Protective Effect of Selenoarginine against Oxidative Stress in D-Galactose-Induced Aging Mice

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Received August 11, 2008; Accepted February 28, 2009; Online Publication July 7, 2009 [doi:10.1271/bbb.80558]

The protective effect of selenoarginine against oxidation resistance was investigated in D-galactose (D-gal)-induced aging mice. The mice were divided into four groups (n = 15): a normal group, a model group, a low-dose selenoarginine group (8.35 μg of Se/kg b.w./d), and a high-dose selenoarginine group (16.78 μg of Se/kg b.w./d). The aging model was induced by s.c. injection D-galactose dissolved in 0.9% normal saline of a dose of 150 mg/kg/d for 6 weeks. The mice in the normal group received s.c. injection of sterile normal saline at the same dose and frequency. The results showed that oxidative stress in the liver, kidney, and brain tissues and the serum of the mice was induced by D-galactose, but selenoarginine had an obviously protective effect against D-galactose-induced aging mice. Low-dose selenoarginine performed better than high-dose selenoarginine. The protective effect of selenoarginine on D-galactose-induced aging mice can be attributed to elevation of the activity of antioxidase and enhanced antioxidant defenses.

Key words: selenoarginine; D-galactose; oxidative stress; protective effect

Aging is a natural phenomenon always accompanied by the occurrence of several neurodegenerative diseases, such as cognitive impairment, schizophrenia, Parkinson’s and Alzheimer’s diseases, and so on. At present, there are several assumptions concerning aging, of which the well-known theories are the free radical theory and the immune theory. Increasing evidence suggests that oxidative stress plays a central role in the process of biological aging.1

Selenium is an essential micronutrient for humans and animals. As an key component of a number of functional selenoproteins required for normal health, selenium has a large number of biological functions. It is perhaps mediated through the glutathione peroxidases (GPx) that remove potentially damaging lipid hydroperoxides and hydrogen peroxide. At least five of these peroxidases have now been identified in different cell and tissue compartments.2,3) Selenium can act as an antioxidant in extracellular space, the cell cytosol, in cell membranes, and specifically in the gastrointestinal tract, with potential to influence immune processes.4) Many diseases are associated with selenium deficiency,5,6) such as chronic heart failure,7,8) skeletal muscle myopathy, and neurogenic disease, and cretinism (in iodine-deficient populations), and more marginal deficiencies may contribute to reduced immune function, some cancers, and viral diseases.9)

Generally, organic selenides have better biological security, absorption, and utility than inorganic ones. Therefore, it is important to seek secure organic selenides for human health. Previous studies in our laboratory demonstrated that selenoarginine synthesized by our researchers possessed better antioxidation effects than SeO2 in alcohol-induced liver injury mice.10) In order to confirm ulteriorly selenoarginine’s protective effect of antioxidation, we exploited D-gal treated mice as an experimental senile model to investigate the antioxidation effect of selenoarginine in aging mice, including Superoxide dismutase (SOD), malondialdehyde (MDA), and GSH-Px in serum sample and tissues of the liver, kidney, and brain.

Materials and Methods

Preparation of selenoarginine. The preparation of selenoarginine was based on an unreported synthesis method (applying for patent), as shown in Scheme 1. Selenoarginine was synthesized by a reaction of L-arginine and selenium dioxide under low-temperature vacuum circumstances.

Animals. Kunming strain mice (equal numbers of males and females, 8 weeks old, 20 ± 2 g) were purchased from the Laboratory Animal Center, Academy of Military Medical Sciences (Beijing, China). They were acclimated to our laboratory environment for one week before the experiment. They were housed under standard conditions (25°C, 12 h light, 12 h dark), and were allowed free access to food and water during the experimental period. All the experiments were carried with the approval of the animal ethics committee of Tianjin University of Science and Technology.

Animals treatments. All the mice were randomly divided into five groups of 15 each: a normal group (N group), a model group (M group), a low-dose selenoarginine group (LD-Se group), and a high-dose selenoarginine group (HD-Se group). The aging model was induced by s.c. injection D-galactose dissolved in 0.9% normal saline a dose of 150 mg/kg/d for 6 weeks. The mice in the normal group received s.c. injections of sterile normal saline at the same dose and frequency. In addition to receiving D-galactose, the mice in the LD-Se groups and the HD-Se groups were fed a diet supplemented simultaneously with 8.35 μg of selenium/kg/d and 16.78 μg of selenium/kg/d from selenoarginine (the selenium content was 10 times and 20 times the minimum human recommended dose of 50 μg Se/d). The low-selenium diet (0.02 mg Se/d). The low-selenium diet (0.02 mg Se/d).
SeO$_2$ + Arg $\rightarrow$ (Arg)$_2$H$_2$SeO$_3$

Scheme 1. Preparation of Selenoarginine.

They were weighed weekly in order to adjust the dose of selenoarginine. After 6 weeks of administration, the mice were killed and blood was collected from the retrobulbar venous plexus, and the kidney, liver, and brain were harvested and stored immediately at $-80^\circ$C for biochemical measurements.

