Biochemical Studies on the Effect of Selenium and α-Difluromethylornithine on the Elevated Polyamines in mice

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Abstract: The present study was designed to investigate the effect of selenium (Se) administration (as sodium selenite: 0.5 or 1 mg kg⁻¹ body weight) or/and α-difluromethylornithine (DFMO: 2 mg kg⁻¹ body weight) on the elevated polyamine levels. For this purpose the polyamine (putrescine, spermidine, spermine) levels were elevated in female Swiss albino mice by adding 2% of both L-arginine and L-ornithine to the drinking water for four weeks. The elevated putrescine and spermidine levels were decreased significantly by administration low and high dose of Se. The DFMO abolished the concentrations of both putrescine and spermidine in liver and kidney of the experimental mice. The combination of Se and DFMO normalized the polyamine levels. On the other hand, the spermine level was increased in liver and kidney by administration of Se or/and DFMO. To elucidate the effects of the elevated polyamines on the oxidative enzymes, the activity of glutathione peroxidase (GSH-PX), glutathione reductase (GSH-R) and superoxide dismutase (SOD) in the liver and the kidneys of the experimental mice were determined. The GSH-Px, GSH-R and SOD activities were increased by Se administration compared to the control. Conversely, DFMO produced an inhibition in the activity of antioxidative enzymes. These results suggest that the combination between Se and DFMO appear to be additive chemopreventive effect to reduce the elevated polyamine levels. This combination will protect the tissues from the deleterious effect of high polyamine levels and improve the activities of the cellular antioxidative system.

Keywords: Polyamines, Se, DFMO, arginine, ornithine, glutathione peroxidase, glutathione reductase, superoxide dismutase

INTRODUCTION

Polyamines are ubiquitous low molecular weight naturally occurring organic alicyclic polycations (Müller et al., 2008; Kashiwagi et al., 1993). These polycations are widely distributed in both prokaryotic and euukaryotic cells and are essential to maintenance cell growth and functions (Bagari and Tassoni, 2001; Moinard et al., 2005). Polyamines are known by the names of spermidine, spermine and their diamine precursor, putrescine. The chemical structure of these polyamines is as follows, spermidine \(\text{H}_2\text{N}-(\text{CH}_2)_2\text{-NH}-(\text{CH}_2)_3\text{-NH}_2\)\(^+\), spermine \(\text{H}_2\text{N}-(\text{CH}_2)_2\text{-NH}-(\text{CH}_2)_2\text{-NH}-(\text{CH}_2)_3\text{-NH}_2\)\(^+\) and putrescine \(\text{H}_2\text{N}-(\text{CH}_2)_2\text{-NH}_2\)\(^+\). At cellular pH, polyamines are fully protonated and carry a positive charge on each nitrogen atom, which facilitates their strong binding to negatively charged molecules like nucleic acids, affecting RNA translation, DNA conformation, chromatin structure and gene expression and transcription (Tabor and Tabor, 1999; D’Agostino et al., 2005). Mutants that can not synthesis polyamines usually have growth problems like a double mutant in E. coli which cannot synthesis putrescine and spermidine that shows an inhibition in growth which is reversible upon exogenous polyamine supply (Tabor and Tabor, 1999). In some tumor cells, polyamines synthesis are uncontrolled resulting in higher polyamines concentrations than those in the normal cells (Casero and Marton, 2007; Pegg, 1988). Therefore, inhibition of polyamines biosynthesis can be considering as chemotherapeutics for tumor (Davidson et al., 1999).

The polyamines biosynthesis starts with the cleavage of arginine to ornithine, an amino acid that is produced as part of the urea cycle, by the action of arginase enzyme (Fig. 1). Ornithine is then decarboxylated into putrescine by the action of ornithine decarboxylase enzyme (ODC, EC 4.1.1.17) (Hebly, 1981). The spermidine and spermine are formed via successive transfer of two aminopropyl groups by the action of spermidine synthase (SDNS) enzyme to convert putrescine into spermidine. Moreover,
Fig. 1: Polyamine biosynthetic pathway

Spermine synthase (SPNS) enzyme is needed to form spermine from spermidine (Ranya et al., 2006; McGann and Pegg, 1992). The aminopropyl donor for these reactions is decarboxylated S-adenosylmethionine, which is formed by the action of S-adenosylmethionine decarboxylase (AdoMetDC: EC 4.1.1.50) on S-adenosylmethionine (Yerlikaya and Stanley, 2006). In addition to ornithine, polyamines can be synthesized from an agmatine (Fig. 1). Agmatine is an amine that is formed by decarboxylation of L-arginine by the enzyme arginine decarboxylase (ADC) and hydrolyzed by the enzyme agmatinase to putrescine (Grimbble and Grimble, 1998).

