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Effect of selenium nanoparticles with different sizes in primary cultured intestinal epithelial cells of crucian carp, *Carassius auratus gibelio*

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Yanbo Wang
Xuxia Yan
Linglin Fu

Marine Resources and Nutrition Biology Research Center, Food Quality and Safety Department, Zhejiang Gongshang University, Hangzhou, People's Republic of China

Abstract: Nano-selenium (Se), with its high bioavailability and low toxicity, has attracted wide attention for its potential application in the prevention of oxidative damage in animal tissues. However, the effect of nano-Se of different sizes on the intestinal epithelial cells of the crucian carp (*Carassius auratus gibelio*) is poorly understood. Our study showed that different sizes and doses of nano-Se have varied effects on the cellular protein contents and the enzyme activities of secreted lactate dehydrogenase, intracellular sodium potassium adenosine triphosphatase, glutathione peroxidase, and superoxide dismutase. It was also indicated that nano-Se had a size-dependent effect on the primary intestinal epithelial cells of the crucian carp. Thus, these findings may bring us a step closer to understanding the size effect and the bioavailability of nano-Se on the intestinal tract of the crucian carp.

Keywords: selenium nanoparticle, intestinal epithelial cell, crucian carp, primary culture

Introduction

After the nutritional essentiality of selenium (Se) was first shown to prevent diet-induced liver necrosis in laboratory animals,[1] a growing number of studies have indicated that Se plays an important role in many aspects of health.[2–11] Indeed, Se, as a component of selenoproteins, has shown metabolic function in preventing oxidative damage to body tissue.[12,13] Nevertheless, the narrow range between its nutritional dosage and the tolerable upper intake level severely compromises wide applications. It is well-known that supplemental Se may be acquired through diet, but Se bioavailability depends on the source.[14] Therefore, dietary advice concerning the improvement of Se bioavailability depends on the Se forms in Se-containing food/feed sources. The novel selenium form, Se nanoparticle (nano-Se), was developed for this investigation.[10,15–17]

Recently, nano-Se has attracted widespread attention, due to its high bioavailability and low toxicity[11,18,19] since nanometer particulates exhibit novel properties, such as high-surface activity, great specific surface area, high catalytic efficiency, a lot of surface active centers, and a strong adsorbing ability.[20] Ball and Garwin[21] pointed out that most nano chemical compounds have a size effect; when the size decreases, the physical, chemical, and biological properties change. Huang et al also reported that there was a potential size-dependent effect on scavenging various free radicals and protecting DNA in vitro by nano-Se.[22] However, little research has been performed to incorporate nano-Se with different sizes into the intestinal tract of the crucian carp (*Carassius auratus gibelio*), one of the most important freshwater finfish cultured in Europe and Asia.[19] To mimic intestinal effects, the primary cultured intestinal epithelial cells of the crucian carp were applied in the present study. Therefore, the

Correspondence: Linglin Fu
Food Quality and Safety Department, Zhejiang Gongshang University, 149 Jiaogong Road, Hangzhou, 310035, Zhejiang Province, People's Republic of China
Tel +86 571 8807 1024 ext 8595
Email fulinglin@mail.zjgsu.edu.cn

objective of this study was to assess and compare the effect of nano-Se with different sizes on related enzyme activities in the intestinal epithelial cells.

Materials and methods

Nano-Se preparation and cell culture

Nano-Se in different sizes was prepared as described previously[15] by adding bovine serum albumin (BSA) to the redox system of selenite and glutathione (GSH). One milliliter of 25 mM sodium selenite was mixed with 4 mL 25 mM GSH containing BSA (200, 20, and 10 mg) for the preparation of the nano-Se with different sizes. The pH of the mixture was adjusted to 7.2 with 1.0 M sodium hydroxide, when the red elemental Se and oxidized glutathione (GSSG) formed. The red suspension was dialyzed against doubly distilled water for 96 hours, with the water changed every 24 hours to separate GSSG from the nano-Se. The final suspension containing the nano-Se and the BSA was lyophilized and stored at room temperature. The sizes of red elemental Se were determined using the Mastersizer particle size and zeta potential analyzer (Malvern Instruments, Malvern, UK), with the average sizes being 13 nm (T-1), 42 nm (T-2), and 92 nm (T-3). All the chemicals used were purchased from Sigma-Aldrich (St Louis, MO, USA) and Sangon Biotech and Bio Basic (Toronto, ON, Canada).

