Toxicology

Cellular and nephrotoxicity of selenium species

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ABSTRACT

Project: Beside its useful functions at very low concentrations, selenium including supplementary Se sources pose a potential toxicological risk. The toxicity of selenium species was tested in HaCaT cell culture and related nephrotoxicity in mice.

Procedure: The apoptotic shrinkage and necrotic expansion of cells were measured by time-lapse image microscopy. Acute nephrotoxicity was estimated upon administration of various selenium species to mice for two weeks. To confirm or to refute the accumulation of Se in the kidney and its potential chronic effect, Se concentration in kidney tissue and histopathology were tested.

Results: The comparison of selenium species showed that organic lactomicroSe did not affect cell growth at 5 ppm, but inorganic nanoSe severely hampered it at lower concentration (1 ppm). The in vivo Se treatment (0.5, 5, 50 ppm, corresponding to 4, 40 and 400 µg/kg) was misleading as it did neither affect the outward appearance nor the weight of the kidney. Se accumulation was observed after selenite, selenite, SelPlex, selenite and nanoSe administration, while lactomicroSe caused no traceable accumulation. In vivo, ex vivo and in vitro experiments reflected this order of selenium toxicity: selenite > selenite > SelPlex > nanoSe > lactomicroSe.

Conclusion: Within the tested species lactomicroSe was the only non-nephrotoxic selenium source recommended for nutritional Se supplementation.

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Introduction

Similarly to other biologically important heavy metals, Se in trace amounts is a micronutrient essential for biological function [1,2]. Selenoproteins provide defense against oxidation, are involved in hormone function and immune defense [3–5]. Selenium as a nutritional trace element is essential to human health, found in meat, seafood, egg, milk, nuts, cereals, mushrooms, etc. [6]. Earlier the maximum safe dietary intake of Se was 800 µg/day (∼15 µg/kg body weight), then reduced to 400 µg/day [7]. The declining blood selenium level in Europe raised some concern about selenium deficiency with potential health implications and incidence of diseases [8,9]. In spite of the general understanding regarding the beneficial role of selenium, there remained much debate about its supplementation. Major concern of Se administration is related to nephrotoxicity as Se accumulation could exceed its toxicological limit (15 µg/L) [10].

Characteristic features of metal nephrotoxicity are lesions that predominate in specific regions of the nephron within specific cell types [11]. The presence of selenocompounds in kidney concerns podocytes responsible for the accumulation of Se. X-ray microanalysis demonstrated that large electron-dense granules referred to as lysosomes are the main target organelles in these cells [12]. Most renal pathologies that lead to kidney disease originate within the glomerulus. It has been established that the depletion of podocytes, the visceral epithelium of capillary convolute is central in this process [11].

Originally podocytes were used to measure cellular toxicity, but these cells undergo a series of major phenotypic modulations [13], thus podocytes were replaced with epithelial HaCaT cells. Although, an earlier subacute toxicological study did not show significant changes in kidney weight at 50 ppm Se concentration [14],
transitory accumulation during its excretion could occur. The aim of this study was to avoid nephrotoxicity and to select the least toxic selenium compound as a nutritional trace element. We have compared selenium levels in serum and kidney tissue, as well as histopathological alterations caused by selenium species in murine kidney tissue.

**Materials and methods**

**Chemicals**

Chemical were purchased from Sigma–Aldrich, Budapest, Hungary. MRS (de-Man, Rogosa and Sharpe) medium was bought from Oxoid Limited, Basingstoke, UK. Entellan rapid mounting medium for glass slide coverslips was purchased from Merck Millipore, Hungary, Budapest.

**Selenium sources**

Inorganic mineral forms of selenate and selenite are known feed supplements. Sodium selenate (Na$_2$SeO$_4$, referred to as selenate) and sodium hydroseleinite (NaHSeO$_3$, or selenite) were purchased from CEDA Chemicals, Knutsford, UK. SelPlex is the organic form of selenium yeast produced by Saccharomyces cerevisiae. SelPlex was obtained from Alltech, Hungary Kft, Budapest.

**Production of elemental nanoSe**

NanoSe of 100–500 nm diameter was produced as described [15]. Briefly, the Se source and the inoculum (probiotic yogurt bacteria) were added to 1 L of MRS medium. As Se source, sodium hydroseleinite (NaHSeO$_3$) stock solution (10 g/L) was used. Then, 20 mL of selenite stock solution was added to 980 mL of MRS medium, for a final concentration of 0.2 g/L selenite. The mixture (10 mL) of strains containing Lactobacillus acidophilus, Streptococcus thermophilus, and Lactobacillus casei served as inoculum. After these additions to the medium, fermentation was carried out for 48 h in a rotary shaker.

