**Inhibition of DMBA Induced Rat Mammary Duct Damage by Novel Synthetic Organoselenium Compounds**

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**Abstract:** The balance between prooxidants and antioxidants is crucial to the survival and functioning of aerobic organisms. Partially reduced derivatives of oxygen, which are produced in aerobic organisms as part of normal physiological and metabolic processes, are toxic species, oxidizing numerous biomolecules, which initiate tissue injury and cell death. DMBA (7,12-dimethylbenz[a]anthracene) is a polycyclic aromatic hydrocarbon (PAH) known to cause tumors in rats. DMBA is known to generate DNA-reactive species, which may enhance oxidative stress in cells, during its metabolism. Besides the formation of DNA adducts, oxidative products derived from mutagen metabolism, such as DMBA, might impair vital cellular functions by damaging proteins and lipid membranes. Synthetic organoselenium compounds inhibit the initiation phase of carcinogenesis by inhibiting DMBA-DNA adduct formation in the target organ in vivo. Because of the health problems induced by many environmental pollutants, many efforts have been undertaken to evaluate the relative antioxidant potential of selenium and synthetic organoselenium compounds. 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 mammary duct damage. In this study, adult female Wistar rats were treated with DMBA and the novel organoselenium compounds (Sel and Sell) in determined doses. In DMBA-treated rats, the effects of the organoselenium compounds on malondialdehyde (MDA) levels and histological changes in the rat mammary lactiferous duct were studied. The ability of the organoselenium compounds to prevent oxidative damage induced by DMBA in rat mammary ducts was demonstrated. Protection against lipid peroxidation measured as MDA in the Sel and Sell treated groups was provided by the novel synthesized organoselenium compounds. Sel and Sell both provided chemoprevention against DMBA-induced oxidative stress in the rat mammary duct.

**Key words:** DMBA, histology, lactiferous duct, MDA, synthetic organoselenium compounds

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Reactive oxygen species (ROS) are known to play an important role in mutagenesis and carcinogenesis, particularly in tumor promotion [3]. They are produced as by-products of normal cellular metabolism, generated by chemicals in the environment, and are present in the air we breathe and the food we eat [16]. ROS have been implicated in the etiology and pathophysiology of many human diseases. They induce strand breaks in DNA, and also cause that oxidative modification of DNA bases which leads to mutagenic and carcinogenic effects [3]. Endogenous targets which are readily accessible to ROS when they are formed and are rapidly affected by free radicals, especially the group of polyunsaturated fatty acids (PUFAs) which is highly susceptible to reactions with free radicals. Peroxidations of lipids in fatty acids may lead to a radical chain reaction [6]. Malondialdehyde (MDA) is the final product of lipid peroxidation, and the concentration of MDA provides direct evidence of toxic processes caused by free radicals [17].

DMBA (7,12-dimethylbenz[a]anthracene) is a polycyclic aromatic hydrocarbon (PAH) known to cause tumors in rats [5]. PAHs, such as petroleum and petroleum derivatives are common organic pollutants in the environment, through oil spills and incomplete combustion of fossil fuels. Since most PAHs persist in the environment for a long period of time, bioaccumulation has likely taken place which causing environmental pollution and drastic effects on the biological equilibrium [4].

Various components have been shown over the years to influence DMBA carcinogenesis in this model system including age, endocrine status, composition of the diet and dose of DMBA [13, 20].

Cancer chemoprevention is currently being focused on and is being investigated as a means of cancer control and prevention by inhibition, 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 cancers have a multifactorial etiology and evolve through several molecular and cellular events occurring over many years following exposure to carcinogens [3].

Searching for optimal diets and for naturally occurring agents in routinely consumed foods that may inhibit cancer development, although challenging, constitutes a valuable and plausible approach to finding ways to control and prevent cancer. To date, the use of the micronutrient, selenium, in human clinical trials is limited, but the outcomes of these investigations indicate that selenium is a most promising agents [8]. The trace element selenium has been implicated in chemoprevention and drug-resistance through the reduction of oxidative stress. Selenium prevented damage to the unsaturated fatty acids of subcellular membranes by lipid peroxidation induced by free radicals [17].

In recent years several inorganic or organic forms of selenium have received wide attention as possible cancer chemopreventive agents. They have been found to inhibit or delay the process of carcinogenesis induced by chemical carcinogens such as DMBA in different tissues in animals [3].