ODC is the first and rate-limiting enzyme in polyamine biosynthesis. Increased ODC activity and concomitantly increased levels of polyamines usually exist in proliferating cells such as those exist in embryos and cancer cells (Krause et al., 2000; Pegg et al., 1987). Therefore, inhibition of ODC activity might induce a depletion of intracellular polyamines, providing an effective anticancer treatment strategy.

Difluoromethylornithine (DFMO) also, is known as α-Difluoromethylornithine an ornithine analog that inhibits ODC enzyme activity irreversibly (Metcalf et al., 1978). In the presence of both ODC and DFMO an intermediate carbonic species is generated from the decarboxylation process to DFMO by an enzymatic mechanism. With the loss of fluorine the intermediate carbamic species alkylates a nucleophilic residue in adjacent or near or at the active site, resulting in covalent binding of inhibitor to the enzyme (Manni et al., 2007; Metcalf et al., 1978). DFMO has been found to significantly suppress many tumor formations (Meyskens and Gerner, 1999).

Selenium is an essential trace element in the nutrition for humans and other animals and is required for the growth of mammalian cells in culture (Zeng, 2002). The metabolic basis of this nutritional function remained obscure, however, until it was identified that the enzyme glutathione peroxidase possesses Se as a vital element in its catalytic center (Rotruck et al., 1973). These selenoproteins include glutathione peroxidases and thioredoxin reductases, which have important antioxidant and detoxification functions. The selenite can be converted in vivo into selenodiglutathione, a form of Se which appears to regulate apoptosis (Ganter, 1999). The other an important Se forms are selenomethionine and selenocysteine which are anticarcinogenic property. The link between Polyamines biosynthesis and Se metabolism is that both of them required S-adenosylmethionene as a cofactor (Kajander et al., 1990).

The aim of the present research was to study the effects of Se and/or DFMO on the high polyamine levels compounds induced in the experimental mice utilizing both L-arginine and L-ornithine.

MATERIALS AND METHODS

Chemicals: All the chemicals used were AR grade and obtained from Sigma Chemical Co. and BDH Chemicals Ltd. DFMO dosing solution was dissolved in deionized distilled water and delivered in 0.2 mL. Sodium selenite (Na₂SeO₃) was prepared with the required amount in deionized distilled water and stored at 4°C. DFMO and sodium selenite were administered individually once daily by intraperitoneal (ip) injection.

Animals: Female Swiss albino mice (kindly provided by Veterinary Research Centre and Animal Production, King Faisal University, Saudi Arabia) at 6 weeks of age, 24±2 g body weight (b.wt.). They were allowed free access to feed a standard diet and water with a 12 h light/dark cycle at controlled temperature.

Study groups and sampling: Polyamines were induced in mice by adding both L-arginine (2%) and L-ornithine (2%) in the drinking water for 4 weeks before starting the experimental according the method described by Teixeira et al. (2002). The mice were randomly distributed into seven groups (each group n = 8), housed individually in stainless steel cages and checked daily for death and weekly for infections.

Group 1: Normal controls receive only drinking water free of any chemical additives

Group 2: Received both L-arginine (2%) and L-ornithine (2%) in drinking water to elevate the polyamine level

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Group 3: As group 2 and received seven doses of DFMO (2 mg kg\(^{-1}\) b.w.t.) by ip injection

Group 4: As group 2 and received seven doses of Se (0.5 mg kg\(^{-1}\) b.w.t.) by ip injection

Group 5: As group 2 and received seven doses of both Se (0.5 mg kg\(^{-1}\) b.w.t.) and DFMO (2 mg kg\(^{-1}\) b.w.t.) by ip injection

Group 6: As group 2 and received seven doses of Se (1 mg kg\(^{-1}\) b.w.t.) by ip injection

Group 7: As group 2 and received seven doses of both Se (1 mg kg\(^{-1}\) b.w.t.) and DFMO (2 mg kg\(^{-1}\) b.w.t.) by ip injection

Homogenate preparation: Liver and kidney tissues were scraped, weighed and homogenized individually by glass-glass homogenizer in cold buffer A (25 mM Tris-HCl buffer, pH 7.4) on ice and the homogenates were centrifuged for 30 min at 3000 rpm. The tissues supernatant were diluted with a nine fold volume of buffer A containing 0.1 mM EDTA and 0.25 mM dithiothreitol. The diluted tissue homogenate was used as a source in the biochemical parameter determinations.