**Production of lactomicroSe**

This selenium species also referred to as lactomicroSe (100–500 nm) was produced from probiotic (“friendly” bacteria) yogurt and sodium nitrite by Bionanoferm Ltd., Debrecen as described [15]. All yogurts are required to be made with two cultures, namely Lactobacillus bulgaricus and Streptococcus thermophilus, which convert pasteurized milk to yogurt during fermentation. In addition, some yogurts contain Lactobacillus acidophilus, Bifidus and other cultures. For the production of lactomicroSe solid sodium nitrate and for inoculation a mixture of three yogurt strains L. acidophilus (0.5–0.6 × 1–2.5 μm), L. casei (0.7–1.1 × 2–4 μm) and S. thermophilus (0.82 μm), were used. Fermentation took place in a rotary shaker at 37 °C for 48 h. At the end of the fermentation process, an Se-rich pink- or red-colored yogurt was obtained. The yogurt was centrifuged for 5 min at 2000 × g to get rid of most of the water. After decantation, the solid phase was placed into a 50–60 °C dryer for 16 h. Grinding was followed by mixing lactoselenium into the feed. The final concentration of lactomicroSe was approximately 2.5 g/kg, with >95% of Se in the form of nanoparticles and <5% as organic Se.

**Cell culture**

In vitro spontaneously immortalized HaCaT keratinocytes were derived from the histologically normal skin of a Caucasian male [16]. Cells were grown in Dulbecco’s modified Eagle’s medium (DMEM; Sigma–Aldrich, Budapest, Hungary) supplemented with 2 mM l-glutamine, 23 mM bicarbonate, antibiotics (100 IU/mL penicillin and 100 μg/mL streptomycin) to avoid infection during long-term incubation and 10% heat-inactivated fetal bovine serum (FBS).

**Image processing and analysis**

Cellular and nuclear surfaces were calculated from cell and nuclear perimeters. The relative surface changes of those cells were observed that did not leave the visual area of the inverse microscope. These surface variations were due to small out-of-focus displacements, with much smaller oscillations than the major cell surface changes caused by the presence of selenium species. The steps of image processing have been described earlier [18]. Edges of cells were detected using a diamond-shaped pixel pattern. Perimeters, cellular and nuclear surfaces were calculated and given in μm$^2$. The growth curves presented are characteristic to the selenium species and their concentrations.

**Animals**

Selenium species were administered by adding them at 0.5, 5, or 50 ppm to the diet of 10-week-old BDF1 male mice obtained from National Institute of Oncology, Budapest weighing an average of 26 g. Labdiet (mouse diet 5015) was obtained from Charles River. Its 0.26 ppm Se content was within the physiological range (0.1–1 ppm) and was subtracted from the administered Se doses. Animal care followed the criteria outlined in the U.S. National Institutes of Health Guide and European Community’s guiding principles for the Care and Use of Laboratory Animals. The experimental protocol has been approved by the National Ethics Committee for Animal Research (1/2009 DE MAB). Each group of animals consisted of six mice.

**Selenium treatment of mice**

The control group received no additional Se beside the basal diet. Fifteen groups of mice were administered Se species. Mice were fed ad libitum without measuring food consumption. The treatment lasting for 14 days, is summarized in Table 1.
Table 1
Treatment of mice with selenium species.

<table>
<thead>
<tr>
<th>Selenium species</th>
<th>No. of mice treated with</th>
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<td></td>
<td>0.5 ppm</td>
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<tr>
<td></td>
<td>5.0 ppm</td>
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<td></td>
<td>50 ppm</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
</tr>
<tr>
<td>NanoSe</td>
<td>6</td>
</tr>
<tr>
<td>LactomicroSe</td>
<td>6</td>
</tr>
<tr>
<td>SelPlex</td>
<td>6</td>
</tr>
<tr>
<td>Selenite</td>
<td>6</td>
</tr>
<tr>
<td>Selenate</td>
<td>6</td>
</tr>
</tbody>
</table>

* The control group consisting of six mice did not get selenium. Fifteen groups of mice (six each) were administered selenium species by mixing them proportionally at increasing concentrations (0.5, 5, or 50 ppm) into the diet of mice. Treatment lasted for two weeks. Diets containing 50 mg Se/kg were not readily consumed by mice due to a temporary diet aversion caused by Se. Selenate was present as sodium selenate (Na$_2$SeO$_4$) and selenite as sodium hydroselenite (NaHSeO$_3$). Mice were subjected to cervical dislocation on day 14.

