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ORIGINAL RESEARCH

In vivo toxicologic study of larger silica nanoparticles in mice

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Abstract: Silica nanoparticles (SiNPs) are being studied and used for medical purposes. As nanotechnology grows rapidly, its biosafety and toxicity have frequently raised concerns. However, diverse results have been reported about the safety of SiNPs; several studies reported that smaller particles might exhibit toxic effects to some cell lines, and larger particles of 100 nm were reported to be genotoxic to the cocultured cells. Here, we investigated the in vivo toxicity of SiNPs of 150 nm in various dosages via intravenous administration in mice. The mice were observed for 14 days before blood examination and histopathological assay. All the mice survived and behaved normally after the administration of nanoparticles. No significant weight change was noted. Blood examinations showed no definite systemic dysfunction of organ systems. Histopathological studies of vital organs confirmed no SiNP-related adverse effects. We concluded that 150 nm SiNPs were biocompatible and safe for in vivo use in mice.
Keywords: in vivo, mice, silica nanoparticle, nanotoxicity

Introduction

Due to their widespread distribution and abundance,[1] as well as their chemical and physical properties, silicon-based materials have been used in many industries, including construction or building, electronics, food industry, consumer products, and medical uses.[2] Many products containing silicon have been manufactured for human use, which can be applied on the skin or inside the body, such as bandages, lens, dietary supplements, dental fillers, catheters, and implants.[3–5] In addition, micro/nanoscale silicone-based materials were used to manufacture consumer products. Due to their basic features, such as size, high specific surface area, low density, optical properties, capacity for absorption, encapsulation capacity, biocompatibility, and low toxicity, silica nanoparticles (SiNPs) attained an important role in the rapidly growing nanotechnologies.[6] These characteristics of SiNPs result in their wide utilization as an inert substance entrapping or supporting matrix.[7] Consequent research on biomedical applications using SiNPs was undertaken intensively through decades, including diagnosing and controlling disease, identifying and correcting genetic disorders, and increasing longevity.[8] SiNPs were used to innovate newer biomedical applications, such as biosensors,[9] enzyme supporters,[10] controlled drug release and delivery,[11,12] and cellular uptake.[12]

As these particles are being applied to humans, concerns about biocompatibility and harm to body health raise. These abovementioned macroscopic devices, including silica and other materials, are generally known to be safe and biocompatible. When the size of particles was decreased to nanoscale, toxicity has been discovered and reported, such as silver and gold, which have been earlier utilized in biomedical field. Owing to its antibacterial property, silver is used for the production of SiNPs containing medical products, such as wound dressings, devices, and catheters, to lower the incidence of bacterial infections.[13] However, Paddle-Ledinek et al[14] found that extracts from wound dressings containing SiNPs were more toxic to keratinocytes among those nanomaterials tested. SiNPs are well known to be toxic to various tissues, such as lung, liver, brain, vessels, and reproductive organs.[15] Gold is inert and considered as biocompatible, and its nanoparticles are used in medical applications, including drug carrier, biosensor, tumor detector, photothermal agent, and dose enhancer in radiotherapy,[16] but a study had shown that gold ions caused suicidal death of erythrocytes.[17] Hematological alterations, a common hallmark of toxicity, had been demonstrated in mice that were intravenously given gold nanoparticles (AuNPs).[18] Cytotoxic effect was noted in both SiNP- and AuNP-treated mice by Shrivastava et al,[19] and increased reactive oxygen species resulting in oxidative stress damage was demonstrated to be the reason for the noxious effect. However, a recent study performed by Fraga et al[20] to observe the short- and long-term toxicities after a single-dose intravenous AuNPs to rats showed no severe acute or delayed toxicity. Size-dependent cytotoxicity of AuNPs was reported, and 1.4 nm nanoparticles induced necrosis of the studied cells, but 15 nm nanoparticles exhibited no toxicity with up to 60-fold higher concentration.[21]

Although some data found that SiNPs are biocompatible, a recent in vitro study with various cell lines showed side effects to some investigated cells depending on nanoparticle size and cell type as well as dosing of the particles.[22] Inflammatory responses presenting as elevated interleukin-1β were elicited more by smaller particles when different size, dose, concentration, and surface area mixtures of SiNPs were internalized by mouse bone marrow-derived macrophages.[23] Sohaebuddin et al[24] reported that SiO_2 nanoparticles of 30 nm diameter induced apoptosis of the cocultured cells with increasing percentages in 3T3 fibroblasts, human bronchiolar epithelial cells, and RAW macrophages, reaching ~10%, 50%, and 90%, respectively; however, little necrosis was observed in these studied cells. In contrast, limited cytotoxicity, measured as global metabolism activity, was seen when human epithelial intestinal HT-29 cells were exposed to both 25 and 100 nm nano-SiO_2 particles for 24 hours.[25] Surprisingly, larger nanoparticles in lower dose resulted in a more unfavorable effect in terms of genotoxicity in the abovementioned study, which was opposite to the concept that smaller particles in higher concentration are usually more toxic to studied subjects.

In in vivo studies, in which SiNPs were administered to animals, discordant results have been reported in various experiments. Inhalation of SiNPs was noted to result in pulmonary tract inflammation and myocardial ischemia in rats, especially in old individuals.[26] Oxidative stress biomarkers and proinflammatory cytokines were found in rat lungs when 30 nm SiNPs were instilled in the trachea for 5 weeks by Lin et al,[27] and oxidation damage with inflammation was thought to be the rationale of pulmonary toxicity caused by the nanoparticles. Pulmonary inflammation was detected by microcomputed tomography in mice lungs after intratracheal injection with the SiNPs of 30 or 3,000 nm, and smaller particles caused more severe complications.[23] Many studies demonstrated that most porous SiNPs deposited in liver and spleen when administered via blood and most of them were cleared in $<$1 month.[28,29] Delayed clearance was noted when nonporous amorphous SiNPs were given intravenously to mice, and elevated aminotransferases, increased inflammatory cytokines, and hepatocytic necrosis were also found.[30] SiNPs accumulated in various organs in mice, but no sign of toxicity to those organs was seen with the particle sizes of 20–25 and 80 nm while giving 3 and 2 mg/kg to mice, respectively.[31,32] A study by Liu et al[33] in 2011, administering 110 nm SiNPs to Institute of Cancer Research mice, reported that 2 of the 10 mice that received $>$1,000 mg/kg of the SiNPs died, but this lethal dose was much higher than that given to mice receiving 64 nm SiNPs.[34] Moreover, as in the report mentioned earlier published in 2012, 100 nm SiNPs produced more genotoxic effect to human epithelial intestinal HT-29 cells than did 25 nm SiNPs;[25] it is doubtful whether larger SiNPs are really safer when administered in vivo.

