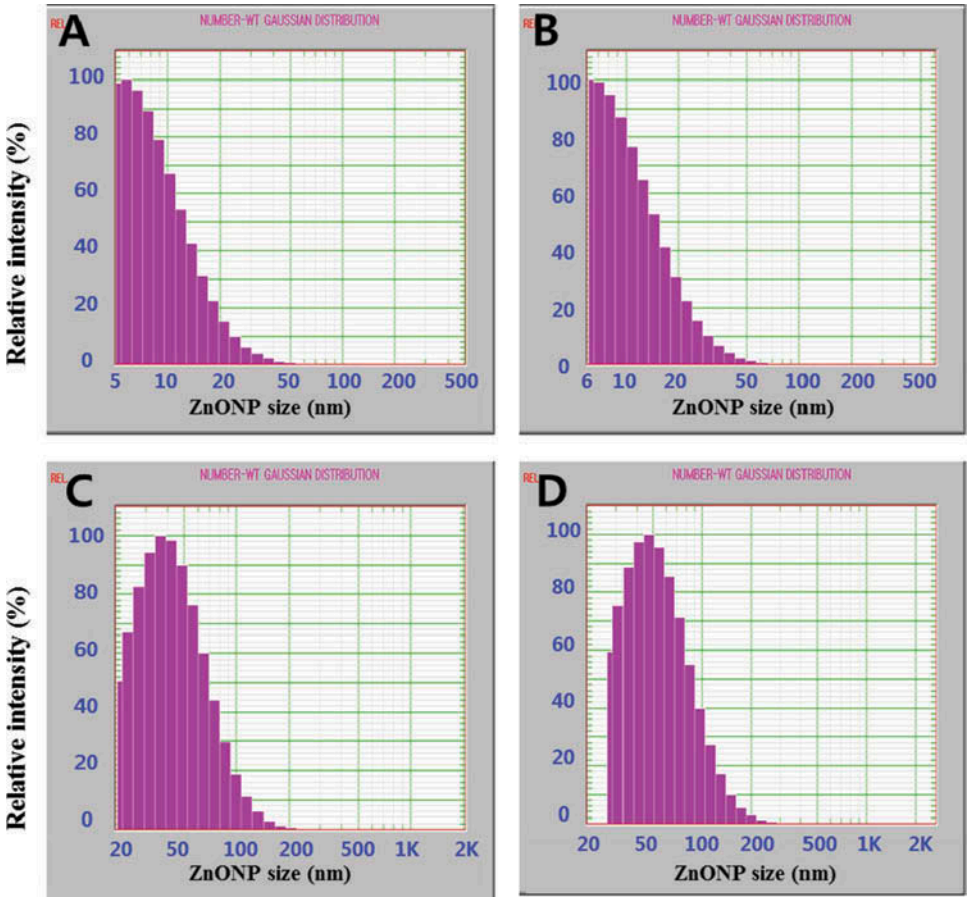


**FIGURE 2.** Size distribution of ZnONP in the mixture solution of fetal bovine serum and 5% glucose solution (9:1). Size distribution of 100–15,000 ppm ZnONP in the mixture of serum and 5% glucose solution (9:1) was measured at 24 h after preparation: (A) 100 ppm, (B) 1000 ppm, (C) 10,000 ppm, and (D) 15,000 ppm. In high concentration of 15,000 ppm, average size of ZnONPs was about 50 nm. The highest concentration of 15,000 ppm was based on the dosage of 30 mg/2 ml/kg for intravenous injection.

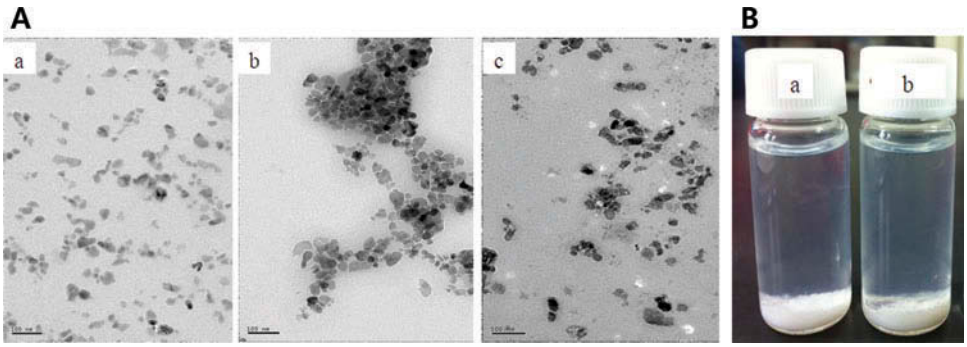


**FIGURE 3.** TEM images of ZnONP in the vehicles and photos in artificial gastric juice. (A) TEM images (400,000×) were shown in 5% glucose solution (a), in deionized water (b), and in a mixture solution (serum:5% glucose = 9:1) (c). (B) For the simulation of gastric environment after oral administration, ZnONP (10,000 ppm) were suspended in the mixture of artificial gastric juice (pH 2) and vehicle (9:1 volume). As shown in the photos, ZnONP were aggregated and precipitated within 5 min after mixing with artificial gastric juice. In the bottom of the glass vial, white precipitates were shown. Mixture of artificial gastric juice and deionized water (a), and 5% glucose solution (b).

of each ZnONP suspension was adjusted to 15 mg/ml (15,000 ppm) for the high-dose group (30 mg/kg), and was injected to rats as 2 ml/kg body weight. For the low-dose group (3 mg/kg), final concentration of ZnONP was adjusted to 1.5 mg/ml (1500 ppm) and the prepared suspension was injected to rats as 2 ml/kg body weight. Control group was treated with 5% glucose vehicle without ZnONP. In the case of oral administration, the high dose (30 mg/kg) and low dose (3 mg/kg) were also prepared in 5% glucose and applied as 10 ml/kg body weight. Generally, four rats were used in each group. All animals were cared for in accordance with the principles outlined in the "Guide for the Care and Use of Laboratory Animals" issued by the Animal Care and Committee of the National Veterinary Research and Quarantine Service (NVRQS).

### Sampling and Zn Analysis

Approximately 200 μl blood was obtained from the jugular vein of rat using heparinized tube at 0 min (before treatment), 5, 10, and 30 min, and 1, 2, 6, 12 h, as well as at 1, 2, 3, 4, 5, 6, and 7 d after oral administration or iv injection. Blood samples were also collected from nontreated controls at 0 min (before treatment) and 7 d. For biochemistry and hematological assays, blood was collected 1 d after dosing. Tissues (liver, kidneys, lung, spleen, thymus, brain, and testes) were obtained at 1 d and 7 d after treatment. Feces and urine from treated rats were collected daily for 7 d, while those of untreated control rats were collected for the first 24 h and at 7 d.