Organ tissue samples

At the end of each set of experiments at day 14, mice were sacrificed by cervical dislocation. Organs including kidneys were excised from dissected mice and rinsed in ice-cold saline. The weight of organs was determined. Organ tissue samples were stored at –30 ºC before sectioning.

Histological sections

Sections of kidneys were prepared from dissected mice. Protocols involved fixation, dehydration, embedding, slicing into 15-mm-thick sections, hematoxylin staining of sectioned preparations, and viewing under a microscope. Fixation of tissue samples took place under standard condition in 10% paraformaldehyde solution for 12 h. This step served to stop the bacterial decay and contamination of samples, and to preserve them for a longer period of time. Fixation was followed by dehydration at increasing concentrations of alcohol from 50% ethanol solution through 60, 70, 80, 90, 96%, and absolute ethanol. Each dehydration step lasted for 30 min. After the last step of fixation, xylene was used to remove alcohol. Following dehydration and removal of alcohol the samples were embedded in paraffin. This process lasted for 24 h and consisted of three separate phases: (1) xylene–paraffin mixture (1:1) for 6 h; (2) first paraffin embedding for 6 h; and (3) second embedding in paraffin for 12 h. The embedding took place in a 56 ºC thermostat. Final embedding in paraffin lasted for 24 h prior to sectioning. A sliding microtome (Reichert, Diversified Equipment) was used to cut tissue slices of 15 mm corresponding to the thickness of approximately three cell layers.

Hematoxylin–eosin staining

Paraffin was removed from tissue slices in two consecutive steps by keeping samples in xylene for 10 min each. For rehydration, the opposite gradient of ethanol concentrations was used (96, 90, 80, 70, 60, 50%) for 10 min and finally distilled water for 5 min. After rehydration, the samples were placed in a staining solution that contained alum hematoxylin and methanol. The nuclear staining lasted for 5 min. The samples were treated with acidic-alcohol (1 mL HCl dissolved in 99 mL 70% ethanol) for 2 s. The samples were washed in tap water for 5 min in two consecutive steps, and then in running tap water for 45 min. After washing, samples were dehydrated in 50% ethanol, followed by 80 and 70% ethanol for 5 min each. Samples were background-stained with eosin–staining solution (containing 70% ethanol). Final dehydration was in 80, 90, 96%, and absolute ethanol, each for 3 min. The alcohol was removed with xylene in two steps for 5 min each. Slides containing tissue samples

Fig. 2. Changes in cytoplasmic and nuclear surface areas. Measurements were carried out after 10 h incubation in the presence and absence of selenium species as described in the Methods. (A) Cytoplasmic surface changes and (B) nuclear surface alterations.

Fig. 1. Characteristic HaCaT cell growth curves in the absence and presence of nanoSe species. (A) Control cells, (B) cell growth in the presence of 5 ppm lactomicroSe, (C) growth curve in the presence of 1 ppm nanoSe and (D) inhibition of cell growth at 5 ppm nanoSe.
were embedded in one drop of Entellan, covered with coverslips, and used for microscopy.

Analytical procedures

Determination of selenium content

Diets, serum, and the various tissues were analyzed for their Se contents using a Millenium Merlin Atomic Fluorescence Spectrometer (PS Analytical, Orpington, Kent, UK) with the following settings: 15 L/min argon carrier gas flow rate, 40 s measurement, 40 s wash time, gain 100×. The instrument was calibrated using Charlau standards, and we used quality control (QC) standards for every 5 samples. For the hydride generation reaction we used 3 M HCl as the acid solution and 1.4 (w/v%) NaBH₄ in 0.1 M NaOH as the reducing agent.

Digital image analysis

Digital images of color photography were combinations of three primary colors. Red, green, blue channels of the acquired 24-bit bitmap images from a standard digital camera were separated. The blue channel was used to generate the thresholded binary image of hematoxylin staining. Binary images were used for particle shape, size, distribution analysis. Red channel was used for the quantification of eosin staining.