Healthy crucian carp (body weight 32.5 ± 3.4 g) were obtained from a local hatchery (Fish Hatchery of Xiaoshan, Hangzhou, People's Republic of China) and anesthetized in diluted MS-222 (ethyl 3-aminobenzoate methanesulfonate [Tricaine] 1:2,500; Sigma-Aldrich). The intestinal epithelia from the carp were isolated and cultured in 24-well plates, as described elsewhere,[23] with minor modification. In brief, the carps were decapitated, and the intestines were excised from the bodies and rinsed twice, 10 minutes each rinse, with 10 mL phosphate buffered saline (PBS) (without Ca^{2+} and Mg^{2+}), which contained 200 μg/mL penicillin/streptomycin, 400 μg/mL gentamicin, and 250 μg/mL Fungizone®. The intestines were then transferred to 5 mL of a trypsin solution (PBS without Ca^{2+} and Mg^{2+}, 0.05% trypsin, 0.02% EDTA [ethylenediaminetetraacetic acid]) and incubated on a rotating wheel (test tube HZ-9201 K rotator, Taicang Experimental Equipment Factory, Taicang, People's Republic of China) for 20 minutes. The cell suspension was aspirated from the tubes and filtrated through a 100 μm nylon cell strainer into a stopping solution (PBS [Gibco/Life Technologies, Carlsbad, CA, USA] containing 10% fetal bovine serum [FBS; Gibco]). Thereafter, the cell pellet was resuspended in culture medium (Dulbecco's Modified Eagle's Medium [Gibco], supplemented with 2 mmol/L L-glutamine, 5% FBS, 100 μg/mL penicillin/streptomycin, and 200 μg/mL gentamicin). The cells were maintained in 24-well plates (EMD Millipore, Millipore, Billerica, MA, USA) at 28°C in a 5% CO_2 atmosphere. The cells were rinsed twice with PBS after 24 hours of incubation to remove unattached cells, and the medium was changed every second day. After 72 hours, all of the cells grown in the same medium supplemented with different sizes of nano-Se (T-1, T-2, and T-3, respectively) were incubated at the above conditions for a period of 48 hours. In addition, three treatments groups (T-1, T-2, and T-3) were all used at the same nano-Se concentrations (0.0, 0.5, 1.0, 2.0, 3.0, and 5.0 μg/mL) in the present study.

Related enzyme assay

Lactate dehydrogenase (LDH) activity in the culture medium was measured as previously described.[24] To assess the cellular enzyme activity, the cells were rinsed with cold PBS and homogenized in an ultrasonicator in 10 mM sodium maleate buffer (pH 6.5).[25] The homogenate was spun at 15,000 g at 4°C for 20 minutes. The supernatant was analyzed for enzyme activity and protein concentration. Protein concentrations in the cell extracts were determined by a Bradford dye-binding assay (Bio-Rad Laboratories, Hercules, CA, USA), using the BSA as a standard. The glutathione peroxidase (GSH-Px) activity was assayed by the described method.[26] Oxidation of nicotine adenine disphosphonucleotide, reduced, was continuously monitored, using a GSH–GSSG glutathione reductase model at 340 nm with an ultraviolet spectrophotometer (Hitachi Ltd, Tokyo, Japan). The nonenzymatic reaction rate was correspondingly assayed by replacing the cell lysate with a phosphate buffer. The activity of GSH-Px was expressed as specific activity (U mg^{-1} protein). To determine the activities of the sodium–potassium adenosine triphosphatase (Na^+/K^+-ATPase) and the superoxide dismutase (SOD), the corresponding testing kit was purchased from Nanjing Jiancheng Biochemical Co, Ltd (Nanjing, People's Republic of China), and the activities were also expressed as U mg^{-1} protein. For the assay of GSH in the cultured cells, GSH reductase was used for the quantification of GSH (mg g^{-1} protein), based on the instruction of a Glutathione Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA).

Statistical analysis

All experiments were replicated three times or more, and the data are given as the mean ± standard deviation (SD) of n experiments. Statistically significant differences ($P<0.05$) were determined with the Student's *t*-test if the overall

analysis of variance was statistically significant. All calculations were performed using the analytical software Statistical Product and Service Solutions (SPSS) for Windows, version 11.5 (IBM Corporation, Armonk, NY, USA).

Results

Addition of the nano-Se with different sizes (13 nm, 42 nm, and 92 nm, designated as T-1, T-2, and T-3, respectively) to the primary cultured intestinal epithelial cells of the crucian carp changed the LDH activities in the medium (Table 1). At the same supplemented nano-Se concentration (5.0 μg/mL), the highest LDH activity ($P<0.05$) was obtained in T-1, compared to the other groups (T-2 and T-3). However, there were no significant differences among these three treatments groups at the other supplemented nano-Se concentrations. In addition, no difference was noted in the LDH activities between T-2 and T-3. As for the T-1 group (nano-Se 13 nm), the LDH activities increased by adding concentrations from 0.0–5.0 μg/mL. A significant difference ($P<0.05$) was only found in the cell medium treated at 5.0 μg/mL. Different concentrations of nano-Se with sizes of 42 nm and 92 nm did not significantly affect the LDH activities.

