

The Investigation of the Antioxidative Properties of the Novel Synthetic Organoselenium Compounds in Some Rat Tissues

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DMBA (7,12-dimethylbenz[a]anthracene) is a polycyclic aromatic hydrocarbon (PAH) known to cause tumors in rats. Selenium is an essential element with physiological non-enzymatic antioxidant properties. Because of the health problems induced by many environmental pollutants, many efforts have been undertaken in evaluating the relative antioxidant potential of selenium and synthetic organoselenium compounds. In this study, adult female Wistar rats were treated with DMBA and the novel organoselenium compounds (1-isopropyl-3-methylbenzimidazole-2-selenone [Sel] and 1,3-di-*p*-methoxybenzylpyrimidine-2-selenone [Sell]) in the determined doses. The protective effects of novel synthetic organoselenium compounds (Sel and Sell) against DMBA-induced changes in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) activities and total glutathione (GSH) and malondialdehyde (MDA) levels of rat heart and brain were investigated. It was determined that Sel and Sell fully or partially restored enzyme activity. It was also found that lipid peroxidation was also decreased in Sel and Sell treated groups. Consequently, it was determined that novel synthetic organoselenium compounds (Sel and Sell) provided protection of antioxidant activity, and protection against lipid peroxidation measured as MDA in Sel and Sell treated groups was provided by novel synthesized organoselenium compounds. The ability of the organoselenium compounds to prevent

oxidative damage induced by DMBA in rats was rationalized. *Exp Biol Med* 233:575–579, 2008

Key words: DMBA; brain; heart; oxidative stress; rat; synthetic organoselenium compounds

Introduction

Environmental sources of free radicals and reactive oxygen species (ROS) include some pollutants, organic solvents, pesticides, tobacco smoke, anesthetics, hyperoxia, and certain drugs (1). Oxygen-derived free radicals as highly reactive chemical species have importance in the aging process and they are also, either directly or indirectly, involved in various clinical disorders, such as atherosclerosis, reperfusion injury, cancer, etc. (2, 3).

For minimizing such damage, cells have defense systems consisting of both enzymatic and nonenzymatic processes. Examples of important components in the enzymatic process are antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR), and examples of components in nonenzymatic processes are antioxidant molecules such as total glutathione (GSH) (4). When the natural protective systems targeting ROS are insufficient, exogenous antioxidative compounds must be delivered. Consequently, the search for new antioxidants as potential drugs is an active field of medicinal chemistry (5). Selenium is a structural component of several enzymes with physiological antioxidant properties, including GPx and thioredoxine (6). The first attempt to research the effectiveness of aromatic selenium compounds in cancer chemoprevention was carried out by Karam El-Bayoumy in the 1980s (7). After that time, clinical trials in humans revealed beneficial effects of organoselenium compounds such as ebselen in pathological situations (8). The concept that

This work was supported by the Technological and Scientific Research Council of Turkey (TUBITAK TBAG-2259) (102T185).

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Received July 17, 2007.
Accepted December 31, 2007.

DOI: 10.3181/0707-RM-191
1535-3702/08/2335-0575\$15.00
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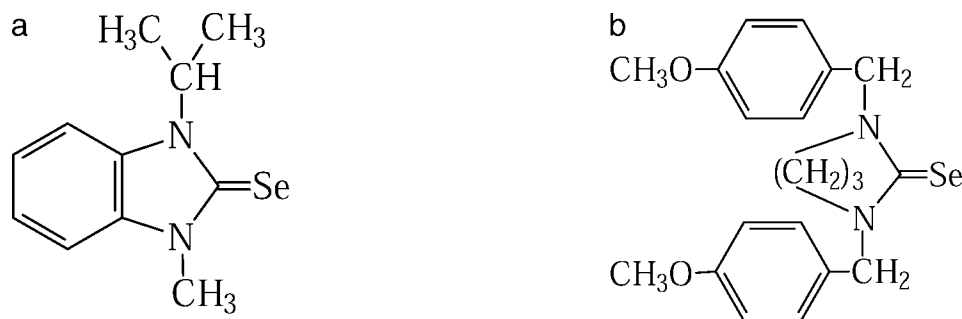


Figure 1. Novel synthetic organoselenium compounds. (A) 1-isopropyl-3-methylbenzimidazole-2-selenone (Se I). (B) 1,3-di-*p*-methoxybenzylpyrimidine-2-selenone (Se II).