Owing to the diverse results of various investigations, we carried out an in vivo study to evaluate the toxic events as well as the dose-related effects of intravenous SiNPs in mice. In this study, we gave regimens in different concentrations of 150 nm SiNPs to the subjects and attempted to determine the lethal dose. In order to determine the general toxicity of SiNPs, we observed for any depressed activity, disability, or disorder in these treated animals. Multiple blood and serum indicators were measured to detect the systemic dysfunction caused by SiNPs in different dosages. Histopathological analysis of various vital organs was performed to understand the extent of cellular effects produced by the nanoparticles.

Methods

Preparation of SiNPs

SiNPs were synthesized by tetraethylorthosilicate (TEOS), ammonium hydroxide, ethanol, and deionized water according to the methods previously reported.[35,36] At first, 3 mL of ammonium hydroxide (30%, J.T. Baker, Center Valley, Pennsylvania, USA) was added to deionized water and 50 mL of ethanol (EtOH, 99.8%; Sigma-Aldrich, St. Louis, Missouri, USA) to make an ammonium–water–EtOH solution, and the solution was stirred vigorously for 5 minutes after admixture. Then, 1.5 mL of TEOS (Sigma Aldrich) was added dropwise to the ammonium–water–EtOH solution with stirring for 1 hour, giving a milky white suspension. This mixture was stood for 17 hours to coarsen the SiNPs. The solidified SiNPs were collected after centrifugation and ethanol washing.

Animal and treatment

Thirty male Balb/C mice aged 7 weeks were purchased from the National Laboratory Animal Center (Taiwan). All mice were housed to a 12-hour light/dark cycle at the animal care center; and environmental condition was maintained at a constant temperature of 22°C±1°C and the humidity of 55%±10%. Water and autoclaved food (Laboratory Autoclavable Rodent Diet 5010; LabDiet, St. Louis, Missouri, USA) were provided ad libitum. The mice were acclimatized for 1 week prior to the experiments. After 1 week, the mice weight was between 24 and 26 g. They were divided into eight groups, each group consisting of three to four mice. A series of doses (1, 2.5, 5, 10, 100, 200, and 300 mg/kg) were set to process the in vivo toxicologic study of SiNPs. The mice received 100 μL of SiNPs in different concentrations by intravenous injection via the tail vein. The control group received 100 μL saline instead of SiNPs, ie, the 0 mg/kg group. Every day, the mice were weighed and behavioral changes were assessed. Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of MacKay Memorial Hospital, Taiwan (IACUC Number: MMH-A-S-101-43). The guidelines of animal welfare were instituted by the National Laboratory Animal Center (Taiwan) and supervised by the Laboratory Animal Center of MacKay Memorial Hospital.

Sample collection

To detect any systemic dysfunction, we examined the blood for changes in hematological and biochemical parameters. Blood samples were collected via the ocular vein 14 days after the exposure to SiNPs. Complete blood counts were analyzed by HEMAVET® (Drew Scientific, Dusseldorf, Germany). Blood samples were centrifuged at 3,000 rpm for 15 minutes in order to obtain serum. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, total protein, albumin, blood urea nitrogen (BUN), and creatinine were measured by FUJI DRI-CHEM 4000i (FujiFilm, Dusseldorf, Germany).

To examine the cellular effect or pathological events, all mice were sacrificed after blood withdrawal. Organs, including brain, heart, lungs, liver, spleen, and kidneys, were harvested. The organs were fixed in 10% formalin and subjected to further histopathological examinations by the Research Center for Animal Medicine, National Chung Hsing University (Taiwan).

Statistics

The quantitative data were expressed as mean ± standard deviation (SD) for measurements. Statistical analyses were performed with independent *t*-test using SPSS 12.0. Statistical significance was defined as a *P*-value of <0.05.

Results

Size of synthesized nanoparticles

The size and size distribution of the synthesized nanoparticles were analyzed by a dynamic light scattering (DLS) particle size analyzer (Zetasizer NANO-ZS90; Malvern Instruments, Malvern, Worcestershire, UK). The nanoparticle size was 153.8 nm in average (peak 164.4 nm and width 43.58 nm) (Figure 1A). TEM images showed that the SiNPs exhibited a near-spherical shape and dispersed well, with the average size of 148.18±16.01 nm (Figure 1B).

Passive behaviors and toxic symptoms

Thirty male Balb/C mice were divided into eight groups, each group consisting of three or four mice. Each group of mice received different dosages of SiNPs (0, 1, 2.5, 5, 10, 100, 200, and 300 mg/kg) through the tail vein. All mice tolerated one dose injection of SiNPs from 1 to 300 mg/kg. No mice showed any passive behavior, such as hypopnea, tremor, and arching of back, or any symptoms of poisoning, such as loss of appetite, diarrhea, and vomiting, to various dosages up to 14 days after treatment. Faintness was noted on the first day of observation when the mice received >100 mg/kg of SiNPs, but the symptom did not occur on the following days (Table 1). All mice survived before they were sacrificed for histopathological analysis after the observation period of 14 days. During the experimental period, no significant difference in body weight change was noted among those mice (Table 2).

