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Effect of Sodium Selenosulfate on Restoring Activities of Selenium-Dependent Enzymes and Selenium Retention Compared with Sodium Selenite In Vitro and In Vivo

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ABSTRACT

Sodium selenosulfate has been extensively used as a precursor of selenide ions in the preparation of nano Se-containing compounds. Its biological properties remain completely unknown. Sodium selenosulfate and sodium selenite were added to the medium of HepG2 cells and administered intraperitoneally at a dose of 0.1 mg Se/kg body weight to selenium-deficient mice, respectively. Both of the selenium compounds could increase the activities of glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) in a dose-dependent manner in cells and efficiently restore selenium retention and activities of GPx and TrxR in mice. All of the variables were in correlation with the Se supply. There was no distinction in elevating activities of GPx and TrxR between selenosulfate and selenite in vitro. After a 2-d supply of selenosulfate, the activity of GPx in the liver was 65% ($p < 0.001$) and Se accumulations in the liver, kidney and blood were 64%, 86%, and 65%, respectively, of those treated with selenite (all $p < 0.01$). With the 7-d selenosulfate supplementation, the activity of GPx in the kidney and activities of TrxR in the liver and kidney were 88%, 75%, and 78%, respectively, of those treated with selenite (all $p < 0.01$); Se retentions in the liver and kidney were 85% and 93%, respectively of those supplemented with selenite (both $p < 0.01$). These facts indicated that selenosulfate could be absorbed and utilized in the biological system. No difference in vitro demonstrated that selenosulfate could be absorbed and generate reduced selenide as efficiently as selenite. The differences between the two compounds in vivo were the result of other factors that affected selenosulfate utilization in tissues.

Index Entries: Sodium selenosulfate; selenite; Se-dependent enzyme; Se retention.

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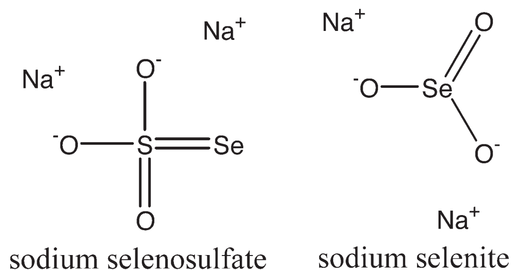


Fig. 1. The chemical structures of sodium selenosulfate (Na_2SeSO_3) and sodium selenite (Na_2SeO_3).

INTRODUCTION

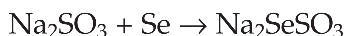
Selenium (Se) is an essential nutrient for both humans and animals. The basis for Se essentiality is its role at the catalytic site of multiple Se-dependent enzymes, such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) *(1).* The bioavailability to be absorbed and utilized by body for specific physiologic functions is often considered as one of the functions of Se compounds *(2).* Se is known to be taken up in inorganic forms (e.g., sodium selenite [Na_2SeO_3]) or organic forms such as selenocysteine (SeCys) and selenomethionine (SeMet) mainly from the food supply. Sodium selenosulfate (Na_2SeSO_3) (Fig. 1), as an inorganic Se, has been extensively used as a precursor of selenide ions in material science for the preparation of selenium-containing nanocompounds *(3–5).* Up to now, whether selenosulfate could act as a precursor of selenide in restoring activities of Se-dependent enzymes and Se retention in biological system is completely unknown.

The bioavailability of different Se compounds has been intensively compared by activities of Se-dependent enzymes and Se accumulation in cells and Se-deficient animals *(6–8).* In this study, the effect of sodium selenosulfate on restoring Se retention and activities of Se-dependent enzymes was determined in cells culture and in Se-deficient mice.