Estimation glutathione reductase activity: The glutathione reductase (GSH-R) activity was determined spectrophotometrically by measuring the rate of NADPH oxidation at 340 nm according to the method of Karni et al. (1984). The reaction mixture consisted of 0.1 M potassium phosphate, pH 7.0, 1 mM EDTA, 0.1 mM NADPH, 1 mM oxidized glutathione and tissue samples. The decrease in absorbance at 340 nm at 30\(^{\circ}\)C was recorded. Because the decrease of absorbance of the control reaction mixture without oxidized glutathione was less than 0.002, contribution of spontaneous NADPH oxidation and other reductases in the samples was ignored. One unit of glutathione reductase activity was defined as the amount of enzyme that catalyzes the oxidation of 1 \(\mu\)mol of NADPH per minute.

Estimation glutathione peroxidase activity: Glutathione peroxidase (GSH-Px) activity was assayed according to the method of Rotruck et al. (1973). One unit of enzyme is expressed as \(\mu\)mol of GSH utilized per minute.

Estimation of superoxide dismutase activity: The activity of superoxide dismutase (SOD) was assayed following the method described by Kakkar et al. (1984).

Total proteins were determined according to the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Polyamine determinations: Aliquots of liver and kidney supernatant were extracted with 0.6 N perchloric acid for 1 h at 4\(^{\circ}\)C prior to centrifugation at 10,000 rpm for 15 min. The supernatant was used for polyamine determination. The polyamine levels were determined according to the method described by Manni et al. (2002). The concentration of polyamines was determined by graphical analysis relative to that obtained from a standard curve generated for each polyamine.

Statistical analysis: All the values are represented as Means±SD (n = 8). Student’s t-test was applied to calculate the significance of difference between groups. The level of significance was set at p<0.05.

RESULTS

Effect of L-arginine and L-ornithine supplementation: Arginine is a substrate for ornithine synthesis and the ornithine is the precursor of the polyamines. Therefore, the effect of 2% L-arginine and 2% L-ornithine on the polyamines production were determined. Body weights of mice treated with L-arginine and L-ornithine did not affected significantly between various groups in the experiment. The levels of putrescine, spermidine and spermine in the liver of group 1 (not treated with L-arginine or L-ornithine) were increased significantly (p<0.001) from 4.01±0.23, 3.58±0.15 and 2.60±0.49 to 6.82±0.71, 6.09±0.73 and 2.88±0.35 respectively, for the polyamine levels in the liver of group 2 (Table 1). Moreover, the polyamine levels were increased in the kidney of group 2 experimental animals compared to group 1 (Table 1). These results indicate that the addition of both L-arginine and L-ornithine to the drinking water can cause a significant elevation in the polyamine levels in liver and kidney.

