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NUTRITION

Synergistic effect of Se-methylselenocysteine and vitamin E in ameliorating the acute ethanol-induced oxidative damage in rat

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ABSTRACT

The present study was conduced to investigate the synergistic effects of combined treatments with Se-methylselenocysteine (SeMSC) and vitamin E (Vit E) in reversing oxidative stress induced by ethanol in serum and different tissues of rats. Sixty female rats were randomly divided into six groups for 30 days' consecutive pretreatments as followed: control (I), physiological saline (II), 2.8 μg kg^{-1} Se as SeMSC (III), 2.8 μg kg^{-1} Se as sodium selenite (Na_2SeO_3, IV), 5 mg kg^{-1} α-tocopherol as α-tocopherol acetate (Vit E, V), 5 mg kg^{-1} α-tocopherol as α-tocopherol acetate and 2.8 μg kg^{-1} Se as SeMSC (VI). All animals in groups II–VI were treated by ethanol treatment to cause oxidative stress. After 6 h of ethanol treatment, the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), the contents of total antioxidant capacity (T-AOC), malondialdehyde (MDA), glutathione (GSH) and carbonyl protein (CP) in the serum, liver, heart and kidney were measured. The result showed that the individual SeSMC, Na_2SeO_3 and vitamin E could effectively increase the SOD, T-AOC, GSH-Px and GSH contents as well as significantly decrease the MDA and CP concentrations in the tissues of ethanol-induced rats. At the same dose on different forms of Se, SeMSC showed greater antioxidant activity than Na_2SeO_3. Moreover, group VI (SeMSC and α-tocopherol acetate) showed much better antioxidant activity than individual group III (SeMSC) and V (α-tocopherol acetate) due to the synergistic effect.

INTRODUCTION

Selenium (Se) has been proven to be associated with reduced risks of a number of chronic diseases [1–3]. The protective effects of Se seem to be primarily associated with its presence in the seleno-enzymes, which could protect DNA, protein and other cellular components from the oxidative damage [4]. Garlic and onion could accumulate the Se from soil and convert the inorganic selenium into organic selenium (Se-methylselenocysteine, SeMSC). In this situation, SeMSC in the garlic and onion is naturally occurring organic selenium [5]. Among different forms of Se, organic selenium such as SeMSC has been considered more effective than inorganic selenium (sodium selenite, Na_2SeO_3). Because SeMSC could decompose to methylselenol, which is hypothesized to be a critical selenium metabolite for anticancer activity *in vivo*, and differential chemopreventive effects of methylselenol on cancerous and noncancerous cells may play an important role. However, there are two alternative metabolic routes for methylselenol and the dose is the key factor deciding whether the effects will be antioxidant or prooxidant [6]. In recent years, increasing attention has been paid to SeMSC due to its beneficial effects such as antitumorigenic [7], antioxidant activity [8] and chemoprevention [9]. Vitamin E (Vit E) could significantly reduce the oxidative stress, lipids peroxidation and oxygen species in biological systems [10]. Vit E works synergistically with other antioxidants to quench free radicals and peroxides. Moreover, Se and Vit E combination was found to be effective against free radicals [11]. There have been a large number of animal and human studies clearly demonstrating the antioxidant and anticancer properties of Se [12,13]. However, some reports have also shown there are no functional effects of Se on the epidemiological study. For example, the Selenium and Vitamin E Cancer Preventive Trial showed that Se in the form of selenomethionine (200 μg/d) or Vit E (400 IU/d), alone or in combination (for 5.46 years) could not prevent prostate cancer in healthy men [14]. It needs to be noted that monomethylated selenoamino

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acids such as SeMSC is different from selenomethionine and SeMSC showed better bioactivity than selenomethionine and selenite [6]. For now, less study was reported on the synergistic effects of SeMSC and Vit E against the *in vivo* acute oxidative damage.

Ethanol-induced acute oxidative stress is directly involved in the production of oxygen species (ROS), which form an environment favourable to oxidative stress. ROS are involved in a variety of physiological and pathological processes [15,16]. The certain doses of ethanol could cause the imbalance between prooxidants and antioxidants and the oxidative damage of biomolecules. Antioxidants such as Vit E and SeMSC may ameliorate the oxidative damage by eliminating the ROS.

Therefore, the aim of the present study was designed to investigate the synergistic antioxidant effects of Vit E and Se (SeMSC) on the acute ethanol-induced oxidative injury in rats. Meanwhile, the antioxidant activity of SeMSC and Na_2SeO_3 were investigated and compared.

MATERIALS AND METHODS

MATERIALS

Vit E acetate, Na_2SeO_3 were purchased from Sigma (Steinheim, Germany), SeMSC of 96% purity was obtained from Chuan Qi Pharmaceutical Corp. Ltd., superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), total antioxidative capacity (T-AOC), reduced glutathione (GSH) and protein carbonyl kits were purchased from Nanjing Jiancheng Biotechnology Company (Nanjing, China).