In animal assays, the chemopreventive effects of inorganic and organic selenium compounds have been observed in the mammary gland, colon, lung, pancreas, and skin [18]. Synthetic organoselenium compounds inhibit the initiation phase of carcinogenesis by inhibiting DMBA-DNA adduct formation in the target organ in vivo. cDNA microarray analysis has indicated that selenium compounds alter genes in a manner that leads to inhibition of cell proliferation and induction of apoptosis, and modulation of apoptosis and cell proliferation can account for chemoprevention during the post-initiation phase of mammary carcinogenesis [8].

In this study DMBA-treated rats were studied for the effects of the novel synthetic organoselenium compounds, 1-isopropyl-3-methylbenzimidazole-2-selenone (SeI) and 1,3-di-p-methoxybenzylpyrimidine-2-selenone (SeII), which were prepared in our laboratories, and also the MDA levels and histological changes in the rat mammary lactiferous duct. The most widely used index of lipid peroxidation is MDA formation, which is often assayed with the thiobarbituric acid (TBA) assay [6].
into 5 groups, each consisting of 6 to 8 animals. Each rat was weighed just before the start of the study. All drugs were administered intraperitoneally (i.p.). DMBA was dissolved in corn oil and rats were i.p. injected with 50 mg/kg. The novel synthetic organoselenium compounds, SeI and SeII, were dissolved in corn oil and rats were injected with 25 µmol/kg. Weight losses, 15 to 20 g, together with a decrease in food consumption were observed among the animals before sacrifice. All animals were sacrificed with an injection of sodium pentobarbital.

The ethical rules described in “Guide for the Care and Use of Laboratory Animals” were obeyed during in this study which was closely scrutinized by the Ethical Commission of the Medical School of Inonu University.

Structure of novel synthetic organoselenium compounds

The novel synthetic organoselenium compounds, SeI and SeII, were synthesized in our laboratories (Fig. 1a and b).

A derivative of 2-seleno benzimidazole, SeI, and a derivative of 2-seleno pyrimidine, SeII, were prepared according to the literatures [1, 10, 11]. The novel compounds synthesized were identified by 1H-NMR (300 MHz), 13C-NMR (75.5 MHz) spectroscopic techniques and FT-IR micro analysis.

DMBA and synthetic organoselenium compounds administration

The rats were divided into five groups. Animals in group I were used as a control. Animals in group II received only the vehicle solution, corn oil for four weeks at two-day intervals. Animals in group III were given a single dose of 50 mg/kg DMBA and were sacrificed four weeks later. Animals in group IV also received DMBA as in group III, but 6 h after DMBA administration, the SeI compound at 25 µmol/kg was administered for four weeks at two-day intervals. Animals in the group V were treated exactly as group IV animals, except that the SeII compound was used instead of SeI. The dose levels were determined based on in vitro studies (7) showing the antioxidative capacities of the SeI and SeII compounds. All the animals were successively sacrificed after anesthetization with 75 mg/kg of sodium pentobarbital.

Preparation of tissues for biochemical analysis

The rat has 3 pairs of thoracic and 3 pairs of abdomino-inguinal mammary lactiferouses. Except for the third thoracic and the first abdomino-inguinal mammary lactiferouses, these were used in biochemical analysis. The first and second thoracic and the second and third abdomino-inguinal mammary lactiferous ducts were divided into two equal parts for homogenization. A homogenizer 9,500 rpm (4 × 10 s at 4°C) was used. The homogenates were centrifuged at 1,000 g for 10 min and the supernatants were collected. The first part was homogenized in a ratio of one part wet tissue to 9 ml of cold 1.15% KCl. From this 1:9 homogenate, 0.5 ml was used for the lipid peroxidation assay. The second part was homogenized in a ratio of one part wet tissue to 4 ml of cold 0.02 M potassium phosphate (pH 7.0) (1:4) as a buffer. The buffered homogenate was used for the total protein assay.

Lipid peroxidation assay

The analysis of lipid peroxidation was carried out as described previously [2] with a minor modification. The reaction mixture was prepared by adding 1 ml homogenate to 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 min. The mixture was cooled to room temperature, centrifuged (10,000 xg for 10 min), and the absorbance was measured at 532 nm.

Fig. 1. Structure of novel synthesized organoselenium compounds (SeI and SeII).
and the absorbance of the supernatant was recorded at 532 nm. MDA results were expressed as nmol mg⁻¹ protein in the supernatant.