The results presented in Table 2 showed the effect of the nano-Se with different sizes on the protein contents of the primary cultured intestinal epithelial cells of the crucian carp. The sizes of the nano-Se had an influence on the cell protein content, and the lowest values were observed in the cells supplemented with a 13 nm nano-Se ($P<0.05$) at 3.0 and 5.0 μg/mL, compared with those groups treated with 42 nm and 92 nm nano-Se at the corresponding concentrations. However, there was no significant difference in the protein content of T-2 and T-3. Besides, the protein contents of the intestinal epithelial cells had been associated with the additional concentrations of nano-Se. In the T-1 group, the highest protein content ($P<0.05$) was observed in the cells supplemented with 2.0 μg/mL nano-Se. As for the groups of T-2 and T-3, the highest protein content was observed in the cells supplemented with 3.0 μg/mL nano-Se.

The Na^+/K^+-ATPase activities in the T-1 treatment are remarkably high ($P<0.05$) at the concentrations of 1.0 and 2.0 μg/mL nano-Se, while those in the T-2 and T-3 groups were high with concentrations of ≥2.0 μg/mL (Table 3). Besides, at the concentration of 1.0 μg/mL, the smaller size of nano-Se has more effective promotion in the Na^+/K^+-ATPase activity, but the contrary phenomenon could be observed when supplemented with 3.0 and 5.0 μg/mL nano-Se. As shown in Table 4, the SOD activity of T-1 significantly decreased ($P<0.05$) at a concentration of 5.0 μg/mL, compared with that of T-2 and T-3. However, within this group (T-1), the SOD activity of the 5.0 μg/mL did not exhibit statistical differences with the groups 0.0, 0.5, or 3.0 μg/mL. With the increase of supplemented nano-Se concentration, the GSH-Px activities are gradually enhanced and then reduced to a stable level in all the groups (Table 5). Obviously, at the concentrations of 3.0 and 5.0 μg/mL, GSH-Px activities in larger size treatment (T-2 and T-3) are remarkably higher ($P<0.05$) than those in the smaller one (T-1). However, there were no differences of GSH contents in cells supplemented with different sizes and concentrations of nano-Se particles (Table 6).

Discussion

The in vitro culture of partial intestinal epithelial cells has been developed to evaluate nutrient safety and to define the specific mechanisms of nutrient metabolism, because it was difficult to control various in vivo environmental parameters of intestine. Numerous experimental studies had suggested that the intestinal epithelial cell as a model of intestinal metabolism had been successfully used for trace elements.[27–30] In the present study, the primary cultured intestinal epithelial cells of the crucian carp, *Carassius auratus gibelio*, were used to evaluate the effect of nano-Se with different sizes and concentrations on related enzyme activities and protein contents.

According to the results determined in the present study, the nano-Se with the different sizes showed the different influence on the intestinal epithelial cells. Similar results

Table 1 Effect of nano-Se with different sizes on the LDH (U/g protein) activity in the medium of primary cultured intestinal epithelial cells of the crucian carp, *Carassius auratus gibelio*

Group	0.0 μg/mL	0.5 μg/mL	1.0 μg/mL	2.0 μg/mL	3.0 μg/mL	5.0 μg/mL
T-1	$40.26^{a,A} \pm 5.18$	$41.37^{a,A} \pm 5.56$	$41.87^{a,A} \pm 5.89$	$42.22^{a,A} \pm 6.04$	$51.67^{a,A} \pm 6.22$	$71.21^{b,A} \pm 7.54$
T-2	$39.37^{a,A} \pm 5.79$	$40.28^{a,A} \pm 5.24$	$40.89^{a,A} \pm 6.13$	$41.18^{a,A} \pm 5.96$	$43.81^{a,A} \pm 4.61$	$49.90^{a,B} \pm 7.09$
T-3	$40.30^{a,A} \pm 5.09$	$40.60^{a,A} \pm 5.69$	$41.58^{a,A} \pm 5.33$	$42.11^{a,A} \pm 5.80$	$43.31^{a,A} \pm 6.29$	$50.71^{a,B} \pm 5.75$

Notes: Treatments groups of T-1, T-2, and T-3 were supplemented with different concentrations (μg/mL) of nano-Se with average sizes being 13 nm, 42 nm, and 92 nm, respectively. Means (± SD) in the same row and column with different lowercase and capital letter were significantly different ($P<0.05$). The lowercase indicates differences between concentrations for the same average size and capitals between sizes for the same Se concentration.
Abbreviations: LDH, lactate dehydrogenase; SD, standard deviation.

