

# Distribution, Translocation and Accumulation of Silver Nanoparticles in Rats

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Silver nanoparticles (SNPs) are widely used in the field of biomedicine, but a comprehensive understanding of how SNPs distribute in the body and the induced toxicity remains largely unknown. The present study was designed to investigate the distribution and accumulation of SNPs in rats with subcutaneous injection. Rats were injected with either SNPs or silver microparticles (SMPs) at 62.8 mg/kg, and then sacrificed at predetermined time points. The main organs of the experimental animals were harvested for ultrastructural analysis by transmission electron microscopy (TEM) and for silver content analysis by inductively coupled plasma mass spectrometry (ICP-MS). Results indicated that SNPs translocated to the blood circulation and distributed throughout the main organs, especially in the kidney, liver, spleen, brain and lung in the form of particles. SMPs, however, could not invade the blood stream, or organ tissues. Ultrastructural observations indicate that those SNPs that had accumulated in organs could enter different kinds of cells, such as renal tubular epithelial cells and hepatic cells. Moreover, SNPs also induced blood-brain barrier (BBB) destruction and astrocyte swelling, and caused neuronal degeneration. The results suggest more cautions needed in biomedical applications of SNPs, in particular, the long term uses.

**Keywords:** Silver Nanoparticle, Distribution, Translocation, Ultrastructure, rat.

## 1. INTRODUCTION

Recent years, silver nanoparticles (SNPs), with stable physiochemical properties have been widely used in the field of medicine, due to their superiority in antibacterial activity over common silver.<sup>1,2</sup>

It is well known that silver ions ( $\text{Ag}^+$ ) can enter the blood circulation, accumulate in tissues and organs, can induce toxicity in the liver and kidney, and even cause human death.<sup>3,4</sup> SNPs are only 1–2 magnitudes larger than silver ions (silver ionic radius 0.126 nm), so it's possible that SNPs can cross some barriers between blood and tissue. It is, therefore, possible that SNPs produce similar toxicity to  $\text{Ag}^+$ . One study has revealed that SNPs can move into the blood circulation system *via* the blood-pulmonary barrier, and can show a systemic distribution.<sup>5</sup> However, the distribution, translocation and accumulation pathways of medically administered SNPs, which do not access the body *via* the respiratory system, may be different. A greater understanding of the translocation,

distribution and accumulation of SNPs in target organs is required. In the present study, we examined the translocation, distribution and accumulation of SNPs in rats administered by subcutaneous injection, and determined the specific target organs of SNPs.

## 2. MATERIALS AND METHODS

### 2.1. Experimental Materials

SNPs and silver microparticles (SMPs), both pure silver, were purchased from Sigma-Aldrich Co. (Beijing, China; Lot: 576832 and 327107, respectively). DMEM (Dulbecco's modified eagle medium, Gibco, Beijing, China).

### 2.2. Characteristics of SNPs and SMPs

After high pressure and high temperature sterilization, SNPs were diluted in the DMEM at a ratio of 5 mg/mL, subjected to ultrasonic dispersion to create a suspension that was then added drop-wise onto a copper screen and dried under vacuum. The size and morphology of the

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SNPs were observed under a Philips EM208S transmission electron microscope (TEM; Royal Dutch Philips Electronics Ltd., Eindhoven, Netherlands).

After high pressure and high temperature sterilization, the size and morphology of SMPs was detected by Hitachi S520 scanning electron microscope (SEM; Hitachi Ltd., Tokyo, Japan).

### 2.3. In Vivo Trial

After high pressure and high temperature sterilization, the weighed SNPs and SMPs were added to DMEM to prepare suspensions, which were dispersed for 30 minutes with a sonic oscillating instrument (600 W; Beijing Jinxing Ultrasonic Equipment Tech Co. Ltd., Beijing, China), before being used for subcutaneous injection.

Ninety Wistar female rats ( $120\text{ g} \pm 5\text{ g}$ ) were randomly divided into three groups: control group, SNPs group, and SMPs group. Each group was treated with its corresponding suspension by a subcutaneous injection, at a dose of  $62.8\text{ mg/kg}$  silver in a volume of 1 mL. Five animals were collected after 2, 4, 8, 12, 18, and 24 weeks, and faeces and urine were harvested within 24 hours at each time point. In addition, 3 ml femoral artery blood was sampled, and then animals were sacrificed to obtain the injection sites, brains, hearts, liver, spleen, lung, kidney, a femur, adrenal gland, ovary and uterus.

Samples from brains, liver, spleen, lung and kidney were processed for electron microscopy by fixation with 2.5% paraformaldehyde, 2.5% glutaraldehyde for 2 hours at  $4\text{ }^\circ\text{C}$ , washing in PBS (pH 7.4), 1% osmic acid fixation for 2 hours at  $4\text{ }^\circ\text{C}$ , then washing with redistilled water, gradient ethanol dehydration, epoxy propane displacement, epoxy resin embedding, cutting, and staining by uranyl acetate/lead citrate. Then thin sections were obtained for the observation of cellular ultrastructure with a TEM, the instrument was equipped with an X-ray microprobe for the elemental analyses by means of an energy dispersive spectrometer (EDS).

