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Antioxidant Enzyme Activities in Ascorbic Acid and Selenium Applied Hepatocellular Carcinoma Cells

Burcu Menekşe BALKAN[1]*, Öğünç MERAL[2], Görkem KISMALI[2], Tevhide SEL[2]

[1]Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Biochemistry, Burdur – TURKEY

[2]Ankara University Faculty of Veterinary Medicine, Department of Biochemistry, Ankara - TURKEY

Abstract: Ascorbic acid and selenium have potential use in the prevention of cancer. The role of antioxidant enzymes against cancer is to prevent oxidative damage. In this study, the effects of L-selenomethionine (234 µM), SeO_2 (100 µM), ascorbic acid (0,313 mM, and 31,3 mM) on antioxidant enzyme activities in HepG2 cell line were studied. GPx, CAT, and SOD activities of the treated cells were measured spectrophotometrically. There was a significant increase in the activity of GPx in HepG2 cells treated with L-selenomethionine (234 µM), SeO_2 (100 µM), and ascorbic acid (0,313 Mm and 31,3 mM). However, the activity of CAT was found significantly decreased in L-selenomethionine (234 µM), SeO_2 (100 µM), ascorbic acid (31,3 mM) treated HepG2 cells. The SOD levels of HepG2 cells treated with ascorbic acid (0,313 mM and 31,3 mM) were found higher than the control cells. The results of the present study indicated that the antioxidant enzyme activities changed depending on L-selenomethionine, SeO_2 application, and CAT activities changed depending on ascorbic acid doses.

Key words: Antioxidant enzymes, Ascorbic acid, HepG2, Selenium

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***Corresponding author. E-mail:** burcualpaslan@yahoo.com.

INTRODUCTION

Free radical species are formed as a result of reactions of oxygen and may play a role in tumor formation (1). On the other hand, overproduction of reactive oxygen species (ROS) can also induce the apoptotic pathway in the tumor cells, and this approach has effectively been used as a critical point for anticancer treatment (2). Depending on tumor type and degree, cancer cells exhibit different redox statuses. Because of these differences, the cell may be affected differently by the oxidants (3). Since oxidative stress may involve the etiology of cancer, agents that can prevent oxidative stress such as exogenous antioxidants have been extensively investigated to prove if they inhibit/reduce oxidative damage and prevent/slow cancer development (4). In addition to their protective effect on cancer, antioxidants like selenium (Se) and ascorbic acid (AA), exhibit pro-oxidant activity in cancer cells (5, 6). Se mediates ROS production in cancer cells, thus induces cytotoxicity (7, 8).

AA, a potent antioxidant, may act as a pro-oxidant when it reacts with metal ions in DNA. High doses of AA may prevent cancer, and it has been shown in limited studies that high dose ascorbate has promising results in the treatment of cancer (9). Because AA contributes to the production of superoxide and

may produce ascorbyl radicals, it is considered as an essential pro-oxidant of free radical reactions (6).

Selenium, which is an essential element for many biological functions, has been shown to have a protective effect against cancer (10, 11). Selenium has various biological activities (12, 13) and can be found as organically bound selenium forms (e.g., selenomethionine, selenocysteine) or inorganic forms (e.g., selenate, selenite) (14). Selenoproteins that contain organically bound selenium are found in living organisms, including humans, and some of their roles are well identified (15, 16, 17).

In the current study, it is aimed to investigate the effect of two different concentrations of AA and two different forms of selenium on antioxidant enzyme activities in the HepG2 cell line.

MATERIALS AND METHODS

Cell Culture

HepG2 cell line was used in this study (ATCC Cat No. HB- 8065). Cells were placed into 75-cm^2 tissue culture flasks and grown in RPMI 1640 Medium containing 10% Fetal Bovine Serum (FBS), 50 mg/l Gentamicin sulfate and 300 mg/l L-glutamine in a cell culture incubator at 37 °C in the presence of 5% CO_2.

For the determination of the effect of compounds in question on antioxidant enzyme activities, 0.313 mM and 31.3 mM AA (Merck, Cat No: 100468), 234 µM L-selenomethionine (Merck, Cat No: 561505), 100 µM SeO_2 (Sigma, Cat no: S-9379) concentrations, which were observed to significantly change the cell viability in previous studies (18, 19), were applied to HepG2 cells. Only RPMI 1640 Medium (containing 10% FBS, 50 mg/l Gentamicin sulfate and 300 mg/l L-glutamine) was applied to the control group.