Table 2 Effect of nano-Se with different sizes on the protein contents (mg mL^{-1}) of the primary cultured intestinal epithelial cells of the crucian carp, *Carassius auratus gibelio*

Group	0.0 μg/mL	0.5 μg/mL	1.0 μg/mL	2.0 μg/mL	3.0 μg/mL	5.0 μg/mL
T-1	$1.42^{a,c,A} \pm 0.09$	$1.44^{a,c,A} \pm 0.10$	$1.54^{a,b,A} \pm 0.07$	$1.55^{b,A} \pm 0.07$	$1.42^{a,c,A} \pm 0.03$	$1.35^{c,A} \pm 0.09$
T-2	$1.41^{a,A} \pm 0.09$	$1.42^{a,b,A} \pm 0.09$	$1.46^{a-c,A} \pm 0.10$	$1.62^{b,c,A} \pm 0.09$	$1.66^{c,B} \pm 0.09$	$1.60^{a-c,B} \pm 0.10$
T-3	$1.40^{a,A} \pm 0.09$	$1.43^{a,b,A} \pm 0.09$	$1.45^{a,b,A} \pm 0.08$	$1.61^{b,c,A} \pm 0.09$	$1.68^{c,B} \pm 0.07$	$1.64^{c,B} \pm 0.07$

Notes: Treatments groups of T-1, T-2, and T-3 were supplemented with different concentrations (μg/mL) of nano-Se with average sizes being 13 nm, 42 nm, and 92 nm, respectively. Means (± SD) in the same row and column with different lowercase and capital letter were significantly different ($P<0.05$). The lowercase indicates differences between concentrations for the same average size and capitals between sizes for the same Se concentration.
Abbreviation: SD, standard deviation.

Table 3 Effect of nano-Se with different sizes on the Na^+/K^+-ATPase activities (U mg^{-1} protein) of the primary cultured intestinal epithelial cells of crucian carp, *Carassius auratus gibelio*

Group	0.0 μg/mL	0.5 μg/mL	1.0 μg/mL	2.0 μg/mL	3.0 μg/mL	5.0 μg/mL
T-1	$0.15^{a,A} \pm 0.03$	$0.16^{a,A} \pm 0.02$	$0.24^{b,A} \pm 0.02$	$0.24^{b,A} \pm 0.03$	$0.16^{a,A} \pm 0.03$	$0.13^{a,A} \pm 0.01$
T-2	$0.16^{a,A} \pm 0.02$	$0.16^{a,A} \pm 0.03$	$0.18^{a,B} \pm 0.02$	$0.26^{b,A} \pm 0.02$	$0.25^{b,B} \pm 0.03$	$0.24^{b,B} \pm 0.02$
T-3	$0.15^{a,A} \pm 0.02$	$0.16^{a,A} \pm 0.02$	$0.17^{a,B} \pm 0.03$	$0.24^{b,A} \pm 0.02$	$0.25^{b,B} \pm 0.03$	$0.24^{b,B} \pm 0.01$

Notes: Treatments groups of T-1, T-2, and T-3 were supplemented with different concentrations (μg/mL) of nano-Se with average sizes being 13 nm, 42 nm, and 92 nm, respectively. Means (± SD) in the same row and column with different lowercase and capital letter were significantly different ($P<0.05$). The lowercase indicates differences between concentrations for the same average size and capitals between sizes for the same Se concentration.
Abbreviations: Na^+/K^+-ATPase, sodium potassium adenosine triphosphatase; SD, standard deviation.

Table 4 Effect of nano-Se with different sizes on the SOD activities (U mg^{-1} protein) of the primary cultured intestinal epithelial cells of crucian carp, *Carassius auratus gibelio*

Group	0.0 μg/mL	0.5 μg/mL	1.0 μg/mL	2.0 μg/mL	3.0 μg/mL	5.0 μg/mL
T-1	$15.39^{a,b,A} \pm 1.48$	$15.99^{a,b,A} \pm 1.29$	$17.68^{a,A} \pm 1.10$	$17.93^{a,A} \pm 1.26$	$15.02^{a,b,A} \pm 1.29$	$13.53^{b,A} \pm 1.19$
T-2	$15.42^{a,A} \pm 1.01$	$15.73^{a,A} \pm 1.51$	$16.44^{a,A} \pm 0.79$	$17.87^{a,A} \pm 1.57$	$17.82^{a,A} \pm 1.46$	$17.65^{a,B} \pm 1.54$
T-3	$15.40^{a,A} \pm 1.46$	$15.54^{a,A} \pm 1.18$	$16.60^{a,A} \pm 0.92$	$17.96^{a,A} \pm 1.20$	$17.86^{a,A} \pm 1.36$	$17.25^{a,B} \pm 1.52$