<p>| Table 1: Levels of polyamines ((\mu)mol/mg protein) such as putrescine, spermidine and spermine in liver, kidney of the control and experimental mice |
|-------------------------------|-------------------------------------------------|-------------------|-------------------|-------------------|-------------------|</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.60±0.49</td>
<td>3.58±0.15</td>
<td>4.01±0.23</td>
<td>2.54±0.29</td>
<td>2.79±0.12</td>
<td>3.41±0.21</td>
</tr>
<tr>
<td>2</td>
<td>2.88±0.35</td>
<td>6.09±0.73</td>
<td>6.82±0.71</td>
<td>3.90±0.38</td>
<td>3.81±0.21</td>
<td>4.99±0.33</td>
</tr>
<tr>
<td>3</td>
<td>4.15±0.42</td>
<td>5.65±0.65</td>
<td>4.44±0.60</td>
<td>3.94±0.76</td>
<td>6.91±0.14</td>
<td>2.99±0.45</td>
</tr>
<tr>
<td>4</td>
<td>2.91±0.54</td>
<td>5.11±0.34</td>
<td>5.84±0.46</td>
<td>2.63±0.17</td>
<td>3.22±0.28</td>
<td>4.14±0.13</td>
</tr>
<tr>
<td>5</td>
<td>3.41±0.87</td>
<td>3.09±0.31</td>
<td>3.65±0.31</td>
<td>3.25±0.20</td>
<td>3.59±0.22</td>
<td>1.37±0.14</td>
</tr>
<tr>
<td>6</td>
<td>2.85±0.51</td>
<td>4.24±0.43</td>
<td>5.02±0.32</td>
<td>2.76±0.32</td>
<td>3.13±0.53</td>
<td>3.84±0.32</td>
</tr>
<tr>
<td>7</td>
<td>4.10±0.32</td>
<td>3.09±0.21</td>
<td>3.36±0.25</td>
<td>3.23±0.47</td>
<td>1.73±0.29</td>
<td>1.88±0.43</td>
</tr>
</tbody>
</table>
Effect of Se and DFMO on the elevated polyamine level:
The supplementation of experimental animals with 0.5 mg Se kg⁻¹ body weight (Table 1, group 4) reduced the elevated putrescine level in the liver and kidney by 14.4 and 17%, respectively. Also, high Se dose 1 mg kg⁻¹ body weight (Table 1, group 6) decreased the putrescine level in liver and kidney by 26.4 and 23% respectively. Group 2 considered as a control.

As represented in Table 1, group 4 low Se dose diminished the spermidine level in both liver and kidney by 16.1 and 15.8%, respectively. Moreover, the high Se dose (Table 1, group 6) depleted the spermidine level in liver and kidney by 30.4 and 17.9%, respectively. The administration of low Se dose did not result a significant changes on spermine level in both liver and kidney (Table 1).

As represented in Table 1, group 3 the DFMO abolished both the putrescine levels in liver and kidney by 64.2 and 94.2% and the spermidine levels by 56.5 and 76.1% in the same order. On the other hand, the DFMO increase the spermine level 44.1% in liver and 35.8% in kidney. The administration of both Se and DFMO tend to normalize the elevated polyamine levels.

Effect of polyamine on GSH-R, GSH-Px and SOD: GSH-Px, SOD and GSH-R were the enzymes selected to evaluate the oxidative damage caused by the elevated polyamine levels on the antioxidant defense system. Figure 2-4 demonstrate the effect of the elevated polyamine levels on the GSH-Px, SOD and GSH-R enzyme activities, respectively.

The supplementation of the experimental mice with Se alone or combined with DFMO increase the GSH-Px activity in liver and kidney as represented in Fig. 2a, b respectively. Group 3 animals that had received DFMO supplementation, the value of GSH-Px activities were lower in the liver but the same as of the control animals in the kidney. DFMO treatments clearly diminish the GSH-Px activity in liver and kidney.

The biochemical changes in the activity of SOD enzyme in both liver and kidney of the experimental mice are illustrated in Fig. 3a, b respectively. Significant increase in the SOD activities in liver and kidney were observed in the Se treated groups. The experimental mice that had received supplement DFMO or combined treatment of Se with DFMO the values of SOD activities were declined compared to the SOD of control group.

![Graph](image1)

Fig. 2: Effect of Se (0.5 and 1 mg kg⁻¹ body weight) or/and DFMO (2 mg kg⁻¹ body weight) on the GSH-Px activity in liver (a) and kidney (b)

![Graph](image2)

Fig. 3: Effect of Se (0.5 and 1 mg kg⁻¹ body weight) or/and DFMO (2 mg kg⁻¹ body weight) on the SOD activity in liver (a) and kidney (b)
DISCUSSION

L-arginine and L-ornithine are important amino acids and are the main sources for putrescine, spermidine and spermine biosynthesis. Arginine is converted to urea and ornithine by the catalytic activity of arginase enzyme (Fig. 1). In the present study putrescine, spermidine and spermine levels increased significantly with supplementation of both L-arginine and L-ornithine in the experimental mice drinking water (Table 1, group 2). Arginine is a common substrate for the synthesis not only polyamines but also, of urea, nitric oxide, agmatine, creatine, glutamate, proline and proteins (Wu and Morris, 1998; Nikolic et al., 2007). L-ornithine, as a product of arginine activity, is necessary for the production of polyamines and proline (Wu and Morris, 1998; Sokolovic et al., 2008). Arginase activity is linked cell growth and connective tissue formation, which is related with polyamines and proline and in ammonia detoxification. Many reports stated that arginine starvation greatly decreases putrescine, spermidine and spermine contents in the rate (Schertel and Eichler, 1991) and mice (Teixeira et al., 2002). It appear, that the combination of L-arginine and L-ornithine induces ODC.