Determination of Antioxidant Enzyme Activities

After treatments with AA, SeO_2 and L-selenomethionine for 24 h, cells were detached using a sterilized scrapper, lysed in ice-cold lysis buffer (0.1 M Tris/HCl, pH 7.4, containing 0.5% Triton X-100, 5 mM β-Mercaptoethanol, 0.1 mg/ml serine proteases inhibitor Phenylmethylsulfonyl fluoride) and centrifuged at 14.000 × g for 5 minutes at +4 °C. The supernatant was collected and kept at −80°C for CAT, SOD, and GPx analysis.

Superoxide dismutase (SOD) activities were determined using the SOD determination kit (Sigma-Aldrich, USA) according to the manufacturer's instructions. GPx activities were measured by spectrophotometric analysis at 340 nm according to the procedure described by Paglia and Valentine 1967 (20). CAT activity was also measured spectrophotometrically by the method stated by Aebi 1983 (21). This method is based on the measurement of the rate of decrease in the absorbance at 240 nm as the catalase enzyme breaks hydrogen peroxide down into water and oxygen. The rate decrease in absorbance is proportional to catalase enzyme activity. The specific activity of catalase is defined as k/g protein. k value was calculated from the equation $k=0.1175/t$ (t= Time in seconds, which is required for reduction of absorbance from 0.45 to 0.40).

Protein concentration in the cell lysate was determined by Bradford assay (22). All enzyme activity values were divided by protein concentration for specific activity determination. All experiments were repeated in triplicate for each group. Mean values were shown in Table 1.

Statistical Analysis

Statistical analysis between treated and control groups was performed with variance analysis (ANOVA), and differences between the groups were determined with the Duncan post hoc test. P values <0.05 were considered statistically significant.

RESULTS

GPx and SOD Enzyme Activities in HepG2 cells

GPx activities were significantly increased in HepG2 cells treated with 0.313 and 31.3 mM AA, 234 µM L-selenomethionine, and 100 µM SeO_2 compared to the control group (Figure 1). SOD activities were significantly increased in HepG2 cells at 0.313 and 31.3 mM of AA and 234 µM L-selenomethionine concentration compared to the control group (Figure 2). These results may indicate that increasing SOD activity 0.313 and 31.3 mM of AA and 234 µM L-selenomethionine have antioxidant effects in this concentration. Also, GPx levels are higher in L-selenomethionine-treated concentration than SeO_2. Hence, we may conclude that L-selenomethionine is less toxic or has more potent antioxidant properties than SeO_2, which is the inorganic form of selenium. The SOD activities of HepG2 cells treated with different doses of L-selenomethionine, SeO_2 and AA in comparison to the control group are presented in Figure 2.

