Size effect of elemental selenium nanoparticles (Nano-Se) at supranutritional levels on selenium accumulation and glutathione S-transferase activity

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Abstract:

It has been shown that 36 nm Nano-Se has lower toxicity than selenite or selenomethionine, but these forms of selenium (Se) all possess similar ability to increase selenoenzyme levels. The size of nanoparticles plays an important role in their biological activity: as expected, 5–200 nm Nano-Se can directly scavenge free radicals *in vitro* in a size-dependent fashion. However, in Se-deficient cells and Se-deficient mice, the size effect of Nano-Se on increasing selenoenzymes and liver Se disappears unexpectedly. We hypothesize that under conditions of Se deficiency, the avidity of Se uptake mechanisms may be increased to maintain the biosynthesis of selenoenzymes, which are fundamental for redox homeostasis. This increased avidity may override the potential advantage of small size Nano-Se seen under Se-replete conditions, thereby eliminating the size effect. Once selenoenzymes have been saturated, Se uptake mechanisms may downregulate; accordingly, the size effect of Nano-Se can then reappear. To test this hypothesis, Se-deficient mice were administered either 36 or 90 nm Nano-Se at supranutritional doses, in both a short-term model and a single-dose model. Under these conditions, Nano-Se showed a size effect on Se accumulation and glutathione *S*-transferase (GST) activity. A size effect of Nano-Se was found in 15 out of 18 total comparisons between sizes at the same dose and time in the two models. Furthermore, the magnitude of the size effect was more prominent on Se accumulation than on GST activity. GST is strictly regulated by transcriptional and translational mechanisms, so its increase in activity normally does not exceed 3-fold. In contrast, the homeostasis of Se accumulation is not as tightly controlled. In the present experiments, GST activity had reached or was approaching saturation, but liver Se was far below saturation. Therefore, our results strongly suggest that the saturation profile of the tested biomarker has an impact on the size effect of Nano-Se. Since both GST and small molecular weight selenocompounds accumulated *in vivo* are important intermediates for chemoprevention by Se, our results also suggest that Nano-Se should be most effective as a chemopreventive agent at smaller particle size.

Keywords: Size effect | Nano-Se | Glutathione *S*-transferase | Selenium

Article:

1. Introduction

It is well known that elemental selenium (Se) at micrometer size and above is black, vitreous, insoluble and biologically inert [1]. Elemental Se nanospheres (300 nm) have been found in certain anaerobic bacteria [2], [3]. Furthermore, it has been reported that the presence of protein can control the aggregation of elemental Se atoms to form elemental Se nanoparticles (Nano-Se) [1], [4]. Various sizes of Nano-Se can be obtained by changing the concentration of bovine serum albumin (BSA). Generally, the higher the BSA concentration, the smaller the resulting Nano-Se particle size [5]. It has been shown that 36 nm Nano-Se is as efficient as selenite or selenomethionine in regard to bioavailability, e.g., in terms of ability to increase glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) enzyme activities [1], [6]. Moreover, 36 nm Nano-Se possesses significantly lower toxicity as compared to selenite or selenomethionine [1], [5], [6], [7], [8].