Samples were stored at –80°C before Zn analysis. The preparation of sample solutions for the analysis of Zn was made by using oxidizing acids, such as nitric acid or hydrogen peroxides. Briefly, the samples were digested in a solution of 7 ml 70% $HNO_3$ and 1 ml 30% $H_2O_2$ using a microwave digestion system (Milestone, Sorisole, Italy) with high temperature and pressure. Samples including tissues, blood, urine, and feces were completely solubilized under this condition. After diluting the acidic digested preparation with deionized water, the Zn concentration was analyzed using inductively coupled plasma–atomic emission spectroscopy (ICP-AES) using an Ultima 2 apparatus (Horiba Jobin, Yvon, France) at the Korean Basic Science Institute (Seoul, Korea). In order to verify the analytical procedures of ICP-AES, the quality control has been made by applying AccuTrace$^{T}$ Reference Standard (IS-21595, lot 210125067). All measurements were triplicate and results averaged. A procedure blank was also used to control the impurities involved during sample preparation and during ICP-AES measurement procedures.

### Toxicity Tests

All animals were examined for the onset of any immediate signs of toxicity and body weight changes were recorded for 7 continuous days. Hematology was analyzed using an Advia 2120 hematological autoanalyzer (Bayer, Whippany, NJ), including total red blood cell (RBC) count, hematocrit (Hct), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), hemoglobin distribution width (HDW), platelet count (PLT), mean platelet volume (MPV), total white blood cell (WBC) count, and differential leukocyte percentage. Serum was employed for the examination of following parameters using a model AV400 biochemistry autoanalyzer (Olympus, Tokyo, Japan): total protein (TP), albumin (ALB), albumin/globulin (A/G) ratio, total bilirubin (TBIL), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (CRE), creatinine phosphokinase (CPK), blood urea nitrogen (BUN), total cholesterol (TCHO), triglyceride (TG), glucose (GLU), calcium (Ca), and phosphorus (IP). The serum electrolytes chloride ($Cl^{-}$), sodium ($Na^{+}$), and potassium ($K^{+}$) were measured using a Rapidchem 744 Na/K/Cl ion autoanalyzer (Rapidchem, Erlarngen, Germany).

Complete gross postmortem examinations were performed on all sacrificed rats. The absolute and relative (organ-to-body weight

ratios) weights of the organs were recorded. Liver, kidney (left), lung, spleen, thymus, brain, and testes were taken from the same three parts of the organs and prefixed in 10% (v/v) neutral buffered formaldehyde for histopathological examination. The fixed tissues were trimmed, dehydrated, embedded in paraffin, sectioned, mounted on glass slides, stained with hematoxylin and eosin, and examined by light microscopy.

### Statistical Analyses

The results are presented as the mean ± standard deviation (SD). Differences in parameters (body weights, organ weights, and the results of the blood biochemistry and hematology) between treated and control group were assessed by a standard two-way analysis of variance (ANOVA). Statistical analyses of blood levels of Zn at each time point and of their distributions in organs were also performed. If statistical significance was observed, Duncan's or Dunnett's multiple-range test was used for comparative analyses among the groups using SPSS version 10.1K (SPSS, Chicago, IL). A $p$ value $<.05$ was considered statistically significant.

## RESULTS

### Size Distribution of ZnONP in 5% Glucose

Size distributions of ZnONP suspended in 5% isotonic glucose are shown in Figure 1. The average diameter of NP at a concentration of 100 ppm was 18.4 ± 10.5 nm. When the concentration was increased to 15,000 ppm, equivalent to the concentration of the high-dose in vehicle (30 mg/2 ml/kg for iv injection), the average diameter remained in the nano-size range (14.4 ± 8.1 nm), and particles were well suspended without aggregation. When NP in 5% glucose were mixed with fetal bovine serum (FBS) (1:9) to simulate the distribution of ZnONP in rat blood after iv injection, the NP were again well dispersed without aggregation, and average size ranged from 7.1 to 53.9 nm at all tested concentrations (Figure 2). TEM images of ZnONP in 5% glucose solution, in deionized water, or in a mixture of 5% glucose solution and FBS (1:9) are shown in Figure 3A. The particles were stable. However, ZnONP suspensions prepared in 5% glucose mixed with artificial gastric juice, which simulated the environment encountered after oral administration, were unstable and precipitated (Figure 3B).

### Blood Concentration of Zn After Single Treatment With ZnONP

Zn concentrations in blood after oral administration or iv injection of ZnONP are shown in Table 1. Mean blood concentrations of Zn increased significantly only in rats injected iv with the high dose: 41.07 ± 7.16 μg/ml at 5 min after injection, which was the peak level of blood Zn, and about 10-fold more than that of untreated rats before treatment (4.45 ± 0.26 μg/ml). The level rapidly decreased to 17.94 ± 2.42 μg/ml within 30 min and decreased further to that of untreated rats within l d. In all other groups, Zn levels in blood were not significantly changed compared to level before treatment. Even in rats treated with the high dose of ZnONP by oral administration, no significant changes in blood Zn were observed.

### Tissue Distribution

Tissue concentrations of Zn at d 1 and 7 after ZnONP administration orally or iv injection were determined in liver, kidneys, lung, spleen, thymus, brain, and testes. As shown in Table 2, no change in distribution was observed in tissues of rats treated following oral administration of the low and high dose. With iv injection, increased distribution was observed mainly in the high-dose group 1 d after injection in the liver, kidneys, lung, and spleen, while no change was found in thymus, brain, and testes. However, the elevated levels were not maintained until 7 d, perhaps due to excretion. In the low-dose group, Zn levels were higher only in liver. Zn levels in liver 1 d after injection were 88.22 ± 4.86 μg/g in the