Blood cells

Blood examination was performed 14 days after the mice were exposed to SiNPs. Increase in total white blood cell (WBC) as well as the differentiated WBCs was noted in all groups, except the neutrophils that showed various elevations. Total WBC was increased most in the 200 mg/kg group when compared

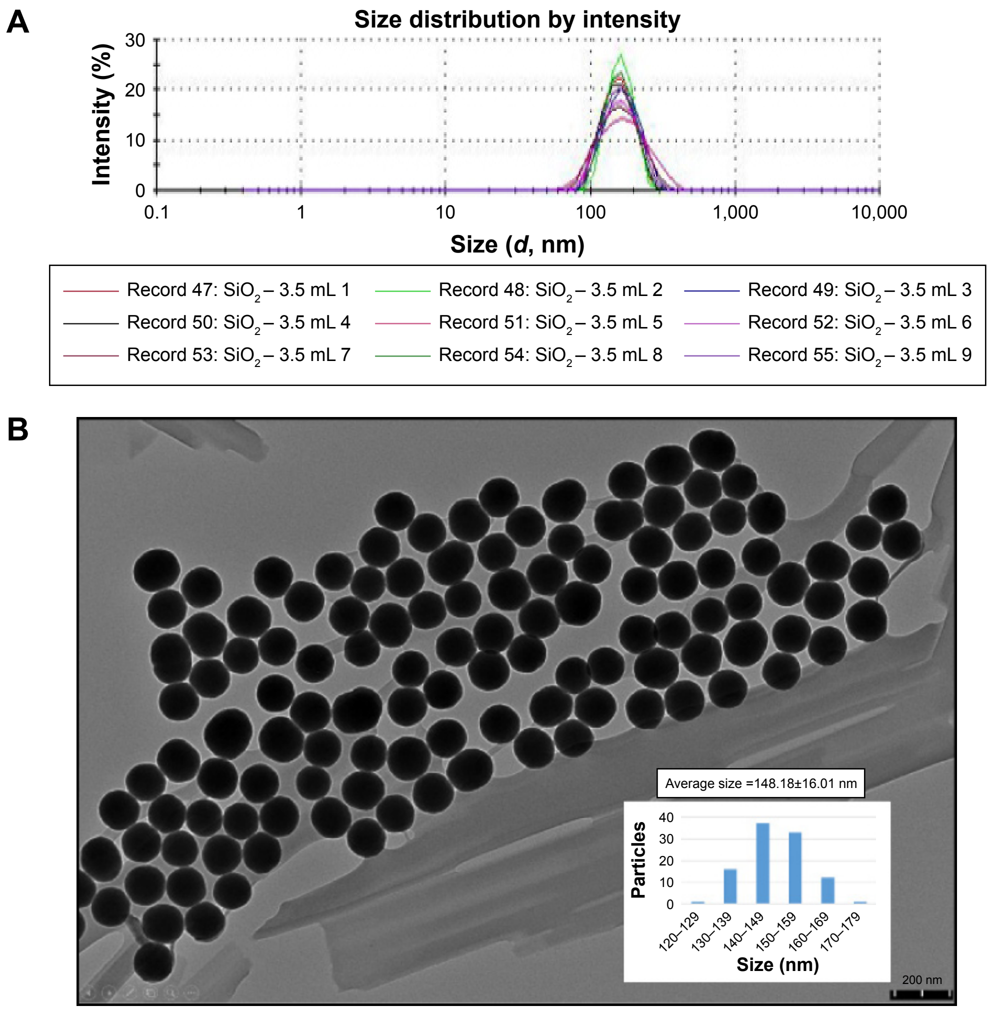

Figure 1 Characterization of SiNPs.

Notes: (**A**) DLS size distribution with an average diameter of ~153.8 nm. (**B**) TEM images showed the SiNPs in near-spherical shape with well dispersibility with an average diameter of ~148.18 nm.

Abbreviations: DLS, dynamic light scattering; SiNPs, silica nanoparticles.

with the control (Table 3), and increased lymphocytes and monocytes were responsible for the result. No obvious difference was noted regarding the red blood cell (RBC) counts, hemoglobin, and hematocrit (Figure 2A–C). Platelet decreased in all groups and significantly in the 1, 5, 100, and 300 mg/kg groups (Figure 2D).

Liver and spleen

Serum AST and ALT, which represent the liver cell damage,[37] were not elevated significantly (Figure 3A). Total bilirubin, total protein, and albumin, which represent the liver function,[37] were not different to the control (Figure 3B and C). These results indicated that SiNPs did not hurt the liver cells.

Table 1 Passive behaviors and acute symptoms of poisoning after the injection of SiNPs with various dosages

Dosage (mg/kg)	Status	Hypopnea	Tremor	Arching of back	Loss of appetite	Diarrhea and vomiting
0	Good	None	None	None	None	None
1	Good	None	None	None	None	None
2.5	Good	None	None	None	None	None
5	Good	None	None	None	None	None
10	Good	None	None	None	None	None
100	Faintness[a]	Faintness[a]	None	None	Faintness[a]	None
200	Faintness[a]	Faintness[a]	Faintness[a]	None	Faintness[a]	None
300	Faintness[a]	Faintness[a]	Faintness[a]	None	Faintness[a]	None

Note: [a]Faintness was noted only on the first day of treatment, and the status was good, as well as no passive behaviors or acute symptom of poisoning was noted on the following days.

Abbreviation: SiNPs, silica nanoparticles.

Table 2 Body weight changes (%) after the injection of SiNPs with various dosages

Dosage (mg/kg)	Day 0	Day 2	Day 4	Day 7	Day 9	Day 11	Day 14
0	100±0.0	101.1±1.2	102.3±0.3	102.2±0.3	104.3±1.4	105.2±0.3	105.2±1.5
1	100±0.0	101.5±0.3	102.9±0.7	102.9±0.9	104.3±0.6	105.9±0.9	107.4±1.5
2.5	100±0.0	100.9±0.4	102.5±0.9	103.5±0.6	105.5±0.6	105.2±0.5	106.3±0.9
5	100±0.0	100.9±0.4	102.4±1.1	103.8±1.1	105.0±0.5	105.1±0.3	105.2±0.5
10	100±0.0	99.7±0.9	103.4±1.3	102.8±1.1	103.8±1.4	105.1±0.9	105.0±1.1
100	100±0.0	99.7±0.3	99.7±1.0	98.5±1.6	100.0±1.1	101.9±0.6*	102.2±0.5
200	100±0.0	99.7±1.3	101.3±0.3	102.7±0.0	103.9±0.3	104.7±0.3	104.9±0.9
300	100±0.0	99.6±1.0	99.1±1.0	100.7±1.3	101.9±1.0	103.2±1.4	101.6±1.7

Notes: $^*P<0.05$ when compared with control group, 0 mg/kg. Data are presented as mean ± standard deviation.
Abbreviation: SiNPs, silica nanoparticles.