The size of nanoparticles plays an important role in their biological activity. Generally, smaller sized nanoparticle are more active than those of larger size [5], [9], [10]. For example, smaller sized regular nanoparticles exert a stronger cytotoxic effect on endothelial cells than those of larger size [11], and smaller sized chitosan nanoparticles have more potent tumor inhibitory effects than those of larger size [12]. As to elemental Se, some properties are indeed dependent on its size. Above micrometer size, it is biologically inert [1]. In contrast, subnano particles of elemental Se have robust cytotoxicity to leukemia cells [4]. It has been reported that Nano-Se has a size effect on redox reactivity [13], and Nano-Se in the range of 5–200 nm has a size-dependent effect in directly scavenging various free radicals *in vitro*, such as 1,1-diphenyl-2-picryhydrazyl (DPPH) and the superoxide anion [14]. Contrary to the phenomenon *in vitro*, the same set of Nano-Se no longer has a size effect on enhancing the activity of GPx, TrxR and phospholipid hydroperoxide glutathione peroxidase (PHGPx) in Se-deficient HepG2 cells; it also has no size effect on increasing the activity of GPx and TrxR in liver, as well as liver Se levels, at doses close to the nutritional level (70 g Se/kg for 7 days) in Se-deficient mice [5]. We speculated that the disappearance of size effect on selenoenzymes may be due to intrinsic mechanisms that counteract or override the potential advantage of smaller sized Nano-Se. It is known that selenoenzymes are fundamental for redox homeostasis. GPx1 knockout mice are more vulnerable to oxidative stress [15], [16], GPx1/GPx2-double-knockout mice have a trend to develop cancer [17], knockout of PHGPx causes early embryonic lethality [18], [19], and knockout of TrxR results in embryonic lethality [19]. Therefore, when cells are in a Se deficient state, the avidity of Se uptake mechanisms may be increased to maintain the biosynthesis of selenoenzymes. Under the circumstance of Se deficiency, cells may upregulate one or more effective pathways for Se uptake, leading to the disappearance of the size effect of Nano-Se on selenoenzyme synthesis.

Se at supranutritional doses exerts a chemopreventive effect via multiple mechanisms, such as the induction of glutathione *S*-transferase (GST) for detoxifying electrophilic compounds generated during xenobiotic metabolism [20], [21], [22], [23], [24] and the accumulation of small molecular weight selenocompounds that can exert cytotoxic effects [24], [25], [26]. Although highly effective in chemoprevention, the supranutritional doses of Se which are 10-

fold more than the normal physiological requirement [6] are in fact approaching the toxic level of Se [24], [27], [28]. Under this circumstance, the cells are less likely to still have the demand to actively absorb Se for the biosynthesis of selenoenzymes, because they have been saturated [29], [30]. In contrast, they may switch to a strategy to prevent the entry of Se, to avoid potential toxicity. We speculated that the potential advantage and superior absorption characteristics of small size Nano-Se would reappear under such conditions. To test this hypothesis, the present study investigated the size effect of Nano-Se at supranutritional dose levels on Se accumulation and GST activity in mice.

2. Materials and methods

2.1. Preparation of Nano-Se

Different sizes of Nano-Se were prepared by adding varying amount of BSA to a redox system of selenite and glutathione (GSH) as previously reported [1], [14].

2.2. Animals and treatments

2.2.1. Se-deficient mice

All mice were purchased from the animal center in Anhui Medical University. 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 selenium.

2.2.2. Short-term model

Forty mice were randomly divided into five groups: the control group was treated with saline; the other groups were administered either 36 nm or 90 nm of Nano-Se at the doses of 0.5 or 2 mg Se/kg for 7 days.

2.2.3. Single-dose model

Forty five mice were randomly divided into nine groups: the control was supplied with saline; the other groups were administered a single dose of either 36 or 90 nm Nano-Se at 5 or 10 mg Se/kg and sacrificed after 24 or 72 h.

All the animals were housed at 22 ± 2 °C and 55% humidity, given a constant 12 h day/12 h night diurnal cycle, and received humane care in accordance with the guidelines of University of Science and Technology of China for the care and use of experimental animals.