**TABLE 1.** Zinc Level (μg/ml) in Blood After Oral Administration and Intravenous Injection of ZnONP ($n = 4$)

| Group | Time 0 | 5 min | 10 min | 30 min | 1 h | 2 h | 6 h | 12 h | 1 d | 2 d | 3 d | 4 d | 5 d | 6 d | 7 d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| po | | | | | | | | | | | | | | | |
| Control | 6.19 ± 0.86 | | | | | | | | | | | | | | 5.39 ± 0.52 |
| 3 mg/kg | 5.56 ± 0.85 | 4.42 ± 1.06 | 5.08 ± 1.06 | 4.82 ± 0.86 | 5.08 ± 0.92 | 4.57 ± 0.42 | 4.99 ± 0.42 | 4.02 ± 0.60 | 2.60 ± 0.07 | 2.63 ± 0.23 | 4.01 ± 0.77 | 3.40 ± 0.10 | 3.26 ± 0.10 | 3.63 ± 0.28 | 5.33 ± 1.33 |
| 30 mg/kg | 7.96 ± 2.55 | 4.81 ± 0.39 | 4.35 ± 0.45 | 5.97 ± 1.00 | 5.88 ± 0.61 | 4.57 ± 0.56 | 5.03 ± 0.81 | 4.45 ± 0.76 | 3.00 ± 0.30 | 3.13 ± 0.53 | 3.61 ± 0.26 | 3.55 ± 0.7 | 3.21 ± 0.27 | 3.98 ± 0.12 | 4.73 ± 0.27 |
| iv | | | | | | | | | | | | | | | |
| Control | 6.90 ± 1.07 | | | | | | | | | | | | | | 5.28 ± 0.26 |
| 3 mg/kg | 9.49 ± 1.15 | 9.90 ± 0.42 | 8.20 ± 0.71 | 6.98 ± 0.46 | 6.90 ± 0.72 | 6.00 ± 0.71 | 4.86 ± 0.82 | 4.39 ± 0.53 | 3.30 ± 0.29 | 1.95 ± 0.27 | 3.66 ± 0.48 | 3.42 ± 0.12 | 3.44 ± 0.39 | 4.25 ± 0.19 | 4.68 ± 0.82 |
| 30 mg/kg | 4.45 ± 0.26 | 41.07 ± 7.16 | 27.26 ± 4.00 | 17.94 ± 2.42 | 16.47 ± 2.06 | 16.24 ± 0.62 | 14.81 ± 2.01 | 10.51 ± 0.87 | 5.58 ± 0.39 | 2.91 ± 0.27 | 4.02 ± 0.25 | 3.57 ± 0.32 | 3.18 ± 0.41 | 4.95 ± 1.29 | 4.86 ± 1.88 |

**TABLE 2.** Tissue Levels of Zn (μg/g) in the Organs of Rats Treated With ZnONP by Oral Administration and Intravenous Injection ($n = 4$)

| | Liver | | Kidney | | Testis | | Spleen | | Thymus | | Lung | | Brain | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | 1 d | 7 d | 1 d | 7 d | 1 d | 7 d | 1 d | 7 d | 1 d | 7 d | 1 d | 7 d | 1 d | 7 d |
| po | | | | | | | | | | | | | | |
| Control | 26.82 ± 5.34 | 32.38 ± 1.97 | 19.25 ± 1.41 | 18.59 ± 0.24 | 17.64 ± 2.55 | 15.23 ± 0.54 | 18.65 ± 3.20 | 16.61 ± 0.64 | 13.92 ± 2.12 | 14.56 ± 0.94 | 17.75 ± 2.09 | 15.68 ± 1.35 | 9.69 ± 1.56 | 10.18 ± 2.55 |
| 3 mg/kg | 25.76 ± 1.06 | 31.07 ± 1.41 | 20.63 ± 1.46 | 16.04 ± 0.74 | 14.36 ± 0.31 | 15.53 ± 2.10 | 20.33 ± 3.34 | 19.25 ± 2.25 | 12.95 ± 1.82 | 14.43 ± 1061 | 15.82 ± 1.65 | 13.72 ± 0.52 | 9.69 ± 1.85 | 11.01 ± 0.78 |
| 30 mg/kg | 25.52 ± 2.40 | 29.84 ± 1.26 | 21.59 ± 2.07 | 17.96 ± 1.10 | 17.84 ± 1.58 | 14.81 ± 1.04 | 17.47 ± 0.50 | 15.80 ± 0.29 | 12.20 ± 0.85 | 15.19 ± 0.23 | 15.04 ± 0.73 | 15.27 ± 0.33 | 8.60 ± 0.28 | 11.69 ± 1.97 |
| iv | | | | | | | | | | | | | | |
| Control | — | 33.07 ± 4.73 | — | 20.98 ± 2.60 | — | 14.43 ± 1.86 | — | 15.93 ± 0.60 | — | 13.67 ± 1.99 | — | 15.72 ± 3.33 | — | 10.92 ± 0.23 |
| 3 mg/kg | 37.68 ± 4.01 | 29.77 ± 0.97 | 19.56 ± 1.03 | 15.27 ± 0.91 | 15.85 ± 5.67 | 14.31 ± 1.40 | 17.24 ± 1.91 | 15.85 ± 0.61 | 14.84 ± 3.13 | 15.06 ± 0.46 | 17.12 ± 3.65 | 12.59 ± 3.04 | 8.98 ± 1.60 | 12.57 ± 3.35 |
| 30 mg/kg | 88.22 ± 4.86 | 32.83 ± 0.90 | 34.63 ± 2.89 | 20.14 ± 1.36 | 17.13 ± 3.88 | 12.11 ± 0.66 | 30.51 ± 3.56 | 16.17 ± 1.64 | 13.73 ± 2.74 | 13.80 ± 3.39 | 391.23 ± 84.99 | 23.87 ± 8.17 | 8.35 ± 0.52 | 9.67 ± 1.10 |

high-dose group and 37.68 ±4.01 μg/g in the low-dose group. The 1-d Zn levels were elevated compared to untreated rats. Zn levels in liver 7 d after injection were not statistically different from vehicle treated controls, in the low-dose and high-dose groups (Table 2). In kidneys, lung, and spleen, increased distribution was found only in the high-dose group. The level of 391.23 ± 84.99 μg/g at 1 d after injection was approximately 4.4-fold greater compared to that of liver, indicating that lung may be the major target of ZnONP after injection. However, the increased distribution of Zn in lung was also cleared and decreased to 23.87 ± 8.17 μg/g, which was comparable to vehicle-treated controls.

### Excretion

Excretion of Zn in feces and urine is presented in Figures 4 and 5, respectively. Absorption of orally administered ZnONP seemed to be poor in the GIT, but they were excreted in feces. Elevated Zn (263.1 ± 60 μg/g) was detected in feces collected for 24 h of oral administration. Zn ranged from 60 to 100 μg/g in feces of untreated rats and from 67 to 115 μg/g in rats orally administered the low dose. As shown in Figure 4A, the elevated Zn levels in feces of rats treated orally with the high-dose of ZnONP returned to the normal range by 4 d after administration. In feces of rats treated by iv injection, Zn reached peak at d 3 (Figure 4B). The excretion through feces was still evident on d 6 and returned to normal at d 7. Although excretion of Zn through feces after oral administration of the low dose did not show a significant difference from the vehicle-treated control, feces excretion was significantly elevated after iv injection of the low dose. Excretion via feces after iv injection suggested the biliary excretion of ZnONP. The accumulated amount excreted in feces for 7 d was 1649.5 ± 171.6 μg/g after oral administration and 2255.9 ± 204.1 μg/g after iv injection of high-dose of ZnONP. The feces Zn levels were higher for 3 d after oral administration compared to iv injection, but feces excretion after iv injection of ZnONP consistently increased during the initial 3 d. Total excreted Zn via feces was similar between oral and iv treatment (Figure 4C). Compared to feces excretion, urine excretion was low. Urine excretion after oral administration was almost negligible (Figure 5A) and did not show any difference from vehicle treated control. When rats were treated with ZnONP by iv injection, urine excretion was observed in the high-dose group. Peak level was reached during the first 24 hr (Figure 5B). The concentration was 4.5 ± 0.7 μg/ml and total amount excreted for the first 24 h was approximately 55 μg. The total amount of excreted Zn via urine was low compared to feces. Excretion via urine was almost negligible after oral administration and 88.9 ± 1.2 μg/ml after iv injection during the whole experimental period (Figure 5C).

