

Comparison of Acute Toxicity of ZnO and Silica-coated ZnO Nanoparticles in Mice after Single Intravenous Injection: Preliminary Experiment to Apply to Biological Imaging

Miki TONGU¹⁾, Hideki HASHIMOTO²⁾, Takaya YAMADA³⁾, Kaori KIMURA-KATAOKA⁴⁾, Toshihiro YASUDA⁵⁾, Hideo AKIYOSHI⁶⁾, Yasuhisa FUJITA⁷⁾, Haruo TAKESHITA⁴⁾ and Junko FUJIHARA⁴⁾

¹⁾*Shin-yamanote Hospital, Higashi-murayama 189-0021, Japan*

²⁾*Organization for the Promotion of Project Research, Shimane University, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan*

³⁾*Department of Experimental Animals, Center for Integrated Research in Science, Shimane University Faculty of Medicine, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan*

⁴⁾*Department of Legal Medicine, Shimane University Faculty of Medicine, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan*

⁵⁾*Division of Medical Genetics and Biochemistry, Faculty of Medical Sciences, University of Fukui, 23-3 Matsuoka Shimoaizuki, Eiheiji-cho, Yoshida-gun, Fukui 910-1193, Japan.*

⁶⁾*Shimane University, Faculty of Life and Environmental Science, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan*

⁷⁾*Shimane University, Interdisciplinary Graduate School of Science and Engineering, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan*

(Received September 11, 2014; Accepted October 23, 2014)

In the present study, acute toxicity of ZnO NPs and silica-coated ZnO NPs following intravenous injection using mice were evaluated. ZnO dispersion (average size: 148 nm) at a dose of 10 mg/kg and 30 mg/kg, and a Silica-coated ZnO (ZnO-SiO₂) at a dose of 10 mg/kg, 30 mg/kg and 130 mg/kg were injected intravenously via tail vein of mouse. All mice ($n = 6$) died within 5 min following intravenous ZnO dose of 10 mg/kg and 30 mg/kg. Impaired motility and convulsion were observed before the death. On the other hand, silica-coating can reduce the toxicity. All mice survived and impaired motility was not observed following intravenous ZnO-SiO₂ (average size: 81 nm) treatment at the same dose of ZnO NPs. At a dose of 130 mg/kg, impaired motility and convulsion was observed

in all mice, one mouse out of 5 mice died after 5 min. Blood coagulation was observed in all died mice, which may be related to the toxicity of ZnO NPs.

Key words: Acute toxicity, Bio-imaging, Nanoparticles, Intravenous injection, ZnO, Silica-coated ZnO

INTRODUCTION

Zinc oxide (ZnO) is a well-known optoelectronic semiconductor material and is applied in light-emitting devices [1] and electron emitters [2]. ZnO nanoparticles (NPs) have been widely used in various commercial products such as sunscreen, antibacterial reagents, rubber additives, paints, and pigments [3]. As ZnO has a wide band gap of 3.37 eV and large excitation binding energy, it ensures efficient UV-blue emission at room temperature and is suitable for bio-imaging applications [4]. Because Zn is essential element, ZnO is considered to be material with low toxicity. Therefore, ZnO NPs are expected to apply to cancer diagnosis and therapy used as a drug delivery carriers. Senthilkumar *et al.* have reported good quality ZnO NPs that disperse in water

Correspondence: J. Fujihara, Ph. D.

Department of Legal Medicine, Shimane University Faculty of Medicine, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan

Tel: +81-853-20-2157

Fax: +81-853-20-2155

E-mail: jfujihar@med.shimane-u.ac.jp

Abbreviation: NPs; Nanoparticles, (ZnO; Zinc oxide)

and organic solvents by a different surface treatment procedure for biomedical applications [5]. Sato *et al.* have successfully prepared non-cytotoxic and visible light-emitting ZnO NPs fluorophores as probes with binding sites to biomolecules on the surface for biological imaging [6, 7]. Although ZnO NPs are expected to apply to wide variety of life science area, no data is so far available on the toxicity of these materials *in vivo*.

To our knowledge, no report is available on ZnO NPs toxicity following intravenous administration. Silica coating is used to increase dispersibility of ZnO NPs for water or saline [8]. Thus, toxicity evaluation of silica-coated ZnO NPs following intravenous injection using mice was also performed in the comparison of ZnO NPs.

MATERIALS AND METHODS

Materials

ZnO NPs were fabricated using a gas evaporation method as reported previously [5]. A Zn metal was melted and evaporated by the arc discharge between a carbon cathode and a Zn metal anode, and the ZnO NPs were formed by the reaction of evaporated zinc with oxygen in the air. The fabrication of ZnO NPs were performed under conditions of arc current of 30 A, the pressure of 81 kPa, and the gas flow rate of 5 L/min, respectively.