Notes: Treatments groups of T-1, T-2, and T-3 were supplemented with different concentrations (μg/mL) of nano-Se with average sizes being 13 nm, 42 nm, and 92 nm, respectively. Means (± SD) in the same row and column with different lowercase and capital letter were significantly different ($P<0.05$). The lowercase indicates differences between concentrations for the same average size and capitals between sizes for the same Se concentration.
Abbreviation: SOD, superoxide dismutase.

Table 5 Effect of nano-Se with different sizes on the GSH-Px activities (U mg^{-1} protein) of primary cultured intestinal epithelial cells of crucian carp, *Carassius auratus gibelio*

Group	0.0 μg/mL	0.5 μg/mL	1.0 μg/mL	2.0 μg/mL	3.0 μg/mL	5.0 μg/mL
T-1	$180.38^{a,c,A} \pm 14.92$	$204.07^{a,c,A} \pm 18.36$	$256.40^{b,A} \pm 18.23$	$253.86^{b,A} \pm 19.87$	$217.02^{a,b,A} \pm 16.38$	$169.29^{c,A} \pm 13.04$
T-2	$180.89^{a,A} \pm 16.68$	$193.16^{a,b,A} \pm 19.43$	$225.62^{b,c,A} \pm 21.32$	$258.34^{c,A} \pm 21.88$	$270.44^{c,B} \pm 22.99$	$252.67^{c,B} \pm 22.80$
T-3	$180.71^{a,A} \pm 15.45$	$193.67^{a,b,A} \pm 17.31$	$231.39^{b,c,A} \pm 22.90$	$256.60^{c,A} \pm 20.97$	$263.08^{c,B} \pm 17.39$	$258.96^{c,B} \pm 15.45$

Notes: Treatments groups of T-1, T-2, and T-3 were supplemented with different concentrations (μg/mL) of nano-Se with average sizes being 13 nm, 42 nm, and 92 nm, respectively. Means (± SD) in the same row and column with different lowercase and capital letter were significantly different ($P<0.05$). The lowercase indicates differences between concentrations for the same average size and capitals between sizes for the same Se concentration.
Abbreviations: GSH-Px, glutathione peroxidase; SD, standard deviation.

Table 6 Effect of nano-Se with different sizes on the GSH contents (mg/g protein) of the primary cultured intestinal epithelial cells of the crucian carp, *Carassius auratus gibelio*

Group	0.0 μg/mL	0.5 μg/mL	1.0 μg/mL	2.0 μg/mL	3.0 μg/mL	5.0 μg/mL
T-1	6.37 ± 0.78	6.55 ± 0.90	7.88 ± 0.96	7.67 ± 0.93	6.93 ± 1.06	6.13 ± 0.82
T-2	6.36 ± 0.81	6.44 ± 0.82	6.54 ± 0.91	7.95 ± 1.06	8.23 ± 0.99	7.87 ± 0.94
T-3	6.35 ± 0.77	6.44 ± 0.91	6.53 ± 0.95	8.07 ± 1.04	7.94 ± 0.92	7.73 ± 0.91

Notes: Treatments groups of T-1, T-2, and T-3 were supplemented with different concentrations (μg/mL) of nano-Se with average sizes being 13 nm, 42 nm, and 92 nm, respectively. There were no significant differences ($P<0.05$) among all the groups.
Abbreviation: GSH, glutathione.

were reported by Huang et al,[22] who proved that nano-Se had potential size-dependent characteristics on scavenging the free radicals, and the smaller size particles had better effects. It was indicated that nano-Se in the small size with the large surface area had more atoms exposed to free radicals for the electron exchanger, with a high efficacy for scavenging multiple free radicals compared to that in the larger size.

It is also necessary, however, to consider the possibility of cell differences, as suggested by Zhang et al.[31] They observed that there was no significant size effect of nano-Se from 5–200 nm in the induction of GSH-Px, phospholipid hydroperoxide GSH-Px and thioredoxin reductase-1 in human hepatoma HepG2 cells. It might be related to the reason that the nano-Se particles in different sizes could be taken and subsequently metabolized into Se-containing enzymes by cultured cells. In addition, the different additional concentrations of nano-Se at the same size also showed the different results in the intestinal epithelial cells. It is suggested that the supplemented concentration of nano-Se can be another factor to influence the enzyme activities of the intestinal epithelial cells from the crucian carp.