2.3. Biomarkers

Blood was centrifuged at 3000*g* for 10 min to isolate plasma, and then stored at −30 °C. Livers were excised immediately and rinsed in ice cold saline, then stored at −30 °C. Livers were homogenized with ice cold saline and centrifuged at 15,000*g* at 4 °C for 15 min. The resulting supernatants were used for the determination of GPx and GST activities. Protein levels were

determined by the Bradford method with BSA as standard. GPx was assayed by the method of Rotruck et al. [31]. GPx activity was expressed as units/mg protein or units/ml plasma; one unit of the activity was calculated in terms of 1 μmol of glutathione oxidized/min. The GST activity was expressed as units/min/mg protein; one unit of the activity was calculated in terms of nmoles 1-chloro-2,4-dinitrobenzene (CDNB) changed [32]. Se was assayed by the diamino–naphthalene method [33]: first, the sample was digested using the mixture of nitric acid and perchloric acid at the molar ratio of 3:1, then reacted with diamino–naphthalene in 60 °C for 30 min, then finally extracted with hexamethylene. The fluorescence intensity excited at 378 nm and recorded at 512 nm was used for calculating Se based on the fluorescence intensity of standard concentrations of Se.

2.4. Statistical analysis

Data are presented as the means ± SD. The differences between the groups, which were considered significant at *p*-values of less than 0.05, were determined by using one-way analysis of variance (ANOVA) test.

3. Results and discussions

Previous studies showed that different sizes of Nano-Se could be prepared by changing the BSA concentration used in the preparation [5], [14]. Generally, the higher the BSA concentration, the smaller the resulting Nano-Se particle size [5]. We prepared two sizes of Nano-Se by adding different concentrations of BSA into the redox system containing selenite and GSH. Transmission electron microscopy (TEM) images are shown in Fig. 1. Both the forms of Nano-Se present as spherical particles, with diameters of 36 ± 6 nm (Fig. 1a) and 90 ± 8 nm (Fig. 1b), respectively. At the same concentration of Se, the number of particles for 36 nm Nano-Se is roughly one order of magnitude more than that for 90 nm Nano-Se, which is similar to the result reported by Chen et al. [34].

The primary purpose of this study was to find whether Nano-Se has a size effect at supranutritional dose levels. Treating mice with Se compounds at supranutritional doses (0.5–1.1 mg Se/kg) for 7 days is a short-term experimental model for assessing the chemopreventive potency of Se [23], [24]. Thus, the mice were administered Nano-Se at doses of 0.5 or 2.0 mg Se/kg for seven consecutive days. Blood Se (Fig. 2a) and liver GST activity (Fig. 3) were size-dependently increased by Nano-Se at both doses. Liver Se (Fig. 2b) was size-dependently increased by Nano-Se at 2 mg Se/kg.

The short-term model gave a cumulative result. However, utilization of Se comprises a series of dynamic processess, for example, Se concentration in liver rapidly increases and then gradually decreases [35]. To see if a size effect exists during these dynamic processes, mice were treated with a single dose of Nano-Se at 5 or 10 mg Se/kg. At 24 h, blood Se and liver Se (Fig. 4a and b) were size-dependently increased by Nano-Se. At 72 h, blood Se (Fig. 4a), liver Se (Fig. 4b) and liver GST activity (Fig. 5) were size-dependently increased by Nano-Se.