ODC is the first rate limiting enzyme in polyamine biosynthesis. Decarboxylation process to ornithine occurs via ODC enzyme to generate putrescine, which is converted into spermidine and spermine by spermidine and spermine synthases enzymes respectively, utilizing aminopropyl residue via AdoMetDC enzyme (Heby, 1981; McCann and Pegg, 1992; Yerlikaya and Stanley, 2006). In normal tissues ODC activity is increased by a variety of environmental and genetic factors associated with carcinogenesis, including ultraviolet light, carcinogenic agents. Increased ODC activity persists in and is associated with a wide variety of epithelial neoplasms including skin, prostate, colon and breast (Gerner and Meyskens, 2004). ODC proto-oncogen is significantly increased in proliferating cells and supplies polyamines required for cell cycle progression and growth. The levels of both polyamines and ODC are elevated in breast cancer compared with normal breast tissue and the increase of polyamine levels are correlated with a less differentiated and more metastatic tumor phenotype (Glikman et al., 1987; Canizares et al., 1999). Polyamines are autoregulated through induction of the inhibitory protein namely ODC-antienzyme, which inhibit both ODC and polyamines (Sutriano et al., 1998; Meyskens and Gerner, 1999). Increased polyamine synthesis has been associated with proliferation and progression of breast cancer and thus, is a potential target for anticancer therapy. Polyamine depletion by α-difluoromethyl-ornithine (DFMO) has been shown to decrease pulmonary and bone metastasis from human breast cancer cell (McCann and Pegg, 1992; Davidson et al., 1999). In the present study 2 mg kg⁻¹ BW DFMO was administrated and that is a safe dose compared with other studies which used high dose of DFMO as 200 and 135 mg kg⁻¹ b.wt in rats and rabbits respectively (Sokolovic et al., 2008). This concentration of DFMO abolishes the elevated putrescine and spermidine levels in both liver and kidney by inhibition the ODC activity. The DFMO effect was associated with a nearly 64.2 and 94.2% suppression of putrescine in the liver and kidney respectively and 56.5 and 76.1% suppression of spermidine in the same order.
Polyamine levels within the cells are tightly regulated and can be changed through modulation of the key enzyme that control the putrescine, spermidine and spermine biosynthesis and interconversion, particularly ornithine decarboxylase, that rapidly responds to several stimuli that are mainly linked with cell growth (Thomas and Thomas, 2001; Ackermann et al., 2003; Wallace et al., 2003). Polyamines are important compounds in the regulation of cell proliferation and differentiation and appear to play an essential role in the metabolic processes involved in cell growth and division, including DNA, RNA and protein synthesis, embryonic development and cell differentiation (Eugene et al., 2004).

The level of spermine, on the other hand, increased by 44% in liver and 36% in the kidney response to 2 mg kg⁻¹ b.wt. of the DFMO treatment. This increase in the spermine level seems to be a compensatory event that takes place following the inhibition of ODC, like increase in S-adenosylmethionine decarboxylase (SAMDC) activity and increased the cellular uptake from the extracellular space (Pegg, 1988; Oredsson et al., 1986; Seiler et al., 1990). DFMO abolishes the putrescine and spermidine levels in liver and kidney of the experimental mice and increases the level of spermine. These results are in agreement with the results of other workers (Halline et al., 1989; Jun et al., 2007). Moreover, it was reported that the DFMO stimulate indirectly S-adenosylmethionine (AdoMet) decarboxylase another rate-limiting enzyme involved in the synthesis of spermidine and spermine (Oredsson et al., 1986; Yerlikaya and Stanley, 2006). This increase in the spermine level by DFMO appear to induced AdoMet decarboxylase activity, together with putrescine and spermidine depletion secondary to ODC inhibition, results in accumulation of decarboxylated AdoMet [S-adenosyl-(5’)-deoxy-(5’)-3’-methylthiopropylamine], the aminopropyl donor for the conversion of putrescine into spermidine and spermine into spermine (Mamont et al., 1982).