Organs, blood and excrement were soaked in digestion solution, consisting of high purity nitric acid and perchloric acid at a ratio of 4:1, for 48 hours. They were then heated at  $60\text{ }^\circ\text{C}$  and diluted to a metered volume using distilled water. ICP-MS was used to determine the content of silver in the metered solution. The total mass of silver in each organ was then determined ( $\mu\text{g}$ ). Results are expressed as mean  $\pm$  standard deviation.

### 2.4. Statistical Analysis

Results were processed with SPSS12.0 software (SPSS Inc., Shanghai, China), and the silver contents in corresponding organs at corresponding times were compared using One-way analysis of variance. Limit states design (LSD) was used to discern multiple comparisons

of equalizing values.  $P < 0.05$  was considered statistically significant.

## 3. RESULTS

### 3.1. Morphology and Size of SNPs and SMPs

Figures 1 and 2 are TEM and SEM photographs of SNPs and SMPs, respectively. Most SNPs are dispersed in the solution, but occasional agglomerated particles of 2–5 SNPs were seen. SNPs observed under TEM were spherical and electron-dense particles, with a 50–100 nm particle diameter. SMPs were irregularly shaped obtuse cubes, with varied sizes and with 2–20  $\mu\text{m}$  particle diameters.

### 3.2. Silver Content in Organs

Table I shows the results of the silver content of different organs of every group.

For both the SNP group and SMP group, most silver particles (>99.8%) accumulated in the injection sites or were excreted through excrements. There were no significant differences between the SNP group and SMP group ( $P > 0.05$ ) in the silver content of injection sites and excrements.

However, the total content of SNPs in different organs was significantly higher than those of SMPs. During the whole trial, the maximal content in the SNPs group was 0.15% (the percentage of the content of silver in organs to the total content of injected silver), whereas that in the SMPs group was no more than 0.02% (Fig. 3).

Compared with SMPs, the SNPs showed a larger amount of translocation and a different distribution *in vivo*. Figure 4 indicates the content of silver in organs at the 4th week, and demonstrates that the contents in the kidney, liver, spleen, brain, lung and blood of the SNPs group were

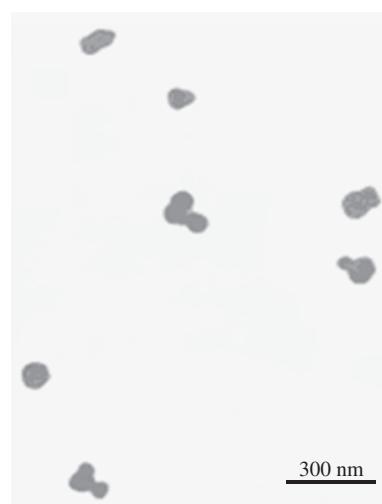


Fig. 1. TEM photo of SNPs.

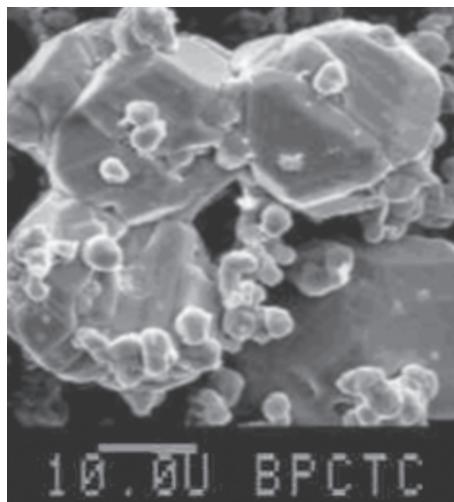


Fig. 2. SEM photo of SMPs.

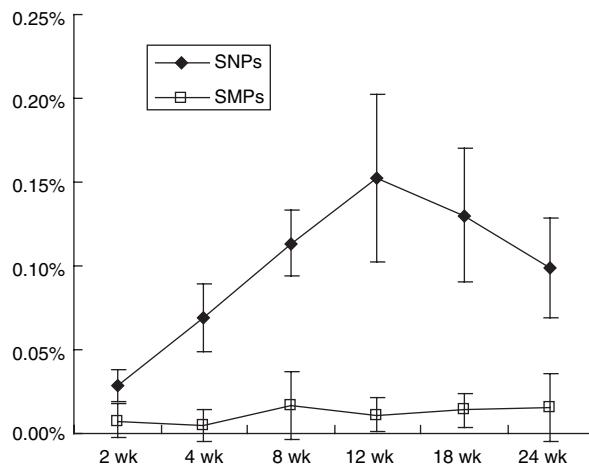
significantly higher than those of the SMPs group and the control group ( $P < 0.05$ ). There was no significant difference between the SMPs group and the control group ( $P > 0.05$ ). No statistical significance in the content of silver among heart, uterus and ovaries, adrenal gland and femur ( $P > 0.05$ ) was found between every group. These results were consistent at 8, 12, 18 and 24 weeks. These indicated that SNPs may accumulate in kidney, liver, spleen, brain, lung and blood for a long period.