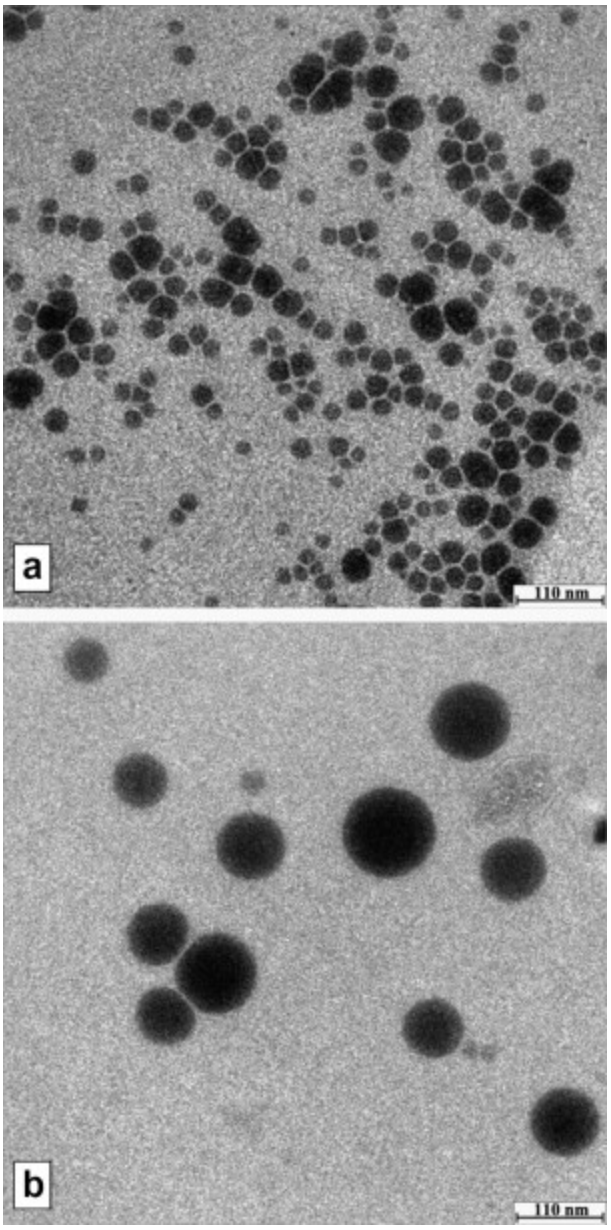

Fig. 1. Transmission electron microscopy images of Nano-Se. (a) 36 nm; (b) 90 nm. The bar in each figure represents 110 nm.

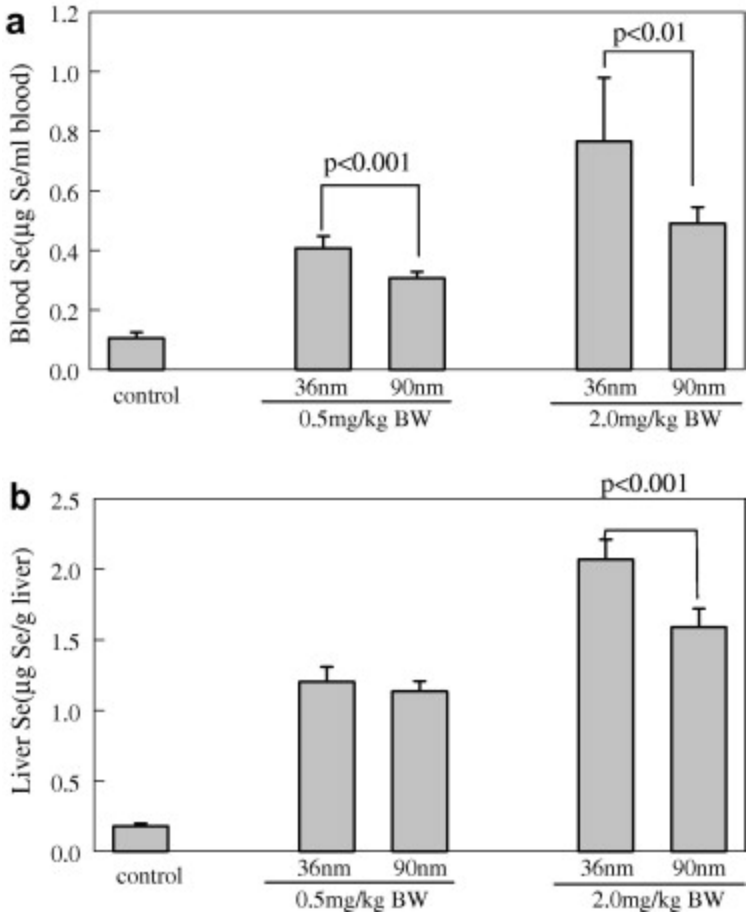

Fig. 2. Size effects of Nano-Se on Se accumulations. (a) Blood Se; (b) liver Se. Mice were administered Nano-Se at doses of either 0.5 or 2.0 mg Se/kg body weight for seven consecutive days (n = 8). The data are expressed as means ± SD. The statistical significance between sizes is marked on each column.