METHODS AND MATERIALS

Materials

Reduced glutathione (GSH), hydrogen peroxide, 5,5-dithionitrobenzoic acid (DTNB), NADPH, insulin, and sodium selenite were all obtained from Sigma (St. Louis, MO, USA). All other agents were analytically pure. Sodium selenosulfate was produced according to the method usually used in material studies *(5,9)* based on the following reaction:



$$Na_2SO_3 + Se \rightarrow Na_2SeSO_3$$

Sodium selenosulfate was used in the form of solution, which was obtained by adding 3.95 g elemental Se powder to 1.0 *M* of 100 mL sodium sulfite (molar ratio, Se : sulfite = 1 : 2), stirring, and refluxing the mixture for 3 h at 90°C *(3,5,9)*. The sulfite ions presented in excess in the selenosulfate solution were of prime importance for the stability of selenosulfate because of their reduction properties [$E°(SO_4^{2-}/SO_3^{2-})$=–0.90 V]. The resulting selenosulfate solution was diluted with phosphate buffer (0.15 *M*, pH 7.4) to the concentration needed. In this system, selenosulfate remained stably for approx 10 h at room temperature; afterward, it was found that selenosulfate could be gradually oxidized to elemental Se. Therefore, selenosulfate was always freshly prepared 1 hr before its supplementation in cells and mice.

Cell Culture and Preparation of Cell Extracts

Human hepatoma HepG2 cells were cultured in modified eagle's medium (MEM) supplemented with fatal bovine serum (10%), penicillin (100 units/mL), and streptomycin (100 µg/mL) in an atmosphere of 5% CO_2 at 37°C. Cells were seeded in 10-cm dishes. When cells reached to about 50% confluence, Se compounds were added into medium for 96 hours culture. Each treatment contained three replications. Adherent cells were washed twice with phosphate-buffered saline (PBS) and harvested using trypsin/ EDTA. Cell extracts for measurement of enzyme activities were obtained by sonication in 0.1 *M* Tris-HCl at pH 7.4, containing 0.1% digitonin and then centrifuged at 15,000*g* at 4°C for 15 min to obtain supernatants.

Animal Treatments

In this experiment, Se-deficient male Kunming mice (body weight: 18–22 g) and the Se-deficient diet (0.01 ppm Se) were purchased from the animal center, Anhui Medical University, People's Republic of China. Forty mice were divided into five groups with eight mice in each group. The mice as control were administered saline; the mice in the other groups were administrated selenosulfate or selenite at the dose of 0.1 mg Se/kg body weight intraperitoneally for 2 or 7 consecutive days. The mice were housed in cages (four mice/each) in a room with controlled temperature (22 ± 2°C) and humidity (50 ± 10%) and a 12-h light/dark cycle, with free access to food and water; each one was marked with trinitrophenol to distinguish them and their body weights were recorded. On the day of the last Se treatment, the mice were fasted overnight and killed to obtain blood and livers. Livers and kidneys were excised immediately, rinsed in ice-cold saline, and stored at –30°C. Tissue samples were homogenized with ice-cold 0.15 *M* NaCl and centrifuged at 15,000*g* at 4°C for 15 min. The resulting supernatants were used for the determination of GPx and TrxR activities.

Biomarkers

Protein levels were determined by the Bradford dye-binding assay with bovine serum albumin as the standard. GPx activity was assayed by