Figures 5 and 6 charts silver content in faeces and urine respectively with time. The silver content of faeces was significantly higher than it is in urine in both the SNPs and SMPs group, which indicated that both SNPs and SMPs were excreted mostly through faeces.

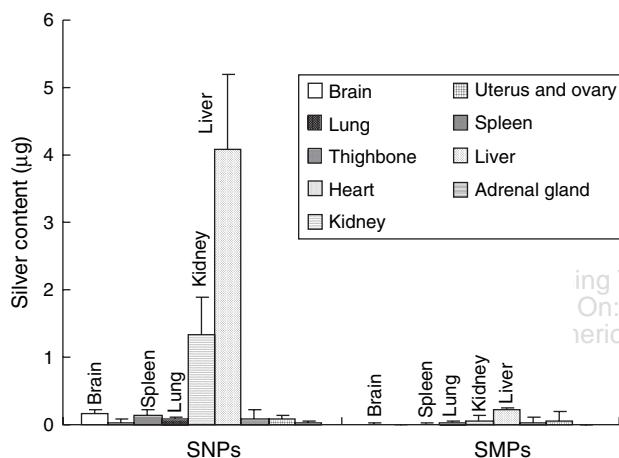
Figure 7 shows the silver content in blood with time, and shows a significant difference in the silver content in blood between the SNPs group and the SMPs group or the control group ( $P < 0.05$ ).

Table I. Mean value and standard deviation (SD) of the silver content in different organs ( $n = 5$ ).

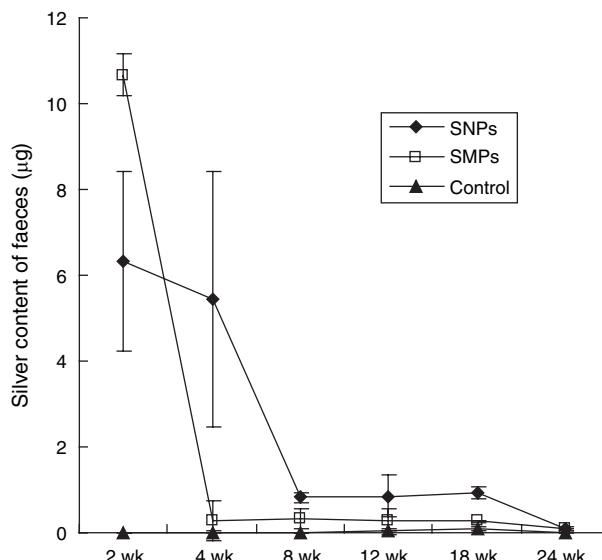
Time points	Group	Mean and SD	The contents of silver in different organs ( $\mu\text{g}$ )								
			Brains	Liver	Spleen	Lung	Kidney	Injection sites	3 ml blood	Faeces at 24 hours	Urine at 24 hours
2 weeks	Control	Mean	0.000	0.000	0.000	0.000	0.000	0.0	0.002	0.000	0.000
		SD	0.000	0.000	0.000	0.000	0.000	0.0	0.003	0.000	0.000
	SNPs	Mean	0.039	2.056	0.040	0.019	0.305	7515.8	0.026	6.322	0.004
		SD	0.018	0.888	0.022	0.017	0.182	3658.2	0.034	2.093	0.008
	SMPs	Mean	0.016	0.212	0.012	0.009	0.267	3538.7	0.001	10.67	0.006
		SD	0.008	0.118	0.011	0.016	0.100	390.9	0.003	0.48	0.013
4 weeks	Control	Mean	0.000	0.000	0.025	0.001	0.001	0.0	0.006	0.012	0.000
		SD	0.001	0.000	0.055	0.002	0.001	0.0	0.003	0.016	0.000
	SNPs	Mean	0.165	4.083	0.143	0.075	1.335	5461.4	0.022	5.427	0.060
		SD	0.071	1.101	0.068	0.047	0.544	2574.1	0.014	2.984	0.091
	SMPs	Mean	0.009	0.230	0.010	0.015	0.051	3113.4	0.008	0.276	0.001
		SD	0.011	0.020	0.022	0.034	0.075	499.4	0.002	0.446	0.002
8 weeks	Control	Mean	0.004	0.016	0.050	0.004	0.022	0.0	0.003	0.005	0.097
		SD	0.001	0.036	0.091	0.001	0.025	0.0	0.003	0.010	0.123
	SNPs	Mean	0.170	4.252	0.317	0.172	4.931	5545.9	0.049	0.823	0.052
		SD	0.111	1.034	0.169	0.073	2.211	1417.0	0.020	0.124	0.041
	SMPs	Mean	0.036	0.783	0.046	0.050	0.384	3042.4	0.015	0.326	0.056
		SD	0.029	0.349	0.031	0.032	0.339	1028.9	0.009	0.254	0.073
12 weeks	Control	Mean	0.004	0.068	0.047	0.008	0.099	83.5	0.009	0.031	0.076
		SD	0.005	0.152	0.076	0.010	0.163	53.8	0.008	0.070	0.124
	SNPs	Mean	0.415	3.869	0.449	0.237	7.181	5519.6	0.057	0.859	0.096
		SD	0.163	2.590	0.340	0.185	3.683	323.3	0.026	0.477	0.051
	SMPs	Mean	0.056	0.350	0.020	0.062	0.254	3285.4	0.013	0.294	0.052
		SD	0.042	0.085	0.016	0.042	0.238	1116.9	0.002	0.258	0.069
18 weeks	Control	Mean	0.088	0.049	0.053	0.067	0.042	52.6	0.006	0.086	0.007
		SD	0.052	0.008	0.036	0.055	0.012	39.5	0.012	0.035	0.003
	SNPs	Mean	0.385	6.718	0.753	0.332	3.006	5251.3	0.035	0.910	0.044
		SD	0.261	2.856	0.243	0.179	0.870	369.5	0.061	0.141	0.065
	SMPs	Mean	0.100	0.749	0.038	0.049	0.119	3472.2	0.005	0.265	0.013
		SD	0.052	0.503	0.021	0.047	0.097	339.5	0.010	0.017	0.014
24 weeks	Control	Mean	0.016	0.000	0.026	0.011	0.009	0.0	0.010	0.000	0.003
		SD	0.023	0.000	0.024	0.024	0.015	0.0	0.006	0.000	0.001
	SNPs	Mean	0.362	4.094	0.818	0.299	2.843	5255.9	0.024	0.092	0.033
		SD	0.120	0.904	0.232	0.127	0.325	655.1	0.015	0.056	0.042
	SMPs	Mean	0.021	0.523	0.080	0.038	0.296	3319.5	0.018	0.072	0.010
		SD	0.012	0.176	0.045	0.015	0.240	690.8	0.011	0.025	0.010