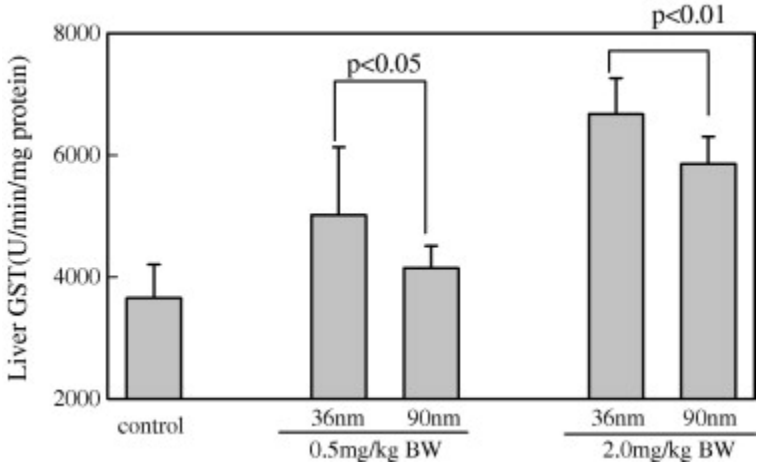

Fig. 3. Size effects of Nano-Se on increasing liver GST activity. Mice were administered Nano-Se at doses of either 0.5 or 2.0 mg Se/kg body weight for seven consecutive days (n = 8). The data are expressed as means ± SD. The statistical significance between sizes is marked on each column.

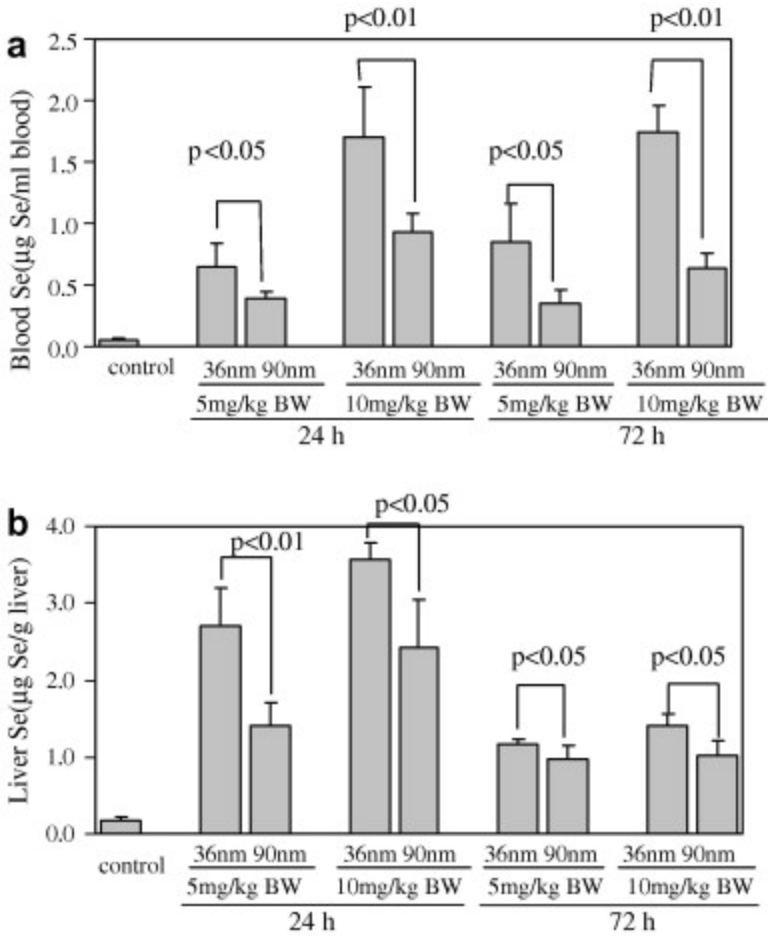

Fig. 4. Size effects of Nano-Se on Se accumulations. (a) Blood Se; (b) liver Se. Mice were administered a single dose of Nano-Se at the level of either 5.0 or 10.0 mg Se/kg body weight (n = 5). The data are expressed as means ± SD. The statistical significance between sizes is marked on each column.