selenium-containing molecules may constitute better nucleophiles (and therefore antioxidants) than classical antioxidants has led to the design of synthetic organoselenium compounds (9). In recent years, several inorganic and organic forms of selenium have been studied as possible cancer chemopreventive agents (10). It was found that the forms were able to inhibit or delay the process of carcinogenesis induced by chemical carcinogens such as DMBA (7,12-dimethylbenz[*a*]anthracene) (11). Because of the health problems induced by many environmental pollutants, many efforts have been undertaken in evaluating the relative antioxidant potential of selenium and synthetic organoselenium compounds (12). Cancer chemoprevention efforts have currently been focusing on and investigating means of cancer control and prevention by inhibition to suppression or reversal of the process of carcinogenesis by administration of naturally occurring or synthetic agents. This approach to cancer prevention is based on the recognition that human cancer has multifactorial etiology and evolves through several molecular and cellular events occurring over many years following exposure to carcinogens (11). Searching for optimal diets and naturally occurring agents in routinely consumed foods that may inhibit cancer development, although challenging, constitutes a valuable and plausible approach for finding ways to control and prevent cancer. To date, the use of the micronutrient selenium in human clinical trials is limited, but the outcome of these investigations indicates that selenium is one of the most promising agents (13). The trace element selenium is called a chemopreventive and antioxidant agent because of the reduction of oxidative stress by selenium. Selenium is thought to prevent damage carried out by lipid peroxidation because of the presence of free radicals in the unsaturated fatty acids of subcellular membranes (14).

Therefore, we undertook the present study to evaluate the chemopreventive potential of the novel synthetic organoselenium compounds (1-isopropyl-3-methylbenzimidazole-2-selenone [SeI] and 1,3-di-*p*-methoxybenzylpyrimidine-2-selenone [SeII]) in the well-established DMBA-treated rat model by monitoring the extent of lipid peroxidation and GSH, and the levels of the enzymatic

antioxidants SOD, CAT, Se-GPx, and GR in heart and brain.

Materials and Methods

Structure of Novel Synthetic Organoselenium Compounds. Novel synthetic organoselenium compounds (SeI and SeII) (Fig. 1A and B) were synthesized in our laboratories by methods found in the literatures (15, 16). The novel compounds synthesized were characterized by ^1H -NMR (300 MHz), ^{13}C -NMR (75.5 MHz), and FT-IR spectroscopic techniques and microanalysis.

Setting of Groups. In this study, 35 healthy female Albino Wistar rats (body weight 180–200 g and 3 and 4 months old) were divided into five groups, each consisting of six to eight animals. Each rat was weighed just before the start of the study. All drugs were administered intraperitoneally (ip). DMBA was dissolved in corn oil, and rats were injected with 50 mg/kg body weight. SeI and SeII were dissolved in corn oil, and rats were injected with 25 $\mu\text{mol/kg}$. Animals in Group I were used as a control group. Animals in Group II received only the vehicle solution, (i.e., corn oil at 2-day intervals over 4 weeks). Animals in Group III were given a single dose of 50 mg/kg DMBA and were sacrificed 4 weeks later. Rats in Group IV also received DMBA as in Group III, but after 6 hrs of DMBA application, SeI at 25 $\mu\text{mol/kg}$ was applied at 2-day intervals over 4 weeks. Animals in Group V were also treated exactly as Group IV animals, except that SeII was used instead of SeI.

The rules outlined in the “Guide for the Care and Use of Laboratory Animals” were followed throughout the study, which was closely monitored by the ethical commission of Medicine School of Inonu University.

Preparation of Tissues for Biochemical Analysis. All animals were successively sacrificed after being anaesthetized with 75 mg/kg of sodium pentobarbital. The vena cava was also cut, and 30 ml of 0.9% NaCl was injected into the heart to rinse blood from the body. The heart and brain were removed and frozen in liquid nitrogen. Tissues were stored at -80°C until used.