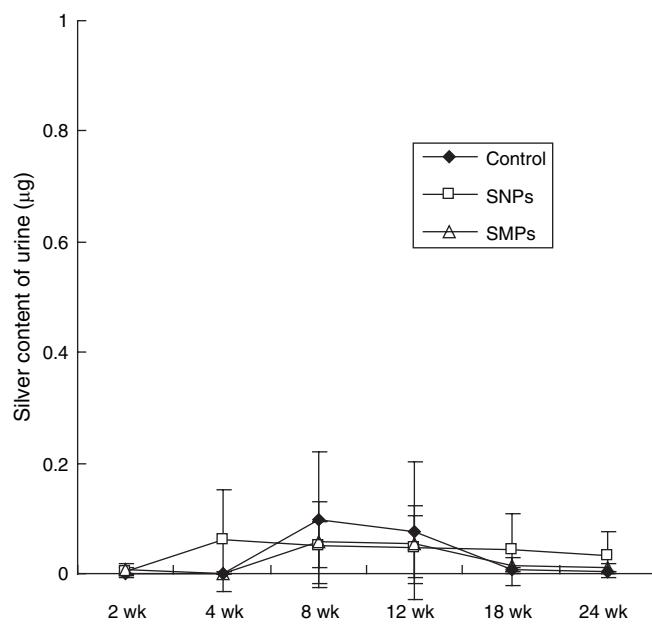
**Fig. 3.** Percentage of silver content in different organs to total silver content.