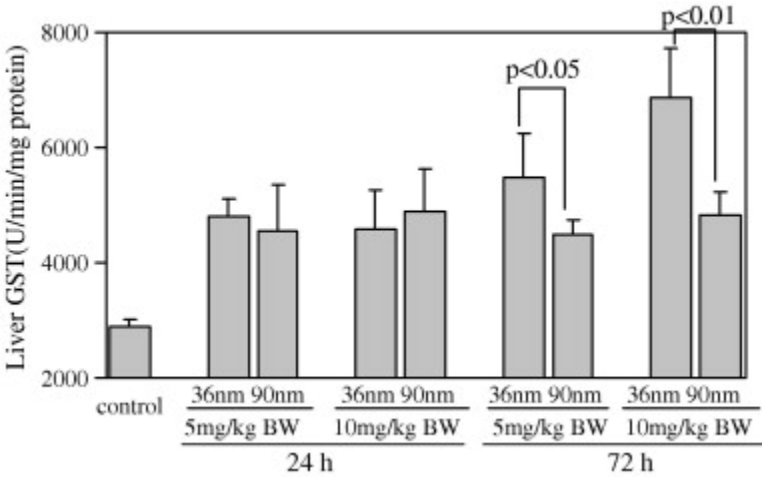

Fig. 5. Size effects of Nano-Se on increasing liver GST activity. Mice were administered a single dose of Nano-Se at the level of either 5.0 or 10.0 mg Se/kg body weight (n = 5). The data are expressed as means ± SD. The statistical significance between sizes is marked on each column.

In summary, the size effect of Nano-Se was verified in two models in the present study. In the short-term model and the single-dose model, each model examined two doses of Nano-Se and two biological parameters (Se accumulation and GST activity); moreover, the latter model included two time points. There were a total of 18 comparisons between sizes at the same dose and time. The size effect of Nano-Se was observed in 15 of them. These results are summarized in Table 1, from which it can be observed that the potency of the size effect of Nano-Se on the tested biomarkers is different, the rank being as follows: blood Se > liver Se > liver GST.

Table 1. Ratio of differences in Se biomarkers between two particle sizes at the same time and dose

	Short-term model		**Single-dose model**				**Mean ± SD**
	7 days	**7 days**	**24 h**	**24 h**	**72 h**	**72 h**	
	0.5 mg/kg	**2 mg/kg**	**5 mg/kg**	**10 mg/kg**	**5 mg/kg**	**10 mg/kg**	
Blood-Se	1.32[a]	1.60[b]	1.66[c]	1.81[b]	2.41[c]	2.74[a]	1.92 ± 0.54
Liver-Se	1.06	1.30[a]	1.94[a]	1.48[c]	1.21[c]	1.39[c]	1.40 ± 0.30
Liver-GST	1.21[c]	1.14[c]	1.06	0.94	1.22[c]	1.42[b]	1.17 ± 0.16

[a] $p < 0.001$.
[b] $p < 0.01$.
[c] $p < 0.05$ indicated significant different between sizes.

