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Research Article

The Effects of Vitamin E on Antioxidant Enzyme Activity in HepG2 Cells [1]

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Abstract

It is aimed to investigate the effect of vitamin E, powerful antioxidant (alpha-tocopherol succinate) on antioxidant enzyme activities in hepatocellular carcinoma (HepG2) cells. The hepatocellular carcinoma cell line HepG2 was used and the cells were cultured in the absence (control) or presence of different dose of vitamin E (50 mM, 50 µM and 10 µM vitamin E) for 24 h. The effect of vitamin E (alpha-tocopherol succinate) on catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzyme activities in hepatocarcinoma cells were measured by spectrophotometry. A significant decrease in GPx activity was detected in 50 mM vitamin E treated HepG2 cells. However a significant decrease occurred in 10 µM and 50µM vitamin E applied HepG2 cells. SOD activity in study groups were lower than in control cells. In addition to this, the decrease in SOD activity in 50 mM vitamin E applied cells was significant. CAT enzyme activity in 50 µm vitamin E applied HepG2 cells was higher and, in 10 µM and 50 mM vitamin E applied HepG2 cells were lower than in control group. It was determined that vitamin E has a dose-dependent effect on antioxidant enzyme activity in HepG2 cells.

Keywords: *Catalase, Glutathione Peroxidase, HepG2, Superoxide dismutase, Vitamin E*

Vitamin E'nin HepG2 Hücrelerinde Antioksidan Enzim Aktivitesi Üzerine Etkileri

Özet

Güçlü bir antioksidan olan vitamin E'nin (alfa-tokoferolsüksinat) HepG2 hücrelerinde antioksidan enzim aktiviteleri üzerine etkisinin araştırılması amaçlanmıştır. Çalışma materyali olarak HepG2 hücre hattı kullanılmıştır. Vitamin E uygulanan hücreler çalışma grubunu vitamin E uygulaması yapılmayan hücreler kontrol grubunu oluşturmuştur. Çalışma grubu hücrelerine 10 µM, 50 µM, 50 mM dozlarda vitamin E uygulaması yapılarak, 24 saat sonunda HepG2 hücrelerinde antioksidan enzimlerden katalaz (CAT), süperoksitdismutaz (SOD) ve glutasyonperoksidaz (GPx) aktiviteleri spektrofotometrik olarak ölçülmüştür. 50 mM vitamin E uygulanan HepG2 hücrelerinde GPx enzim aktivitesinde anlamlı bir artış saptanmıştır. Ancak, 10 µM ve 50 µM vitamin E uygulanan HepG2 hücrelerinde ise anlamlı bir azalma meydana gelmiştir. Vitamin E uygulaması yapılan hücrelerdeki SOD aktivitesi vitamin E uygulaması yapılmayan kontrol grubuna göre daha düşük ölçülürken, 50 µM vitamin E uygulanan HepG2 hücrelerinde SOD aktivitesinde anlamlı azalma tespit edilmiştir. 50 µM vitamin E uygulanan HepG2 hücrelerindeki CAT enzim aktivitesi kontrol grubuna göre daha yüksek bulunurken, 10 µM ve 50 mM vitamin E uygulanan hücrelerdeki CAT enzim aktivitesi kontrol grubuna göre daha düşük tespit edilmiştir. Vitamin E'nin HepG2 hücrelerinde antioksidan enzim aktiviteleri üzerinde doz-bağımlı etkisinin olduğu belirlenmiştir.

Anahtar sözcükler: *Glutatyon peroksidaz, HepG2, Katalaz, Süperoksit dismutaz, Vitamin E*

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INTRODUCTION

Hepatocellular carcinoma (HCC) is common malignant disease associated with high mortality rate [1]. It has been known that Vitamin E (Vit. E), which is a fat-soluble vitamin, is the most effective antioxidant in biological systems. Vit. E converts superoxide and hydroxyl radical, singlet oxygen and lipid peroxides to lesser active form and hence, it protect against lipid peroxidation and oxidative tissue damage [2]. According to the analyses results, it has been found that Vit. E has an anti-oxidant activity and it exists in 8 different forms including four tocopherol and four tocotrienol [3]. Alpha-tocopherol is most common and active form of Vit. E in nature [4].

In humans, the free radicals that are formed as a result of a reaction between polyunsaturated fatty acids in membranes lipids and oxygen may have a role in tumor mechanism [5]. Vit. E may prevent certain tumor formation as a powerful anti-oxidant by protecting cells and DNA from the damage caused by free radicals [5,6]. In the laboratory studies, it has been shown that nutritional antioxidants including Vit. E prevent the growth of cancer cells [7]. The anti-oxidant effect of Vit. E refers researchers to investigate the protective effect of Vit. E in chronic diseases such as cardiovascular diseases, atherosclerosis and cancer. Many epidemiological studies have shown a relationship between high-dose Vit. E intake and cardiovascular diseases [8,9].