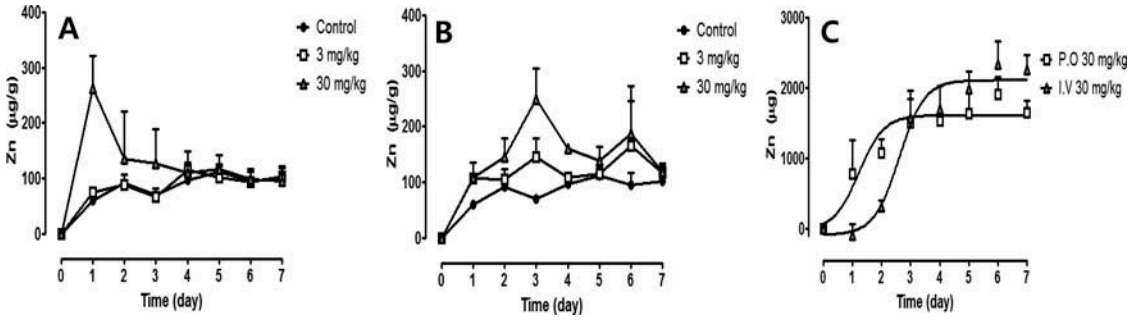


**FIGURE 4.** Concentration–time curves of zinc in feces of rats for 7 d after a single treatment of ZnONP. Feces were collected every day for 24 h from each rat in a metabolic cage ($n = 4$) and were mixed with 10 volumes of deionized water for ICP-AES analysis (1 g → 10 ml). Concentration is presented as in weight of feces and bars show standard deviation. (A) Zinc level after oral administration, (B) zinc level after intravenous injection, and (C) cumulative zinc amount excreted via feces for 7 d.

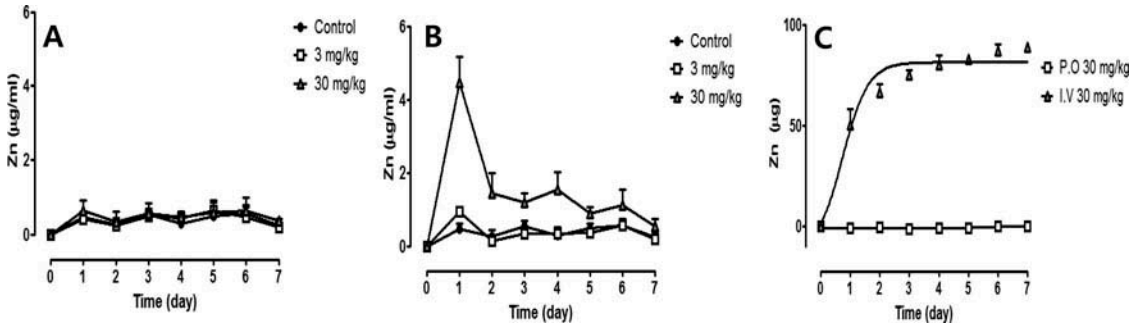


**FIGURE 5.** Concentration–time curves of zinc in urine of rats for 7 d after a single treatment of ZnONP. Urine was collected every day for 24 h from each rat in metabolic cage ($n = 4$) and were homogenized. The aliquots were sampled for ICP-AES analysis. Concentration is presented as in ml volume of urine and bars show standard deviation. (A) Zinc level after oral administration, (B) zinc level after intravenous injection, and (C) cumulative zinc amount excreted via urine for 7 d.

## General Toxicity

No deaths occurred, and no clinical symptoms, including salivation, sedation, abnormal movement, or alopecia, were evident. Differences in body weight gain were not observed. However, significant changes were found in urine volume collected for 24 h in rats receiving the high-dose of ZnONP by iv injection compared to control (Table 3). For the first day, urine volume was decreased, but rose in the second day compared to control. On the second day, urine volume was markedly lower in all groups due to unknown factor. No differences in the amount of feces were observed (data not shown).

## Hematology and Serum Biochemistry

The hematological and serum biochemical findings in rats sacrificed at 24 hr after treatment are given in Tables 4 and 5, respectively. WBC, Hb, and LYM increased in rats orally administered the high dose, but MONO and MPV decreased compared to control (Table 4). Although the rise was significant, it is not biologically relevant as values are within normal range. HDW, NEU, and EOS rose, while LYM and MONO fell in the rats treated with 30 mg/kg iv. Table 5 summarizes the serum biochemical analysis results in rats sacrificed 24 h after high-dose treatment. Significant elevation in levels of AST, CPK, TP, BUN, and CRE were observed in the iv group, while GLU and A/G decreased. However, serum biochemical changes evident at d 1 had disappeared by d 7 (data not shown). No changes in serum biochemical parameters were observed in rats orally administered ZnONP.

## Organ Weights

The absolute and relative organ weights of rats treated with ZnONP by oral or iv route

**TABLE 3.** Urine Volume (ml) of Rats After ZnONP Treatment by Intravenous Injection ($n = 4$)

| | | po | | iv | |
|---|---|---|---|---|---|
| Day | Control | 3 mg/kg | 30 mg/kg | 3 mg/kg | 30 mg/kg |
| 1 | 7.53 ± 0.86 | 6.55 ± 0.39 | 5.18 ± 3.11 | 5.37 ± 2.16 | 3.54 ± 1.91* |
| 2 | 1.08 ± 0.60 | 1.48 ± 0.85 | 2.35 ± 1.19 | 2.33 ± 1.16 | 3.66 ± 1.15* |
| 3 | 6.69 ± 2.08 | 8.32 ± 1.70 | 7.55 ± 3.44 | 9.84 ± 1.39 | 7.25 ± 2.08 |
| 4 | 6.75 ± 0.97 | 7.59 ± 3.02 | 6.34 ± 1.50 | 5.04 ± 1.66 | 4.90 ± 2.12 |
| 5 | 6.63 ± 1.98 | 5.60 ± 2.80 | 7.23 ± 2.47 | 5.50 ± 2.57 | 7.21 ± 0.82 |
| 6 | 7.55 ± 3.93 | 9.51 ± 1.08 | 9.35 ± 1.14 | 5..23 ± 3.88 | 6.49 ± 3.19 |
| 7 | 7.85 ± 1.94 | 5.55 ± 2.84 | 5.47 ± 1.77 | 6.44 ± 2.56 | 6.00 ± 1.79 |

*Significant difference at $p < .05$ compared with vehicle control.