The tissues were separated into two parts for determination of enzymatic activity and lipid peroxidation. The samples for enzymatic activity analysis were homogenized

Table 1. Changes in Heart CAT, SOD, Se-GSH-Px, and GR Activities and GSH and MDA Levels Caused by Administration of Novel Synthesized Organoselenium Compounds in DMBA-Induced Rat^a

	CAT ($\mu\text{mol}/\text{mg.p}$)	SOD ($\text{ng}/\text{mg.p}$)	Se-GSH-Px ($\text{nmol}/\text{mg.p}$)	GR ($\mu\text{mol}/\text{mg.p}$)	GSH ($\text{nmol}/\text{mg.p}$)	MDA ($\text{nmol}/\text{mg.p}$)
Control	59.50 $\pm$ 9.21*	2.28 $\pm$ 0.46**	7.48 $\pm$ 0.63*	0.37 $\pm$ 0.03**	3.20 $\pm$ 0.34*	0.075 $\pm$ 0.009**
Corn oil	65.15 $\pm$ 1.96*	1.97 $\pm$ 0.29**	5.50 $\pm$ 0.67**	0.44 $\pm$ 0.02**	2.93 $\pm$ 0.22*	0.086 $\pm$ 0.008**
DMBA	41.03 $\pm$ 3.99**	0.40 $\pm$ 0.07***	1.30 $\pm$ 0.19***	0.29 $\pm$ 0.01***	2.09 $\pm$ 0.12**	0.22 $\pm$ 0.04*
DMBA+Sel	72.70 $\pm$ 3.26*	4.81 $\pm$ 1.05*	4.04 $\pm$ 0.27**	0.82 $\pm$ 0.18*	3.41 $\pm$ 0.22*	0.074 $\pm$ 0.004**
DMBA+Sell	65.94 $\pm$ 3.05*	6.58 $\pm$ 1.58*	4.32 $\pm$ 0.46**	0.78 $\pm$ 0.16*	3.72 $\pm$ 0.43*	0.082 $\pm$ 0.003**

^a All data points are the average of $n = 8$ with $\pm$ standard deviation.

*,**,***, Statistically significant ($P < 0.05$).

in phosphate-buffered saline (PBS) (pH 7.4) using PCV Kinematica Status Homogenizer (Germany). Homogenized samples were then sonicated for 1.5 mins (30-sec sonications interrupted with a 30-sec pause on ice). Samples were then centrifuged at 17,000 g for 15 mins, and supernatants, if not used for enzyme assays immediately, were kept in the deep freeze at -80°C .

The second part of tissues homogenate was used for lipid peroxidation analysis. Tissue was washed three times with ice-cold 0.9% NaCl solution and homogenized in 1.15% KCl. The homogenates were assayed for malondialdehyde (MDA), the product of lipid peroxidation.

Protein Assay. The protein content of the supernatants for enzyme assays and MDA assay was determined using the colorimetric method of Lowry *et al.* and using bovine serum albumin (BSA) as the standard (17). All analyses were performed in duplicates.

Enzyme assays. The samples were determined spectrophotometrically for CAT ($\mu\text{mol}/\text{mg}$ protein), SOD (ng/mg protein), Se-GSH-Px (nmol/mg protein), and GR ($\mu\text{mol}/\text{mg}$ protein) activities in the supernatant fraction.

CAT Activity. CAT activity was measured at 37°C by following the rate of disappearance of H_2O_2 at 240 nm ($\epsilon_{240} = 40 \text{ M}^{-1} \text{ cm}^{-1}$) (18). One unit of CAT activity was defined as the amount of the degradation of 1 μmol of $\text{H}_2\text{O}_2/\text{min}$ at 37°C by enzyme catalyzing and as the specific activity corresponds to μmol transformation of substrate (H_2O_2) min^{-1} per mg protein.

Cu, Zn-SOD Activity. SOD activity in the supernatant fraction was measured by using the xanthine oxidase/cytochrome *c* method (19), in which one unit of activity was the amount of enzyme needed to cause half-maximal inhibition of cytochrome *c* reduction. The amount of SOD in the extract was determined as ng of enzyme per mg protein, by utilizing a commercial SOD preparative as the standard.

Se-GSH-Px Activity. Se-GSH-Px activity was determined though a coupled assay with GR and by measuring the rate of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidation at 340 nm and by using hydrogen peroxide as the substrate (20). Specific activity is given as nmol NADPH disappearing min^{-1} per mg protein.