Table 3 White blood cells and differentiation at 14 days after the injection of SiNPs

Dosage (mg/kg)	WBC (10^3/μL)	Neutrophil (10^3/μL)	Lymphocyte (10^3/μL)	Monocyte (10^3/μL)	Eosinophil (10^3/μL)	Basophil (10^3/μL)
0	6.29±1.78	1.92±0.70	3.71±0.96	0.34±0.07	0.24±0.14	0.08±0.05
1	8.12±0.61	2.11±0.22	5.09±0.24	0.44±0.06	0.35±0.11	0.13±0.04
2.5	9.31±0.46	2.49±0.14	5.53±0.19	0.61±0.07*	0.51±0.08	0.18±0.04
5	7.96±1.16	1.87±0.28	5.11±0.76	0.42±0.07	0.42±0.08	0.15±0.03
10	7.65±1.27	1.70±0.40	5.00±0.72	0.56±0.06	0.29±0.08	0.10±0.04
100	10.36±1.39	2.78±0.63	6.27±0.58	0.69±0.14	0.46±0.12	0.15±0.04
200	13.49±2.51	3.38±1.10	8.57±1.14*	0.77±0.05*	0.60±0.23	0.18±0.08
300	10.11±1.48	1.95±0.52	6.99±0.68	0.66±0.18	0.39±0.12	0.13±0.03

Notes: $^*P<0.05$ when compared with control group, 0 mg/kg. Data are presented as mean ± standard deviation.
Abbreviation: SiNPs, silica nanoparticles.

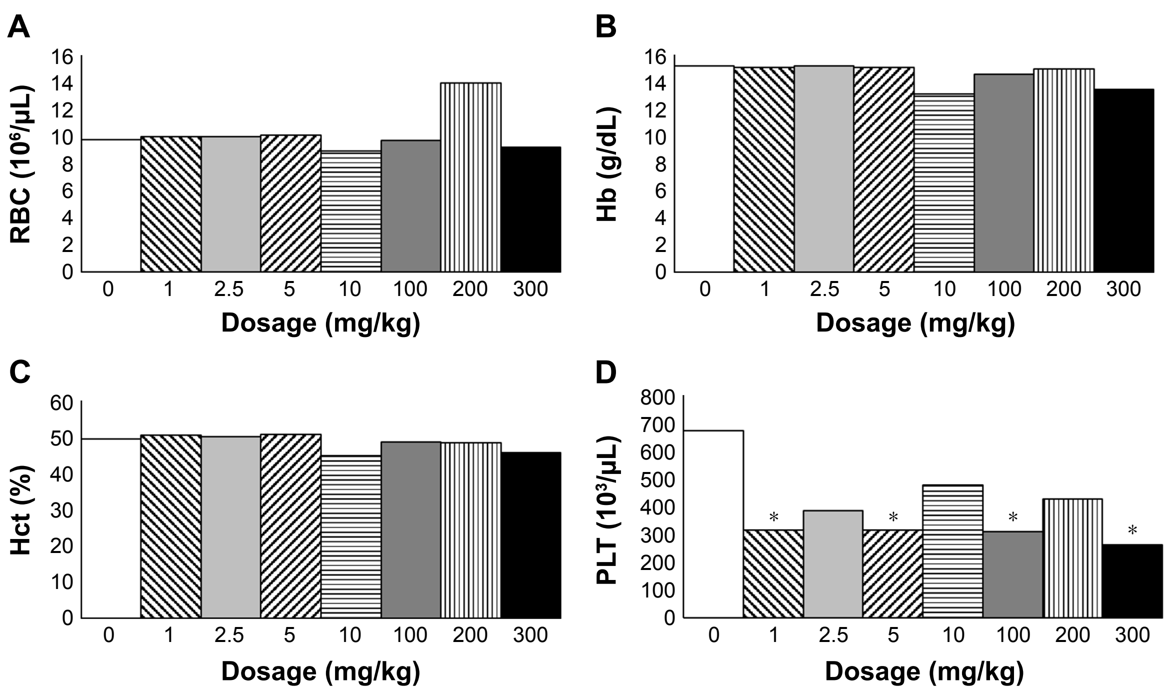

Figure 2 Hemogram of RBCs and PLTs.
Notes: Hemogram at 14 days after the injection of SiNPs: (**A**) RBC count, (**B**) Hb, (**C**) Hct, and (**D**) PLT count. $^*P<0.05$ when compared with control group, 0 mg/kg.
Abbreviations: Hb, hemoglobin; Hct, hematocrit; PLT, platelet; RBC, red blood cell; SiNPs, silica nanoparticles.