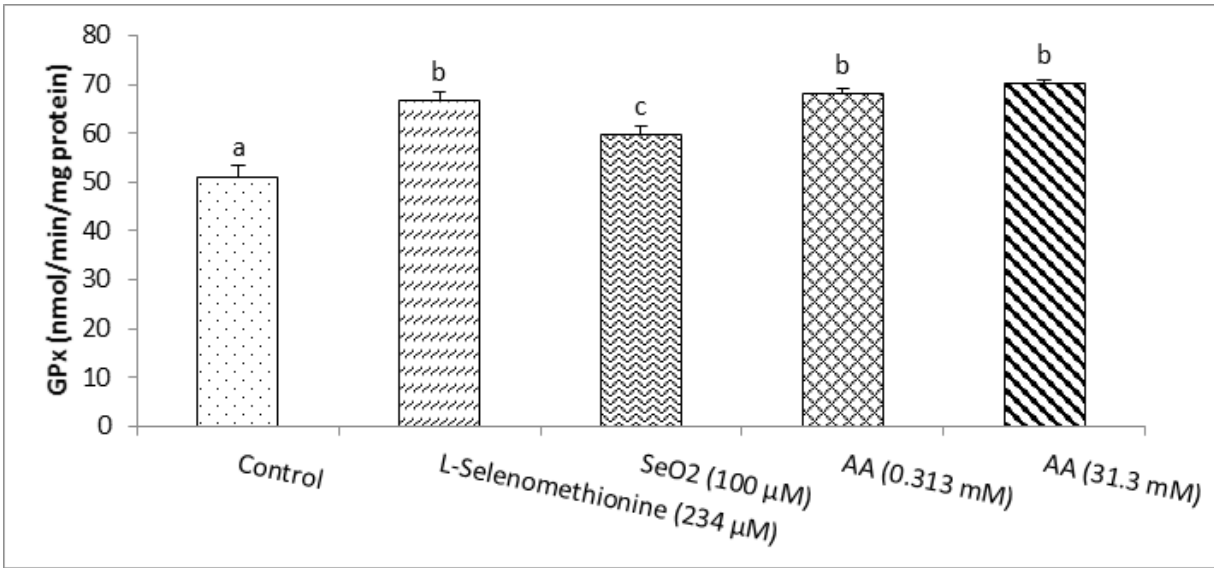

Figure 1. The GPx activities in HepG2 cells treated with 234 µM L-selenomethionine, 100 µM SeO_2, 0.313 mM, and 31.3 mM AA (Different letters indicate the differences between the groups).

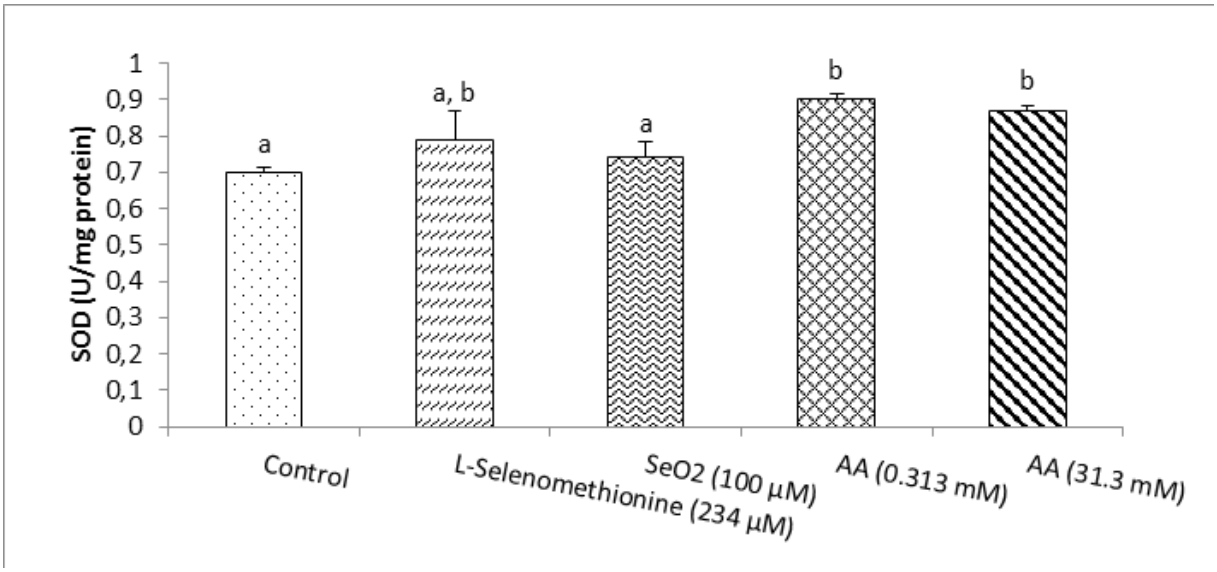

Figure 2. The SOD activities in HepG2 cells treated with 234 µM L-selenomethionine, 100 µM SeO_2, 0.313 mM, and 31.3 mM AA (Different letters indicate the differences between the groups).

CAT Enzyme Activities in treated HepG2 cells

CAT activities were significantly increased in HepG2 cells only at 0.313 mM AA concentration. CAT activities were significantly decreased in 31.3 mM AA, L-selenomethionine, and SeO_2 applied HepG2 cells compared to the control group (Figure 1). These results may indicate that depending on its dose, AA may have either antioxidant or pro-oxidant effects on HepG2 cells. Decreased activity of catalase represents the conversion of hydrogen peroxide to more toxic hydroxyl radicals. The CAT activities of HepG2 cells which were treated with different doses of L-selenomethionine, SeO_2 and AA in comparison to the control group are presented in Figure 3.