**Protein assay**

The protein content of the supernatants for the MDA assay was determined using the colorimetric method of Lowry et al. using BSA as the standard [15]. All analyses were performed in duplicate.

**Histological Analysis**

The third thoracic and the first abdomino-inguinal mammary lactiferous ducts were fixed with 10% neutral buffered formalin and then routinely embedded in paraffin. Serial sections were longitudinally or transversally cut at 6 µm and stained with haematoxylin-eosin (HE). An Olympus BH2 microscope was used to obtain photographs of the prepared tissues samples [19].

The slides were examined and scored as follows:

Assessment of mammary lactiferous duct alteration in each section was conducted by an experienced histologist who was unaware of the treatment. Mammary lactiferous duct damage was scored by grading epithelial hyperplasia and degeneration of the secretory units, nuclear pleomorphism and change in cell size and uniformity, ductal hyperplasia and epithelial degeneration and inflammation with a maximum score of 12. Epithelial hyperplasia and degeneration of the secretory units was scored as 0=absent, 1=mild, 2=moderate, 3=severe. Nuclear pleomorphism and change in cell size and uniformity was scored as 0=absent, 1=rare, 2=moderate, 3=widespread. Ductal hyperplasia and epithelial degeneration was scored as 0=absent, 1=mild, 2=moderate, 3=severe. Inflammation was scored as 0=absent, 1=mild, 2=moderate, 3=severe.

**Statistical Analysis**

The data were analyzed with SPSS 9.0 for Windows 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 \( P<0.05 \).

<table>
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<th><strong>Results</strong></th>
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<td><strong>Lipid peroxidation</strong></td>
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<td>A statistically significant increase ( P&lt;0.05 ) in MDA levels in the nipple of the rats exposed to DMBA was observed (Fig. 2).</td>
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<td>The toxic effect of DMBA caused stress to the rats. However the administration of the novel synthetic organoselenium compounds, SeI and SeII, caused statistically significant decrease in MDA levels ( P&lt;0.05 ) (Fig. 2). The antioxidant activities of SeI and SeII compounds on free radical induced lipid peroxidation has been decreased.</td>
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| **Histological observations** |
| The histopathology of rat lactiferous ducts (Total Damage Scores) is shown in tabular form in Table 1. |
| Preparates of lactiferous ducts and lactiferous sinus belonging to inactive mammary tissues of the control (Fig. 3A) and corn oil (the vehicle solution) groups were to be in a normal histological state (Fig. 3B) The histopathological analysis demonstrated the development of DMBA-induced lactiferous duct proliferation in the rat. There were papillary parts projecting into the lumen of the lactiferous sinus and degenerated tissue remains in the lumen of the DMBA group (Fig. 3C). |
| The epithelium of the lactiferous duct was split completely after degeneration. Secretory units had dense atypical hyperplasia and degeneration (Fig. 3D). |
| However, a normal lactiferous sinus, normal lactiferous duct and hyperplasic area around the lactiferous duct were observed after treatment with the SeI and SeII compounds, (Fig. 3E and 3F). Ducts belonging to the mammary glands were seen in a normal histological state in the DMBA + SeI group, and pyknotic atypical cells were rarely found in the epithelium of the ducts (Fig. 3E). |
| Ducts belonging to the mammary glands were also seen to be in a normal histological state in the DMBA + SeII group, but small hyperplasic areas were also found (Fig. 3F). |

| **Discussion** |
| An increase in the MDA level was observed in the rat lactiferous ducts of rats exposed to DMBA \( P<0.05 \) |
DMBA is a potent oxidizing agent that induces oxidative stress, with subsequent oxidation of changes to intracellular and cellular membranes through lipid peroxidation. The toxic effect of DMBA causes stress in rats. The use of biochemical methods provides valuable knowledge about physiological reactions occurring with changing environmental conditions. Especially, an understanding of the physiological and biochemical changes occurring at sublethal toxicity helps in the prediction of the possible level of threat to life. PAHs, heterocyclic aromatic amines and nitro polycyclic aromatic hydrocarbons have been shown to induce mammary cancer in rats [9]. One well-established model system for the study of mammary tumour development involves the use of a single oral dose of DMBA to initiate mammary cancer in young rats [12], and interacting components have been studied for their ability to alter the growth of the tumours in this model [13, 20].