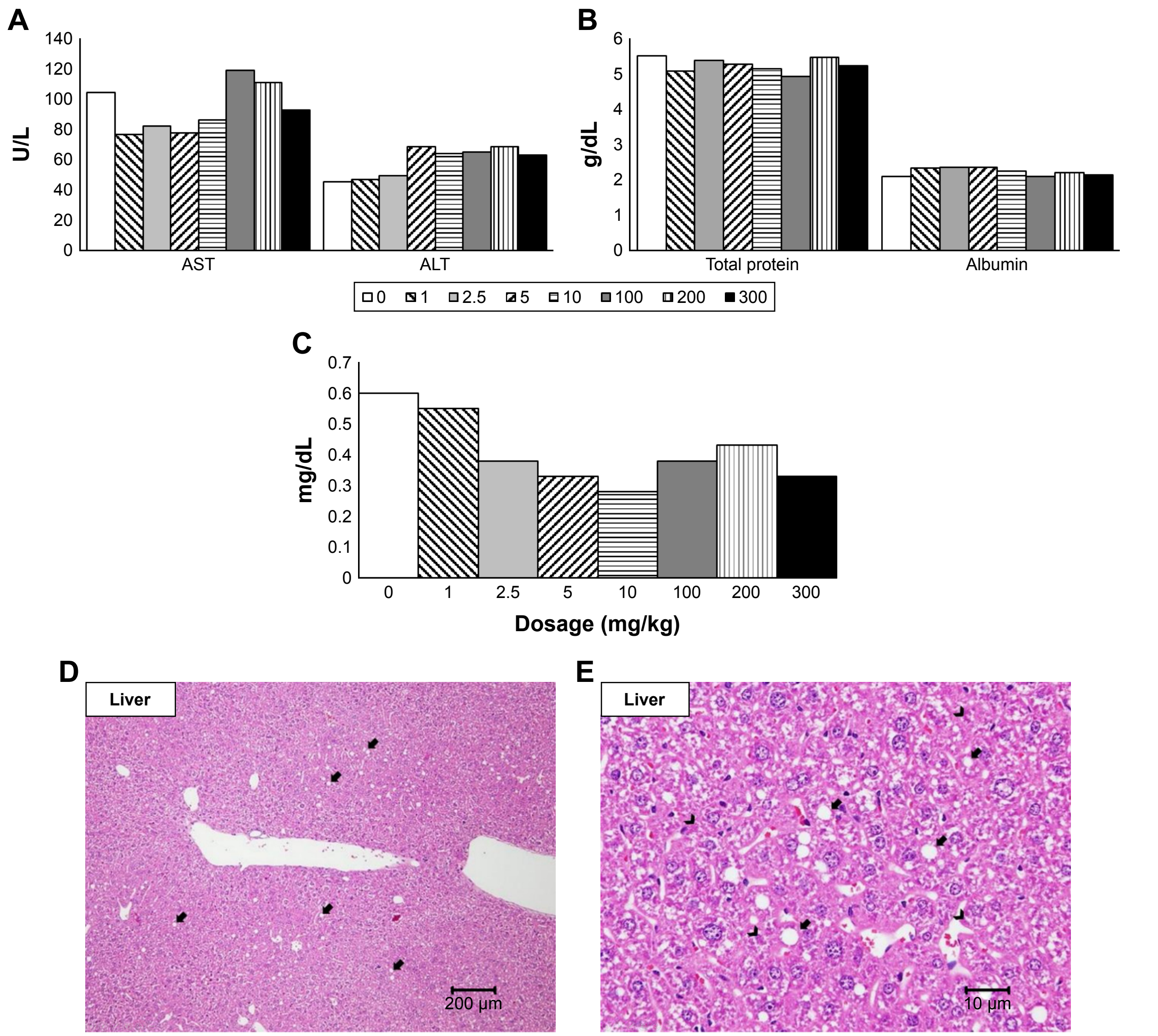

Figure 3 Blood biochemistry test and histopathological analysis of liver.

Notes: Serum biochemical parameters indicating liver cell damage with (**A**) AST and ALT, liver function with (**B**) total protein and albumin and (**C**) total bilirubin at 14 days after the injection of SiNPs. Histopathological analysis with H&E staining of liver at (**D**) 20× and (**E**) 400× magnifications with mild focal fat deposition (arrow) and mild glycogen deposition (arrow head).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; SiNPs, silica nanoparticles.

There was no gross pathological lesion seen in the livers and in the spleens. However, in the livers, various degrees of diffuse glycogen infiltration were seen in all groups and minimal-to-mild focal infiltration of fat was seen in all groups except the 200 and 300 mg/kg groups under the microscopic examination, which was thought to be due to nonfasting status before sacrifice (Figure 3D and E).[38] No microscopic abnormality was seen in the spleens.

Kidneys

BUN and creatinine were not increased after the injection of SiNPs, indicating no kidney dysfunction (Figure 4A and B).[37] No gross lesion was seen within the kidneys. Microscopically, minimal to mild focal infiltration of mononuclear cells as well as focal tubular regeneration was seen in all groups except the 200 mg/kg group (Figure 4C and D).

Heart and lungs

Grossly, no lesions were noted in the hearts and in the lungs. Under the microscopic examination, mild-to-moderate focal fibrosis and mild focal fibrotic embolism were seen in some individual hearts and epicardial mineralization was seen in all groups but not in the 10 and 200 mg/kg groups (Figure 5). No pulmonary lesion was seen in all mice except one mouse in the 1 mg/kg group showing moderate focal inflammation in lungs (Figure 6).

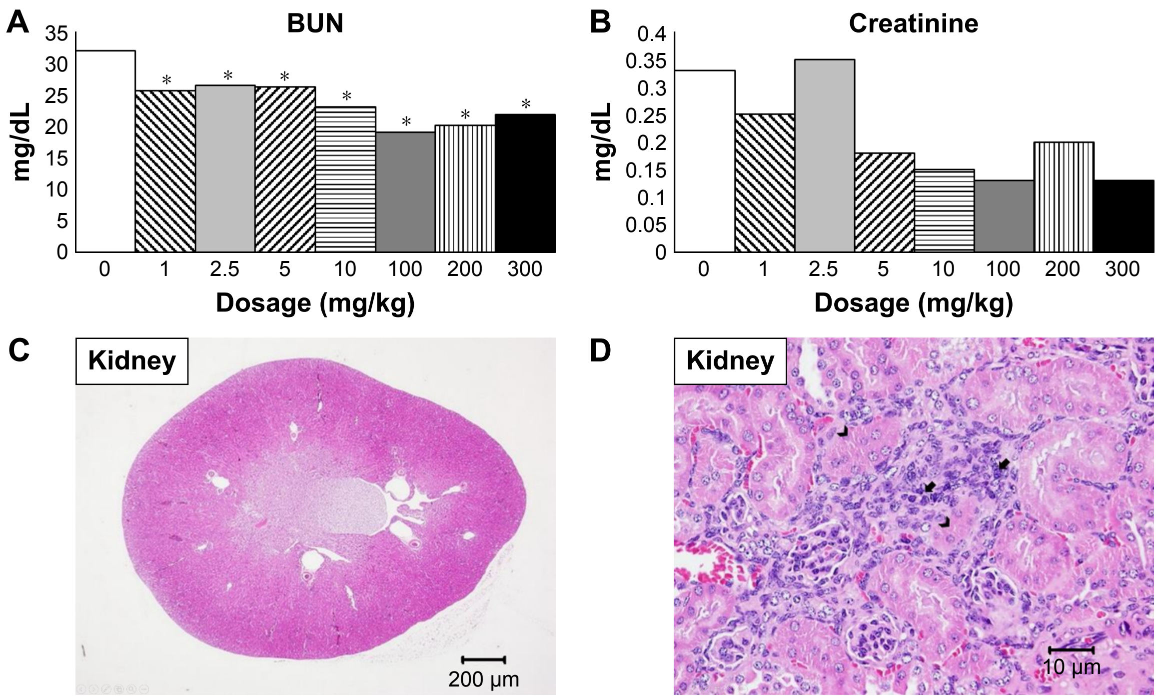

Figure 4 Blood biochemistry test and histopathological analysis of kidney.