0.313 mM and 31.3 mM AA (Different letters indicate the differences between the groups). The results of the study are summarized in Table 1.

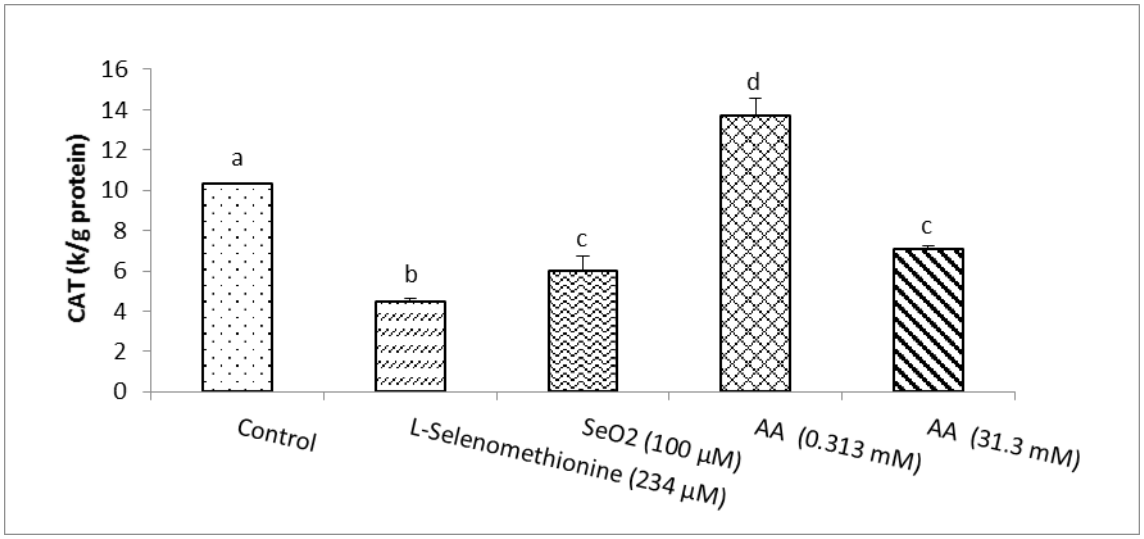

Figure 3. The CAT activities in HepG2 cells treated with 234 µM L-selenomethionine, 100 µM SeO_2, 0.313 mM and 31.3 mM AA (Different letters indicate the differences between the groups)

Table 1. The GPx, SOD and CAT enzyme activities of HepG2 cells treated with 234 µM L-selenomethionine, 100 µM SeO_2, 0.313 mM and 31.3 mM AA.

GROUPS	GPx (X±SD)	SOD (X±SD)	CAT (X±SD)
Control group	51.1±2.32[a]	0.70±0.014[a]	10.3±0.035[a]
L-Selenomethionine (234 µM)	66.7±1.85[b]	0.79±0.077[a,b]	4.47±0.16[b]
SeO_2 (100 µM)	59.7±1.79[c]	0.74±0.042[a]	5.99±0.714[b]
AA (0.313 mM)	68.1±0.9[b]	0.9±0.014[b]	13.7±0.86[d]
AA (31.3 mM)	70.1±0.9[b]	0.87±0.011[b]	7.09±0.15[c]

Data are presented as (Mean±SD). SD: Standard deviation. Statistical analysis was performed using one-way ANOVA with post hoc Duncan test. P values<0,05 were considered statistically significant (GPx : nmol/min/mg protein; SOD: U/mg of protein; CAT: k/g of protein). Different letters in the same column are statistically significant.

DISCUSSION AND CONCLUSION

As oxidative stress causes DNA damage, it has a role in cancer pathogenesis (23). The antioxidant enzymes CAT and GPx can scavenge hydrogen peroxide, which is produced by SOD from superoxide radical. In some cases, hydrogen peroxide is converted to highly toxic hydroxyl radicals by Fenton reaction due to the low CAT level (24). For the normal cellular function, there should be an appropriate balance between Cu/Zn-superoxide dismutase (Cu/Zn-SOD), catalase, and selenium-glutathione peroxidase activities. CAT and SOD activity could demonstrate the positive or negative balance of the antioxidant defense system (25).