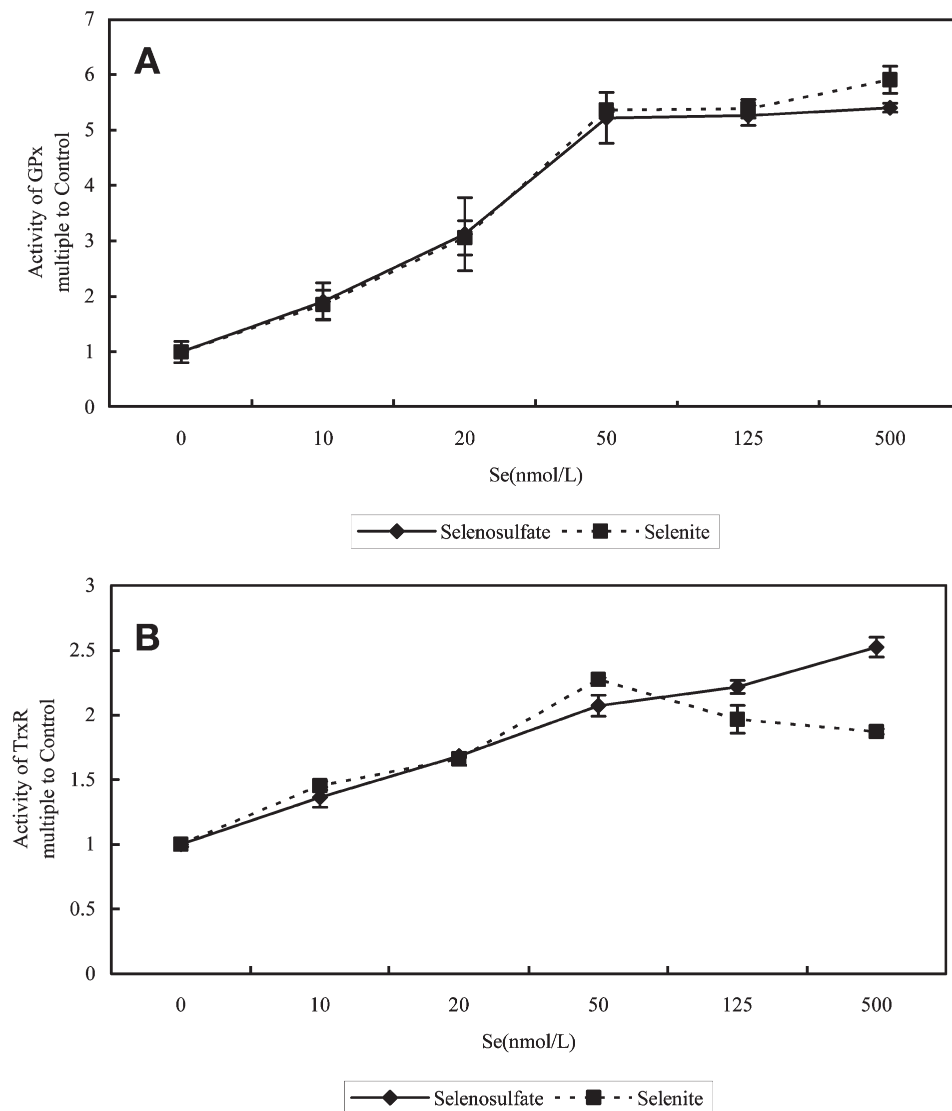


Fig. 2. The activities of Se-dependent enzymes in HepG2 cells ($n = 3$): **(a)** activity of GPx; **(b),** activity of TrxR. The basal activities of GPx and TrxR in HepG2 cells were 96.2 ± 18.4 U/mg protein and 34.0 ± 0.6 mU/mg protein, respectively.

the method of Rotruck et al. *(10).* The activity of GPx was expressed as units per milligram of protein; a unitper was defined as 1 μmol of GSH oxidized per minute. TrxR activity was measured using insulin as a substrate *(11,12).* A stock mixture was made using 50 μL of 1.0 *M* HEPES buffer (pH 7.6), 10 μL of NADPH (40 mg/mL), 10 μL of 0.2 *M* EDTA, and 125 μL of insulin (10 mg/mL). In a 96-well plate, 7 μL of stock mixture, 3 μL of 2 mg/mL Trx, 40 μL of 50 m*M* HEPES (pH 7.6), and 10 μL tissue