Determination of SOD, MDA, and GSH-PX levels in different tissues. Blood samples were allowed to clot for 2–3 h, and serum was separated by centrifugation at 2,200 x g for 10 min and stored at 4°C for biochemical analysis. The tissues were rinsed and weighed, and then put in tubes with 9 volumes of 9 g/l normal saline. Then the tissue samples were homogenized (10% w/v) in icky 50 mM phosphate buffer (pH 7.4). After centrifugation at 3,000 x g at 4°C for 10 min, the supernatant was used to assay enzyme activities. Protein concentrations, the MDA level, GPs, and SOD activities in the tissue homogenates supernatant and the serum samples were measured with an assay kit (Nanjing Jiancheng, Nanjing, China) according to the providers’ instructions.

Statistical analysis. All data were expressed as mean ± SD. Statistical analysis of the data was performed by ANOVA followed by post hoc multiple comparisons made by Tukey’s test. The criterion of significance was $p < 0.05$ or $p < 0.01$ in all statistical evaluations.

Results

Synthesis and characterization of selenoarginine

In our previous studies, the structure and physicochemical properties of selenoarginine were characterized. The results of fourier transform infrared (FTIR) and electrospray ionization mass spectrometry (ESI-MS) and other relevant informations of selenoarginine were as in Table 1.

Effects of selenoarginine on the contents of MDA in aging mice

Mice MDA levels in serum and tissue homogenates increased more significantly in the model groups than in the normal group ($p < 0.01$). After administration of selenoarginine, the contents of MDA significantly decreased, more than in the model group ($p < 0.05$ and $p < 0.01$), but no significant difference was found between the normal group and the selenoarginine groups (LD-Se and HD-Se). In two selenoarginine groups, the MDA levels in kidney, brain, and serum in the LD-Se group were lower than in the HD-Se group, but no significant decrease was observed, and the content of MDA in liver significantly increased ($p < 0.05$, Fig. 1). The results showed that the lipid peroxide process in mice was noticeably accelerated by injection d-galactose. However, selenoarginine was effective in reducing the MDA content in the tissues and serum, and had good scavenging effects on free radicals.

Effects of selenoarginine on GPX activities in aging mice

The activity of GPX is an important index of the level of selenium. Glutathione peroxidase activities were studied to evaluate changes in antioxidant status in different tissues. The results showed that GPX activity decreased markedly in the model group compared with the normal group and the selenoarginine treated groups ($p < 0.05$ and $p < 0.01$). Compared with the normal group, the levels of GPX in the liver, kidney, and brain were no statistically significant, but the serum GPX significantly decreased in the LD-Se group ($p < 0.01$). In the LD-Se group, the activity of GPX in the brain and serum was significantly higher than in the HD-Se group ($p < 0.05$), but no obvious change was observed in the kidney or liver (Fig. 2).

Effects of selenoarginine on SOD activity in aging mice

SOD plays a crucial role in the balance of oxidation and antioxidation. We found that the activity of SOD significantly decreased in the model group ($p < 0.05$ and $p < 0.01$). The SOD levels were significantly increased after administration of selenoarginine for 6 weeks ($p < 0.05$ and $p < 0.01$). In the LD-Se group, the activity of SOD in the brain was remarkably higher than in the normal group or the HD-Se group ($p < 0.05$), but the SOD levels in the serum, liver, and kidney increased

Table 1. Relevant Information on Selenoarginine

<table>
<thead>
<tr>
<th>Relevant informations</th>
<th>Selenoarginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>C$<em>{12}$H$</em>{26}$N$<em>{6}$O$</em>{5}$Se</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>457.34</td>
</tr>
<tr>
<td>IR</td>
<td>1575(COO−) 1258(C−N) 1047(Se–O)</td>
</tr>
<tr>
<td>ESI-MS theory value (M):</td>
<td>1048(C−N)</td>
</tr>
<tr>
<td>Translucent rate (%)</td>
<td>99.2</td>
</tr>
<tr>
<td>Specific rotation</td>
<td>+17.6°</td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>126.2</td>
</tr>
</tbody>
</table>

Fig. 1. Levels of MDA in Different Tissues of Aging Mice.

All values are mean ± SE (n = 15). *$p < 0.05$, **$p < 0.01$ as compared to the normal group; ´$p < 0.05$, ´´$p < 0.01$ as compared with the model group, and ´´´$p < 0.05$ as compared with the LD-Se group. N, normal group; M, model group (150 mg/kg b.w.); LD-Selenoarginine (8.35 µg Se/kg b.w.) + d-gal (150 mg/kg b.w.); HD-Selenoarginine (16.78 µg Se/kg b.w.) + d-gal (150 mg/kg b.w.).
selenoarginine in maintaining the activity of SOD had better performance than high doses. All the results showed that low doses of selenoarginine slightly, and there was no statistical significance among the three groups. All the results showed that low doses of selenoarginine had better performance than high doses of selenoarginine in maintaining the activity of SOD in D-gal-induced aging mice (Fig. 3).