On the hepatic metastasis model, Heukamp et al. (26) found that vitamins A (retinol), C (ascorbic acid) and E (alpha-tocopherol) increased GPx and SOD activities and decreased thiobarbituric acid-reactive substances (TBARS) levels. They concluded that these vitamins prevent oxidative stress in hepatocytes. In a study on young and aged rats, they found that the AA level decreased due to the increase in lipid peroxidation and MDA levels (27).

Although it is considered to be an antioxidant agent, at pharmacological doses (0.3-20 mM) AA, have been reported to be selectively toxic to cancer cells by producing ascorbate radicals, and inducing H_2O_2 formation (28). AA has also been reported in other studies to have cytotoxic effects on various types of cancer cells through a hydrogen peroxide generation (5, 29, 30, 31). Studies showed that specific protein transcription factors significantly decreased, and iron metabolism disrupted after AA treatment in colon cancer cells that are involved in cancer progression (32, 33). In

Ryszawy et al. (34) study, sodium ascorbate showed anti-tumor activity in glioblastoma multiforme cells. Gao et al. (35) demonstrated that AA could lead to liver cancer cells' death via intracellular reductive stress.

Increasing ROS production and oxidation, Se is known to induce apoptotic cell death in cancer cells (36, 37). Se, which is an essential element for many biological functions, has been shown to have cancer-protective effects in epidemiological and experimental animal studies. In addition to its protective effect against cancer, Se shows prooxidant activity and increased oxidative stress in cancer cells (38).

Wu et al. (39), demonstrated the therapeutic effect of selenite on peritoneal cancer in a mouse model. They indicated that selenite induced ROS production depending on its dose in the H22 cells and 4 mg Se/kg could be more effective than that of cisplatin in killing cancer cells. The mechanism of Se is related to endogenously formed selenium nanoparticles and its effect to hijack the Trx- and Grx-coupled GSH systems to produce ROS to kill cancer cells. Some organic Se components such as selenoamino acids, selenoproteins, and synthetic organic Se compounds have been reported to have cancer-protective effects (40, 41). Many preclinical, epidemiological studies and experimental animal models supported that Se compounds play a protective role against cancer. To date, studies have demonstrated the importance of the dose, which is administered, and the chemical structure of Se, which is used to determine the protective activity of Se components from cancer (42).

It is thought that there are many mechanisms in the anticancer activity of Se (43). But it was revealed that cell deaths that are related to Se applications were associated with ROS production (37, 38, 44, 45), and apoptotic cell death due to increased ROS production was observed through the caspase-independent apoptotic pathway (46). Estevez et al. (47) investigated the effects of chitosan-stabilized selenium nanoparticles, which were compared with other selenium-containing species on cell proliferation, apoptosis, and cell cycle pattern in HepG2 cells. They compared the effect of different type of Se compounds and concluded that cells which were exposed to Se(IV) showed strong induction of apoptosis and a significant population of cells when they are compared to control cells.

In our study, while the effects of AA on CAT activity were found to be dose-dependent, no changes were found in GPx and SOD activities. GPx activities increased, but CAT activities decreased in L-selenomethionine, and SeO_2 applied to HepG2 cells when they are compared to the control group. L-selenomethionine generated a significant increase in GPx activity than SeO_2. There were no statistical differences in SOD activity between L-selenomethionine and SeO_2 applied HepG2 cells. Compared to the control group: GPx activities increased in HepG2 cells treated with L-selenomethionine, SeO_2, and different doses of AA, but CAT activities decreased in all groups but increased in 0.313 mM AA applied cells compared to the control group.

The results of this study indicated that L-selenomethionine, SeO_2, and AA application increase GPx enzyme activities in HepG2 cells. L-selenomethionine and SeO_2 decrease CAT enzyme activities but do not chance SOD enzyme activities. SOD enzyme activities are increased in both 0.313 mM and 31.3 mM AA concentrations. Depending on its doses, AA effects CAT enzyme activities. 0.313 mM AA increases, and 31.3 mM decreases CAT enzyme activities in HepG2 cells.

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