LDH was a soluble cytosolic enzyme that was released into the culture medium following the loss of membrane integrity, resulting from either apoptosis or necrosis.[32] LDH activity, therefore, could be used as an indicator of cell membrane integrity and serve as a general means to assess cytotoxicity resulting from chemical compounds or environmental toxic factors.[33] In the present study, the significantly higher LDH activities were only observed in the intestinal epithelial cells treated with 5.0 μg/mL nano-Se at the average size 13 nm. Based on a previous study, Se could be toxic at a certain level that is not much higher than the beneficial dose, although it is an essential trace element.[34] In addition, the present study also showed that nano-Se had the potential size-dependent characteristic on the regulation of LDH activity, and it could be associated with the nano-Se sizes. At the same treatment concentration (5.0 μg/mL), only the small nano-Se significantly increased LDH activity in the culture medium. It could be explained that the smaller nano-Se passed more easily through the cell membrane and thus more nano-Se particles accumulated inside the cells beyond the processing capabilities of the cells themselves. However, while no significant differences were observed between treatments, there was a tendency for differences. It is suggested that a certain concentration of nano-Se with the average sizes of 42 nm and 92 nm had no influence on the membrane integrity and viability of the intestinal epithelial cells of the crucian carp.

Protein synthesis was the molecular biological basis of cell growth and, thus, the increase of intracellular protein was one of the important indicators to evaluate cell growth conditions.[35] In the present study, a certain concentration of nano-Se increased the protein contents of intestinal epithelial cells, and it could be associated with the biological functions of Se as a component of the amino acids selenocysteine and selenomethionine. In addition, significantly higher protein contents were observed in T-2 and T-3 – compared with that of T-1 – and this could be explained by the changes in the partial cells' membrane integrity and the viability indicated through the results of LDH activities. As for the Na^+/K^+-ATPase, its activity provided the driving force for a secondary active transport of solutes, such as amino acids, phosphate, vitamins and, in epithelial cells, glucose.[36] According to previous studies, intestinal digestion products, such as glucose and amino acids, are dependent on the Na^+/K^+-ATPase; thus, its activity could reflect the level of cell metabolism at a certain extent.[37] The results of the Na^+/K^+-ATPase activities obtained in the present study were consistent with the determined LDH activities and protein contents. The relatively higher Na^+/K^+-ATPase activities were shown in groups at a certain concentration of nano-Se, and it was indicated that the increased enzyme activities improved the intestinal epithelial cell metabolism and thus accelerated the metabolism of Se in the cells. In other words, the accumulation and the toxicity of Se decreased in the cells.

The quantity of Se in the cells is an important regulator of the GSH-Px activity; it was proved that the activity of this enzyme could be increased up to 1,000× in the presence of selenocysteine in the enzyme active site.[38] The in vitro experiment showed that the activity of GSH-Px was markedly enhanced by increasing the Se concentration in the culture medium of primary rabbit hepatocytes.[39] We also demonstrated that the addition of Se could increase the activities of GSH-Px of the primary intestinal epithelial cells of the crucian carp. Similar results were also observed by Ebert et al,[40] who reported that GSH-Px activity was enhanced 1.8-fold in bone marrow stromal cells that were cultured in the presence of 100 nm Se, supplied as Na_2SeO_3. As for the sizes of nano-Se, significantly higher GSH-Px activities were found in the groups of T-2 and T-3 than that of T-1. It was also suggested that in primary intestinal epithelial cells, both the nano-Se concentration and size were the critical regulation factors of GSH-Px activities. In addition, the SOD activities of cells supplemented with nano-Se with different sizes at 5.0 μg/mL were shown the similar trends with GSH-Px activities. Indeed, SOD was an

important antioxidant defense in most kinds of cells exposed to oxygen. Therefore, a certain concentration of nano-Se with average sizes of 42 nm and 92 nm could have potential beneficial effects to the cells.

A ubiquitous thiol-containing tripeptide, GSH is one of the most potent cellular antioxidants and is mainly associated with the thiol group of the cysteine residue.[41] According to the report of Wu et al,[42] the incubation of bovine hepatocytes for 24 hours with Se supplied as selenomethionine, Na_2SeO_3, or kappa selenocarrageenan at doses of 0.5–5.0 μmol/L, reduced intracellular GSH concentrations compared with controls. Previous research has also indicated that increasing the Na_2SeO_3 concentration in the culture medium decreased the level of intracellular GSH in rabbit hepatocytes.[39] However, based on the findings of our study, no significant differences were obtained across all the groups. The discrepancy might be attributed to the different effects on GSH activity of cell culture between nano-Se and the other Se forms.

