Elemental Selenium at Nano Size (Nano-Se) as a Potential Chemopreventive Agent with Reduced Risk of Selenium Toxicity: Comparison with Se-Methylselenocysteine in Mice

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Selenium (Se) is an essential trace element with a narrow margin between beneficial and toxic effects. As a promising chemopreventive agent, its use requires consumption over the long term, so the toxicity of Se is always a crucial concern. Based on clinical findings and recent studies in selenoprotein gene-modified mice, it is likely that the antioxidant function of one or more selenoproteins is responsible for the chemopreventive effect of Se. Furthermore, upregulation of phase 2 enzymes by Se has been implicated as a possible chemopreventive mechanism at supranutritional dietary levels. Se-methylselenocysteine (SeMSC), a naturally occurring organic Se product, is considered as one of the most effective chemopreventive selenocompounds. The present study revealed that, as compared with SeMSC, elemental Se at nano size (Nano-Se) possessed equal efficacy in increasing the activities of glutathione peroxidase, thioredoxin reductase, and glutathione S-transferase, but had much lower toxicity as indicated by median lethal dose, acute liver injury, survival rate, and short-term toxicity. Our results suggest that Nano-Se can serve as a potential chemopreventive agent with reduced risk of Se toxicity.

Key Words: Nano-Se; Se-methylselenocysteine; toxicity; bioavailability; selenoenzymes; glutathione S-transferase.

The trace element selenium (Se) appears to have cancer-preventive properties based on a converging body of evidence from epidemiologic, clinical, and experimental studies (Whanger, 2004). By using transgenic mice that carry a mutant selenocystine transfer RNA gene, which causes reduced selenoprotein synthesis, recent studies suggest that selenoenzymes, including glutathione peroxidase (GPx) and thioredoxin reductase (TrxR), play a role in the ability of Se to protect against cancer (Diwadkar-Navsariwala et al., 2006; Irons et al., 2006). Cancer-protective effects of Se in normal animals occur at a level that is 10-fold higher than what is required to maximize the activity of selenoenzymes. The higher intake of Se can induce phase 2 enzymes, such as glutathione S-transferase (GST). It has been demonstrated that Nrf-2 knockout mice, in which hepatic GST activity is reduced by 50% compared with wild-type mice, are more susceptible to benzo[a]pyrene carcinogenesis (Ramos-Gomez et al., 2001); on the other hand, high expression of GST has been shown to be protective against tumor development (Hayes et al., 2005). Modulation of the metabolism of carcinogens by phase 2 enzymes is one of the most effective and well-established strategies for protecting animals and their cells against the toxic and neoplastic effects of carcinogens (Talalay, 2000).

Elemental Se powder in the redox state of zero is not soluble and is generally considered to be biologically inert. Nanotechnology holds promise for medication and nutrition because materials at the nanometer dimension exhibit novel properties different to those of both isolated atoms and bulk material (Albrecht et al., 2006). With bovine albumin protein as dispersing agent, nascent elemental Se atoms formed by reducing selenite with GSH can aggregate into particles in sizes of 20–60 nm (Zhang et al., 2001). Our earlier studies demonstrated that elemental Se at nano size (Nano-Se) has comparable efficacy to selenite in upregulating selenoenzymes and tissue Se levels, but is less toxic (Zhang et al., 2001; Zhang et al., 2005). These results challenged the long-held dogma that elemental Se has no biological activities, and stimulated us to further compare Nano-Se with selenomethionine (SeMet), which has excellent bioavailability and lower toxicity among various Se forms. In comparison with SeMet, Nano-Se has lower toxicity and possesses equal efficacy in increasing the activities of selenoenzymes. Furthermore, the efficacy of GST induction by Nano-Se was higher than that of SeMet at supranutritional levels (Wang et al., 2007). These results indicate Nano-Se can serve as an antioxidant with reduced risk of Se toxicity and a potential chemopreventive agent if the induction of GST by Se is a crucial mechanism for its chemopreventive effect (Wang et al., 2007).

Use of Se in chemoprevention requires that the element to be considered as a pharmacological agent, whose clinical utility

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may require long-term administration at supranutritional levels, in which case the safety margin and potential toxic effects of Se are important considerations (Reid et al., 2004). Selenomethionine (SeMSC), a precursor of methylselenol, is one of the most effective Se compounds for chemoprevention, yet surprisingly, its potential toxicity has not been well investigated. Recently, it was found that methylselenol precursors were highly effective inducers of GST in cells (Xiao and Parkin, 2006). The comparison regarding the capacity of both Nano-Se and SeMSC in inducing GST in animals is of particular interest. Given that Nano-Se and SeMSC had similar potency in inducing GST, it would be important to know which one was more approaching toxicity of Se.

Therefore, the present study compared Nano-Se and SeMSC in inducing GST, and their toxicity in mice; in addition, we also compared their bioavailability which is the fundamental property of Se. Results herein demonstrate that, as compared with SeMSC, Nano-Se has lower toxicity and possesses equal efficacy in increasing the activities of selenoenzymes and GST.