**Fig. 4.** Silver particles distribution in different organs at 4th week.



**Fig. 5.** Silver content in the faeces.

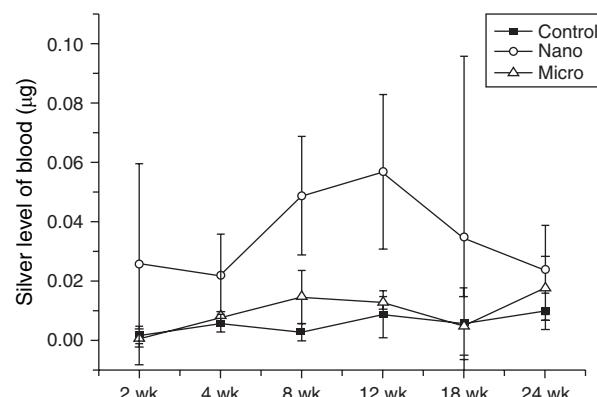


**Fig. 6.** Silver content in the urine

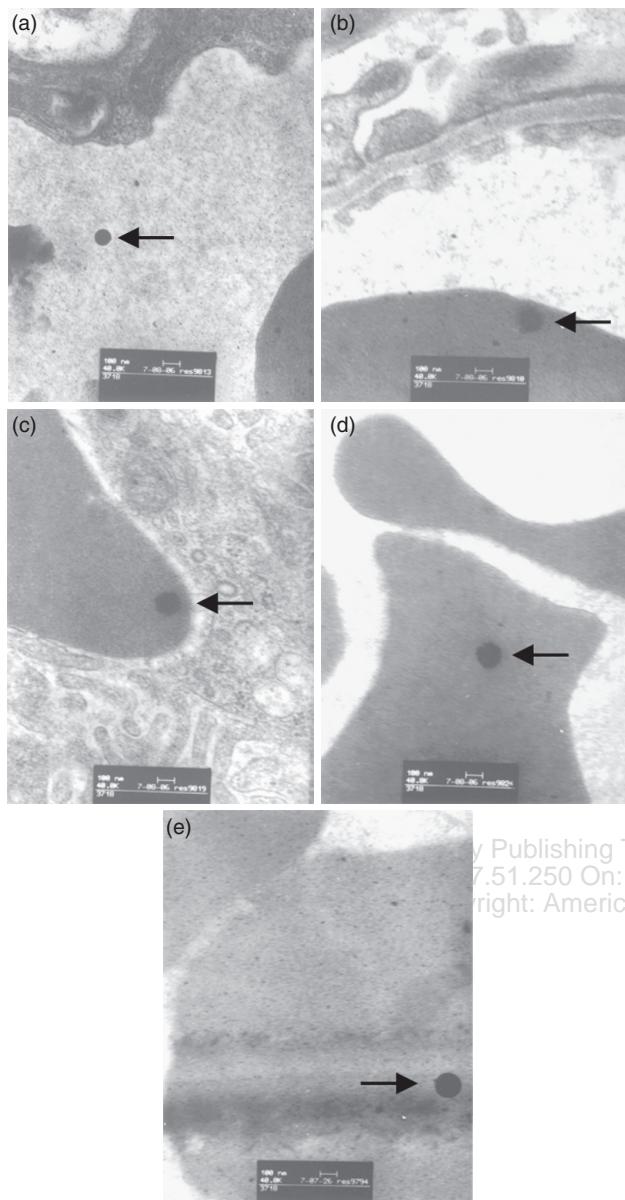
### 3.3. Ultrastructure Observations of Organs *In Vivo*

In the SNPs group, electron-dense spherical substances appeared in the lumen of blood vessels in the kidney, liver, spleen, brain and lung (Fig. 8). EDS was carried out in the areas where these spherical substances exist. The results showed that these substances were mainly composed of elemental silver, and were presumed to be SNPs. No abnormal substances were detectable in the control group or in the SMPs group during the whole observation period (2–24 weeks).

Electron-dense spherical substances were observed by EDS in the cytoplasm of renal tubular epithelial cells of the SNPs group, and are assumed to be SNPs (Fig. 9). SNPs were also found in the renal corpuscle and the foot processes of podocytes, at the margin of renal capsule (Fig. 10), in hepatocytes (Fig. 11), hepatic sinusoidal and perisinusoidal space (Fig. 12), lymphocytes in the



**Fig. 7.** Silver content in blood.



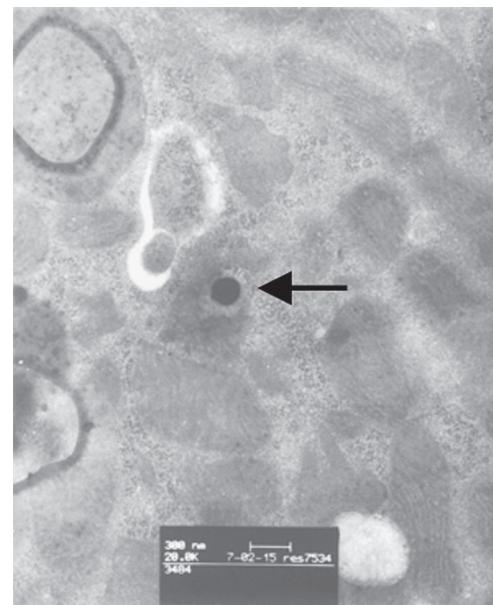
**Fig. 8.** SNPs in the lumen of blood vessels in different organs of animals in SNPs group. (a) brain (b) kidney (c) liver (d) lung (e) spleen.

splenic cord (Fig. 13), cerebral neurons (Figs. 14 and 15), in vascular endothelial cells of the blood-brain barrier (BBB) (Figs. 16 and 17), and in alveolar epithelial cells (Fig. 18).

Ultrastructure of the observed material showed more neuronal pyknosis and apoptosis in the SNPs group during the 2–24 weeks of observation (Fig. 14). Further, the BBB was abnormal, with a manifestation of astrocyte swelling out of the BBB (Figs. 16 and 17).

#### 4. DISCUSSION

According to the results, we found that the silver content in kidney, liver, spleen, brain and lung of the SNPs group