Statistical analysis

Experiments were replicated three time and data expressed as mean ± standard error of mean. Statistically significant differences (*p < 0.005 and **p < 0.001) were determined with the Student’s t-test when the variance was statistically significant.

![Fig. 3. Time-lapse photography of control HaCaT cells. Cells were grown in the absence of selenium for 900 min. Photography of growing cells was taken every min by our custom-built video camera attached to the microscope and connected to the computer. To improve the visibility of cells only one quarter (upper left segment) of the visual field is shown in each panel. White numbers indicate the time of photography. Exposures were converted to videofilm by speeding up the projection to 30 exposures/s (supplementary video 1).](image-url)
Results

Inhibition of cell growth by nanoselenium

With the exception of lactomicroSe all selenium sources inhibited cellular growth at 1 ppm concentration (results not shown). The growth curves of two selenium species, namely the inorganic nanoSe and lactomicroSe obtained by time-lapse image analysis are presented in Fig. 1. Relative to the control, where continuous growth was measured for 40 h (Fig. 1A), 5 ppm lactomicroSe treatment did not exert inhibition (Fig. 1B). In the presence of 1 ppm nanoSe cell growth was inhibited for 8 h followed by a moderate growth (Fig. 1C). Cellular damages are reflected by the fluctuations of the growth curve at 5 ppm nanoSe concentration followed by a steep, but short virtual rise after 9 h of incubation (Fig. 1D).

Nuclear and cytoplasmic shrinkage and expansion upon selenium treatment

Selenate turned out to be the only selenium compound that caused an ~34% apoptotic shrinkage of the nucleus. All other selenium species that are derivatives of selenite caused the expansion of cells. By regarding the nuclear expansion as a measure of damage, the increasing level of toxicity order relative to the control (100 ± 18.3%) is: lactomicroSe (103.4 ± 16.3%), SelPlex (121.6 ± 23.4%), selenite (132.9 ± 24.7%), nanoSe (136.4 ± 23.1%), selenate 66.0 ± 30.6%) (Fig. 2A). Deviations from cytoplasmic surface area (shrinkage and expansion) reflected a different order of Se toxicity: control (100 ± 26.0%), lactomicroSe (98.5 ± 23.4%), nanoSe (117.1 ± 23.5%), SelPlex (106.2 ± 22.2%), selenite (86.0 ± 16.8%), selenate (53.1 ± 26.1%) (Fig. 2B). LactomicroSe was the only selenium species that caused non-significant nuclear or cytoplasmic changes.

Cellular damages caused by Se and followed by time-lapse videomicroscopy

We have followed and monitored the growth of individual cells with our long-term scanning device in control HaCaT cells and after subjecting cells to 1 and 5 ppm Se incubation. After sedimentation and attachment of cells the growth of the control population started at around 180 min and 100% confluency of the monolayer was reached after 900 min (Fig. 3).

When HaCaT cells were incubated in the presence of 5 ppm lactomicroSe, the growth rate was similar to that of control cells and complete confluency was obtained within 900 min (Fig. 4).

Higher growth inhibition was observed when HaCaT cells were treated with 1 ppm nanoSe. Extremely elongated cells appeared already after 60 min of incubation (Fig. 5). At 5 ppm nanoSe concentration moderate increase of cell growth lasted for 10 h, then complete disintegration of cell morphology started (Fig. 6) that appeared in the cell surface increase as virtual cell growth seen in Fig. 1D.

Fig. 4. Monitoring HaCaT cell growth in the presence of 5 ppm lactomicroSe. Cells were grown as described in the Materials section and under Fig. 3. The whole visual field is shown in each panel. White numbers at the bottom of each panel refer to the time passed in minutes from the recording. Bar, 50 μm for each panel (supplementary video 2).
Acute toxicity of selenium species in mice

Subacute toxicity studies by oral administration of Se species lasted for 14 days and aimed to determine concentrations spanning from subacute to lethal doses. These concentrations ranged between 0.5 and 50 ppm. All mice survived lower doses (0.5 and 5 ppm). Survival data of higher toxic concentrations show that two-thirds of animals died after selenate, one-third after selenite treatment. SelPlex and nanoSe were less toxic and 83% of the mice survived these administrations. All animals survived lactomicroSe treatment (Table 2).