Notes: Serum biochemical parameters indicating kidney function with (**A**) BUN and (**B**) creatinine. BUN was significantly lower in all treatment groups. Histopathological analysis with H&E staining of kidney at (**C**) 20× and (**D**) 400× magnifications with mild focal monocyte infiltration (arrow) and tubular regeneration (arrow head). *$P<0.05$ when compared with control group, 0 mg/kg.

Abbreviation: BUN, blood urea nitrogen.

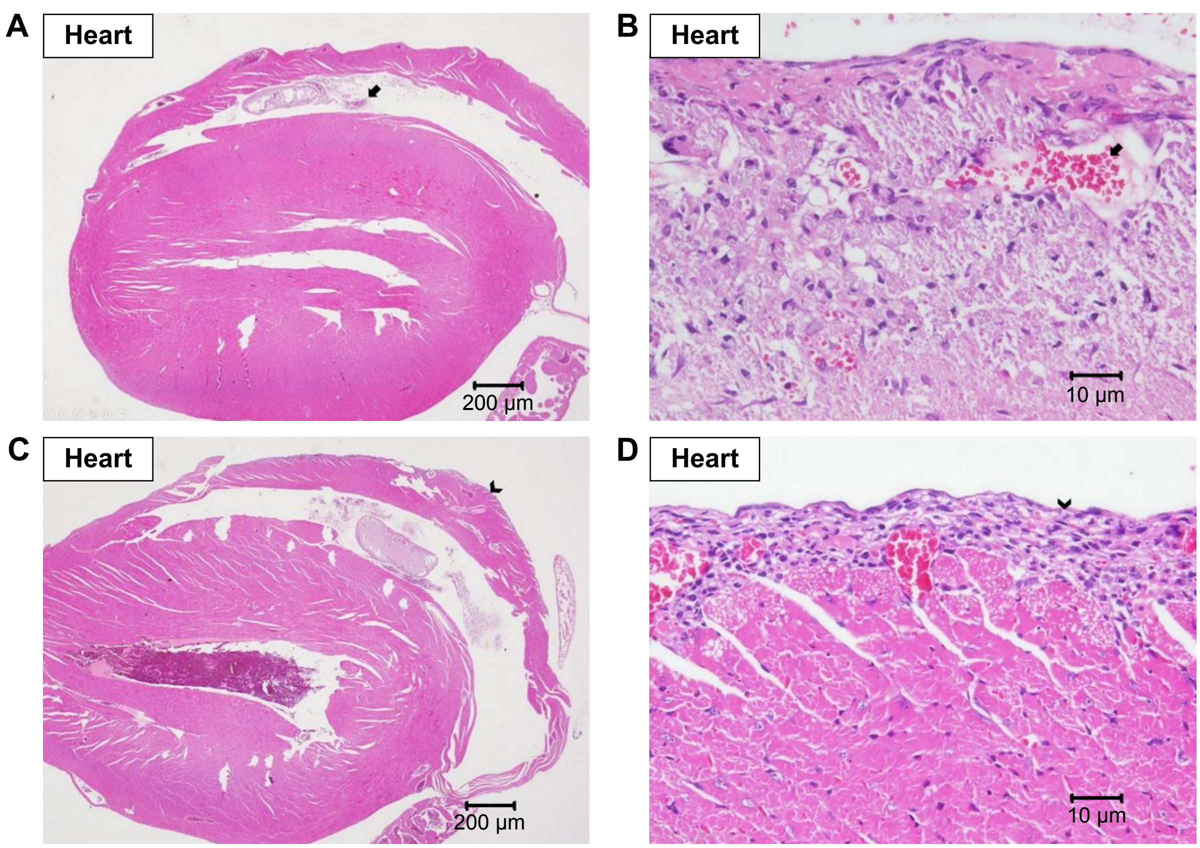

Figure 5 (*Continued*)

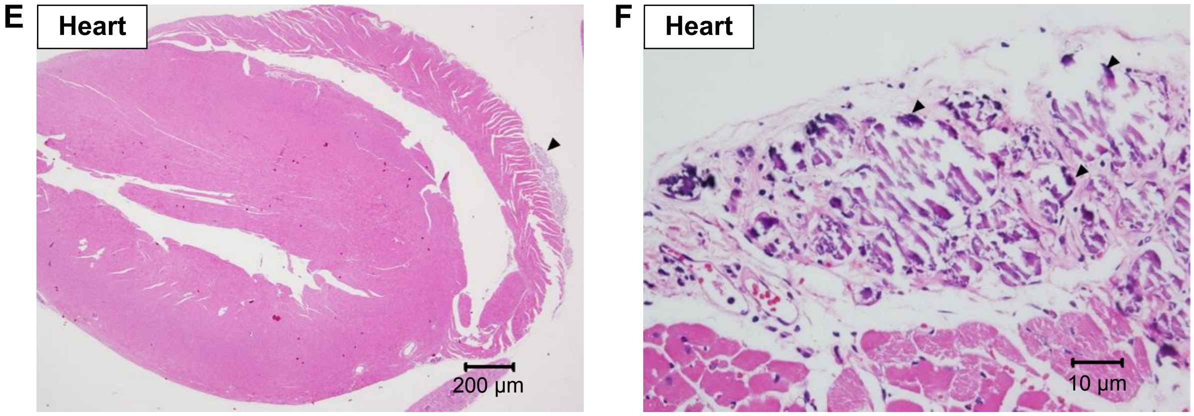

Figure 5 Histopathological analysis of heart.
Note: Histopathological analysis with H&E staining of heart at (**A**) 20× and (**B**) 400× magnifications with focal fibrotic emboli (arrow), at (**C**) 20× and (**D**) 400× magnifications with focal fibrosis (arrow head), and at (**E**) 20× and (**F**) 400× magnifications with epicardial mineral deposition (triangle).

Brain

No gross lesion was seen in the brain. Mild-to-moderate focal hemorrhage was seen in some brains of all groups, which was thought to be resulted due to the method of their sacrifices (Figure 7A and B).[39] One mouse in the 100 mg/kg group showed moderate granuloma in cerebral cortex (Figure 7C and D).

These lesions found in the hearts, lungs, and kidneys were related to aging rather than to adverse effects from SiNPs. The incidence of the microscopic lesions in the organs is listed in Table 4.