To prepare ZnO NPs dispersion, ZnO particles of 0.1 g were put into a 2 mL microtube with ultrapure water of 1 mL and 0.1 mm diameter zirconia beads of 3 g, and ZnO particles was dispersed by shaking for 2 hours by using a homogenizer CD-1000 (EYELA, Tokyo, Japan) at 2500 rpm. The dispersions were then centrifuged at 3000 g for 1 min at room temperature in order to remove zirconia beads and large/aggregated particles from the dispersion. The supernatant was collected, and the ZnO concentration was determined by its absorbance at 370 nm using Ultra-violet and visible spectrophotometer UV-3600 (SHIMADZU, Kyoto, Japan).

Silica-coated ZnO NPs (ZnO-SiO₂) were prepared by coating the ZnO NPs as mentioned above with silica derived from tetraethoxysilane (TEOS: Si (OC₂H₅)₄) [9]. ZnO NPs of 0.1 g were dispersed in ethanol (KANTO CHEMICAL, Tokyo, Japan) of

20 mL by using an ultrasonic homogenizer VCX-500 (SONICS & MATERIALS, Newtown, CT, USA). The dispersions were centrifuged at 3000 g for 1 min at room temperature in order to remove large/aggregated particles. ZnO dispersion of 12.5 mL was put into 30 mL vial container with magnet stirrer, and 28 % aq-NH₄OH (KANTO CHEMICAL, Tokyo, Japan) of 0.1 mL, ultrapure water of 0.9 mL, and TEOS (KANTO CHEMICAL, Tokyo, Japan) of 1 mL were added into the ZnO dispersion, and the mixture was stirred for 24 hours at room temperature. Following centrifugation, the precipitate was washed with ethanol and ultrapure water and was dispersed in saline by using an ultrasonic homogenizer. The average size of prepared particles was analyzed by the dynamic light scattering particle size analyzer LB-550 (HORIBA, Kyoto, Japan).

Animal experimental procedures

Twenty-four female 7-week-old ICR mice (B.W. 27.0-34.2 g) purchased from CLEA Japan, Incorporated (Tokyo, Japan) were allowed to acclimatize to their environment for 1 week before treatment. A ZnO dispersion in doses of 10 mg/kg and 30 mg/kg and ZnO-SiO₂ in doses of 10 mg/kg, 30 mg/kg, and 130 mg/kg were injected intravenously via tail vein. Doses of 30 mg/kg for ZnO and 130 mg/kg for silica-coated ZnO NPs are the maximum dose that can be dispersed. For good dispersion, ultrapure water was used in ZnO, though saline was used in silica-coated ZnO NPs. The summary of treatments for each group is described in Table 1. The experimental protocols were approved by the Shimane University Animal Experimental Committee.

Statistical analysis

Survival distributions were compared by the Log-Rank test utilizing χ^2 analysis in SPSS IBM 19 (IBM, Armonk, New York).

RESULTS

Intravenous single dose of ZnO NPs

The average size of ZnO NPs used in the experiment was 148 nm (Fig. 1A). Mice died within 5 min following intravenous ZnO doses of 10 mg/kg and 30 mg/kg (Table 1, Fig. 2). The dose-

effect relationship was observed regarding mortality with ZnO NPs administration. Impaired motility and convulsion were observed before mouse death. The mice were immediately dissected, and the taking of blood was attempted. However, the blood could not be taken due to coagulation.

The average size of ZnO-SiO₂ NPs used in the experiment was 81 nm (Fig. 1B). Following intravenous ZnO-Silica NPs doses of 10 mg/kg and 30 mg/kg, all mice survived, and impaired motility was not observed. At a dose of 130 mg/kg, impaired

motility and convulsion were observed in all mice; one mouse died after 5 min (Table 1, Fig. 3). As with the ZnO NPs treatment, blood could not be taken due to coagulation in the dead mouse. Impaired motility and piloerection were observed in four surviving mice after a dose of 130 mg/kg. The health status of surviving mice was monitored until 2 weeks following the treatment. Abnormality in appearance (hair coat, nasal cavity, ear, eye, oral cavity, and anus), behavior, and body temperature were not observed.