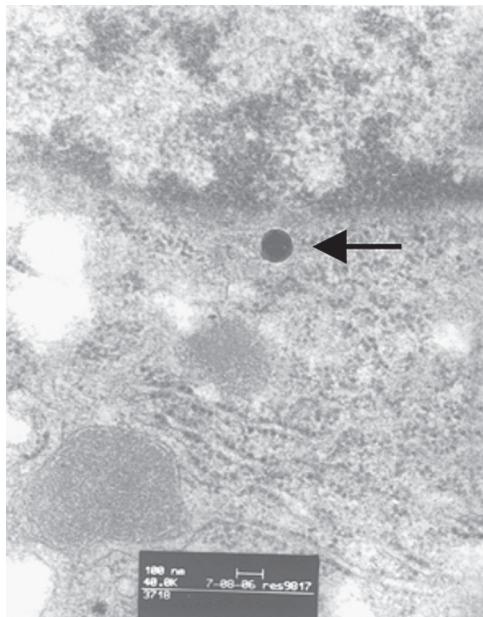


**Fig. 9.** SNPs in the renal tubular endothelial cells of animals in the SNPs group.

is significantly higher than those of SMPs group. Figure 7 shows a significant difference in the silver content in blood between the SNPs group and the SMPs group or the control group ( $P < 0.05$ ). The silver contents in blood were in accordance with those in different organs, as can be seen by comparing Figures 3 and 7. Silver content rose during 0–12 weeks and then decreased up to 24 weeks. It is reasonable to suggest that SMPs never translocate into the blood, while the injected SNPs do translocate into kidney,



**Fig. 10.** SNPs in the renal corpuscle which are hindered by the filtration membrane.

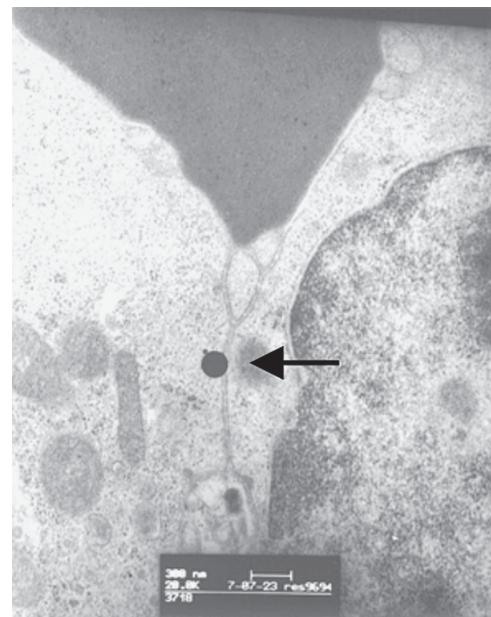


**Fig. 11.** SNPs in hepatocytes of animals in the SNPs group.

liver, spleen, brain and lung *via* blood circulation, which would explain the significantly different distributions of the two *in vivo*.

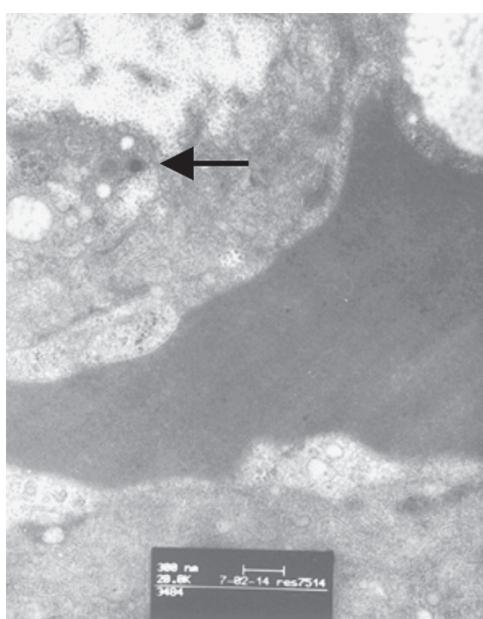
SNPs *in vivo* may translocate throughout the body by two approaches. Firstly, SNPs dissolved in body fluid may produce systemically distributed  $\text{Ag}^+$ . Secondly, SNPs could interact with some proteins and be distributed evenly in the body through protein metabolism.<sup>6</sup>

Although the silver content in kidney, liver, spleen, brain and lung of the SNPs group is significantly higher than those of SMPs group, it is not sure that SNPs could