**MATERIALS AND METHODS**

**Chemicals and Drugs**

Nicotinamide adenine dinucleotide (reduced), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, insulin, 5,5′-dithiobis (2-nitrobenzonic acid), thioreredoxin (Escherichia coli), thiorereductase (E. coli), guandine hydrochloride, reduced glutathione (GSH), bovine serum albumin, hydrogen peroxide, and 1-chloro-2,4-dinitrobenzene (CDNB) were all purchased from Sigma (St Louis, MO). SeMSC was obtained from PharmaSe, Inc. (Lubbock, TX). Nano-Se in the size range of 20–60 nm was prepared as described previously (Zhang et al., 2001).

**Animals and Treatments**

Male Kunming mice (body weight of 22–24 g) were used in this study. They and their diet were purchased from the animal center, Anhui Medical University, P.R. China. The mice were housed in plastic cages (5–7/each) in a room with controlled temperature (22 ± 1°C) and humidity (50 ± 10%) and 12-h light/dark cycle. The mice were allowed ad libitum access to diet and water. The protocol complied with the guidelines of University of Science and Technology of China for the care and use of laboratory animals.

**Acute lethal dose.** One hundred mice were randomly divided into 10 groups with 10 mice per group. A bolus of SeMSC or Nano-Se dissolved in saline was administered orally at the doses indicated in Table 2. Cumulative mortality within 14 days after the treatment was used for the calculation of LD₅₀.

**Acute toxicity at non lethal dose.** Forty mice were randomly divided into nine groups with six mice per group. They, respectively, received saline (as control), a bolus of SeMSC or Nano-Se at the dose of 10 mg Se/kg orally. Mice in Se-treated groups were sacrificed at 6, 12, 24, and 48 h.

**Determine the presence of Nano-Se particles.** To determine if intact Nano-Se particles are present in vivo, 10 mice were administered a bolus of 10 mg Se/kg Nano-Se orally, and sacrificed at 6 h (five mice) and 48 h (five mice) after the treatment. The livers and red blood cell (RBC) lysate from each group of five mice were pooled for the assay of intact Nano-Se.

**Survival rate.** Thirty mice were randomly divided into three groups with 10 mice per group. The control mice were orally administered saline; the mice in the other groups were orally administered SeMSC or Nano-Se at the dose of 10 mg Se/kg for 7 consecutive days. Survival of the mice was monitored for 14 days.

**Short-term toxicity.** Thirty mice were randomly divided into three groups with 10 mice per group. The control mice were orally administered saline; the mice in the other groups were orally administered SeMSC or Nano-Se at the dose of 5 mg Se/kg for 7 consecutive days. Animals were all sacrificed 24 h after the last dose.

**Bioavailability.** Mice and their offspring in the animal center had been continuously fed with a Se-deficient diet (< 0.02 μg Se/g diet) for more than one generation to deplete Se. When GPx activity in the liver was around or under 150 U/mg protein, the mice could be considered as Se deficient, because normally its activity is approximately 1500 U/mg protein. Fifty-six Se-deficient mice were randomly divided into seven groups with eight mice per group. They were orally administered saline (as control), SeMSC, and Nano-Se at the doses of 35, 70, and 1000 μg Se/kg once daily for 7 consecutive days. The Se-deficient diet was still used during this period. Animals were all sacrificed 24 h after the last dose.

**Distribution of blood Se.** Twenty-eight mice were randomly divided into seven groups with four mice per group. The mice were treated with saline or Nano-Se or SeMSC as the modality of bioavailability, and were sacrificed 24 h after the last dose.

**Potential of GST enhancement at supranutritional levels.** Forty-two Se-deficient mice were randomly divided into seven groups with six mice per group. They were orally administered saline (as control), SeMSC, and Nano-Se at the doses of 500, 1000, and 1500 μg Se/kg once daily for 7 consecutive days. Animals were all sacrificed 24 h after the last dose.

**Blood and tissue preparation.** At the end of each set of experiments, the mice were sacrificed by cervical dislocation. Peripheral blood from ophthalmic veins was collected into heparinized tubes to obtain whole blood or plasma and RBC after centrifugation, or into nonheparinized tubes to obtain serum after centrifugation. Liver and kidney were excised and rinsed in ice cold saline. Tissue and blood samples were stored at −30°C before being analyzed.