In conclusion, our results suggested that different sizes and doses of nano-Se have varied effects on the protein contents and activities of secreted LDH, intracellular Na^+/K^+-ATPase, GSH-Px, and SOD. However, the detailed metabolic pathways of nano-Se with different sizes were still not well-investigated, and this would be of further interest.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Schwarz K, Foltz CM. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J Am Chem Soc*. 1957;79(12): 3292–3293.
2. Beck MA, Levander OA, Handy J. Selenium deficiency and viral infection. *J Nutr*. 2003;133(5 Suppl 1):1463S–1467S.
3. Finley JW, Grusak MA, Keck AS, Gregoire BR. Bioavailability of selenium from meat and broccoli as determined by retention and distribution of 75Se. *Biol Trace Elem Res*. 2004;99(1–3):191–209.
4. Dumont E, Vanhaecke F, Cornelis R. Selenium speciation from food source to metabolites: a critical review. *Anal Bioanal Chem*. 2006;385(7): 1304–1323.
5. Wang Y, Han J, Li W, Xu Z. Effect of different selenium source on growth performances, glutathione peroxidase activities, muscle composition and selenium concentration of allogynogenetic crucian carp (*Carassius auratus gibelio*). *Animal Feed Science and Technology*. 2007;134(3):243–251.
6. Navarro-Alarcon M, Cabrera-Vique C. Selenium in food and the human body: a review. *Sci Total Environ*. 2008;400(1–3):115–141.
7. Saad MB, Gertner LR, Bona TD, Santin E. Selenium influence in the poultry immune response – review. *Recent Pat Food Nutr Agric*. 2009;1(3):243–247.
8. Zhou X, Wang Y, Gu Q, Li W. Effect of different dietary selenium source (selenium nanoparticle and selenomethionine) on growth performance, muscle composition and glutathione peroxidase enzyme activity of crucian carp (*Carassius auratus gibelio*). *Aquaculture*. 2009;291(1–2): 78–81.
9. Toulis KA, Anastasilakis AD, Tzellos TG, Goulis DG, Kouvelas D. Selenium supplementation in the treatment of Hashimoto's thyroiditis: a systematic review and a meta-analysis. *Thyroid*. 2010;20(10): 1163–1173.
10. Zhou X, Wang Y. Influence of dietary nano elemental selenium on growth performance, tissue selenium distribution, meat quality, and glutathione peroxidase activity in Guangxi Yellow chicken. *Poult Sci*. 2011;90(3):680–686.
11. Hu CH, Li YL, Xiong L, Zhang HM, Song J, Xia MS. Comparative effects of nano elemental selenium and sodium selenite on selenium retention in broiler chickens. *Animal Feed Science and Technology*. 2012;177(3–4):204–210.
12. Nève J. Selenium as a 'nutraceutical': how to conciliate physiological and supra-nutritional effects for an essential trace element. *Curr Opin Clin Nutr Metab Care*. 2002;5(6):659–663.
13. Yan L, Johnson LK. Selenium bioavailability from naturally produced high-selenium peas and oats in selenium-deficient rats. *J Agric Food Chem*. 2011;59(11):6305–6311.
14. Finley JW. Bioavailability of selenium from foods. *Nutr Rev*. 2006; 64(3):146–151.
15. Zhang JS, Gao XY, Zhang LD, Bao YP. Biological effects of a nano red elemental selenium. *Biofactors*. 2001;15(1):27–38.
16. Zhang J, Wang H, Yan X, Zhang L. Comparison of short-term toxicity between Nano-Se and selenite in mice. *Life Sci*. 2005;76(10): 1099–1109.
17. Zhang J, Wang X, Xu T. Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: comparison with se-methylselenocysteine in mice. *Toxicol Sci*. 2008;101(1):22–31.
18. Shi LG, Yang RJ, Yue WB, et al. Effect of elemental nano-selenium on semen quality, glutathione peroxidase activity, and testis ultrastructure in male Boer goats. *Anim Reprod Sci*. 2010;118(2–4):248–254.
19. Rezvanfar MA, Rezvanfar MA, Shahverdi AR, et al. Protection of cisplatin-induced spermatotoxicity, DNA damage and chromatin abnormality by selenium nano-particles. *Toxicol Appl Pharmacol*. 2013;266(3):356–365.
20. Wang H, Zhang J, Yu H. Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice. *Free Radic Biol Med*. 2007;42(10):1524–1533.
21. Ball P, Garwin L. Science at the atomic scale. *Nature*. 1992;355(6363): 761–766.
22. Huang B, Zhang J, Hou J, Chen C. Free radical scavenging efficiency of Nano-Se in vitro. *Free Radic Biol Med*. 2003;35(7):805–813.
23. Song Z, Wu T, Pan X. [Primary culture methods of intestinal epithelial cells of crucian carp (*Carassius auratus*)]. *Freshw Fish*. 2008;38(1): 67–71. Chinese [with English abstract].
24. Wroblewski F, Ladue JS. Lactic dehydrogenase activity in blood. *Proc Soc Exp Biol Med*. 1955;90(1):210–213.
25. Nano JL, Czerucka D, Menguy F, Rampal P. Effect of selenium on the growth of three human colon cancer cell lines. *Biol Trace Elem Res*. 1989;20(1–2):31–43.
26. Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller DJ. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney. Possible implications in analgesic neuropathy. *Biochem Pharmacol*. 1984;33(11):1801–1807.
27. Movileanu L, Flonta ML, Mihailescu D, Frangopol PT. Characteristics of ionic transport processes in fish intestinal epithelial cells. *Biosystems*. 1998;45(2):123–140.
28. Zödl B, Zeiner M, Paukovits P, Steffan I, Marktl W, Ekmekcioglu C. Iron uptake and toxicity in Caco-2 cells. *Microchem J*. 2005;79(1–2): 393–397.