homogenate containing 20–30 μg of protein were added to each cell and incubated at 37°C for 20 min. The reaction was stopped by the addition of 240 μL of 0.2 mg/mL DTNB/6 *M* guanidine hydrochloride in 0.2 *M* Tris-HCl (pH 8.0). A nonenzyme reaction containing all components except Trx, which was substituted by same volume of water, was used as the control. The 96-well plates were read at 412 nm. The absorbance of the control was subtracted from the absorbance of the sample. A background control, which was the subtraction of absorbance with and without Trx in the absence of tissue homogenate, was further subtracted from all samples. TrxR activity was calculated based on the standard curve prepared with pure TrxR. Se retention was assayed by the fluorescent method. The tissues were prepared by following sequences: digested using the blend of nitric acid and perchloric acid at the ratio of 3 : 1, reacted with diamino-naphthalene in 60°C for 30 min and extracted with hexamethylene. At the excitation wavelength of 378 nm, the fluorescence intensity at the emission wavelength of 520 nm was recorded and the Se level was calculated by comparing with the fluorescence intensity of Se standard *(13).*

Statistical analysis

Data were presented as the mean ± SD; the differences between the groups were examined using analysis of variance (ANOVA). A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Comparison of GPx and TrxR Activities In Vitro

Selenite and selenosulfate were diluted in medium and added to HepG2 cells at indicated concentrations and cultured for 96 h. Both Se compounds significantly increased the activities of GPx and TrxR. Compared with the control, there was a dose-dependent increase in activities of GPx and TrxR (Fig. 2a,b) treated with selenite under the dose of 50 n*M.* While the doses were above 50 n*M*, platforms *(14)* were found in both enzymes' activities of the cells treated with selenite (Fig. 2a,b). No difference was found in the activities of GPx and TrxR between the two Se compounds under the concentration of 50 n*M.*

The dose effect in enhancing activities of two Se-dependent enzymes could be easily found in both of Se treatments (Table 1). The activity of GPx had significant correlation with the doses of the Se compounds (both $r > 0.99$; $p < 0.01$). TrxR activity had a lower but significant correlation with the doses of the Se compounds (both $r > 0.96$; $p < 0.05$). It could be concluded that there was no difference in restoring activities of Se-dependent enzymes between selenosulfate and selenite in vitro.

Selenite is metabolized to selenide via selenodiglutathione (GSSeSG, SDG) and glutathione selenopersulfide (GSSeH) *(15–17).* Selenide is

Table 1
Correlation of Activities of Se-Dependent Enzymes
to Dose of Se (0–50 n*M*)

	Linear Regression Coefficient (R Value)	
	Selenosulfate(0~50 nM)	Selenite(0~50nM)
GPx	0.993**	0.996**
TrxR	0.963*	0.983*

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

generally regarded as the precursor for supplying Se in the active form for the synthesis of Se-dependent enzymes *(18)*. Selenosulfate might be transferred to selenide through a redox pathway, which was oxidized to red elemental Nano-Se *(19–21)* and then, reduced to selenide. Selenide had two potential metabolic pathways; one pathway resulted in the formation of Se-dependent enzymes *(18)*. No difference between selenosulfate and selenite in vitro means that both of the Se compounds could be incorporated into the Se pool in the cells, and the suggested pathway of selenosulfate did not depress the metabolic efficiency.

Comparison of Activities of GPx, TrxR, and Se Retention In Vivo

The aforementioned in vitro results had clearly shown that there was no difference between selenosulfate and selenite in the induction of Se-dependent enzymes; furthermore, we wanted to know whether there was still no difference in vivo. Two- or 7 consecutive days of Se supply with sodium selenosulfate and sodium selenite to mice at 0.1 mg Se/kg body weight by intraperitoneal pathway was used; the data of the Se-dependent enzymes' activities and Se status are showed in Fig. 3.