**Biochemical Parameters**

The serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were estimated by spectrophotometry using kits obtained from Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China. Livers and kidneys were homogenized with ice cold saline and centrifuged at 15,000 × g at 4°C for 15 min. The resulting supernatants were used for biochemical assessments. Protein level was determined by the Bradford dye-binding assay with bovine serum albumin as standard. Malondialdehyde (MDA) was determined according to the method of Ohkawa et al. (1979). The level of MDA was expressed as nmol/mg protein. GST activity was chemically determined using CDNB as substrate. One unit of GST activity was calculated in terms of nmol CDNB changed/min/mg protein (Habig et al., 1974). GPx was assayed by the method of Rotruck et al. (1973). The activity of GPx was expressed as units/mg protein, with a unit being defined as 1 μmol of glutathione oxidized/min. TrxR activity in animal tissues was measured using insulin as substrate. The activity of TrxR was calculated based on the standard curve prepared with pure TrxR (Zhang et al., 2003). Se in blood and tissues was determined by a fluorescent method (Mehlman et al., 1975). The total antioxidant capacity (T-AOC) was measured by the method of Benzie and Strain (1996). One T-AOC unit is defined as 0.01 increase of absorbance value caused by 1 mg protein in 1 min at 37°C.

**Nano-Se Particles Analysis**

It has been known that black powder of elemental Se can be reduced by a fourfold molar ratio of sodium sulfite to form selenosulfate (Na₂SeSO₃) according to the reaction: Na₂SO₃ + Se → Na₂SeSO₃ (Garcia et al., 1999; Rodriguez-Lazcano et al., 2005). As indicated in Table 1, 98.7% pure Nano-Se particles (20–60 nm) could be separated by centrifugation at 15,000 × g for 30 min (treatment 2 to treatment 1). Pure Nano-Se could be reduced by a fourfold molar ratio of sodium sulfite into Na₂SeSO₃ at 37°C for 20 min, which could not be separated from solution phase by centrifugation (treatment...
Based on the data presented in Table 2, the LD50 of SeMSC caused 100% mortality at a dose of 27.5 mg Se/kg. However, Nano-Se caused only 10% mortality at a dose of 36 mg Se/kg and 70% mortality at a dose as high as 150 mg Se/kg. Based on the data presented in Table 2, the LD50 of SeMSC was 14.6 mg Se/kg (with 95% confidence limits of 13.1–16.2) and 92.1 mg Se/kg (with 95% confidence limits of 71.1–131.1), respectively. The acute toxicity of Nano-Se was sixfold that of Nano-Se based on Se dose. For Nano-Se, the LD50 is consistent with the results that we previously reported (Zhang et al., 2001).

**Acute Lethal Effect of SeMSC and Nano-Se by Single Oral Administration in Kunming Mice (n = 10)**

<table>
<thead>
<tr>
<th>SeMSC Se dose (mg Se/kg)</th>
<th>Mouse mortality (%)</th>
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<tr>
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<td>100</td>
<td>150.0</td>
<td>70</td>
</tr>
<tr>
<td>20.6</td>
<td>100</td>
<td>150.0</td>
<td>70</td>
</tr>
<tr>
<td>15.5</td>
<td>60</td>
<td>73.5</td>
<td>20</td>
</tr>
<tr>
<td>11.6</td>
<td>30</td>
<td>51.5</td>
<td>20</td>
</tr>
<tr>
<td>8.7</td>
<td>0</td>
<td>36.0</td>
<td>10</td>
</tr>
</tbody>
</table>

**RESULTS**

3 to treatment 1). Furthermore, after intact Nano-Se was added into liver homogenate, 93.2% Nano-Se still could be separated by centrifugation, and 6.8% Nano-Se could be reduced by liver homogenate itself (treatment 6 to treatment 4). In a liver homogenate system, sodium sulfite still could fully reduce Nano-Se into Na2SeSO3 (treatment 7 to treatment 4). However, a 2000-fold molar ratio of sodium sulfite was needed to fully reduce Nano-Se into Na2SeSO3, probably due to the presence of oxidants and peroxides in tissue homogenate which consume sulfite. Therefore, if tissues contain intact Nano-Se, it could be detected via the following method: two parallel samples were prepared, to one was added sufficient sodium sulfite for the reduction of Nano-Se, to the other was added water of equal volume, followed by centrifugation to harvest the supernatants to test their Se concentrations. The difference in Se concentrations between the two samples was the content of Nano-Se.

Based on these properties, the liver homogenate or RBC lysate was divided into three portions. Portion I without any further treatment was used for testing total liver Se or total Se in RBC lysate. Portions II and III with exact same volume were added water and sufficient sodium sulfite, respectively, then were incubated at 37°C for 20 min, and then were centrifuged at 15,000 × g for 30 min. In order to avoid resuspension of Nano-Se pellet, top supernatants (approx. one fourth of supernatants) were carefully taken out for Se assay. Nano-Se concentration was calculated by subtracting the Se concentration of portion II from that of portion III. Portion I was used for the calculation of Nano-Se percentage in liver or RBC.

**Histopathological Studies**

Tissues were fixed in buffered formaldehyde (10% in phosphate-buffered saline) for more than 8 h. The fixed organs were dehydrated in graded ethanol and embedded in paraffin. Four-micrometer tissue sections were stained with the hematoxylin and eosin and then observed under light microscope by a pathologist in a blind fashion.