It has been suggested that the rank of the size effects of Nano-Se on the tested biomarkers may be associated with the different saturation profiles of the tested biomarkers. Our previous study showed that 36 nm Nano-Se at the dose of 5 mg Se/kg for 7 days increased liver Se to a level of 10 μg Se/g [6]. In the present study, liver Se was increased to 2.1 μg Se/g in the short-term model and increased to 3.6 μg Se/g in the single-dose model by 36 nm Nano-Se, both of which are far below 10 μg Se/g, demonstrating that liver Se in the present experiments did not approach saturation. Although GST could be modulated by higher dose of Se [22], [23], [24], its synthesis undergoes strict control at the transcriptional and translational levels [23], [24], [36]. Normally, its maximum activity is approximately 2–3-fold higher than the control [22], [37], [38]. In the present work, GST activities were maximally increased to 1.8- and 2.4-fold higher than the control in the short-term model and the single-dose model, respectively. The results demonstrate that GST had reached or was approaching saturation. All these data indicate that liver GST is more easily saturated than liver Se, leading to compromised size effect on GST as compared with liver Se (Table 1). Thus, the saturation profile of a tested biomarker has an obvious impact on the size effect of Nano-Se on the biomarker.

It is generally accepted that when a biomarker is approaching its saturation status, the dose-dependent response will compromise or disappear, and verse versa. Thus, the intensity of the dose dependency of a biomarker can be used to estimate the saturation status of the biomarker. For this reason, we calculated the ratio of Se biomarkers between two doses at the same time and particle size (Table 2). The sequence of dose dependency of the biomarkers is as follows: blood Se > liver Se > liver GST. This sequence indicates that, within the dose range used herein, liver Se is more difficult to saturate than GST, a result that is consistent with the deduction described above. Also this sequence implies that blood Se is the hardest to saturate among the three biomarkers. Since saturation profile has an obvious impact on size effect of Nano-Se, the size effect of Nano-Se on blood Se ought to be more marked compared with liver Se or liver GST. Indeed, the size effect of Nano-Se on blood Se is the most prominent among the three

biomarkers (Table 1). These observations consistently support the conclusion that the saturation profile of the tested biomarker has an obvious impact on the size effect of Nano-Se.

Table 2. Ratio of differences in Se biomarkers between two doses at the same time and particle size

	Short-term model			Single-dose model			Mean ± SD
	7 days	7 days	24 h	24 h	72 h	72 h	
	36 nm	90 nm	36 nm	90 nm	36 nm	90 nm	
Blood-Se	1.88[a]	1.60[a]	2.62[b]	2.40[a]	2.07[c]	1.82[b]	2.06 ± 0.38
Liver-Se	1.72[a]	1.40[a]	1.32[c]	1.73[c]	1.21[c]	1.05	1.41 ± 0.27
Liver-GST	1.41[a]	1.33[a]	0.95	1.07	1.25[c]	1.08	1.18 ± 0.18

[a] $p < 0.001$.
[b] $p < 0.01$.
[c] $p < 0.05$ indicated significant different between doses.