In our earlier study based on the organ toxicity more organs were affected by selenite, but the lower survival rate suggested that selenate was generally more toxic than selenite leading to the conclusion that the order of selenium species was: selenate > selenite > nanoSe > SelPlex > lactomicroSe [13]. Compared to liver, the kidney weight did not change significantly even at higher (50 ppm) Se concentrations. Lowest reduction was observed upon lactomicroSe administration (1.5%), followed by the weight loss caused by selenate (4%), SelPlex (7%), nanoSe (11%) and selenite.

<table>
<thead>
<tr>
<th>Selenium species</th>
<th>Treatment</th>
<th>Survival in days</th>
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<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>0 2 4 6 8 10 12 14</td>
</tr>
<tr>
<td>Selenate</td>
<td>50</td>
<td>6 6 6 6 5 5 3 2 2</td>
</tr>
<tr>
<td>Selenite</td>
<td>50</td>
<td>6 6 6 6 6 6 6 6 5</td>
</tr>
<tr>
<td>SelPlex</td>
<td>50</td>
<td>6 6 6 6 6 6 6 6 5</td>
</tr>
<tr>
<td>Nanoselene</td>
<td>50</td>
<td>6 6 6 6 6 6 6 6 5</td>
</tr>
<tr>
<td>LactomicroSe</td>
<td>50</td>
<td>6 6 6 6 6 6 6 6 6</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>6 6 6 6 6 6 6 6 6</td>
</tr>
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</table>

Note: Toxicity test was carried out as described under Materials and methods.
(12%) treatment. This toxicity order is similar to the liver toxicity order of weight loss: selenate (5%), lactomicroSe (6%), SelPlex (11%), nanoSe (20%) and selenite (36%). Each value of liver weight loss is higher than the weight loss of kidneys caused by Se, a clear indication that nephrotoxicity of selenium species is lower than their liver toxicity.

**Absorption of lactomicroSe**

As the low nephrotoxicity of lactomicroSe could reflect low absorption, renal and serum Se concentrations were measured after treatment with high concentrations (50 ppm) of selenium species. The effects of dietary Se sources on Se concentrations in serum and in kidney tissue are shown in Fig. 7. The serum Se concentration was low after lactomicroSe treatment followed by increasingly higher Se serum levels after sodium selenite, sodium selenate, nanoSe and SelPlex treatment of mice (Fig. 7A). Higher Se concentrations were measured in kidney tissue, but the order of Se concentrations were the same, with lactomicroSe treated mice containing the lowest Se concentrations followed by selenite, selenate, nanoSe and SelPlex (Fig. 7B). The differences in kidney Se levels could be influenced by selenium particle size. However, the comparison of the nanoSe particle size (140–300 nm) (Fig. 7C) with the somewhat larger lactomicroSe particles (180–360) (Fig. 7D) did not explain the higher absorption of nanoSe particles.

The Se levels after 50 ppm lactomicroSe treatment were also measured in other tissues, relative to the kidney Se level taken as 100%. Se levels were low in brain (13.5%) and heart (30.1%), and not much higher in spleen (104.6%) and liver (122.7%). The lower Se levels in kidney could explain the lower toxicity of lactomicroSe. To confirm the lower nephrotoxicity of lactomicroSe histopathological examinations were performed.
Kidney histopathology of selenium species

Hematoxylin–eosin staining of liver tissue at day 14 after 5 ppm administration to mice has shown that largest tissue disruptions occurred after selenate treatment, blurred disruptions by selenite, uniform and smaller disruptions by Sel-Plex, small local disruptions by nanoSe and few occasional disruptions by lactomicroSe. The controversy between the toxicity based on organ weight reduction and histopathology combined with the observations of others that the kidney may accumulate Se prompted our histopathological examinations in kidney tissue after 0.5 and 50 ppm selenium treatment of mice (Fig. 8).

Histopathology of nanoSe

The control kidney tissue representing healthy morphology is seen at lower (Fig. 8a and b) and at larger (Fig. 8c–f) magnifications. Mice were treated with 0.5 and 50 ppm nanoSe for 14 and kidney tissue sections were stained with hematoxylin–eosin (Fig. 8c–f). Relative to the control kidney tissue (Fig. 8a and b), deposition of Se was suspected at 0.5 ppm treatment visible at larger (40×) magnification (Fig. 8d). Higher (50 ppm) nanoSe caused a relatively even distribution of precipitated proteins seen as small dots both at lower (10×) and higher (40×) magnification (Fig. 8e and f). The precipitation of proteins was confirmed by using the blue channel for generating the thresholded binary image of hematoxylin staining (Fig. 8g and h). Another characteristic feature was the turning of normal morphology of kidney cells to cobblestone phenotype (Fig. 8e and f).