**Statistical Analysis**

Data are presented as the mean ± SD. The differences between the groups were examined using the standard one-way ANOVA. SPSS for Windows 13.0 software package (SPSS Inc., Chicago, IL) was used. p value < 0.05 indicated statistical significance.

**Acute Lethal Dose**

Table 2 shows the results of acute Se toxicity in mice. SeMSC caused 100% mortality at a dose of 27.5 mg Se/kg. However, Nano-Se caused only 10% mortality at a dose of 36 mg Se/kg and 70% mortality at a dose as high as 150 mg Se/kg.

**Acute Liver Injury**

Based on the results of the acute lethal dose study above, a bolus of SeMSC and Nano-Se at the dose of 10 mg Se/kg did not cause any mortality in mice.

**TABLE 1**

**Reduction and Separation of Nano-Se by Sodium Sulfite and Centrifugation, Respectively**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Se in supernatant (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 4 μg Se/ml Nano-Se, 1 ml</td>
<td>3.98 ± 0.17</td>
</tr>
<tr>
<td>2. 4 μg Se/ml Nano-Se, 1 ml, centrifugation</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>3. 4 μg Se/ml Nano-Se, 1 ml, adding 25.2 μg sodium sulfite, incubating at 37°C for 20 min, then centrifugation at 15,000 × g for 30 min</td>
<td>3.97 ± 0.08</td>
</tr>
<tr>
<td>4. Liver homogenate, 1 ml</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>5. Liver homogenate, 1 ml, adding 4 μg Nano-Se</td>
<td>4.11 ± 0.10</td>
</tr>
<tr>
<td>6. Liver homogenate, 1 ml, adding 4 μg Nano-Se, centrifugation at 15,000 × g for 30 min</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>7. Liver homogenate, 1 ml, adding 4 μg Nano-Se, adding 12.6 mg sodium sulfite, incubating at 37°C for 20 min, then centrifugation at 15,000 × g for 30 min</td>
<td>4.09 ± 0.04</td>
</tr>
</tbody>
</table>

**Note.** Liver homogenate: livers were homogenized with saline (1:10 wt/vol); Se assay of triplicate.

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**Acute Liver Injury**

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**TABLE 2**

**Acute Lethal Effect of SeMSC and Nano-Se by Single Oral Administration in Kunming Mice (n = 10)**

<table>
<thead>
<tr>
<th>SeMSC Se dose (mg Se/kg)</th>
<th>Mouse mortality (%)</th>
<th>Nano-Se Se dose (mg Se/kg)</th>
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</tr>
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<td>10</td>
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</tbody>
</table>
not cause death within 48 h; thus, this dose and this duration were chosen to investigate their toxic effects on liver by kinetic analysis.

**Time-related changes of the hepatic metabolic enzymes.** Under liver injury, hepatic metabolic enzymes, such as ALT, AST, and LDH, are released from liver into serum. As shown in Figs. 2A–C, with similar time course, the levels of ALT, AST, and LDH were sharply elevated at 12 h after the administration of SeMSC, whereas the changes were largely compromised in Nano-Se–treated mice.

**Time-related changes of liver MDA.** As shown in Fig. 2D, compared with the control, significant increase of MDA was observed in SeMSC-treated mice from 6 to 24 h, whereas only at 6 h in Nano-Se–treated mice; in addition, MDA at 12 and 24 h in SeMSC-treated mice was higher than that in Nano-Se–treated mice (p < 0.001 and 0.05, respectively). The higher accumulation and longer duration of MDA in SeMSC-treated mice might indicate that these mice incurred more severe and persistent status of oxidative stress.

**Time-related changes of liver GST activity and T-AOC.** As shown in Fig. 2E, GST activity was significantly increased by Nano-Se from 12 to 48 h and by SeMSC at 48 h compared with control. GST activity at 12 and 24 h in Nano-Se–treated mice was higher than that in SeMSC-treated mice (p < 0.01 and 0.001, respectively). T-AOC was decreased significantly by SeMSC from 6 to 24 h compared with control (all p < 0.001), whereas there was no differences between Nano-Se–treated mice and control at all time (Fig. 2F).

**Time-related changes of liver Se concentration.** Both liver Se concentrations examined reached plateaus at 6 h after the administration of the two Se origins (Fig. 2G), and SeMSC caused stronger escalation of Se at 6 and 12 h as compared with Nano-Se (p < 0.05 and 0.001, respectively).

In order to test whether there is intact Nano-Se particles in tissue and cells, mice were administered with Nano-Se at the dose of 10 mg Se/kg. Consistent with the Se content change as shown in Fig. 2G, we still found that liver Se increased at 6 h, then decreased at 48 h (Table 3). After sodium sulfite treatment...
(portion III), Se content in liver homogenate significantly increased as compared with that without the treatment of sodium sulfite (portion II), demonstrating that intact Nano-Se existed in liver. There was 14.9% and 9.8% Nano-Se in liver at 6 and 48 h after the treatment of 10 mg Se/kg, respectively. Nano-Se was not found in RBC at these two time points. Therefore, there seems no intracellular intact Nano-Se, and tissue contains a little portion of intact Nano-Se.