GR Activity. GR is a ubiquitous enzyme, which catalyzes the reduction of oxidized glutathione (GSSG) to GSH. This assay is based on the oxidation of NADPH to nicotinamide adenine dinucleotide phosphate (NADP), which is catalyzed by limiting a concentration of GR. One GR activity unit is defined as the amount of enzyme catalyzing the reduction of 1 μmol of GSSG per minute at pH 7.6 and 25°C . One molecule of NADPH is consumed for each molecule of GSSG reduced. Therefore, the reduction of GSSG was determined indirectly by the measurement of the consumption of NADPH, demonstrating decreases in absorbance at 340 nm (A_{340}) as a function of time (21).

GSH Assay. The formation of 5-thio-2-nitrobenzoate (TNB) is followed spectrophotometrically at 412 nm (22). The amount of GSH in the extract was determined as nmol/mg protein by utilizing a commercial GSH (Sigma, St. Louis, MO) as the standard.

Lipid Peroxidation Assay. The analysis of lipid peroxidation was carried out as described by Beuge and Aust with a minor modification (23). The reaction mixture was prepared by adding 1 ml homogenate into 4 ml reaction solution (15% trichloroacetic acid, 0.375% thiobarbituric acid, 0.25 N NaOH; 1:1:1, w/v) and heated at 100°C for 10 mins. The mixture was cooled to room temperature, centrifuged (10,000 g for 10 mins), and the absorbance of the supernatant was recorded at 532 nm. MDA results were expressed as nmol/mg protein in the supernatant.

Statistical Analysis. The data were analyzed with SPSS 9.0 for Windows (SPSS Inc., Chicago, IL) by using one-way analyses of variance (ANOVA). Differences between means were determined using Duncan's multiple range test in which the significance level was defined as $P < 0.05$.

Results

Changes in biochemical parameters—specifically in Se-GSH-Px, SOD, CAT, and GR activity and GSH and MDA levels in the heart tissue of rats, caused by administration of novel synthesized organoselenium compounds in DMBA-induced rats—are shown in Table 1. A statistically significant decrease ($P < 0.05$) in CAT, SOD, and Se-GSH-Px, GR activity, and GSH level was found in heart tissue of DMBA-induced rats in comparison with the

Table 2. Changes on Brain CAT, SOD, Se-GSH-Px, and GR Activities and GSH and MDA Levels Caused by Administration of Novel Synthesized Organoselenium Compounds in DMBA-Induced Rat^a

	CAT ($\mu\text{m}/\text{mg.p}$)	SOD ($\text{ng}/\text{mg.p}$)	Se-GSH-Px ($\text{nm}/\text{mg.p}$)	GR ($\mu\text{m}/\text{mg.p}$)	GSH ($\text{nm}/\text{mg.p}$)	MDA ($\text{nm}/\text{mg.p}$)
Control	$9.53 \pm 1.26^{**}$	$3.031 \pm 0.56^{***}$	$9.72 \pm 0.37^*$	$0.79 \pm 0.04^*$	$1.95 \pm 0.31^{**}$	$0.56 \pm 0.05^{**}$
Corn oil	$9.33 \pm 0.57^{**}$	$4.56 \pm 0.60^{***}$	$7.21 \pm 0.53^*$	$0.72 \pm 0.05^*$	$2.88 \pm 0.37^{**}$	$0.61 \pm 0.04^{**}$
DMBA	$7.51 \pm 0.40^{***}$	$6.67 \pm 0.44^{**}$	$3.21 \pm 0.29^{**}$	$0.23 \pm 0.04^{***}$	$1.02 \pm 0.16^{***}$	$0.83 \pm 0.04^*$
DMBA+SeI	$15.10 \pm 0.38^*$	$11.90 \pm 0.72^*$	$7.82 \pm 0.45^*$	$0.60 \pm 0.05^{**}$	$4.76 \pm 0.26^*$	$0.49 \pm 0.04^{**}$
DMBA+SeII	$12.34 \pm 0.66^*$	$9.34 \pm 1.98^*$	$8.84 \pm 0.38^*$	$0.51 \pm 0.04^{**}$	$4.13 \pm 0.37^*$	$0.48 \pm 0.09^{**}$

^a All data points are the average of $n = 8$ with $\pm$ standard deviation.

***, ***, Statistically significant ($P < 0.05$).

control group, but there was a statistically significant increase in MDA level in heart tissue of DMBA-induced rat ($P < 0.05$) (Table 1).