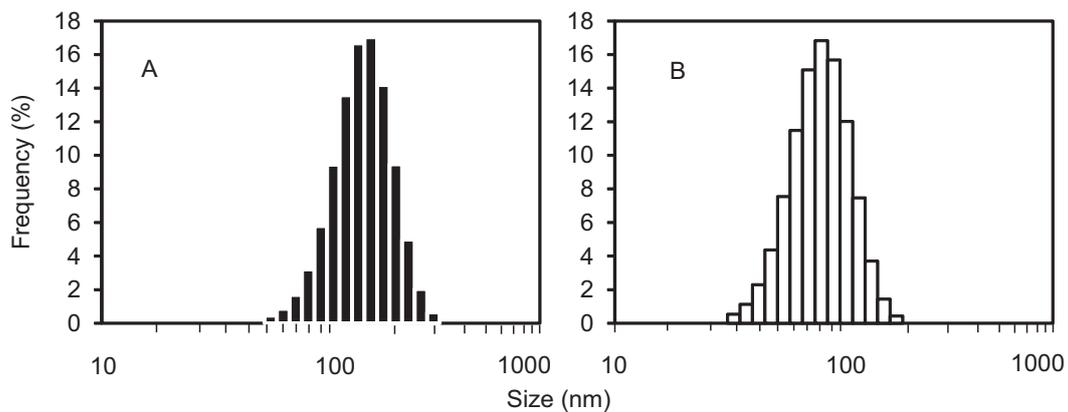


Fig. 1. Size distribution of the ZnO NPs (A) and ZnO-SiO₂ NPs (B).

Table 1. Summary of animal experiment

	Dose	n	Impaired motility	Death
	(mg/kg)			
ZnO	0	3	-	0/3
	10	3	-	3/3
	30	3	-	3/3
ZnO-SiO ₂	0	5	-	0/5
	10	5	0/5	0/5
	30	5	0/5	0/5
	130	5	5/5	1/5

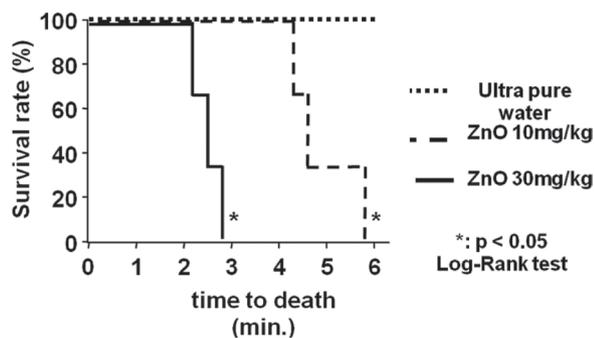


Fig. 2. Survival rate following intravenous single injection of ZnO NPs in mice.

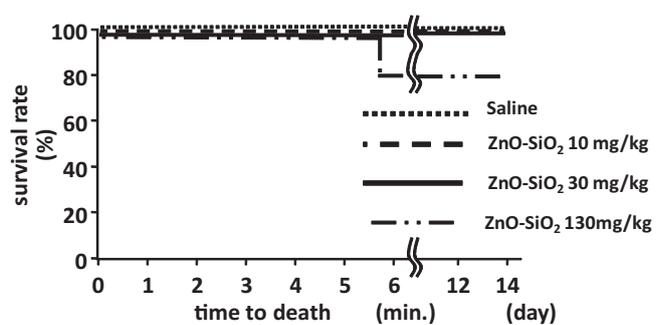


Fig. 3. Survival rate following intravenous single injection of ZnO-SiO₂ NPs in mice.

DISCUSSION

We consider medical application of ZnO for bio-imaging such as cancer diagnosis, and it is important to evaluate the toxicity before the medical application. Several previous studies *in vivo* have shown the toxicity of ZnO NPs. Gao *et al.* have shown that ZnO NPs induced significant damage to the rat olfactory epithelium, and they suggested that the possible toxicological mechanism might involve cellular energy metabolic dysfunction [10]. Fukui *et al.* have conducted intratracheal instillation of ZnO NPs to rat lungs and shown that ZnO NPs induce strong oxidative stress in the lung in the acute phase [11]. Esmaeillou *et al.* have investigated the oral toxicity of ZnO NPs following a single oral dose and shown by histopathological examination that the liver, kidney, and lungs were damaged [12]. In the present study, the toxicity of ZnO NPs following intravenous administration was evaluated for the first time. Silica coating of ZnO NPs are effective for UV stability and the stability of dispersion. In the present study, the toxicity following intravenous administration of silica-coated ZnO NPs were also investigated.

At first, we did not predict death following intravenous ZnO NPs treatment: no report is available on the acute toxicity and lethal effect of ZnO NPs. However, single intravenous administration of ZnO NPs produced a lethal effect with 5 min (Table 1, Fig. 2). In the present study, blood coagulation was observed in all of the dead mice. This may be related to the cause of death following intravenous injection of ZnO NPs. On the other hand, silica-coated ZnO NPs are less toxic than ZnO NPs (Table 1, Fig. 3). Only one mouse died at a dose of 130 mg/kg.