**TABLE 4.** Hematological Data at 1 d After ZnONP Treatment (30 mg/kg) ($n = 4$)

| Tests | Units | Control | po | iv |
|---|---|---|---|---|
| RBC | $10^3/\mu l$ | 7.26 ± 0.51 | 7.73 ± 0.19 | 7.41 ± 0.13 |
| HCT | % | 44.2 ± 1.90 | 46.6 ± 1.20 | 45.0 ± 1.40 |
| HGB | g/dl | 14.1 ± 0.50 | 14.9 ± 0.30* | 14.4 ± 0.30 |
| MCV | fl | 61.1 ± 1.80 | 60.3 ± 1.00 | 60.7 ± 1.60 |
| MCH | pg | 19.5 ± 0.80 | 19.3 ± 0.20 | 19.5 ± 0.30 |
| MCHC | g/dl | 31.8 ± 0.30 | 32.1 ± 0.40 | 32.1 ± 0.70 |
| RDW | % | 11.5 ± 0.30 | 11.4 ± 0.30 | 11.7 ± 0.30 |
| HDW | g/dl | 1.92 ± 0.02 | 1.94 ± 0.06 | 2.20 ± 0.11* |
| RET | % | 3.80 ± 0.90 | 3.06 ± 0.24 | 4.23 ± 0.47 |
| PLT | $10^3/\mu l$ | 1242.80 ± 133.00 | 1274.30 ± 219.80 | 999.50 ± 76.00 |
| MPV | fl | 5.80 ± 0.20 | 4.9 ± 0.10* | 6.40 ± 0.60 |
| WBC | $10^3/\mu l$ | 8.94 ± 0.41 | 11.26 ± 1.57* | 9.81 ± 1.05 |
| NEU | % | 9.40 ± 3.50 | 6.50 ± 1.70 | 30.3 ± 1.90* |
| LYM | % | 84.9 ± 3.00 | 89.4 ± 2.00* | 66.1 ± 1.30* |
| MONO | % | 4.73 ± 1.26 | 3.08 ± 0.48* | 2.08 ± 0.64* |
| EOS | % | 0.28 ± 0.10 | 0.33 ± 0.10 | 0.60 ± 0.22* |
| BASO | % | 0.18 ± 0.05 | 0.23 ± 0.05 | 0.18 ± 0.05 |
| LUC | % | 0.58 ± 0.05 | 0.53 ± 0.05 | 0.73 ± 0.17 |

*Significant difference at $P<0.05$ compared with vehicle control.

**TABLE 5.** Serum Biochemistry Data at 1 d After ZnONP Treatment (30 mg/kg) ($n = 4$)

| Tests | Units | Control | po | iv |
|---|---|---|---|---|
| AST | U/L | 71.80 ± 12.90 | 72.10 ± 4.00 | 140.10 ± 13.40* |
| ALT | U/L | 38.70 ± 3.30 | 37.10 ± 2.50 | 37.80 ± 3.60 |
| ALP | U/L | 205.30 ± 18.90 | 194.80 ± 33.70 | 212.40 ± 6.00 |
| CPK | U/L | 240.00 ± 52.90 | 225.00 ± 37.40 | 581.80 ± 73.00* |
| TBIL | mg/dl | 0.14 ± 0.01 | 0.13 ± 0.01 | 0.16 ± 0.01 |
| GLU | mg/dl | 153.50 ± 16.30 | 145.9 ± 8.50 | 87.00 ± 11.20* |
| TCHO | mg/dl | 93.50 ± 2.40 | 76.80 ± 13.20 | 97.80 ± 17.60 |
| TG | mg/dl | 42.00 ± 5.10 | 42.00 ± 7.00 | 45.50 ± 9.00 |
| TP | g/dl | 5.09 ± 0.08 | 5.13 ± 0.28 | 5.51 ± 0.12* |
| ALB | g/dl | 3.00 ± 0.04 | 2.99 ± 0.14 | 2.98 ± 0.05 |
| A/G | ratio | 1.45 ± 0.07 | 1.40 ± 0.03 | 1.18 ± 0.04* |
| BUN | mg/dl | 9.35 ± 1.30 | 9.00 ± 3.30 | 15.70 ± 4.20* |
| CRE | mg/dl | 0.19 ± 0.08 | 0.27 ± 0.08 | 0.36 ± 0.04* |
| IP | mg/dl | 8.18 ± 0.18 | 8.52 ± 0.25 | 8.19 ± 0.22 |
| $Ca^{2+}$ | mg/dl | 9.81 ± 0.40 | 9.92 ± 0.33 | 10.19 ± 0.15 |

*Significant at $p < .05$ compared with vehicle control group.

are presented in Table 6. The absolute or relative weights of the kidneys, spleen, and lung were increased only in 30 mg/kg-treated rats 1 d after iv injection but not in the orally treated group. These changes correlated with Zn levels in blood or tissues. Relative weight of liver was decreased both in orally treated and iv-treated rats, but an absolute fall in liver weight was shown only in iv treated rats. All weight changes disappeared by d 7 (data not shown).

## Necropsy and Histopathological Findings

A histopathological examination was performed in the control group and the high-dose group. A summary of the histological findings is presented in Table 7. In liver, increased mitotic figures were observed in all 4 rats treated iv (4/4), but no specific lesion was found in controls (Figure 6). Infiltration of mononuclear cells was minimally in the liver of 1 of 4 rats using either route of ZnONP administration (1/4), and in 1 rat in the vehicle-treated control

**TABLE 6.** Organ Weight of Rats at 1 d After Treatment of ZnONP (30 mg/kg) ($n = 4$)

| Parameters | Control | po | iv |
|---|---|---|---|
| Body weight (g) | 197.84 ± 7.58 | 196.38 ± 8.52 | 194.59 ± 6.22 |
| Kidney (L), abs | 0.73 ± 0.06 | 0.72 ± 0.05 | 0.78 ± 0.04* |
| Kidney (L), rel | 0.37 ± 0.02 | 0.37 ± 0.01 | 0.40 ± 0.02 |
| Kidney (R), abs | 0.73 ± 0.04 | 0.72 ± 0.04 | 0.76 ± 0.04 |
| Kidney (R), rel | 0.37 ± 0.01 | 0.37 ± 0.01 | 0.39 ± 0.02* |
| Liver, abs | 7.59 ± 0.19 | 7.14 ± 0.42 | 6.35 ± 0.17* |
| Liver, rel | 3.84 ± 0.09 | 3.63 ± 0.06* | 3.26 ± 0.04* |
| Thymus, abs | 0.44 ± 0.03 | 0.40 ± 0.03 | 0.46 ± 0.06 |
| Thymus, rel | 0.22 ± 0.01 | 0.20 ± 0.01 | 0.24 ± 0.03 |
| Testis (L), abs | 1.47 ± 0.07 | 1.47 ± 0.03 | 1.40 ± 0.08 |
| Testis (L), rel | 0.74 ± 0.01 | 0.75 ± 0.03 | 0.72 ± 0.03 |
| Testis (R), abs | 1.43 ± 0.13 | 1.46 ± 0.02 | 1.42 ± 0.10 |
| Testis (R), rel | 0.72 ± 0.04 | 0.75 ± 0.03 | 0.73 ± 0.04 |
| Spleen, abs | 0.49 ± 0.05 | 0.49 ± 0.09 | 0.63 ± 0.05* |
| Spleen, rel | 0.25 ± 0.03 | 0.25 ± 0.04 | 0.32 ± 0.03* |
| Lung, abs | 10.5 ± 0.05 | 1.09 ± 0.04 | 1.27 ± 0.05* |
| Lung, rel | 0.53 ± 0.02 | 0.56 ± 0.03 | 0.66 ± 0.03* |
| Brain, abs | 1.63 ± 0.08 | 1.64 ± 0.05 | 1.55 ± 0.08 |
| Brain, rel | 0.83 ± 0.04 | 0.84 ± 0.03 | 0.80 ± 0.06 |

*Note.* Relative, rel; absolute, abs. L, left; R, right.
*Significant difference at $P < 0.05$ compared with vehicle control.