Opposite to its antioxidant activity, pro-oxidative effects of vitamin E are also observed *in vitro*. It has to be considered that vitamin E, like every redox-active compound, may exert anti- and pro-oxidative effects [10]. In a study performed by Heisler et al.[11], it was stated that Vit. E does not inhibit the growth of pancreatic cell lines. In another study performed on animals, it has been indicated that the mechanism of reduction of liver metastasis in pancreas cancer may be affected in accordance with increased GPx and SOD activities and decreased levels of thiobarbituric acid-reactive substances (TBARS).According to result of study, it was concluded that antioxidant vitamins prevent oxidative stress in hepatocytes [12]. It has also been reported that Vit. E intake has a protective effect on the progression of certain cancer types [13,14]. Vit. E has been shown to protect liver damage induced by oxidative stress in animal experiments [15,16], but effects of Vit. E on cancer cells it has not been well studied. Thus, in the present study, it is aimed to investigate the effect of Vit. E on antioxidant enzyme activity in hepatocellular carcinoma (HepG2) cells.

MATERIAL and METHODS

The HepG2 cell line was used in the present study (ATCC Cat No. HB- 8065). Cells were 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.

According to cell viability test, the optimum proliferation conditions for HepG2 cell were determined as 100.000 cell/well for 24 h. The HepG2 cells were incubated with Medium containing different doses of Vit. E (50 mM, 50 µM and 10 µM) for 24 h; whereas Vit. E-free medium was added in cells of control group. The enzyme activities of SOD, CAT and GPx in cell lysates were measured at the end of 24 h.

Determination of Superoxide Dismutase (SOD) Activity

SOD activity was measured by the method developed by Sun et al.[17]. This assay involves inhibition of nitroblue tetrazolium reduction, with xanthine-xanthine oxidase. In the assay, xanthine-xanthine oxidase used as a superoxide generator.

Determination of Catalase (CAT) Activity

CAT activity was measured by the method stated by Aebi [18]. According to the method, decrease in absorbance at 240 nm of a reaction mixture consist of H_2O_2, in phosphate buffer, and sample is determined. The decrease in absorbance is proportional to enzymes activity in sample.

Determination of Glutathione Peroxidase (GPx) Activity

GPx activity was measured by the method stated by Paglia and Valentine [19]. Glutathione peroxidase (GPx) reduces the CumeneHydroperoxide while oxidizing glutathione (GSH) to oxidized glutathione (GSSG). The generated GSSG is reduced to GSH with consumption of NADPH by glutathione reductase (GR). The decrease in NADPH absorbance measured at 340 nm during the oxidation of NADPH to NADP+ is indicative of GPx activity and the decrease of NADPH is proportional to GPx activity.

Bradford method was used for protein quantitation in cell lysates [20].

The Bradford assay is a protein determination method that involves the binding of CoomassieBrilliant Blue G-250 dye to proteins. This blue protein-dye form is detected at 595 nm by a spectrophotometer or microplate reader.

Statistical Analysis

Statistical comparison between treated and control groups were performed using one-way ANOVA with post hoc Duncan test. P values <0.05 were considered statistically significant.

RESULTS

The SOD activities of HepG2 cells treated with different Vit. E doses in comparison to the control group are presented in *Fig. 1*. The SOD activity in Vit. E-treated cells was found to be lower in comparison to control group. A significant decrease was determined in SOD activity of cells treated with 50 µM Vit. E.