Recent study *in vitro* has shown that ZnO NPs cause erythrocyte aggregation and activation of human platelets [13]. The platelet aggregation may be induced by the Zn^{2+} [15]. Further, Fukui *et al.* have shown that the significant correlation between the intracellular reactive oxygen species (ROS) levels and the intracellular Zn^{2+} levels, and they also suggested that Zn^{2+} is an important factor in the cytotoxicity ZnO NPs [11]. Blood coagulation observed in the present study may be caused by the similar mechanism. In general, silica-coating may pre-

vent Zn^{2+} ion release to body fluids, and coating NPs is effective to prevent ROS [8]. This may be related to the mortality difference between ZnO NPs and silica-coated ZnO NPs. Further investigation is needed to clarify the mechanism of toxicity of ZnO NPs.

In conclusion, this study is the first to show the acute toxicity of ZnO NPs via intravenous injection and to suggest that silica coating can reduce the toxicity. Acute toxicity of the ZnO NPs may be caused by the erythrocyte aggregation and prothrombogenic. Further studies are needed to clarify the appropriate dose of ZnO NPs and the appropriate modification of ZnO NPs for application in the biomedicine field.

ACKNOWLEDGMENTS

This work was supported by the Foundation for Priority Research Project (S-Green & Life Nanomaterials Project) of Shimane University.

REFERENCES

- 1) Tsukazaki A, Kubota M, Ohtomo A, Onuma T, Ohatani K, Ohno H, Chichibu SF and Kawasaki M (2005) Blue light-emitting diode based on ZnO. *Jpn J Appl Phys* 44: L 643-L 645.
- 2) Kim YJ, Yoo J, Kwon BH, Hong YJ, Lee C and Yi G (2008) Position-controlled ZnO nanoflower arrays grown on glass substrates for electron emitter application. *Nanotechnology* 19: 315202.
- 3) Kotecha M, Veeman W, Roche B and Tausch M. (2006) NMR investigation s of silane -coated nano sized ZnO particles. *Microporous Mesoporous Mat* 95: 66-75.
- 4) Senthilkumar O ,Yamauchi K, Senthilkumar K, Yamane T, Fujita Y and Nishimoto N (2008) UV-blue light emission from ZnO nanoparticles. *J Korean Phys Soc* 53: 46-49.
- 5) Senthilkumar K, Senthilkumar O ,Yamauchi K, Sato M, Morito S, Ohba T, Nakamura M and Fujita Y (2009) Preparation of ZnO nanoparticles for bio-imaging applications. *Phys Status Solidi B-Basic Solid State Phys* 246: 885-888.
- 6) Sato M, Harada, H Morito S, Fujita Y, Shimosaki S, Urano T and Nakamura M (2010) Preparation, characterization and properties of novel covalently

- surface-functionalized zinc oxide nanoparticles. *Appl Surf Sci* 256: 4497-4501.
- 7) Sato M, Shimatani K, Iwasaki Y, Morito S, Tanaka, H, Fujita Y and Nakamura M (2011) New surface-modified zinc oxide nanoparticles with aminotriethylene oxide chains linked by 1, 2, 3-triazole ring: Preparation, and visible light-emitting and noncytotoxic properties. *Appl Surf Sci* 258: 786-790.
- 8) Nohynek GJ, Lademann J, Ribaud C and Roberts MS (2007) Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. *Crit Rev Toxicol* 37: 251-77.
- 9) Nakamura M, Shono M and Ishimura K (2007) Synthesis, characterization, and biological applications of multifluorescent silica nanoparticles. *Anal Chem* 79: 6507-6514.
- 10) Gao L, Yang ST, Li S, Meng Y, Wang H and Lei H (2013) Acute toxicity of zinc oxide nanoparticles to the rat olfactory system after intranasal instillation. *J Appl Toxicol*, in press.
- 11) Fukui H, Horie M, Endoh S, Kato H, Fujita K, Nishio K, Komaba LK, Maru J, Miyauhi A, Nakamura A, Kinugasa S, Yoshida Y, Hagihara Y and Iwahashi H (2012) Association of zinc ion release and oxidative stress induced by intratracheal instillation of ZnO nanoparticles to rat lung. *Chem Biol Interact* 25: 29-37.
- 12) Esmaeillou M, Moharamnejad M, Hsankhani R, Tehrani AA and Maadi H (2013) Toxicity of ZnO nanoparticles in healthy adult mice. *Environ Toxicol Pharmacol* 35: 67-71.
- 13) Šimundić M, Drašler B, Šuštar V, Zupanc J, Štukelj R, Makovec D, Erdogmus D, Hägerstrand H, Drobne D and Kralj-Iglič V (2013) Effect of engineered TiO₂ and ZnO nanoparticles on erythrocytes, platelet-rich plasma and giant unilamellar phospholipid vesicles. *BMC Vet Res* 9: 7
- 14) Spaet TH and Zucker MB (1964) Mechanism of platelet plug formation and role of adenosine diphosphate. *Am J Physiol* 206: 1267-1274.