**TABLE 7.** Histopathological Findings in Rats at 1 d After Treatment of ZnONP (30 mg/kg)

| Organs | Findings | Control | po | iv |
|---|---|---|---|---|
| Liver | No specific lesion | 3 | 3 | 0 |
| | Mitotic figures, increased | | | |
| | Minimal | | | 3 |
| | Mild | | | 1 |
| | Infiltration, mononuclear cells, multifocal (minimal) | 1 | 1 | 1 |
| Spleen | No specific lesion | 4 | 4 | 4 |
| Kidney | No specific lesion | 0 | 0 | 0 |
| | Basophilic tubules, multifocal (minimal) | 3 | 1 | 1 |
| | Focal nephropathy (minimal) | 3 | 1 | 1 |
| | Hyaline nephropathy, (multi)focal (minimal) | | | 2 |
| | Focal glomerulonephropathy, focal (minimal) | 1 | | 1 |
| | Hyaline cast, (multi)focal (minimal) | 2 | | 1 |
| | Tubular dilatation and fibrosis, focal (minimal) | | 1 | 1 |
| Lung | No specific lesion | 4 | 4 | |
| | Black deposit in alveolar capillaries with/without acute injury | | | 4 |
| Brain (cerebrum and cerebellum) | No specific lesion | 4 | 4 | 4 |
| Thymus | No specific lesion | 4 | 4 | 4 |
| Testis | No specific lesion | 4 | 4 | 4 |

*Note.* The number indicates the number of rats showing positive findings. Total number of rats is 4.

group (1/4). In the kidney, multifocal basophilic tubules, focal nephropathy, hyaline nephropathy, focal glomerulonephropathy, hyaline cast, tubular dilation, and fibrosis were observed randomly in treated and nontreated rats. Abnormal changes seemed to be more frequent in rats receiving iv injection (Figure 7). The serum biochemistry of increased BUN and CRE in the iv-treated group supports histological changes in kidney (Table 5). In lung, black deposits in alveolar capillaries were noted in rats iv injected with ZnONP, while no deposits were evident in the orally treated and nontreated control groups. Sites with the black deposits showed acute injury of lung cells (Figure 8). TEM of liver tissue from control and

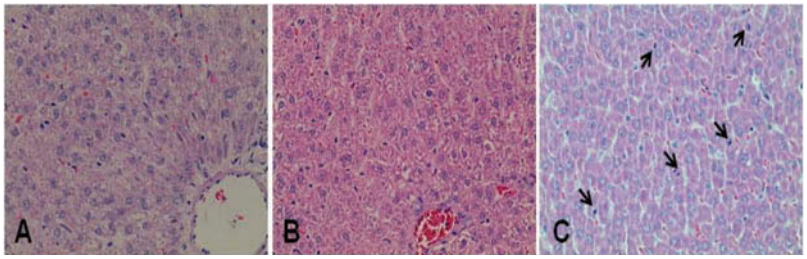


**FIGURE 6.** Histopathological changes in liver of rats treated with ZnONP. Rats were treated with 30 mg/kg-ZnONP by oral administration and intravenous injection, respectively. Rats were sacrificed at 1 d after treatment. Hematoxylin and eosin were used for histological staining (×200). (A) Vehicle-treated control group, (B) orally treated group, and (C) intravenously injected group. Arrows show increased mitotic figures in intravenously ZnONP-treated rats.

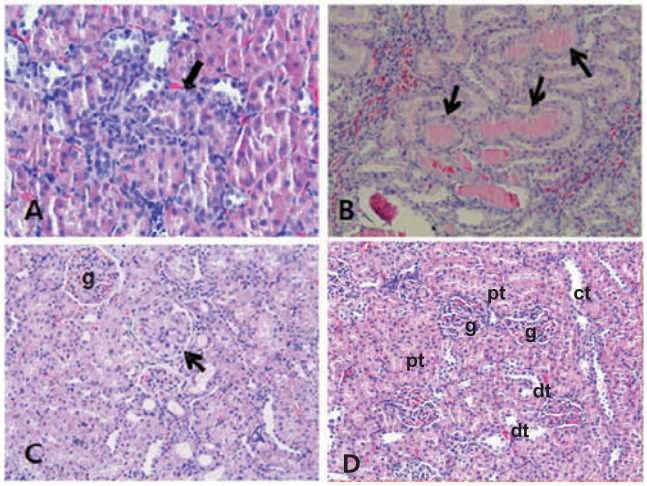


**FIGURE 7.** Histopathological changes in kidney of rat treated with ZnONP. Rats were treated with 30 mg/kg ZnONP by oral administration and intravenous injection, respectively. Rats were sacrificed at 1 d after treatment. Hematoxylin and eosin were used for histological staining. (A) Arrows show the focal nephropathy in orally treated group (400×). (B) Arrows show hyaline nephropathy in intravenously treated group (200×). (C) Arrow shows glomerulonephropathy in intravenously injected group (200×). (D) Vehicle-treated control group (200×). g, Glomerulus; pt, proximal tubule; dt, distal tubule; ct, collecting tubule.

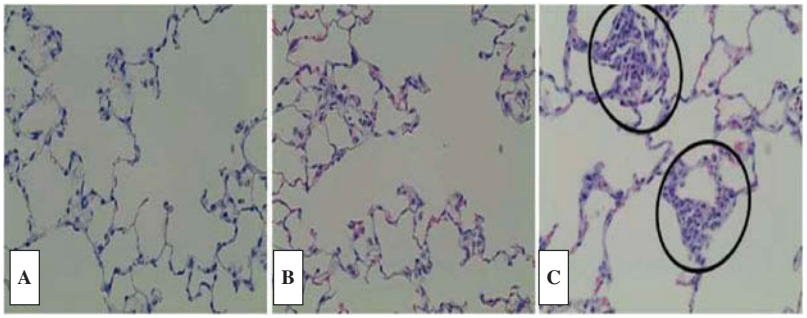


**FIGURE 8.** Histopathological changes in lung of rat treated with ZnONP. Rats were treated with 30 mg/kg ZnONPs by oral administration and intravenous injection, respectively. Rats were sacrificed at 1 d after treatment. Hematoxylin and eosin were used for histological staining (×200). (A) Vehicle treated control group. (B) Orally treated group. (C) Intravenously injected group. Multifocal acute injuries with dark brown pigment were noted (circle in C).