29. Zha LY, Xu ZR, Wang MQ, Gu LY. Chromium nanoparticle exhibits higher absorption efficiency than chromium picolinate and chromium chloride in Caco-2 cell monolayers. *J Anim Physiol Anim Nutr (Berl)*. 2008;92(2):131–140.
30. Fang W, Goldberg ML, Pohl NM, et al. Functional and physical interaction between the selenium-binding protein 1 (SBP1) and the glutathione peroxidase 1 selenoprotein. *Carcinogenesis*. 2010;31(8):1360–1366.
31. Zhang J, Wang H, Bao Y, Zhang L. Nano red elemental selenium has no size effect in the induction of seleno-enzymes in both cultured cells and mice. *Life Sci*. 2004;75(2):237–244.
32. Wolterbeek HT, van der Meer AJ. Optimization, application, and interpretation of lactate dehydrogenase meaurements in microwell determination of cell number and toxicity. *Assay Drug Dev Technol*. 2005;3(6):675–682.
33. Haslam G, Wyatt D, Kitos PA. Estimating the number of viable animal cells in multi-well cultures based on their lactate dehydrogenase activities. *Cytotechnology*. 2000;32(1):63–75.
34. Bell RR, Nonavinakere VK, Soliman MR, Early JL 2nd. Effect of in vitro treatment of rat hepatocytes with selenium, and/or cadmium on cell viability, glucose output, and cellular glutathione. *Toxicology*. 1991;69(2):111–119.
35. Sikder HA, Devlin MK, Dunlap S, Ryu B, Alani RM. Id proteins in cell growth and tumorigenesis. *Cancer Cell*. 2003;3(6):525–530.
36. Sweeney G, Klip A. Regulation of the Na^+/K^+-ATPase by insulin: why and how? *Mol Cell Biochem*. 1998;182(1–2):121–133.
37. Bird TA, Kyriakis JM, Tyshler L, Gayle M, Milne A, Virca GD. Interleukin-1 activates p54 mitogen-activated protein (MAP) kinase/stress-activated protein kinase by a pathway that is independent of p21ras, Raf-1, and MAP kinase kinase. *J Biol Chem*. 1994;269(50):31836–31844.
38. Burk RF. Selenium, an antioxidant nutrient. *Nutr Clin Care*. 2002;5(2):75–79.
39. Müller AS, Pallauf J. Effect of increasing selenite concentrations, vitamin E supplementation and different fetal calf serum content on GPx1 activity in primary cultured rabbit hepatocytes. *J Trace Elem Med Biol*. 2003;17(3):183–192.
40. Ebert R, Ulmer M, Zeck S, et al. Selenium supplementation restores the antioxidative capacity and prevents cell damage in bone marrow stromal cells in vitro. *Stem Cells*. 2006;24(5):1226–1235.
41. Curello S, Ceconi C, Cargnoni A, Cornacchiari A, Ferrari R, Albertini A. Improved procedure for determining glutathione in plasma as an index of myocardial oxidative stress. *Clin Chem*. 1987;33(8):1448–1449.
42. Wu X, Huang K, Wei C, Chen F, Pan C. Regulation of cellular glutathione peroxidase by different forms and concentrations of selenium in primary cultured bovine hepatocytes. *J Nutr Biochem*. 2010;21(2):153–161.