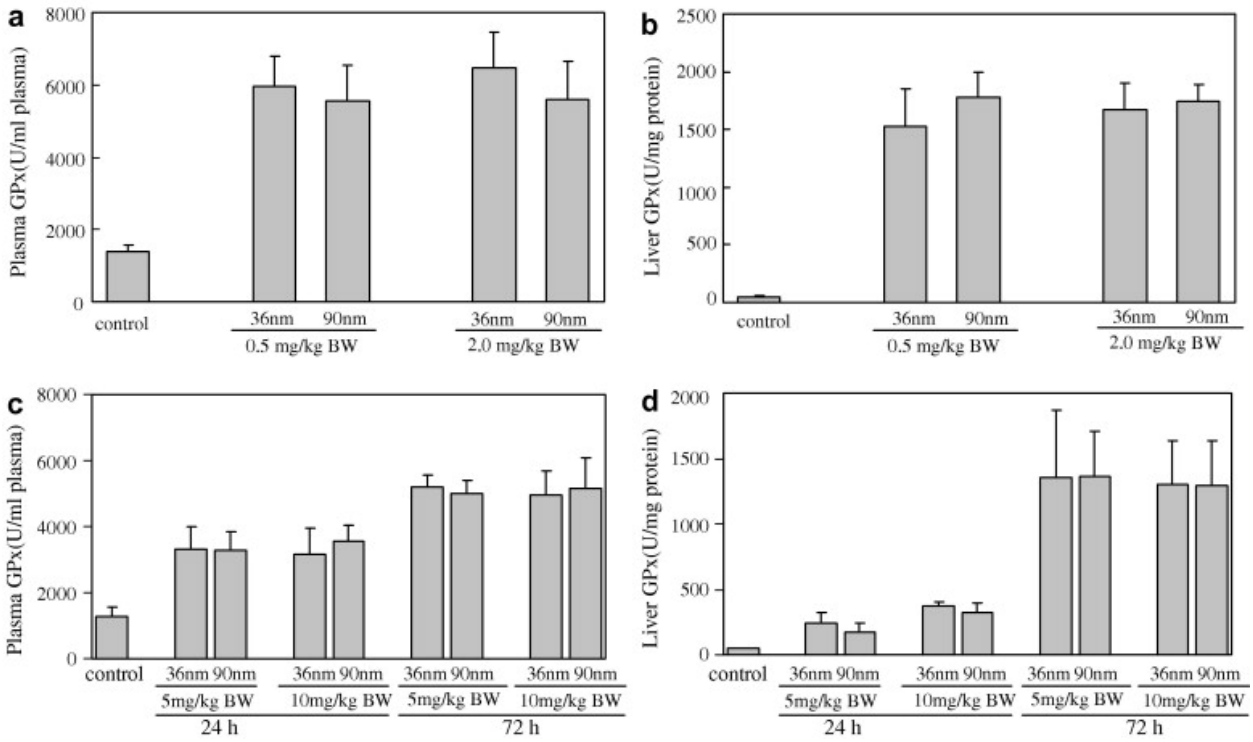

Fig. 6. The effect of two sizes of Nano-Se on GPx activity. (a) GPx in liver in a short-term model. (b) GPx in plasma in a short-term model. (c) GPx in liver in a single-dose model. (d) GPx in liver in a single-dose model. Mice in the short-term model were administered Nano-Se at the doses of either 0.5 or 2.0 mg Se/kg body weight for seven consecutive days ($n = 8$); Mice in the single-dose model were administrated with a single dose of Nano-Se at the level of either 5.0 or 10.0 mg Se/kg body weight ($n = 5$). The data are expressed as means ± SD.

To further support this conclusion, we compared the size effect of Nano-Se on GPx activity in the present experimental models. It is well known that GPx activity can be saturated at nutritional levels [6], [39] and that further supplementation with supranutritional levels of Se does not further increase its activity [24], [29], [30], suggesting GPx is an easily saturated biomarker compared to Se accumulation and GST. According to the conclusion that the saturation profile of the tested biomarker has an impact on the size effect of Nano-Se, the size

effect of Nano-Se on GPx ought to be less prominent as compared with liver GST or liver Se or blood Se. As expected, as shown in Fig. 6, none of the total 12 comparisons related to GPx activity between two sizes showed a size effect.

The present study not only shows that Nano-Se has a size effect in mice, but also establishes that the saturation profile of the tested biomarker has an obvious impact on the size effect. Since the levels of small molecular weight selenocompounds accumulated *in vivo* and GST are size-dependent, and these are important intermediates for the chemoprevention of Se [6], [22], [26], our results also suggest that Nano-Se should be most effective as a chemopreventive agent at smaller particle size, despite the lack of a size effect on selenoenzymes.

4. Abbreviations

ANOVA	one-way analysis of variance
BSA	bovine serum albumin
CDNB	1-chloro-2,4-dinitrobenzene
DPPH	1,1-diphenyl-2-picryhydrazyl
Nano-Se	elemental Se nanoparticles
GPx	glutathione peroxidase
GSH	glutathione
GST	glutathione *S*-transferase
PHGPx	phospholipid hydroperoxide glutathione peroxidase
Se	selenium
TEM	transmission electron microscopy
TrxR	thioredoxin reductase

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