Jacoby et al. (1996) reported that interferon has inhibitory effect on the ODC activity, possibly by stimulating a polyamine dependent phosphokinase which phosphorylates ODC, thus inhibit the ODC to catalyze the putrescine formation. Also, interferon depresses the polyamine synthesis by inhibiting the SAMDC activity, which is an enzyme that catalyzes the transfer of isopropylamine groups in the formation of spermidine from putrescine and spermine from spermidine (Singh et al., 1993).

The administration of Se 0.5 or 1 mg kg⁻¹ b.wt. was safe and effective to decrease the elevated polyamine levels. Other studies applied high selenium doses over 2 mg kg⁻¹ b.wt. close to the LD₅₀, which are equal to 3.5 mg kg⁻¹ body weight per day (Battel et al., 1998). The low Se concentration appears to be effective in reducing the elevated levels of polyamines. Selenium supplementation has recently been shown to decrease total cancer incidence. Selenium is able to reduce the risk for liver cancer even when it is used only during a short period of time covering the promotion phase of the carcinogenic process (Bergman et al., 2005). Chemically induced hepatocarcinogenesis may be prevented by selenium supplementation both during promotion and progression phase (Zeng, 2002). However, the mechanism of action of selenium as an anticarcinogenic agent has not been elucidated (Redman et al., 1998).

In the present study, Se low or high dose combined with DFMO were administrated ip. The results of these combinations are represented in this study provide insight into the two agents administration are more effective than either alone. The polyamine levels can be modulated by administration of Se combined with DFMO. The precise mechanism of action of these two comprehensive agents is still obscure and required more work to be elucidated.

Utilization of inhibitors of other parts of the polyamine biosynthetic pathway, such as SAMDC inhibitor SAM 486A and/or polyamine-deficient diets in combination with DFMO, may induce a more complete depletion of polyamine pools and clarify whether the more preserved tissue polyamine levels are responsible for the lack of the antimetastatic effect of DFMO in cancer cells (Marni et al., 2005).

Reactive Oxygen Species (ROS) compounds are derivatives of molecular oxygen occurs in vivo and consists of hydrogen peroxide, superoxide anions and hydroxyl radicals. The release of ROS in vivo can cause proteins, DNA and lipids damage and eventually cell death (Fleury et al., 2002). Superoxide anion radical can be rapidly eliminated and converted to free hydroxyl radical (OH⁻), which can diffuse through membranes and initiate lipid peroxidation. Several reports have suggested that polyamine toxicity is a direct result of ROS that produced by polyamine catabolism and can damage macromolecular compounds (Ivanov et al., 1998). Spermine was reported to act as a potent antioxidant either by scavenging oxygen radical or by chelating the Fe⁺ which catalyze the OH⁻ generating via the Fenton reaction (Ha et al., 1998). Polyamines, among other functions, are considered to act as a free radical scavenger and antioxidant in brain and exert relevant roles in the physiology of cells (Belle et al., 2004).

In animals and humans, the highest level of Se occurs in the kidney and the major sites of the synthesis of the selenoenzyme GSH-Px are liver and kidney. An increase in
Se supplementation has been generally reported to result in an elevation in GSH-Px activity. In the present study, liver and kidney GSH-Px activity was found to increase significantly at 0.5 and 1 mg Se kg\(^{-1}\) body weight. Sodium selenite supplementation was expected to increase GSH-Px activity. It was also anticipated that the increase in the GSH-Px activity would provide a further defense against the elevated polyamine levels and result in a reduction in occurrence of various tumors. Polyamine deficient cell accumulate the ROS resulting an inhibition in the SOD expression \textit{vice versa} (Chattopadhyay et al., 2006). The mechanism by which polyamine decreases the ROS levels in the cells is still not clear. Several \textit{in vitro} studies suggested that polyamines can protect DNA from H\(_2\)O\(_2\) or singlet oxygen and that polyamines can directly react with oxygen radicals as scavengers \textit{in vitro} (Tkachenko et al., 2001; Jung and Kim, 2003).

In conclusion, this study showed that the addition of arginine and ornithine to the drinking water of the experimental models will increase the polyamines levels in the tissues. Regulating the abnormality of polyamine biosynthesis is a main strategy for cancer chemoprevention. The administration of Se and DFMO are required to decrease oxidative stress and higher levels of polyamines. The inhibition of polyamine biosynthesis with Se and DFMO markedly suppressed tumor cells.

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