Discussion

According to the literature, SiNPs were considered safe and biocompatible.[8,31,32,40] The lethal dose 50 (LD50) of single injected 64 and 110 nm SiNPs that have been demonstrated in ICR mice were 262.45±33.78 and >1,000 mg/kg, respectively.[33,34] No mice died in our study received a single injection of 150 nm SiNPs up to 300 mg/kg. Acute toxicity, even death, was encountered with very large amount of application of SiNPs, easier in those with a single dose with larger amount.[33] In addition, smaller nanoparticles caused death easier than larger nanoparticles.

According to the result from the in vitro test that lysis of mice erythrocyte, hemolysis, occurred while those cells were incubated with SiNPs, the authors supposed that SiNPs might lead to anemia when administered in vivo.[41] Such condition was not observed in our study as there was no obvious change with the data about RBCs. Platelet aggregation was demonstrated by Corbalan et al[42] applying SiNPs to isolated human platelets, and they also stated that the effects on platelet aggregation were inversely proportional to the nanoparticle size. The effect of SiNPs on platelet aggregation was noted to be induced through the thromboxane A2-mediated and matrix metalloproteinase-2-mediated pathways.[43] In our study, platelet count decreased in those mice received SiNPs, which was supposed to be compatible

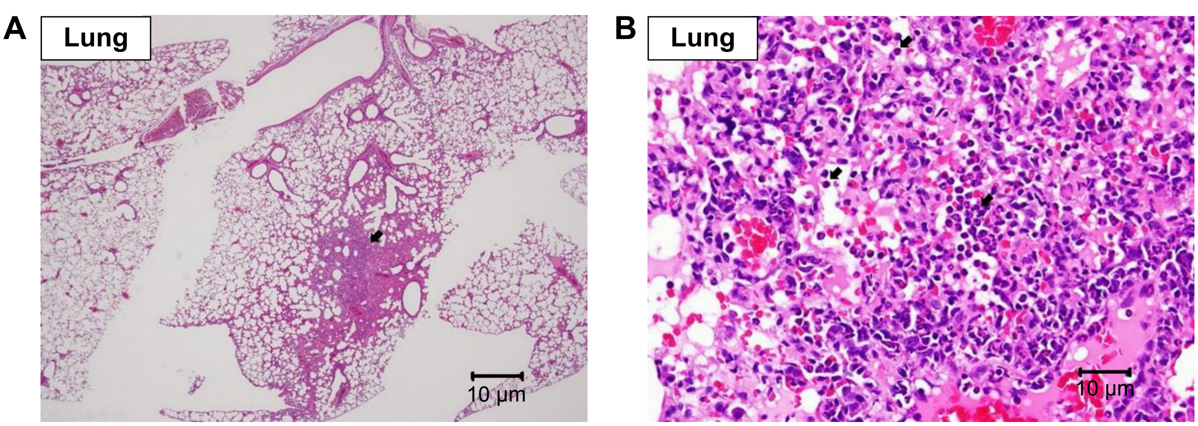

Figure 6 Histopathological analysis of lung.
Note: Histopathological analysis with H&E staining of lung showing moderate focal inflammation (arrow) at (**A**) 20× and (**B**) 400× magnifications.

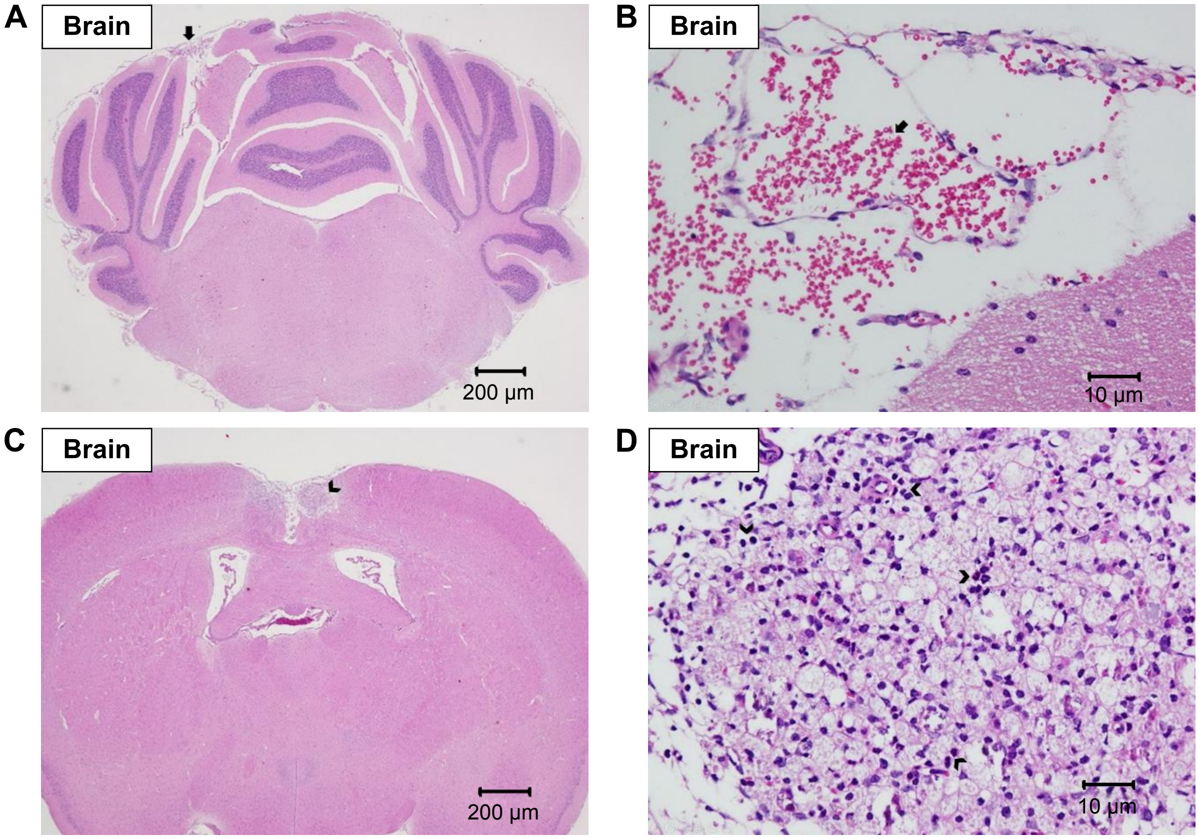

Figure 7 Histopathological analysis of brain.
Note: Histopathological analysis with H&E staining of brain showing focal hemorrhage in the cortex of cerebellum (arrow) at (**A**) 20× and (**B**) 400× magnifications and focal granuloma in the cortex of the cerebrum (arrow head) at (**C**) 20× and (**D**) 400× magnifications.