Selenite could enhance the activities of Se-dependent enzymes. With a 2- or 7-consecutive-day selenite supply, the activities of GPx in the liver and kidney could be significantly increased to 4.48 and 11.95 times or 2.26 and 5.42 times, respectively (Fig. 3a,b; all $p<0.001$), whereas the activities of TrxR in the liver and kidney could only be enhanced 1.54 and 1.69 times or 1.38 and 1.65 times, respectively, compared to the control (Fig. 3c,d; all $p<0.001$). The activities of GPx and TrxR treated with sodium selenite were both in linear correlation with the quantities of the Se supply (Table 2).

The Se retentions in the liver, kidney and blood were investigated. Se accumulations in tissues were relevant to quantities of the Se supply (Table 2). The basal Se concentrations were 0.233 ± 0.032 μg Se/g liver (Fig. 4a), 0.4450 ± 0.051 μg Se/g kidney (Fig. 4b), and 0.106 ± 0.008 μg Se/mL blood (Fig. 4c). After supplementation with selenite for 2 and 7 d, the Se retentions in the liver, kidney, and blood were increased to 2.68 and 5.25 times, 1.57 and 2.15 times, and 1.81 and 2.75 times, respectively (all $p<0.01$, compared with the control) (Fig. 4).

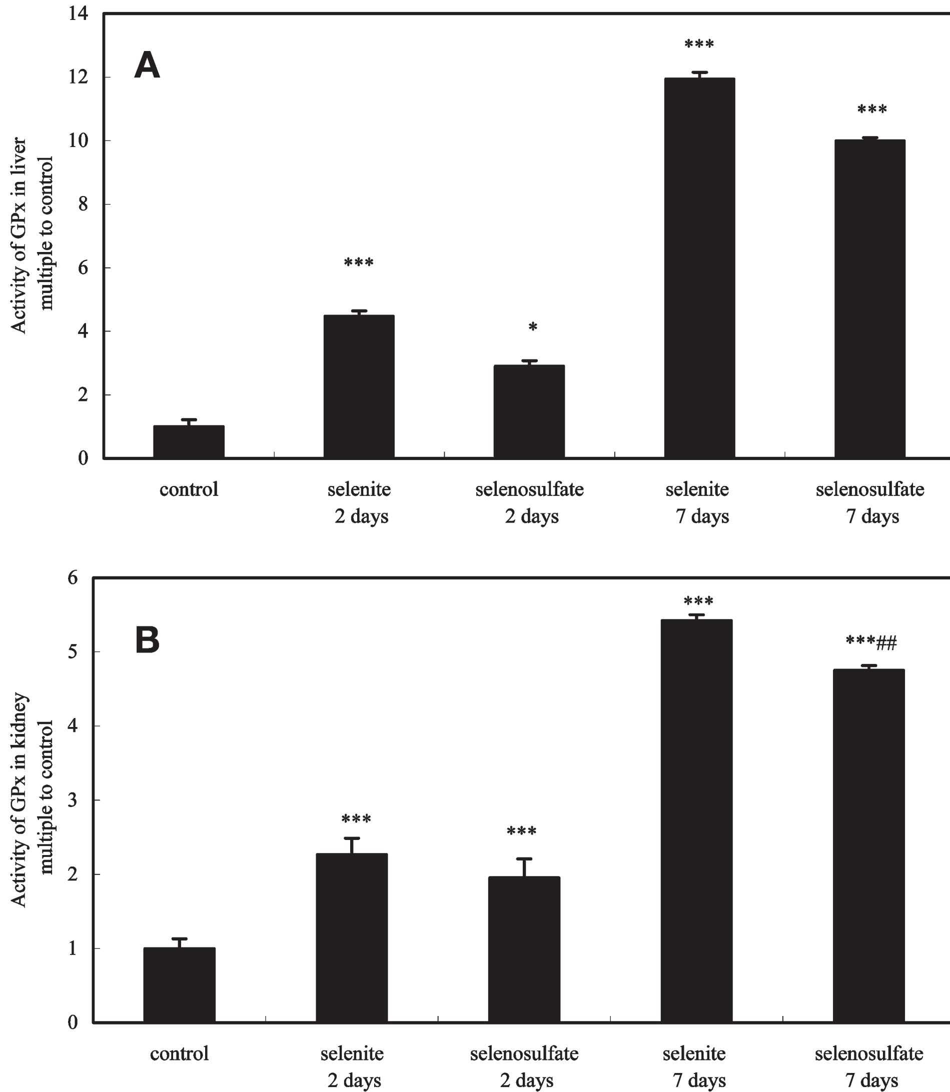


Fig. 3. Comparison of selenosulfate in restoring activities of GPx and TrxR with selenite in vivo (n = 8): **(a)** activity of GPx in the liver; **(b)** activity of GPx in the kidney; **(c)** activity TrxR in the liver; **(d)** activity of TrxR in the kidney. The basal activities of GPx in the liver and kidney were 159 ± 35 and 176 ± 23 U/mg protein, respectively; the basal activities of TrxR in the liver and kidney were 38 ± 13 and 29 ± 7 mU/mg protein, respectively; *Comparison of the treatments of Se compounds to the control; #comparison between selenosulfate and selenite on the same supply procedure. $^{*,\#}p<0.05$; $^{**,\#\#}p<0.01$; $^{***,\#\#\#}p<0.001$. *(Figure continues.)*

Selenosulfate was inferior to selenite in increasing the activities of Se-dependent enzymes and Se depositions in tissues. After a 2-d supply of selenosulfate, the activity of GPx in the liver (Fig. 3a) and Se accumulations in the liver, kidney, and blood (Fig. 4a–c) were 65% ($p<0.001$) and