Over all, compared with Nano-Se, acute treatment with SeMSC potently disturbed redox homeostasis as evidenced by MDA and T-AOC, led to less adaptive response as manifested by GST, and therefore caused more robust acute liver injury as displayed by AST, ALT, and LDH. These results suggest that repetitive administration of such a dose of SeMSC could result in lethal consequences, which was confirmed in a survival study as follows.

**Survival Rate**

Mice were orally administered SeMSC and Nano-Se daily for 7 days at the dose of 10 mg Se/kg and then they were further observed for another 7 days to compare the survival rate. The survival curve (Fig. 3) shows that SeMSC caused 80% death ($p < 0.001$ compared with control), whereas Nano-Se caused only 10% death ($p < 0.01$ compared with SeMSC).

**Short-term Toxicity**

Mice were orally administered SeMSC and Nano-Se daily for 7 days at the dose of 5 mg Se/kg to compare their short-term toxicity.

**Suppressing growth.** As shown in Fig. 4A, Nano-Se displayed growth suppression on the fifth day and thereafter as compared with control ($p < 0.05$). However, SeMSC began to suppress growth as early as on the third day and thereafter as compared with control ($p < 0.01$), furthermore, SeMSC caused 20% mortality.

**Liver injury.** Both Se forms caused liver injury, but SeMSC was more potent in this regard. As compared with normal liver architecture (Fig. 4B), SeMSC caused irreversible and serious pathological change in the forms of pyknosis and necrosis (Fig. 4C), whereas Nano-Se caused hydropic degeneration which is a reversible and moderate pathological change (Fig. 4D). In addition, the increases of serum ALT and LDH (Table 4) in SeMSC-treated mice were higher than those in Nano-Se–treated mice ($p < 0.05$).

**Liver GST and T-AOC.** Both Se forms all significantly increased liver GST activity (Table 4), and GST activity in Nano-Se–treated mice was higher than that in SeMSC-treated mice ($p < 0.05$). T-AOC was decreased by SeMSC compared with control ($p < 0.05$), whereas there were no differences between Nano-Se–treated mice and control (Table 4).

Taken together, the major profile of short-term toxicity largely resembled the changes found in acute liver injury, and Nano-Se throughout manifested lower toxicity.

**Bioavailability**

Nutritional levels of Se at the doses of 35 and 70 µg Se/kg were used to compare bioavailability and supranutritional level of Se at the dose of 1000 µg Se/kg was used to indicate saturated activities of selenoenzymes.

**Se accumulation.** Both SeMSC and Nano-Se caused significant increase of Se in whole blood, liver, and kidney compared with control ($p < 0.01$) in a dose-dependent manner (Figs. 5A, D, and E). At nutritional levels, there was no

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**TABLE 3**

Detection of Intact Nano-Se Particles after an Oral Dose of 10 mg Se/kg Nano-Se

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Portion I (total Se)</th>
<th>Portion II (supernatant)</th>
<th>Portion III (supernatant + Na2SO3)</th>
<th>Nano-Se contents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver Se 6 h</td>
<td>4.50 ± 0.17</td>
<td>2.26 ± 0.18</td>
<td>2.93 ± 0.20*</td>
<td>14.9</td>
</tr>
<tr>
<td>(µg/g liver)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Se 48 h</td>
<td>1.80 ± 0.17</td>
<td>0.84 ± 0.024</td>
<td>1.01 ± 0.027*</td>
<td>9.4</td>
</tr>
<tr>
<td>(µg/g liver)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC Se 6 h</td>
<td>1.89 ± 0.22</td>
<td>1.82 ± 0.062</td>
<td>1.86 ± 0.23</td>
<td>0</td>
</tr>
<tr>
<td>(µg/ml blood)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC Se 48 h</td>
<td>2.47 ± 0.035</td>
<td>2.30 ± 0.20</td>
<td>2.52 ± 0.13</td>
<td>0</td>
</tr>
<tr>
<td>(µg/ml blood)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. Se assay of triplicate; compared with portion II, *$p < 0.05$. 

**FIG. 3.** Effects of SeMSC and Nano-Se on the survival rate of mice. Each group consisted of 10 animals. Saline was orally administered to mice as the control. SeMSC and Nano-Se were orally administered to mice at the dose of 10 mg Se/kg once daily for 7 consecutive days. The survival rate was determined daily for 14 consecutive days. ***$p < 0.0001$ indicates significant difference between control and SeMSC group; **$p < 0.01$ indicates significant difference between Nano-Se and SeMSC group.
significant difference in Se accumulation between the two Se forms; however, Se accumulation in SeMSC-treated mice was higher than that in Nano-Se–treated mice at supranutritional level, as indicated by *p* value in corresponding Figures.