ANIMALS AND EXPERIMENTAL DESIGN

The experiments were carried out using Sprague-Dawley rats (female, 180–220 g body weight (BW)). All the rats were maintained alone in stainless cages in a temperature controlled room ($24 \pm 2\,^{\circ}C$) with a controlled photoperiod (12-h light/dark cycle) and relative humidity of $50 \pm 5\%$. The rats were allowed free access to food and tap water. The basal diet consisted of 20% vitamin-free casein, 40% sucrose, 20% corn starch, 5% fibre, 10% fat (soybean oil), 3.5% mineral mix and 1% vitamin mix (without Vit E). All procedures involving animals were conducted in accordance with the guidelines of China for the care and use of laboratory animals.

After 7-day acclimatizing period, the rats were randomly assigned to six groups (10 rats for each). The rats in control group (group I) and model control (group II) were intragastric gavage (i.g.) administered with 1 mL of saline per day. The rats in SeMSC group (group III) were i.g. administrated with 1 mL SeMSC ($2.8\,\mu g\,kg^{-1}$ BW Se, in the form of SeMSC per day). The rats in Sodium selenite (Na_2SeO_3) group (group IV) were i.g. supplemented with 1 mL sodium selenite ($2.8\,\mu g\,kg^{-1}$ BW Se in the form of Na_2SeO_3 per day). The rats in Vit E group (group V) were i.g. fed with Vit E acetate ($5\,mg\,kg^{-1}$ BW Vit E acetate in the form of α-tocopherol acetate per day). The rats in Vit E and SeMSC group (group VI) were fed with Vit E acetate and SeMSC ($2.8\,\mu g\,kg^{-1}$ BW Se in the form of SeMSC and $5\,mg\,kg^{-1}$ BW Vit E acetate in the form of α-tocopherol acetate per day). Generally, the recommended daily dose of Se is 70 μg (http://www.crnusa.org/about_recs.html) and for Chinese adults is around 50 μg [17]. The doses for the rats in our study were chosen based on two-thirds of this recommendation ($50\,\mu g \times 2/3 = 33.3\,\mu g$). This dose (33.3 μg) is for a health adult (around 60 kg), which converts to the rat experiment (conversion coefficient 5–10 is needed, conversion based on body surface area, FDA. Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. 2005.7, website) and the final dose is ($33.3/60 \times 5 = 2.8\,\mu g/kg$). All groups were treated once daily for 30 consecutive days. After 12 h of the last administration, the rats except the control group (group I) were administered orally with 12 mL/kg BW of 50% ethanol solution. Six hours later, all the rats were euthanised (intraperitoneal sodium pentothal and inhalation isoflurane). The study was approved by institutional ethical committee of Nanchang University.

BIOCHEMICAL ASSAY

Pretreatment

After sacrifice, the blood samples of rats were collected immediately and centrifuged at 3000 rpm for 10 min at 4 °C. The tissues of rats including liver, heart and kidney were homogenized with cold saline. The homogenates were centrifuged at $3000 \times g$ for 20 min at 4 °C (Neofuge 15R, Heal Force, Hong Kong), and the supernatants were collected at the extractions, which were stored at −80 °C before analysis. The activities of SOD, GSH-Px and the levels of MDA, GSH and protein carbonyl in serum and tissues were determined using the commercial corresponding kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the enclosed guidelines. The protein content was measured by Lowry method [18].

THE T-AOC ACTIVITY

The total antioxidant capacity (T-AOC) was examined by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing City, China). The spectrometric method was applied to evaluate T-AOC. In the reaction, mixture ferric ion was reduced by antioxidant reducing agents and blue complex Fe^{2+}-TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) is produced. The optical density was measured at 520 nm. Absorbance was taken every 1 min for total of 10 min by spectrophotometer (Spectra max190, Molecular Devices, California). One unit (U) of T-AOC was defined as the amount that increased the absorbance by 0.01 at 37 °C. Fe (II) standard solution was tested in parallel. Data were expressed as U/mg protein (U/mg prot).

THE SOD ACTIVITY

SOD activity in homogenates was measured with a SOD kit based on its ability to inhibit the oxidation of hydroxylamine by the xanthine–xanthine oxidase system [19]. The absorbance at 550 nm was recorded for the calculation of SOD activity. One U of SOD activity was calculated as that inhibiting 50% of the oxidation of hydroxylamine without an enzyme source. SOD activity in homogenates was expressed as units per milligram of protein (U/mg prot).

THE GSH-PX ACTIVITY

GSH-Px activity was measured by oxidation of NADPH at 340 nm coupled with reduction of glutathione oxidized by GSH-Px. The absorbance in optical density at 340 nm was measured. GSH-Px activity was expressed as nmol of NADPH oxidized/min per milligrams of protein, calculated on the basis of an extinction coefficient of $6.22\,mM^{-1}cm^{-1}$ for NADPH. Data were expressed as U/mg of protein (U/mg prot).

THE GSH CONTENT

The GSH content was measured by the procedure of a GSH kit. The absorbance at 412 nm was measured at 10-s interval. From the calibration curve made with commercial GSH, the concentration of