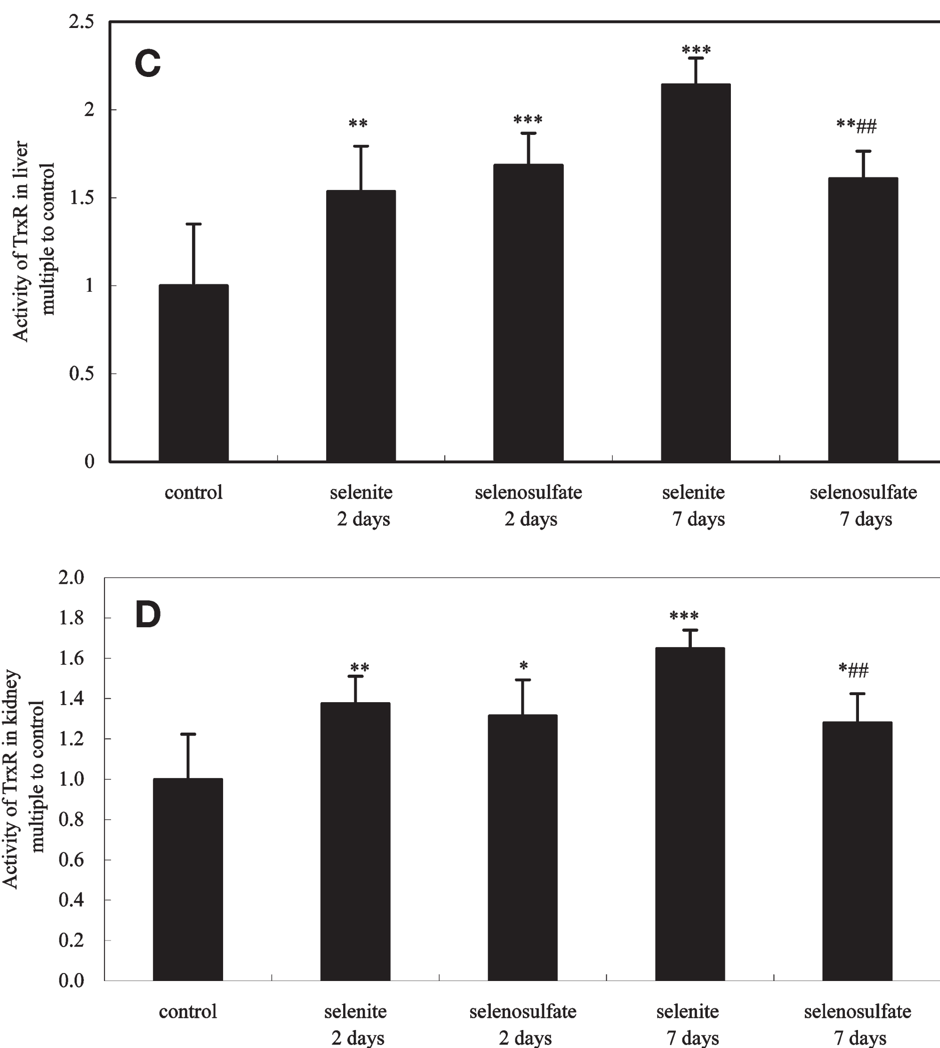


Fig. 3. *(Figure continued.)*

Table 2
Correlation of Se Supplementation with the Se Status and Activities of Se-Dependent Enzymes

	Linear Regression Coefficient (R Value)/P Value	
	Selenosulfate	Selenite
GPx in liver	0.990**	0.952**
GPx in kidney	0.780**	0.980**
TrxR in liver	0.455*	0.789**
TrxR in kidney	0.300	0.983**
Se in liver	0.984**	0.968**
Se in kidney	0.947**	0.934**
Se in blood	0.923**	0.924**