**Blood Se distribution.** There was no difference in plasma Se between the two Se forms at any of tested doses ranging from nutritional to supranutritional levels (Fig. 5B); also there was no difference in RBC Se between the two Se forms at nutritional levels (Fig. 5C); large difference in RBC Se between the two Se forms was identified at a supranutritional level (Fig. 5C), which contributed to the large difference in whole blood between the two Se forms (Fig. 5A). As to Se distribution in blood, most of blood Se was in plasma at nutritional levels, irrespective of the Se forms (64–67%; Fig. 5B). It has been demonstrated that supranutritional or toxicological levels of Se can result in most of Se distributed in RBC (Styblo et al., 1988; Styblo et al., 1991), consistent with these findings, at the dose of 1000 μg Se/kg, 86% and 65% blood Se was in RBC of SeMSC and Nano-Se treated mice, respectively (Fig. 5C).

**Selenoenzymes.** Utilization of Se, after its absorption, involves transformation to selenoenzymes such as GPx and TrxR. Both SeMSC and Nano-Se caused significant increase of GPx activity in plasma, liver, and kidney of Se-deficient mice compared with controls (*p* all < 0.01) in a dose-dependent

### TABLE 4
**Effects of SeMSC and Nano-Se on Biochemical Parameters in Mice Serum and Livers (Oral Dose of 5 mg Se/kg Daily for 7 Days)**

<table>
<thead>
<tr>
<th></th>
<th>Control (<em>n</em> = 10)</th>
<th>SeMSC (<em>n</em> = 8)</th>
<th>Nano-Se (<em>n</em> = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/l)</td>
<td>11.2 ± 3.9</td>
<td>55.7 ± 23.3***</td>
<td>30.1 ± 10.1**</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>23.9 ± 6.6</td>
<td>51.9 ± 15.2***</td>
<td>47.9 ± 9.1***</td>
</tr>
<tr>
<td>LDH (U/ml)</td>
<td>18.0 ± 3.7</td>
<td>28.2 ± 7.8***</td>
<td>19.0 ± 3.2</td>
</tr>
<tr>
<td>T-AOC (U/mg protein)</td>
<td>734.1 ± 188.0</td>
<td>514.7 ± 129.4***</td>
<td>789.7 ± 165.3</td>
</tr>
<tr>
<td>GST (U/mg protein)</td>
<td>4.1 ± 0.8</td>
<td>6.8 ± 0.7***</td>
<td>7.9 ± 0.1***</td>
</tr>
</tbody>
</table>

*Note. Composed with control, *p < 0.05, **p < 0.01, and ***p < 0.001; compared with Nano-Se, †p < 0.05 and ††p < 0.01.

SeMSC caused 20% mice die in the experiment.
manner (Figs. 6A–C). In general, there was no significant difference between SeMSC and Nano-Se in increasing GPx activity. Distinct from the huge dose-dependent increase of GPx activity (maximal increases were 4.0-, 8.5-, and 15.6-fold in plasma, kidney, and liver), TrxR activity was maximally increased to two- to threefold in kidney and liver by both Se forms, which had no significant difference (Figs. 6D and E). Also, it appeared that both Se forms at the dose of 35 µg Se/kg had made TrxR activity reach a plateau, which is consistent with the observation that TrxR is more conserved under Se deficiency and ranks higher in the hierarchy for Se utilization relative to GPx (Behne and Kyriakopoulos, 1993).

**Potential of GST Enhancement at Supranutritional Levels**

In the bioavailability experiment, we observed that both Se forms can equally increase GST activity at the supranutritional level.

![FIG. 5. Se retention of SeMSC and Nano-Se. Se-deficient mice were orally administered SeMSC and Nano-Se at the dose of 35, 70, and 1000 µg Se/kg once daily for 7 consecutive days. Se in blood (A), plasma (B), RBC (C), liver (D), and kidney (E). p values indicate significant difference between SeMSC and Nano-Se at same Se dose.](image)

![FIG. 6. Effect of SeMSC and Nano-Se on selenoenzymes. Se-deficient mice were orally administered SeMSC and Nano-Se at the dose of 35, 70, and 1000 µg Se/kg once daily for 7 consecutive days. Activity of GPx in plasma (A), liver (B), and kidney (C). Activity of TrxR in liver (D) and kidney (E). p values indicate significant difference between SeMSC and Nano-Se at same Se dose.](image)
There was a dose-dependent relationship between 500 and 1000 µg Se/kg for 7 days. Serum ALT and AST activities indicated both Se compounds did not cause liver toxicity at these doses (Figs. 7A and B). At all investigated doses, both Se forms significantly increased liver GST activity compared with control (p < 0.05 for Nano-Se at 500 µg Se/kg, and p all < 0.01 for others). There was a dose-dependent relationship between 500 and 1000 µg Se/kg (p all < 0.01) and Se dose of 1500 µg Se/kg did not further significantly increase GST activity compared with the dose of 1000 µg Se/kg. There was no significant difference between the two Se forms at equi-Se doses (Fig. 7C), being consistent with what we had observed in the bioavailability experiment at the dose of 1000 µg Se/kg.