Administration of the novel synthetic organoselenium compounds, SeI and SeII, decreased MDA levels ($P<$0.05) (Fig. 2). SeI and SeII both provided chemoprevention against 7,12-DMBA-induced oxidative stress in the rat lactiferous duct. The results of this study agree with those of other studies in the literature. It has been demonstrated that 1,4-phenylenebis (methylene) selenocyanate (p-XSC) and its putative metabolite glutathione conjugate (p-XSeSG) are highly promising agents for the chemoprevention of mammary carcinogenesis in the 7,12-dimethylbenz[a]anthracene (DMBA)-rat mammary tumor model system [8]. 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. Adequate antioxidant defense systems including micronutrient intake may prevent lipid peroxidation. Oxidative factors may markedly increase oxidative cell injury. Selenium has antioxidant properties and is a scavenger of free radicals, thus preventing damage to lipid membranes.

The histopathological analysis has demonstrated that SeI and SeII inhibited the effect of DMBA-induced tumorigenesis in the rats. This effect which leads to tissue injury [5] causes degenerated tissue in the lumen and papillary parts projecting into the lactiferous lumen in the rats. Also, in this study DMBA caused degeneration, splitting of the lactiferous duct epithelium and hyperplasia, and degeneration of the secretory units of the rats (Fig. 3C and 3D).

The effects of SeI and SeII were demonstrated in the DMBA-induced tumors in the lactiferous duct of the rats. Selenium has antioxidant properties and is a scavenger of free radicals, and thus prevented damage to the rat lactiferous ducts. SeI and SeII decreased the effect of DMBA which leads to tissue injury. Atypical cells with pycnotic nuclei were observed in the lactiferous duct of DMBA-treated rats, whereas apoptosis occurred in cells less traumatically and the degeneration took place by a less traumatic route in the lactiferous duct epithelium after treatment with SeI and SeII. After SeI and SeII treatment, partial amelioration of the DMBA-induced effects in the lactiferous duct epithelium was observed.

Oxidative stress increases with DMBA administration and this was demonstrated by the increase in the MDA level which decreased after administration of SeI and SeII as shown in Fig. 2. The decrease in MDA

<table>
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<th>Groups</th>
<th>Total Damage Scores</th>
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<tr>
<td>Control</td>
<td>1.00 ± 0.36*</td>
</tr>
<tr>
<td>Corn oil</td>
<td>1.16 ± 0.40*</td>
</tr>
<tr>
<td>DMBA</td>
<td>7.66 ± 0.49*</td>
</tr>
<tr>
<td>DMBA+SeI</td>
<td>4.16 ± 0.54*</td>
</tr>
<tr>
<td>DMBA+SeII</td>
<td>3.16 ± 0.60*</td>
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Values are mean ± SD. a,b,c=statistically significant ($P<$0.05).
levels with the oxidative stress was also observed histopathologically and here the results are demonstrated in Fig. 3E and 3F.

Various organic and inorganic selenium compounds, generally considered to be antioxidants, produced mixed results when tested in animal models and human subjects [14]. Using mammary adenocarcinomas, the major carcinoma induced by DMBA, El-Bayoumy and Sinha

Fig. 3. A. (Control group): Normal histological images of mammary sinus and secretory units ×10. B. (Corn oil group): Normal histological images of mammary sinus and secretory units ×20. C. (DMBA group): Papillary part projecting into the lactiferous lumen (Thick arrow). Degenerated tissue remains in the lumen (Thin arrow) ×20. D. (DMBA group): Degeneration and splitting in the lactiferous duct epithelium (arrow). Hyperplasia and degeneration in secretory units (star) ×40. E. (DMBA + SeI group): Atypical cells with pyknotic nuclei in the lactiferous duct epithelium (arrow) ×40. F. (DMBA + SeII group): Normal lactiferous sinus (S). Normal lactiferous duct (arrow) and hyperplasic area around the lactiferous duct (star) ×20.
showed that selenium had an impact in the multistep carcinogenic process [8].

Treatment of adult Wistar rats with DMBA and the novel synthetic organoselenium compounds SeI and SeII, with determined doses showed that damage induced by DMBA was prevented by the antioxidative properties of the organoselenium compounds. Our objective was to examine how SeI and SeII supplementation influenced oxidative stress as measured by MDA and the histological changes in the rat lactiferous duct. We report the chemopreventive potential of SeI and SeII against mammary duct damage. Though SeI and SeII differ in chemical structure, they showed similar chemopreventive effects on mammary duct carcinomas in terms of biochemical and histological properties.

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References