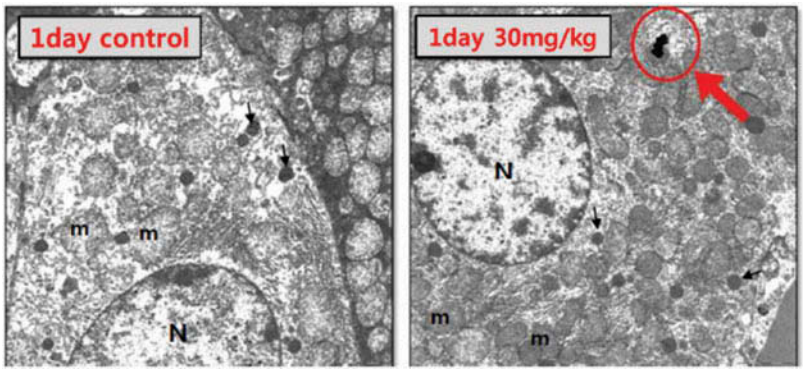


**FIGURE 9.** The ZnONP image of transmission electron microscopy (TEM) in the liver of treated rats. Rats were sacrificed at 1 d after 30 mg/kg ZnONP treatment by intravenous injection. Black spot indicated by a circle with thick arrow was observed in the liver tissue, and may be an aggregate of the nanoparticles. Spots were not found in vehicle-treated control group. 8000×; N, nucleus; m, mitochondria; arrows, microbodies.

iv injected with the high dose are depicted in a representative image (Figure 9). Black spots evident in the treated group may have represented aggregates of ZnONP. Many black spots were observed in the liver. The black spots seemed to be ZnONP but were not chemically identified in this study.

## DISCUSSION

Recently, a few in vivo toxicity tests of ZnONP showed that toxicity appeared only in relatively high-dose groups when animals were treated by oral administration (Pasupuleti et al., 2012; Sharma et al., 2012). Other exposure routes including skin or inhalation produced different results according to the exposure routes (Ho et al., 2011; Surekha et al., 2012). When mice were treated orally with 50 or 300 mg/kg ZnONP once a day for 14 d, ZnONP accumulated in liver and led to cellular damage. ALT and ALP serum levels were increased and pathological lesions were observed in the liver. Lipid peroxidation and DNA damages were also produced by ZnONP. The toxicity was noted in mice treated with a high dose (300 mg/kg), but not with a low-dose (50 mg/kg) (Sharma et al., 2012). Compared to the toxic chemicals, the dose of 300 mg/kg is a relatively high dose based on a 14-d repeated toxicity test. In another study, mice were treated with 500, 1000, or 2000 mg/kg per day ZnONP for 14 d by oral administration, and serum biochemistry, hematology, and histopathological analysis were performed (Patra et al., 2012). No significant changes were found in LDH, CRE, ALP, total protein cholesterol, TG, uric acid, and phosphorus in treated mice. Slight swelling in the renal glomerulus was observed only in 2000-mg/kg-treated mice, which also uniquely displayed an irregular array of veins, loss of sinusoid, and hydrophobic degeneration with fatty liver. The no-observed-adverse-effect level (NOAEL) was 1000 mg/kg. In another study, mice were treated with 333.3 mg/kg of ZnONP for 5 consecutive days by oral gavage. NP produced hepatic injury including necrosis and other pathological observations (Esmaeillou at al., 2013). When ZnONP were orally administered to Sprague-Dawley rats at doses of 67.1, 134.2, 264.8, or 535.8 mg/kg per day for 13 wk, body weight gain was significantly lower only in rats receiving the highest dose compared to control. Mild to moderate pancreatitis also developed in this group. The NOAEL of ZnONP was 268.4 mg/kg per day (Seok et al., 2013). Baky et al. (2013) reported that oral administration of ZnONP (600 mg/kg or 1000 mg/kg per day) for 5 consecutive days induced inflammation, DNA damage, and apoptosis in rat heart, while co-administration of vitamin E was cardioprotective. Reverse dose-dependent toxicity induced by ZnONP was also reported in rats treated orally with 5, 50, 300, 1000,

or 2000 mg/kg (Pasupuleti et al., 2012). According to the TOXNET database, the $LD_{50}$ of zinc is 630 mg/kg when rats are orally treated. It seemed that ZnONP are less toxic compared to Zn after oral administration.

As shown in previous studies, it is common that orally administrated ZnONP induced toxicity at a relatively high dose level. The NOAEL was 1000 or 264.8 mg/kg according to the dosing and toxicity endpoint (Esmaeillou at al., 2013; Seok et al., 2013). Presently, toxicity tests by other routes except oral administration have not been frequently performed. Instead, toxicokinetics or distribution studies after oral administration of ZnONP were investigated. The results from previous studies suggested that bioavailability of ZnONP after oral administration is low. Baek et al. (2012) orally administered rats with ZnONP doses up to 2000 mg/kg; $C_{max}$ was only 179.53 ± 12.62 μg/ml at the highest dose. Since no iv treatment was performed, bioavailability of ZnONP was not calculated in the study. However, it is certain that most administered ZnONP might not be absorbed, but rather excreted via feces, so that toxicity after oral treatment was low. In a study of comparative toxicokinetics between titanium dioxide and ZnONP after repeated oral administration (Cho et al., 2013), ZnO showed higher absorption than titanium dioxide. Repeated oral administration was carried out with 134.2, 268.4, or 536.8 mg/kg ZnONP for 13 wk. Although Zn levels were higher than titanium levels, the approximate levels of 5 μg/g in blood and 60–805 μg/g in liver were low based on the repeated high dosing regimen of ZnONP for 13 wk. Data indicate that the relatively low toxicity of ZnONP after oral administration may reflect low bioavailability. However, inhalation of ZnONP (3.5 $mg/m^3$, 4 h/d) showed an elevated concentration of $Zn^{2+}$ in bronchoalveolar lavage fluid and lung to induce inflammatory responses (Adamcakova-Dodd et al., 2014). Following in vivo nasal exposure of rats to ZnONP, the presence of NP was found in brain, which suggested an olfactory bulb–brain translocation (Kao et al., 2012).

Investigations to compare the toxicities produced by different exposure routes based on the bioavailability have not been performed. In our study, rats were treated by two different routes, oral administration and iv injection, to clarify the relationship between bioavailability and toxicity of ZnONP. A high dose of 30 mg/kg was selected based upon prior knowledge that iv injection of 100 mg/kg was lethal. ZnONP were suspended in 5% glucose, which is isotonic and biocompatible in blood. Glucose is a reliable biocompatible dispersant for citrate-coated silver NP and is the vehicle used for iv injection (Park and Lee, 2013). According to the information provided by the manufacturer, the average size of NP was less than 50 nm. When NP were suspended in 5% glucose, no aggregation occurred and nanosize was always maintained (Figures 3A). This would be suitable for the toxicokinetic or toxicity study by exposure route of iv injection.