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

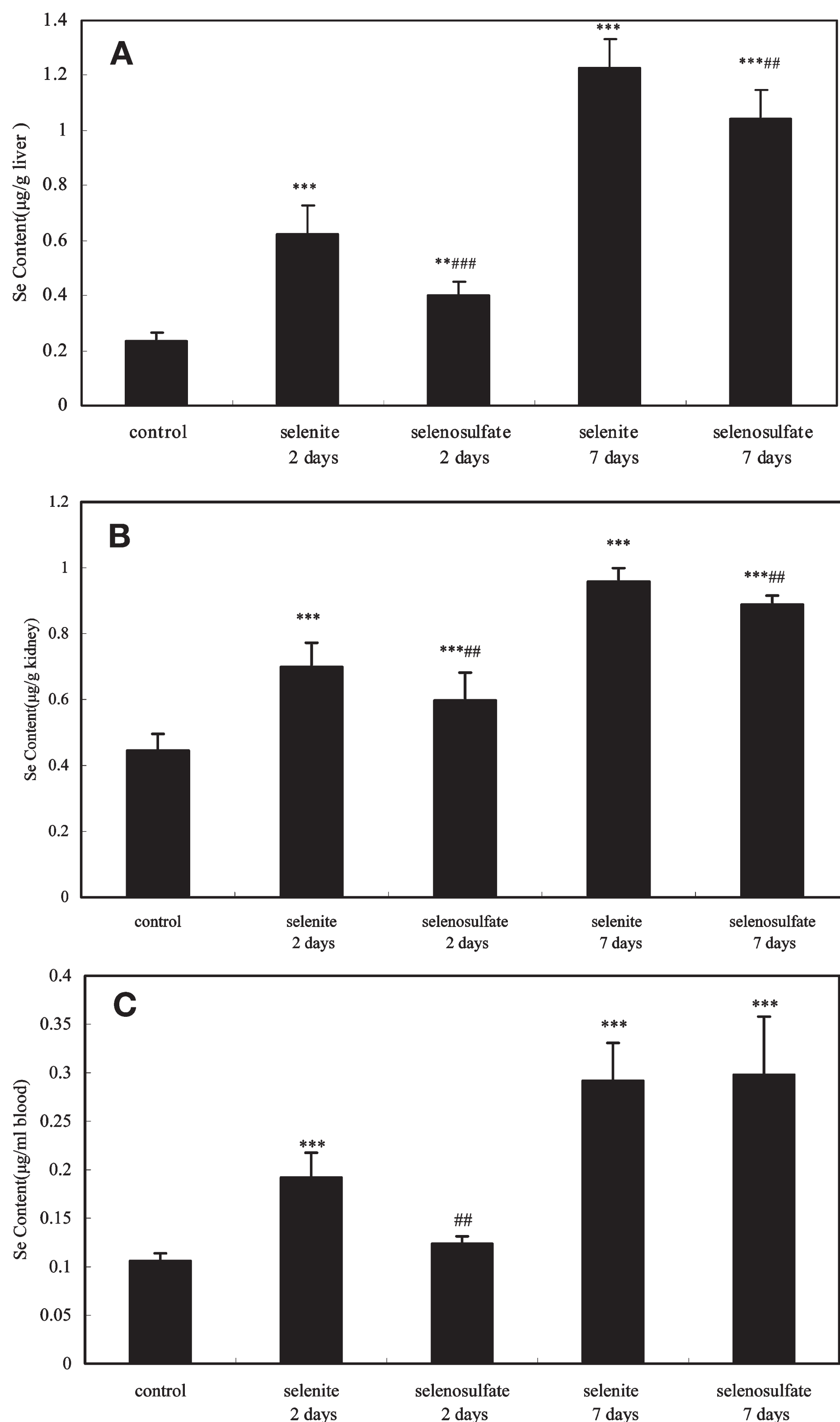


Fig. 4. Comparison of selenosulfate in restoring Se retentions in tissues with selenite in vivo (n = 8): **(a)** Se retention in the liver; **(b)** Se retention in the kidney; **(c)** Se retention in blood. *Comparison of the treatments of Se compounds to the control; #comparison between selenosulfate and selenite on the same supply procedure. $^{*,\#}p<0.05$; $^{**,\#\#}p<0.01$; $^{***,\#\#\#}p<0.001$.

64%, 86%, and 65% (all $p<0.01$), respectively, of those treated with selenite. After a 7-d supply of selenosulfate, the activity of GPx in the kidney, the activities of TrxR in the liver and kidney (Fig. 3b,c,d) were 88%, and 75% and 78% (all $p<0.01$), respectively, and Se depositions in the liver and kidney (Fig. 4a,b) were 85% and 93% (both $p<0.01$), respectively, of those treated with selenite. The activity of Se-dependent enzymes, except TrxR in the kidney, and Se retentions in these tissues supplemented with selenosulfate correlated with the quantities of the Se supply.

The data showed that selenosulfate was less available than selenite in the restorations of GPx and TrxR and in depositions of Se in tissues. However, there was no difference between the two Se compounds in incorporation into GPx and TrxR in the cell culture; these results hinted that there were some other factors in vivo that affected selenosulfate utilization in tissues. Selenosulfate could act not only as the precursor of selenide ions in material science but also a kind of Se source in vitro and in vivo.

The bioavailability of Se was not correlated with anticarcinogenic potential, such as SeMet was highly bioavailable *(18)* but weak in anticancer activity, whereas methylselenocysteine (MSeCys) was to the contrary *(22)*. It had been found that Se from selenized garlic *(15,16,23)* or broccoli *(18)*, which were metabolized by methylation, were equally or less bioavailable than Se from selenite and selenate when bioavailability was based on repletion of tissue Se concentrations or GPx activity, but they were superior to selenite or selenate alone for the reduction of colon or mammary cancer *(18)*. Furthermore, selective modulation of Se in the therapeutic efficacy of anticancer drugs was not relevant to Se bioavailability either. It was found that SeMet, which was higher than MSeCys in bioavailability, was equal to MSeCys as an ameliorator of irinotecan-induced toxicity in nude mice *(22)*. Therefore, selenosulfate, which was less bioavailable than selenite in vivo, should have probable latency in anticancer activity. Corresponding research is being undertaken.

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