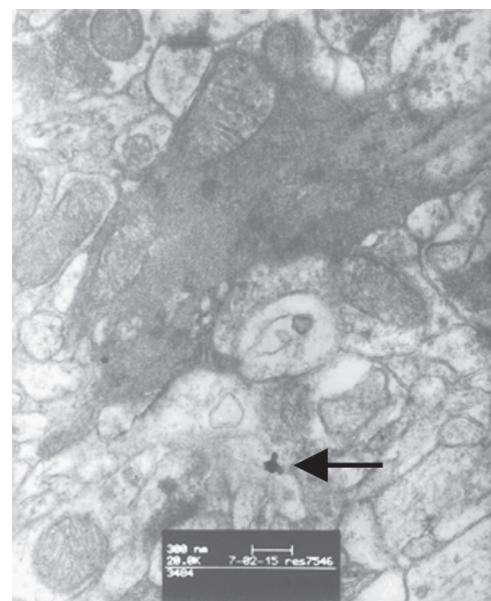


**Fig. 13.** SNPs in spleen lymphocytes of animals in the SNPs group.

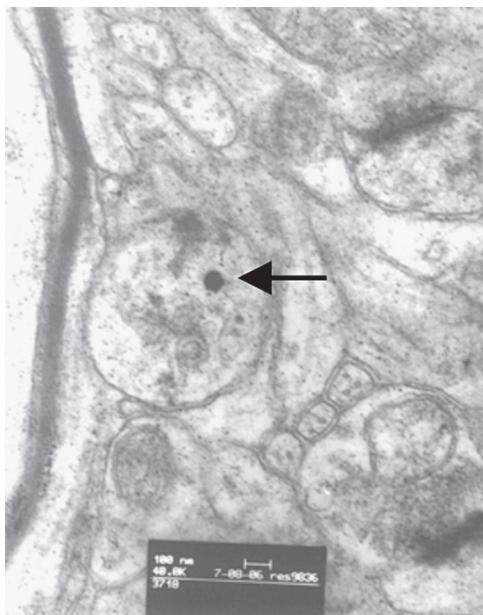
translocate *in vivo* in the form of particles. Using only ICP-MS it was impossible to distinguish whether the silver *in vivo* exists as ions or as particles. So the results, that silver content in organs is higher in the SNPs group, may be because SNPs can dissolve more  $\text{Ag}^+$  due to its surface effect (SNPs are likely to possess a larger specific surface area, a higher Gibbs energy and an incomplete surface structure compared with SMPs at the same dose. These factors together may result in SNPs *in vivo* dissolving more  $\text{Ag}^+$  than SMPs). To determine the state



**Fig. 12.** SNPs in hepatic sinusoid and perisinusoidal space.



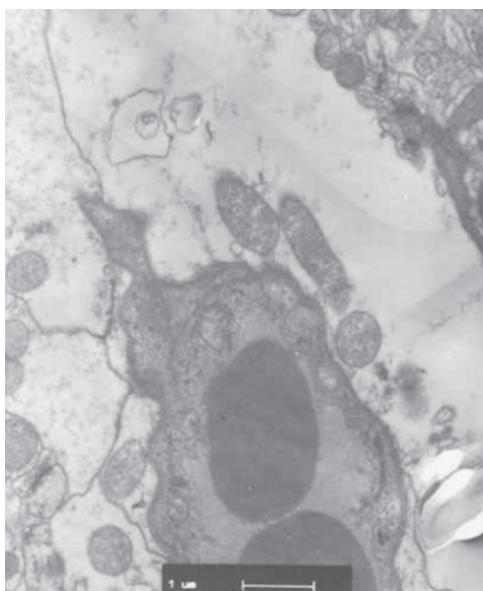
**Fig. 14.** SNPs in pyknotic neurons.



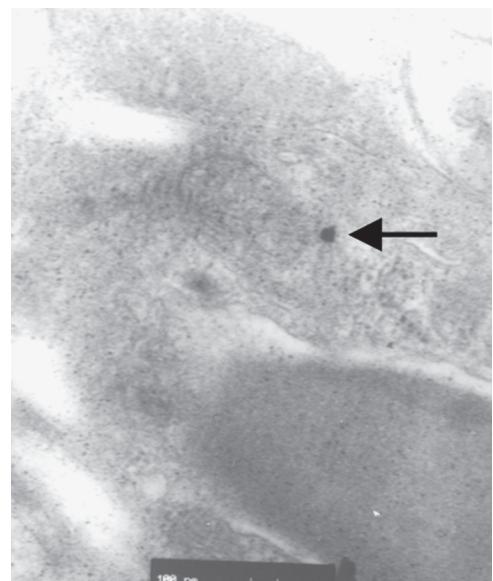
**Fig. 15.** SNPs in normal neurons.

of SNPs *in vivo*, the ultrastructural analysis was conducted by TEM.

In the SNPs group, SNPs appeared in the lumen of blood vessels in the kidney, liver, spleen, brain and lung of animals (Fig. 8). But no similar substances were found in the SMPs group. We can, therefore, conclude that the subcutaneously injected SNPs translocate into the blood vessels as particles. But SMPs could not enter into blood vessels. This outcome authenticates the presumption by Takenaka et al., which maintains that silver is a bio-inert material, unreactive to the oxygen in body fluids, and could not dissolve completely *in vivo*. Why can SNPs enter