with the aggregation of platelets. In a cell model utilizing mice platelets, larger dose of SiNPs aggregated more platelets,[44] but dosing effect of the SiNPs on platelet aggregation was not observed in current experiment. The discrepancy may be due to the difference of in vitro and in vivo, as SiNPs contribute to system organs following injection in the blood stream, resulting in a loss of the nanoparticles from the circulation.[32] Further studies should be performed to understand if both conditions, erythrocyte hemolysis and platelet aggregation, may be caused by the administration of SiNPs. SiNPs of 70 nm had been demonstrated to induce coagulopathy as well as fatality in Balb/C mice but not larger sizes, including 100, 300, and 1,000 nm;[45] no such disaster was seen in our mice that received nanoparticle size of 150 nm.

Table 4 The case number of histopathological lesions of various organs in each group

Organ	Histopathological lesions	Dosage (mg/kg)							
		0 (3)[a]	1 (4)[a]	2.5 (4)[a]	5 (4)[a]	10 (4)[a]	100 (4)[a]	200 (3)[a]	300 (4)[a]
Liver	Glycogen infiltration, diffuse, mild to moderate	2	4	3	4	4	4	1	1
	Fat infiltration, focal, minimal to mild	1	1	2	1	1	3	0	0
Spleen	No lesion	–	–	–	–	–	–	–	–
Kidneys	Mononuclear cell infiltration, focal, minimal to mild	1	1	3	3	3	1	0	1
	Tubular regeneration, focal, minimal to mild	1	1	3	3	3	1	0	1
Heart	Fibrotic emboli, focal, mild	0	0	0	0	0	0	2	0
	Fibrosis, focal, mild to moderate	0	0	0	0	1	0	1	2
	Epicardial mineralization, focal, mild to moderate	1	3	4	1	0	2	0	1
Lungs	Inflammation, focal, moderate	0	1	0	0	0	0	0	0
Brain	Hemorrhage, focal, mild to moderate	3	1	1	3	2	2	1	1
	Cortical granulation, focal, moderate	0	0	0	0	0	1	0	0

Note: [a]The digit inside the parentheses was the number of mice in each group, and all mice were sacrificed at 14 days after injection of SiNPs and blood sampling.
Abbreviation: SiNPs, silica nanoparticles.

Several studies demonstrated that injected SiNPs accumulated mainly in liver and spleen, accounting ~75% of the injected 20–25 nm multimodal organically modified silica (ORMOSIL) nanoparticles [31] or 80% of the injected rod-shaped mesoporous SiNPs.[29] Most of these nanoparticles were excreted via the hepatobiliary system, and ~100% of the injected ORMOSIL taken up by liver was cleared out in 15 days.[31] However, ~38%–42% of these SiNPs (20 or 80 nm) accumulated in liver and 30%–37% of that accumulated in spleen remained after 30 days followed by a single injection to ICR mice.[32] It is reasonable to consider that SiNPs may damage the liver and spleen when given intravenously. Inflammatory cell infiltration was found in the liver tissue of rats[46] and mice[32] when these animals were injected 15 and 20 nm and 80 nm SiNPs, respectively. After injection into the blood stream, these substances may diffuse through the endothelial wall of capillaries into tissues or may be blocked by the endothelial barrier. Other than larger molecules, nanoparticles passed through the gaps between the cells of endothelium.[47] Small NPs passed through more easily than large NPs and, thus, accumulated more in certain tissues.[48] In liver, hepatocytes did not uptake SiNPs, but the Kupffer cells, macrophage in the liver, endocytosed the nanoparticles.[32] Kupffer cells were activated, and the released cytokines induced inflammatory responses in liver.[32,46] However, most of the in vivo studies showed no or minimal toxicity to these two organs,[2] as in our current study.

Most of the in vivo studies investigating the biodistribution of SiNPs found little renal accumulation of such particles.[29,31,49] It is because kidney is not a reticuloendothelial system (RES) organ, but excretion via kidney was an important way for the clearance of injected SiNPs from the body.[29,49] Borak et al[50] reported that 36% of the introduced amount would be excreted from urine in rats administered with 150 nm SiNPs. Another report by He et al[49] stated that most of the mesoporous SiNPs were excreted in 30 minutes after an intravenous injection to ICR mice, then the excretion rate lowered gradually, and a total of up to 54% of these SiNPs were excreted in urine after 30 days. Usually, there was no or minimal damage to kidney when SiNPs were administered in vivo.[40,49,51]

It had been reported that SiNPs induced cardiotoxicity to zebrafish embryo because these particles inhibited angiogenesis and disturbed heart formation and development;[52] neutrophil-mediated cardiac inflammation[53] was assumed to be the cause. However, no definite cardiac injury was noted in the studies[40,49,51] of mature mice. Studies investigating the adverse effects about inhalation or intratracheal instillation with SiNPs had been performed, and pulmonary inflammation was common.[27,54,55] As the lung is an RES organ, SiNPs may be endocytosed by the pulmonary macrophages and accumulation of SiNPs in lungs was noted in oral,[56] inhaled, and intravenous[32] administrations. In contrast, least complication was found in those mice receiving SiNPs intravascularly.[31,32]

SiNPs were shown to be able to pass through the blood–brain barrier of mice when injected into the carotid artery[57] or instillated into the nasal cavities,[58] and inflammation of the brain was proposed in these studies. However, when administered intravenously, only scanty amount of SiNPs was deposited in mice brain[59] and no studies reported brain damage in the tested animals.[31,51]

All the histopathological abnormal findings in our study were nonspecific, and the incidence did not correlate to the dosage of the SiNPs administered proportionally.

Conclusion

No definite toxic effect was noted in our study. The lesions seen in those vital organs were not contributed by SiNPs injected because those lesions might also be seen in mice received no nanoparticles in this study and were not closely related to the given amount of nanoparticles. We concluded that SiNPs of 150 nm diameter were safe to Balb/C mice when administered intravascularly.

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Disclosure

The authors report no conflicts of interest in this work.

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