Discussion

The current research revealed different potential applications of antioxidant/free radical manipulations in the prevention and control of disease. Increasing evidence demonstrates that impairment of antioxidant defense mechanisms is the pathogenesis of over 50 human diseases. Antioxidants including the plant extracts, vitamin E and selenium, used in the treatment of many diseases have the capacity to protect the integrity of unsaturated bonds of membrane phospholipids by reducing free radical-mediated damage and delaying the progression of lipid oxidation. Selenium is an active immunomodulator, a much more potent but more toxic anti-oxidant than vitamins E, C, A, or beta-carotene. Selenium performs its vital functions through selenoproteins, including the families of glutathione peroxidases, deiodinases, and thioredoxin reductases.

It has been suggested that aging is the result of oxidative stress. With aging, the activity of antioxidant enzymes and the ability of antioxidant defenses decrease, which results in accumulated intracellular free radicals. Meanwhile, the chain reaction of lipid peroxidation accelerates. These changes accelerate the process of neuro-inflammation, resulting in several kinds of neuro-degenerative diseases.

Schroeder et al. reported that cellular aging may be partially caused by superoxide radical-induced release of immature mRNA from its intranuclear binding site, resulting in the appearance of immature messengers in the cytoplasm. This may cause changes of protein synthesis. Thus, aging may be associated not only with the expression of genes coding for proteins not characteristic of the proper state of differentiation of a given cell, but also with impaired maturation of the primary gene transcripts due to the interference of superoxide radicals, not sufficiently eliminated by antioxidant mechanisms with age, in RNA-matrix attachment.

In recent years, the D-galactose model has been widely used in studying. Previous studies showed that chronic injections of D-gal subcutaneously into mice induced changes similar to those in natural aging. The model showed neurological impairment, decreased activity of anti-oxidant enzymes, and poor immuneresponses. Therefore, we exploited D-gal treated mice as an experimental aged model to investigate the anti-aging effect of selenoarginine.

MDA is a metabolic product of lipid peroxidation. The levels of MDA are often used as a marker of oxidative damage and as an indicator of aging. GPx and SOD are the important antioxidant enzymes in vivo. GPx catalyzes the dismutation of superoxide to oxygen and hydrogen peroxide, which is catalyzed next by GPx with GSH as electron donor. The activities of GPx and SOD decrease with aging. Thus SOD and GPx are two important indicators in aging assessment. Our results suggest that the level of MDA increased and that SOD and GPx activities decreased in the liver, kidney, and brain in D-gal-induced aging mice as compared with the normal group, in accordance with previously reported data. The results showed that oxidative stress in mice tissues was induced by D-gal with the formation of lipid peroxide.

After administration of selenoarginine, we observed that the contents of MDA significantly decreased, while the activities of Glutathione peroxidase and SOD increased more in the liver, kidney, brain, and serum of D-galactose-induce aging mice than in the model group. Compared with the HD-Se group, the levels of MDA in the serum, kidney, and brain decreased, but the activities of glutathione peroxidase increased in the LD-Se group, but just the reverse in the liver. At the same time, the activity of SOD in mouse tissues and serum in the LD-Se group were higher than in the HD-Se group.

All the results showed that selenoarginine successfully inhibited lipid peroxidation induced D-gal and possessed potent antioxidant activity in different tissues.
of mice. Low doses of selenoarginine showed better performance than high doses of selenoarginine in maintaining the activity of GPs and SOD in D-gal-induced aging mice. In conclusion, the present results indicate that selenoarginine possesses potent antioxidant properties and an anti-aging effect on D-gal-treated mice through regulating its major Se-containing antioxidant enzyme, GPs, and raising SOD and free radical scavenging activities.

Evidence has emerged suggesting that organic selenium maintains the antioxidant defense system more efficiently than inorganic selenium. As a toxic element, inorganic selenium is not suitable for animals or humans. Mahmoud examined the GSH–GPX system in broiler chickens, and determined that chickens fed organic selenium as Sel-Plex, a selenized yeast, had elevated GPX activity in both the blood and liver in a thermoneutral environment and after heat distress. More importantly, the ability to reduce oxidized glutathione (GSSG to 2 GSH) was enhanced by the maintenance of glutathione reductase activity. Organic selenium-fed chickens were less affected by mild heat distress than inorganic selenium-fed chickens.28) Our previous study indicated that selenoarginine maintained lower contents of MDA and higher activity of GPs and SOD, and possessed better antioxidation effects than SeO3 in liver-injury mice.10)

This study preliminarily confirms the antioxidant function of selenoarginine, and provides a basis for the study of this newly synthesized organic selenium source. Further studies should be focused on comparison in antioxidant activities of selenoarginine and other organic selenium sources and confirming the interrelationships between the dose and the activity of the antioxidant enzyme to help reinforce current observations in order to define more clearly the exact antioxidative mechanism of selenoarginine. Therefore, selenoarginine with its antioxidant properties is a promising antioxidant and can be used as a dietary supplement to delay the process of aging.

Acknowledgments

This work was supported by grant from the National Natural Science Foundation of China (no. 20576101) and Recruited faculty research start-up foundation in Tianjin University of Science and Technology (no. 0200060).

References