As shown in Table 1, no change in Zn levels compared to control group were observed when rats were treated orally. The low absorption may result from precipitation in the acidic condition of the stomach because positive charge of ZnONP could be disturbed at low pH. In other studies (Baek et al., 2012; Cho et al., 2013), rats were treated with high doses of ZnONP, (2000 mg/kg or 536.8 mg/kg, respectively), where elevated Zn levels in blood were evident. In our study, the lack of elevated blood Zn levels may reflect the single oral treatment with the relatively low dose of 30 mg/kg. However, the blood Zn levels after a single iv injection of 30 mg/kg was higher than previously reported (Cho et al., 2013) where rats were repeatedly orally administered with 536.8 mg/kg, but is comparable to Baek et al. (2012) where the highest dose was 2000 mg/kg. In this study, Zn level was measured in blood, not in plasma. When plasma was separated from blood sample, NP may be precipitated by the centrifugal force. Then, plasma concentration of NP may be measured incorrectly, which is the basis for Zn measurement in blood and not plasma.

No alterations were presently observed in all tissues tested following oral treatment

(Table 1). However, Zn levels were significantly elevated in liver, kidneys, spleen, and lung 1 d after iv injection. The highest distribution was observed in lung followed by liver. Zn levels in lung were about 4.4-fold higher than liver and 11-fold higher than kidneys and spleen. This is consistent with a scenario in which NP injected through the tail vein reach the lung and are captured in lung macrophages or other cells. The distributed ZnONP seemed to be excreted before d 7 because the elevated Zn levels in lung, liver, kidney, and spleen returned to control levels. The decrease of Zn level in tissues was attributed to the increase of Zn excretion via biliary excretion to feces (Figure 4C). The ZnONP dose of 3 mg/kg seemed to be too small to detect kinetically, even with iv injection. Zn was only numerically elevated in blood and liver in rats treated iv with 3 mg/kg ZnONP.

Urine excretion of Zn was not detected after oral administration (Figure 5), due to the low level of Zn in blood or in tissue, which was due to low absorption rate after oral administration. When rats were treated by the oral route, the excretion via feces was significant (Figure 4). In the urine of rats treated with ZnONP by iv injection, Zn was elevated. This finding differed from the groups treated by oral administration. Zn levels in urine were higher compared to the control throughout the experimental period in the iv-treated group. However, total excreted Zn in urine after iv injection (up to d 7) was relatively scant compared to total excreted Zn in feces. Excretion via feces after iv injection indicates biliary excretion of ZnONP. As shown in Figures 4B and 4C, total Zn in feces after iv injection was significantly increased until d 4 and reached almost the same level or a higher level compared to the level after oral administration. This indicates iv-injected Zn was excreted through the biliary excretion pathway. Calculation of mass balance is important in toxicokinetic study. If 30 mg/kg of ZnONP was administered to a 250-g rat, about 7.5 mg ZnONP (7.5 mg ZnO equals 6 mg Zn) was administered to a rat. As shown in Figure 4, accumulated excretion of Zn was about 1.6 mg in feces and 2.3 mg in urine, respectively. It was 27% and 38% of total administered Zn, respectively. The rest of the undetected Zn should be measured in the rat but the mass balance needs further effort.

Based on previous results, low bioavailability and feces excretion were expected after oral administration, and urine excretion and biliary excretion to feces were expected after iv injection. The bioavailability of ZnONP may affect in vivo toxicity. Thus, the toxicities of ZnONP were compared after treatment by two different exposure routes, a single oral administration and a single iv injection. No death was found in all groups due to ZnONP. Urine volume was changed only in the iv high-dose-treated group (Table 4). Data suggest kidney damage by ZnONP. This interpretation is supported by increased concentration of BUN and CRE (Table 5). However, the results of unexpected up- and downregulation in urine volume during the early phase of d 1 and d 2 may not be explained in this study. Regardless of the urine volume, kidney damage by histopathological analysis was also noted in 30-mg/kg-treated rats following iv injection. As shown in Table 5, biomarkers for liver damage including AST level were elevated only in iv high-dose-treated rats. The liver damage was also detected by histopathological analysis (Figure 6). CPK rise may support damage to muscles, including the heart. Zn is essential in insulin action, which is important in the metabolism of glucose. It is interesting that blood glucose levels significantly decreased following iv injection of high-dose ZnONP (Table 6). The effect of ZnONP on the insulin action or glucose metabolism was not clearly elucidated in this study, but ZnONP may increase glycolysis because no change in diet consumption was observed in the high-dose iv group. ZnONP showed antidiabetic activity in streptozotocin-induced type 1 and type 2 diabetic rats (Umrani and Paknikar, 2014). In that study, oral administration of ZnONP improved glucose tolerance, increased serum insulin level (70%), reduced blood glucose (29%), lowered nonesterified fatty acids (40%), and decreased TG (48%).

All the abnormal signs of ZnONP were observed only in rats iv administered the high

dose, and these disappeared by d 7, which correlated with Zn levels in blood and tissues (Tables 1 and 2). The changes in absolute or relative weights were also mainly evident in the iv group (Table 6). A summary of the histopathological analyses (Table 7) indicates that most abnormalities were in the iv group, which also supports the relationship between elevated level of ZnONP and observed toxicities. No deposit of ZnONP was observed in brain, testes, and thymus. Another report on the distribution of ZnONPs also showed an absence of deposits in brain and testis, even in rats orally treated with a high dose of 2000 mg/kg (Baek et al., 2012). In the latter study, Zn levels increased about twofold in liver and maximally sevenfold in lung after an oral treatment with 2000 mg/kg. In our study, Zn levels rose by about threefold in liver and more than 20-fold in lung after iv injection of 30 mg/kg, although the treated dosage of NP was 1.5%. This comparison may suggest the low absorption rate of ZnONP in GIT. Further, most toxicity tests after oral administration need to be considered based on systemic concentration of ZnONP after treatment. Evidence indicates low risk of ZnONP after oral exposure compared to other exposure routes.

In summary, this study provides information on the toxicokinetics of ZnONP and their toxicities. Rats were treated with ZnONP by two different exposure routes, oral administration and iv injection. Zn was elevated in blood and tissues including liver, lung, kidneys, and spleen only in the group treated iv with a dose of 30 mg/kg. ZnONP were not absorbed readily from GIT but were excreted via feces. The iv injected ZnONP were excreted in urine but the main excretion was found to be by biliary route and then feces. Toxicity determined by serum biochemistry and histopathological analysis occurred mainly in the rats treated iv with a high dose of ZnONP.

## FUNDING

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