**DISCUSSION**

Compared with SeMSC, one of the most noticeable features of this comparative study is that Nano-Se has lower toxicity and possesses equal efficacy in increasing the activities of selenoenzymes and GST. Another prominent profile revealed herein is that tissue Se accumulation is largely compromised with Nano-Se at supranutritional level, but not at nutritional levels.

Animal studies have demonstrated that the liver is the major target organ of Se toxicity (Diskin *et al.*, 1979). In the present study, we found both Se forms caused liver injury, but SeMSC was more potent as evidenced by serum levels of ALT, AST, and LDH (Figs. 2A–C; Table 4) and the changes of liver architecture (Figs. 4B–D). Moreover, an oral dose of 5 mg Se/kg for 7 days, SeMSC caused 20% mice death and suppressed mice growth earlier than Nano-Se (Fig. 4A); an oral dose of 10 mg Se/kg for 7 days, SeMSC and Nano-Se caused 80% and 10% mice death, respectively (Fig. 3). In addition, Nano-Se was 6-fold higher in LD50 value than SeMSC (Table 2). The mechanism for the toxic effects of Se has been suggested to occur due to its prooxidant ability to catalyze the oxidation of thiols and simultaneous generation of reactive oxygen species (ROS), which can damage cellular components by lipid peroxidation (Spallholz, 1994). MDA is the product of lipid peroxidation caused by ROS. Both Se forms caused MDA increase, but its accumulation and duration in liver tissues of SeMSC-treated mice was higher and longer than that for Nano-Se (Fig. 2D) which implied the extent of oxidative stress was different. In parallel to this, T-AOC, a sum of the activities of the various antioxidative substances, is significantly decreased by SeMSC but not by Nano-Se (Fig. 2F and Table 4); GST, responsible for detoxifying harmful compounds and scavenging lipid peroxidation (Hayes *et al.*, 2005), was induced much earlier and higher by Nano-Se as compared with SeMSC (Fig. 2E and Table 4).

Empiric deduction suggests that Nano-Se has an impaired bioavailability due to the decrease in toxicity, especially to its background of elemental Se, which has long been thought to be biologically inert. Therefore, a comparison of bioavailability was conducted by orally administering SeMSC and Nano-Se to Se-deficient mice once daily for 7 consecutive days. Our results herein showed that the investigated Se forms were equally able to increase GPx and TrxR (Fig. 6).

At nutritional levels of Se intake, Se accumulation was comparably efficient between the two Se forms (Fig. 5); however, at a supranutritional level the Se accumulation associated with Nano-Se was significantly lower compared with SeMSC in addition to plasma (Fig. 5). The Se species accumulated from SeMSC at supranutritional level may include methylselenol, an assumed reactive Se species involved in the anticaarcinogenic effects of Se (Itoh and Suzuki, 1997). Multiple studies have demonstrated the ability of methylselenol to affect the cell cycle and induce apoptosis in cancer cell lines (Kim *et al.*, 2001; Medina *et al.*, 2001). Therefore, the potential higher accumulation of methylselenol by SeMSC at supranutritional level may lead to argue for its advantage over Nano-Se for chemoprevention. Although the actual chemopreventive mechanisms of Se are still not fully understood, selenoenzymes, phase 2 enzymes, and the cytotoxic effect of Se are all likely to be involved. Therefore, the presumed advantage of SeMSC over Nano-Se is largely dependent on the assumption of a selective modulation of cancer cells by Se and a predominant position occupied by cytotoxic pathway over other mechanistic pathways, such as selenoenzymes and phase 2 enzymes.

**FIG. 7.** Effect of SeMSC and Nano-Se on GST activity. Se-deficient mice were orally administered SeMSC and Nano-Se at the dose of 500, 1000, and 1500 µg Se/kg once daily for 7 consecutive days. Activity of ALT in serum (A). Activity of AST in serum (B). Activity of GST in liver (C).
Regarding selectivity, several investigators have reported that Se compounds preferentially inhibit growth and induce apoptosis in cancer cells compared with normal cells (Ghose et al., 2001; Watrach et al., 1984). However, surprisingly, in the case of liver, the major target of Se toxicity in animals (Diskin et al., 1979), it was found that the cytotoxic effect of Se was at least equally or even more potent in normal hepatocytes compared with hepatic carcinoma cells (Weiller et al., 2004). Consistent with this study, in the case of prostate, an extensively investigated tissue in Se chemoprevention, nontumorigenic prostate cells are highly sensitive to Se toxicity as compared with prostate cancer cells at physiologically relevant concentrations (5–10 µM) (Rebsch et al., 2006).