In groups that were administered DMBA+SeI and DMBA+SeII, it was observed that there were statistically significant differences to the control group values ($P < 0.05$) for CAT, SOD, and Se-GSH-Px, GR activity, and GSH level in the heart tissue of rats. In addition, there was a statistically significant change in MDA values ($P < 0.05$) of rats receiving DMBA+SeI and DMBA+SeII. MDA levels in this group presented a statistically significant difference to control group values (Table 1). As an important indicator of oxidative damage, the MDA level decreased in the heart tissue of rats administered with DMBA+SeI and DMBA+SeII (Table 1). Negative correlation with GSH-Px activity, in which selenium acts as cofactor, and with CAT, SOD, and GR activity was found.

In the brain tissue of DMBA-induced rats, a statistically significant decrease was determined ($P < 0.05$) in the activities of CAT, SOD, Se-GSH-Px, and GR as compared to the control. However, it was determined that there was a statistically significant increase in the MDA levels in rat brain tissue compared to the control ($P < 0.05$) (Table 2).

In the DMBA+SeI and DMBA+SeII groups, it was found that there were statistically significant differences to control group values in respect to CAT, SOD, Se-GSH-Px, and GR activity in the brain tissue of rats ($P < 0.05$). In addition, there was a statistically significant difference in MDA values ($P < 0.05$) of rats administered both DMBA and novel synthesized organoselenium compounds. It was determined that there was a statistically significant difference in MDA levels of this group compared to the control group.

Discussion

Decreases in antioxidant enzyme activities and GSH levels and increases in MDA levels of heart and brain tissues of rats exposed to DMBA were observed (Tables 1 and 2). Administration of novel synthetic organoselenium compounds (SeI and SeII) increased antioxidant enzyme activity and GSH levels (Tables 1 and 2). In addition, the novel organoselenium compounds (SeI and SeII) proved to

be beneficial in decreasing the levels of MDA in tissues (Tables 1 and 2). Novel synthetic organoselenium compounds (Se I and Se II) provided protection against free radicals and lipid peroxidation measured as MDA in tissues. Both SeI and SeII provide chemoprevention against 7,12-DMBA-induced oxidative stress in rats. The results of this study parallel those found in the literature (24–27). Various organic and inorganic selenium compounds are generally considered to be antioxidants, and they have produced mixed results when tested in animal models and human subjects (28).

According to biochemical data from rat tissues (heart and brain), selenium can contribute to the antioxidative defense system of rats in DMBA-induced oxidative stress. This tolerance can be explained by the cofactor nature of selenium for Se-GSH-Px.

Our results suggest that polycyclic aromatic hydrocarbons (PAHs) are capable of inducing biochemical parameters in rats that may cause physiometabolic dysfunction. In conclusion, the results provide direct evidence for the chemopreventative role of selenium on the antioxidative defense system against the toxicity of organic pollutants.

Because of the health problems induced by many environmental pollutants, many efforts have been undertaken in evaluating the relative antioxidant potential of selenium and synthetic organoselenium compounds (12). In recent years, there have been a number of studies carried out on selenium metabolism (29). In most of these studies the external selenium was given to experimental animals in selenite form. Furthermore, using diets rich in selenium could be beneficial in alleviating DMBA toxicity. It has been demonstrated that selenium had prevented cancer and chromosome damage as well as increased resistance to viral and bacterial infections (30, 31).

The presence of selenium with DMBA alleviated its harmful effect on all the above measured biochemical parameters. DMBA has toxic effects on human health. This study demonstrated that selenium administered in combination with DMBA minimized its hazards. Both Se I and Se II provide chemoprevention against DMBA-induced oxidative stress in rat tissues (heart and brain). In the present study, administration of DMBA to the Wistar rats resulted in well-developed oxidative stress. Also, we reported effects of

the chemopreventive potential of novel synthesized organoselenium compounds against oxidative stress.

As many carcinogens produce free radicals *in vivo*, selenium compounds can act as a trap for free oxygen radicals and exert their effect by scavenging free radicals and converting them into stable compounds. Sufficient antioxidant defense systems including micronutrient intake may prevent lipid peroxidation. Oxidative factors may markedly increase oxidative cell injury. Selenium has antioxidant properties and scavenges free radicals, thus it can prevent tissue damage (14).

In summary, we conclude that DMBA treatment induces an increase in oxidative damage in rat tissues. This increase in oxidative production plays a role in DMBA-induced tissue damage. And as a result, it can be concluded that novel organoselenium compounds provide a decrease in oxidative stress caused by DMBA induction.

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