Histopathology of lactomicroSe

After mice were subjected to subacute lactomicroSe diet (0.5 and 50 ppm) for 14 days significant morphological changes were observed neither at 0.5 ppm (Fig. 8i and j), nor at higher (50 ppm) Se concentrations (Fig. 8k and l).

Histopathology of Sel-Plex

Groups of mice were treated with 0.5 or 50 ppm Sel-Plex and sections of kidney tissue were examined microscopically after hematoxylin–eosin staining (Fig. 8m–p). Significant Se deposition and rounded up apoptotic cells were seen at lower and higher Sel-Plex concentrations without major tissue disruptions.

Histopathology of selenate

The treatment of mice took place with 0.5 and 50 ppm selenate (Fig. 8q–t). The accumulation of Se was seen at lower dose (0.5 ppm) as veil-like infiltration separated as small dots and nuclear condensations visible at higher magnification. At higher selenate concentration, beside the deposition of Se as larger dots, tissue disruptions, nuclear condensation and the appearance of apoptotic bodies dominated the pictures.

Histopathology of selenite

Mice were subjected to 0.5 and 50 ppm selenite treatment as before and accumulation of Se was visualized after staining (Fig. 8u–x). Proteins precipitated and accumulated at 0.5 ppm selenite concentration, massive denaturation with tissue disruption were seen after 50 ppm selenite administration.

Discussion

Selenium deficiency may develop: (a) in patients with compromised intestinal function, (b) in patients subjected to parenteral nutrition, (c) after gastrointestinal bypass surgery, (d) in old age (>90 years), (e) in people eating food grown on selenium-deficient soil. Selenium deficiency has been implicated in the development of cardiovascular disease, skeletal muscle myopathy, anemia, increased cancer risk, weakened immune function and is likely to be associated with renal failure as the kidney plays an important role in selenium homeostasis. Selenium is involved in thyroid fibrosis in improved survival of lupus and is essential for normal thyroid function. Elevated selenium intake may be associated with reduced cancer risk, while too much selenium can be toxic. Clinical trials were supposed to confirm or refute this hypothesis, by supplementing the diminishing selenium intake in some parts of the world, notably in some European countries, and in certain regions of US and Canada, where selenium deficiency was common unless supplementation was secured. Although, the health benefits of selenium are not doubted, our knowledge regarding the safe selenium supplementation and the type of selenium species is...
Fig. 8. Hematoxylin–eosin staining of kidney tissue after Se treatment of mice. Treatment of mice, tissue sectioning and staining took place as described in the Methods. (a) Control kidney tissue at lower (10×) magnification, (b) control at higher (40×) magnification, (c) kidney tissue after 0.5 ppm nanoSe treatment at low (10×) magnification, (d) low concentration (0.5 ppm) nanoSe treatment at higher (40×) magnification, (e) NanoSe treatment (50 ppm) at lower magnification, (f) NanoSe treatment (50 ppm) at higher magnification, (g) NanoSe precipitation at 50 ppm concentration seen as small black dots in the hematoxylin–eosin stained tissue after microscopic enlargement at higher (40×) magnification, (h) the same as (g) after blue channel thresholding, LactomicroSe treatment: (i) at 0.5 ppm and 10× magnification, (j) lactomicroSe (0.5 ppm, 40× magnification), (k) lactomicroSe (50 ppm, 10× magnification), (l) lactomicroSe (50 ppm, 40× magnification). SelPlex treatment: (m) 0.5 ppm, 10× magnification, (n) 0.5 ppm, 40× magnification, (o) 50 ppm, 10× magnification, (p) 50 ppm, 40× magnification. Selenite treatment: (q) 0.5 ppm, 10×, (r) 0.5 ppm, 40×, (s) 50 ppm, 10×, (t) 50 ppm, 40×. Selenate treatment: (u) 0.5 ppm, 10×, (v) 0.5 ppm, 40×, (w) 50 ppm, 10×, (x) 50 ppm, 40×. Scale bar, 50 μm each.
insufficient to avoid both acute and chronic toxicity of this essential heavy metal. To answer the question of Se supplementation we have compared the subacute toxicity of different selenium species in mice [14]. The evaluation of the toxicity was based on several criteria involving the changes in the number of bone marrow cells, white blood cells, granulocyte-macrophage colony forming units, body and organ weights and liver histology. The applied murine doses of selenium species were 0.5, 5 and 50 ppm, corresponding to ten times higher and ten times lower doses of the maximum recommended level per day in the US for adults (400 μg/day –5 ppm Se/kg food).