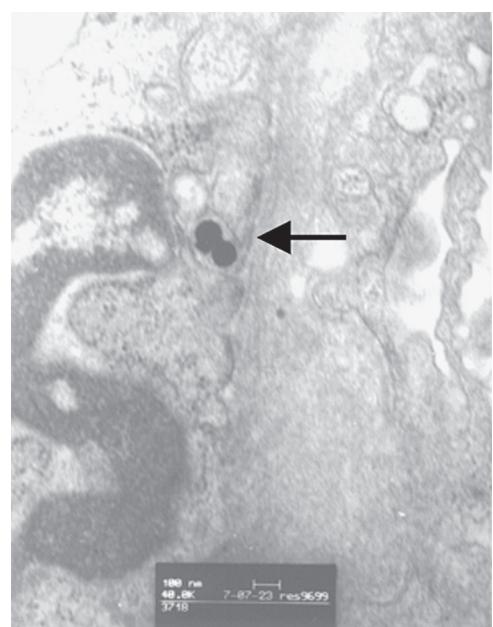


**Fig. 16.** Astrocytic swelling out of the BBB in the SNPs group.



**Fig. 17.** SNPs in the blood-brain barrier of animals in the SNPs group.

the blood, whereas SMPs cannot? We presume that physical size determines the different distributions of the two particles. Blood vessels and lymphatic tissues are plentiful in subcutaneous tissue and dermis, especially capillary vessels. After subcutaneous injection, dispersed SNPs may be phagocytized into vascular endothelial cells, and then enter the blood by exocytosis. Also the injection may result in local inflammation of the subcutaneous tissues, increasing vasopermeability and contracting the endothelial cells. The scattered SNPs can then enter the blood *via* 0.5–1.0 μm crevices between the endothelial cells,



**Fig. 18.** SNPs in the alveolar epithelial cells of animals in the SNPs group.

which are flat, with a width of 10–15  $\mu\text{m}$  and a length of 25–50  $\mu\text{m}$ . But SMPs or agglomerated SNPs could not pass the crevices or be phagocytized by endothelial cells due to their bigger size.<sup>8</sup>

SNPs would translocate systemically through the blood circulation. The ultrastructural photographs of the kidney, liver, spleen, brain and lung showed SNPs. This suggests that SNPs can enter the blood and then distribute systemically in particles. Kidney, liver, spleen, brain and lung were the organs that accumulated SNPs (Fig. 4). Why in these organs?

By ultrastructural photography of the kidney, SNPs were found in the foot processes of podocytes at the margin of the renal capsule, but not inside the renal capsule (Fig. 10). That means that SNPs could not pass through the filtration membrane between renal corpuscle and renal capsule, which is consistent with the absence of SNPs in the urine (Fig. 6), the dominant excretion pathway of SNPs is the faeces. So SNPs would accumulate in filtration membranes for a long period.

Many researchers have reported that the liver and spleen, which have developed endothelial net tissues, can be considered as the organs that accumulate nanomaterials.<sup>9–11</sup> The present study obtained similar outcomes, possibly resulting from the sinusoidal capillary, which is an irregular capillary in the liver and spleen. SNPs could enter surrounding tissues through crevices between vascular endothelial cells. Figures 12 and 13 both display the detection of SNPs in the hepatic sinusoid and in the splenic cord, as well as in hepatic cells and lymphocytes. SNPs in the hepatic sinusoid or splenic cord were presumed to be phagocytized by the adjacent hepatic cells and lymphocytes (Figs. 11 and 13).

There is no data from *in vivo* trials to prove that silver particles in the blood can cross the BBB and, thus, enter the brain. The results of the present study initially validated that SNPs could cross the BBB in particles, then enter and accumulate in the brain *in vivo*. SNPs distributed in cerebral neurons, and the neuronal apoptosis of the SNPs group was obviously more than that of the control group and the SMPs group (Figs. 14 and 15). It is, therefore, possible that SNPs in the brain can enter neurons and produce toxicity, resulting in neuronal degeneration. Subsequently, the neuronal cell membrane would decompose and the SNPs would be released to affect other neurons. The silver content in brain indicated long-term accumulation of SNPs; consequently neuronal apoptosis increased and may have induced pathological changes. The mechanism by which SNPs induce neuronal apoptosis needs *in vitro* verification. Although the BBB may hinder the entry of foreign substances, Figures 16 and 17 revealed that SNPs were present inside the vascular endothelial cells and with astrocytic swelling, most likely resulting from SNPs crossing the BBB. The current literature suggests that there are two mechanisms for the entry of nanoparticles into the brain through the BBB, namely vascular

endothelial cell transcytosis and BBB destruction by loosening of the endothelial junction or by dissolving of the endothelial membrane.<sup>12,13</sup> It is difficult to determine this mechanism only *in vivo*, some research model *in vitro* is needed.

The results shown in Figure 18 reveal the appearance of SNPs in alveolar cells of the lung. According to the study by Takenaka et al.,<sup>5</sup> SNPs invade alveoli and then lung capillary, and even show a systemic distribution at a higher diffusion rate than Ag<sup>+</sup>. In this study SNPs were found to diffuse from lung capillaries into the alveoli. It is probably resulting from the concentration difference between silver in the blood and the alveoli. When SNPs were inhaled, the silver content in the alveoli was higher than that in the blood, and then the SNPs diffused into the blood; following the subcutaneous injection of SNPs, the content in the blood was increased, and the SNPs transferred to the alveoli. Irrespective of the direction of diffusion, the SNPs can cross the capillary wall of the lung and diffuse between the blood and the alveoli. This study confirmed that the SNPs diffused as particles. Further investigations are required to identify the mechanism of SNP-vessel wall transit.

In the end, we want to emphasize that the potential health and environmental benefits of nanotechnologies have been welcomed. But at the same time, concerns should be expressed that the very properties that are being exploited by researchers and industry (such as systemic body distribution for targeted treatment, high surface reactivity and ability to cross cell membranes) might have negative health ramifications, particularly, those that might result in greater toxicity.

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