With respect to the rank of cytotoxic effect among the multiple pathways for chemopreventive effect of Se, to the best of our knowledge, no such a direct comparison has been conducted up to now. On the contrary, in animal studies of Se at supranutritional levels, the modulation of phase 2 enzymes has frequently been reported (El-Sayed et al., 2006; El-Sayed and Franklin, 2006; Ip and Lisk, 1997) and the involvement of selenoenzymes at nutritional levels has again received considerable attention (Diwadkar-Navsariwala et al., 2006; Irons et al., 2006; Rebsch et al., 2006). On the other hand, if the cytotoxic effect of Se is indeed the central mechanism or sole mechanism for its chemopreventive capacity, the risk of Se toxicity seems to be unavoidable unless a superior selectivity to cancer cells is firmly established because ingested supranutritional Se is overwhelmingly taken by normal cells compared with malignant cells in a chemopreventive setting, whereas the converse selectivity, opposite to expectation, that was reported by Weiller et al. (2004) and Rebsch et al. (2006) suggests that one cannot attribute the predominant chemopreventive mechanism of Se to cytotoxicity alone.

In addition, many selenocompounds, such as 1,4-phenylenebis(methylene)selenocyanate and 2-substituted selenazolidine-4(R)-carboxylic acids, whose chemical structures are less likely to be metabolized into methylselenol, still possess convincing chemopreventive effects. A prominent profile of these selenocompounds is their capacity to induce phase 2 enzymes in animals at a level lower than the toxic dose (El-Sayed and Franklin, 2006; Sohn et al., 1999). Induction of phase 2 enzymes is an effective and sufficient strategy for achieving protection against the toxic and neoplastic effects of many carcinogens (Talalay, 2000). Therefore, if taking the toxicity of Se into account, the induction of phase 2 response might represent a more attractive mechanism compared with the cytotoxic mechanism.

Recently, methylselenol precursors were identified to be highly effective selenocompounds for the induction of phase 2 enzymes in Hepa 1c1c7 cells (Xiao and Parkin, 2006). It should be noted that the Se level needed for the induction of phase 2 enzymes can be much lower than those causing cell growth inhibition or cytotoxicity (Xiao and Parkin, 2006). As stated by Xiao and Parkin (2006), methylselenol precursors were highly effective inducers for phase 2 response in cells; these authors suggested that animal studies were needed to assess the gap between effective dose and toxic dose. Our present study demonstrates that SeMSC dose dependently increases hepatic GST activity at supranutritional levels (0.5–1.5 mg Se/kg, daily, for 7 days) without causing toxicity, and that there is no significant difference between SeMSC and Nano-Se at equi-Se doses (Fig. 7). However, further escalated doses of SeMSC (5–10 mg Se/kg, daily, for 7 days) produce more prominent toxicity as compared with Nano-Se.

Significantly lower accumulation of Se at supranutritional levels in mice fed Nano-Se compared with SeMSC is relevant to the size effect of Nano-Se and is a strategy for avoiding toxicity by application of nanotechnology. It has been reported that Nano-Se has a size-dependent effect in scavenging various free radicals; small-size Nano-Se has greater ability to transfer electrons to radicals (Huang et al., 2003). However, the size effect of Nano-Se within 200 nm in terms of induction of selenoenzymes could not be observed in both Se-deficient cultured cells and Se-deficient mice at nutritional level (Zhang et al., 2004). The explanation to this phenomenon has two aspects: intrinsically, an active absorption pathway as driving force to obtain Se may exist when Se-deficient cells are thirsty for Se; extrinsically, even modest Se supplementation can easily saturate selenoenzymes, thereby excluding a size effect under an urgent need. At the supranutritional level, in fact, approaching to the toxic level, whereupon selenoenzymes have already been fully saturated, cells may in turn change to passive absorption of Se. Our unpublished results which compared Se retention of 36 and 90 nm Nano-Se at supranutritional levels showed large size constituted a barrier for Se accumulation. Compared with small molecular weight selenocompounds, for example, SeMSC herein, 36 nm Nano-Se is fairly larger in size. With respect to Se accumulation, SeMSC and 36 nm Nano-Se did not show a difference at nutritional levels, but did show a significant difference at supranutritional levels, which is exactly the same as the rules in size comparison between 36 and 90 nm Nano-Se. Reduced accumulation of Se at supranutritional levels may effectively delay the onset and development of Se toxicity; fortunately, the reduction of Se accumulation does not lead to attenuate the induction of GST.

In summary, based on chemoprevention-related responses in animals, as compared with SeMSC, Nano-Se possesses equal efficacy in increasing the activity of GPx, TrxR, and GST, but has much lower toxicity, suggesting that Nano-Se can serve as a potential chemopreventive agent with significantly reduced risk of Se toxicity.

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