In larger doses, selenium is toxic to virtually all tissues, especially to the liver. Selenium poisoning could also harm kidneys, but more likely as a secondary effect related to liver damage. The highest Se levels of tissue concentrations were found in liver, followed by kidney cortex, and heart [27]. The liver weight of mice was found to be significantly reduced and to different extent affected by selenium species. Short-term high-dose of selenite caused pronounced oxidative stress, greater liver injury, and retardation of growth as compared to nanoSe [28]. Elemental selenium at nano size (nanoSe) possessed equal efficacy with respect to glutathione peroxidase and thioredoxin reductase activities as selenomethionine, but exerted lower acute liver injury, and short-term toxicity [29]. NanoSe was more effective as a chemopreventive agent at smaller particle size [30].

Due to the insufficient information available to offer speculation about the kidney toxicity of Se we have measured and found that the weight of the kidney was hardly affected by Se treatment. It was reported that selenium has a protective effect against cadmium-induced renal toxicity of rats [31]. These observations contradicted the potential nephrotoxicity and accumulation of Se in the kidney described by others [10]. To resolve this contradiction we have extended experiments from cellular toxicity to Se levels in kidney and to the nephrotoxicity of selenium compounds.

Our original plan to use kidney to study the cellular toxicity of Se failed after we have observed major phenotypic transitions in our podocyte cell culture [13]. Cellular damages caused by different Se compounds were visualized by time-lapse videomicroscopy in HaCaT cells. These experiments revealed that contrary to the acute toxicity tests that did not show nephrotoxicity, selenite derivatives (sodium selenite, SelPlex, nanoSe) caused severe morphological alterations in size and shape of HaCaT cells. Cellular distortions turned to an amorphous morphology of broken cells resembling apoptotic bodies. The comparison of cytoplasmic shrinkages and expansions as toxicity indicators with those of the histopathology order of kidney toxicity: lactomicroSe < nanoSe < selenite < SelPlex < selenate showed a closer toxicological relationship than those of nuclear changes. These differences could be accounted for by the less reliable estimation of nuclear surface.

Histological experiments confirmed the accumulation in kidney and precipitation of selenium in kidney slices, and answered the question which selenium compound was causing more damage to the kidney tissue. Largest deposition of Se was caused by selenate and SelPlex, less precipitation was caused by selenite and nanoSe. The absorption of lactomicroSe by the kidney was the lowest and did not cause microscopically visible Se accumulation. This harmless selenium species is recommended for selenium supplementation not only in murine experiments but also for human use.

**Conclusion**

Based on previous observations [14] and recent results acute Se treatment is likely to cause tissue damage in major organs involved in detoxification. The liver toxicity order corresponded to: selenate > selenite > nanoSe > SelPlex > lactomicroSe [14], while the kidney toxicity order was: selenate > SelPlex > selenite > nanoSe > lactomicroSe. These differences indicate that Se compounds may affect different metabolic pathways.

Compared with toxic selenium compounds such as sodium selenite and sodium selenate [32], others have found that nanoSe exhibited much lower toxicities. NanoSe was thus regarded a potential selenium source [33] commonly used in dietary supplements [34]. Among the tested compounds, lactomicroSe proved to be the only harmless selenium, damaging neither cells, nor the tissue of the liver nor that of the kidney. LactomicroSe administration even at high (50 ppm) concentration: (a) did not cause cellular toxicity, (b) hardly affected body weight, (c) did not influence the weight of major organs involved in detoxification (liver, kidney), (d) did not cause Se accumulation in the kidney. LactomicroSe was the only Se species that did neither cause cellular toxicity nor tissue disruptions nor the accumulation of Se in the kidney excluding the possibility of acute or chronic nephrotoxicity. Based on the results it is likely that lactomicroSe has developed to reduce renal toxicity by its lower absorption and to prevent selenium deficiency.

**Conflict of interest**

The authors have no conflict of interest to declare.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jtemb.2014.12.011.

**References**