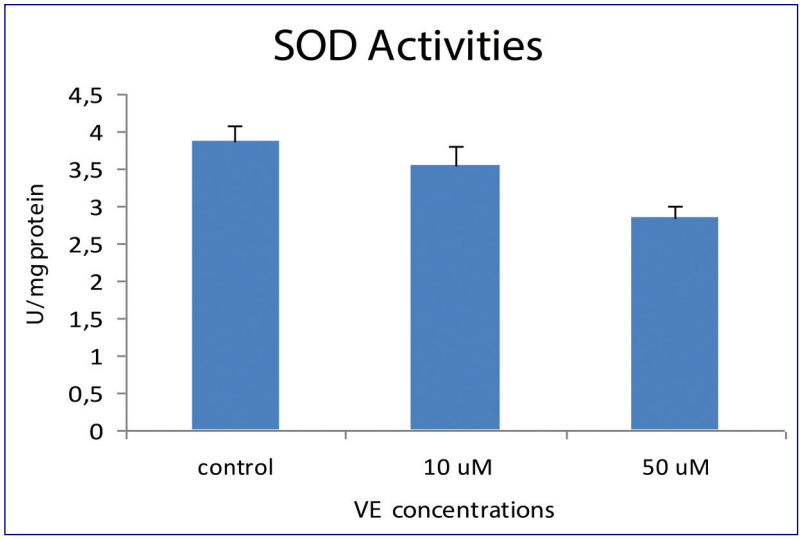
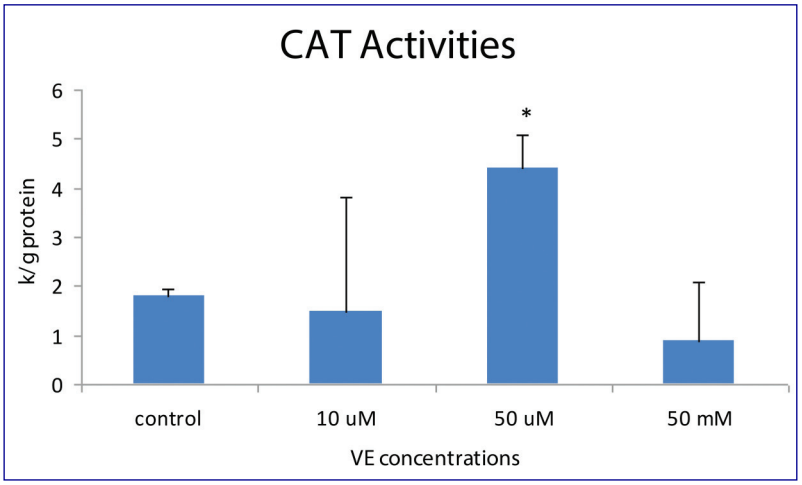


Fig 1. The SOD activities of HepG2 cells treated with different Vit. E doses

Şekil 1. Farklı dozlarda Vit. E uygulanan HepG2 hücrelerinde SOD aktiviteleri

Fig 2. The CAT activities of HepG2 cells treated with different Vit. E doses

Şekil 2. Farklı dozlarda Vit. E uygulanan HepG2 hücrelerinde CAT aktiviteleri



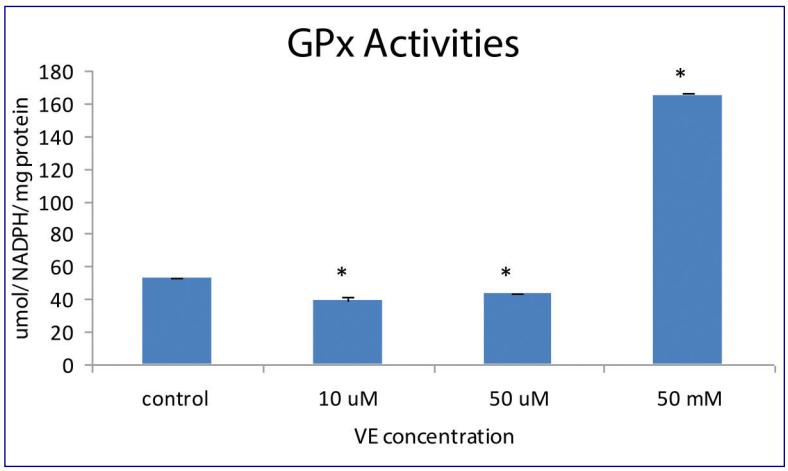


Fig 3. The GPx activities of HepG2 cells treated with different Vit. E doses

Şekil 3. Farklı dozlarda Vit. E uygulanan HepG2 hücrelerinde GPx aktiviteleri

The CAT activities of HepG2 cells treated with different Vit. E doses in comparison to the control group are presented in *Fig. 2*. A significant increase was determined in HepG2 cells treated with 50 μM Vit. E. However, a non-significant decrease was observed in HepG2 cells treated with 10 μM and 50 mM Vit. E.

The GPx activities of HepG2 cells treated with different Vit. E doses in comparison to the control group are presented in *Fig. 3*. Although, the GPx activity in HepG2 cells treated with 50 mM Vit. E was found to be higher in comparison to control group, it was lower than control group in cells treated with 10 μM and 50 μM.

DISCUSSION

It has been known that oxidative damage has a role in cancer pathogenesis. Oxidative damage and ongoing process may lead to DNA damage which is the basis of cancer development [21]. The deficiency at the level of antioxidant defense is important in the defense mechanism against free radicals. Antioxidants are the chemical components preventing the activation and formation of free radicals [22]. Enzymatic and non-enzymatic antioxidants are the first defense mechanism against toxicity generated by free radicals. The balance between prooxidants and antioxidants are important for normal cellular function. Vit. E shows its antioxidant activity by affecting free radicals on cell membranes [23].

CAT and GPx both act to scavenge SOD products, hydrogen peroxide. In the absence of adequate amounts of CAT, hydrogen peroxide might be expected to undergo conversion to highly toxic hydroxyl radicals by way of the Fenton type reaction [24]. Therefore, when the H_2O_2 product of SOD is probably more scavenged by GPx, and CAT, its activity is eventually decreased. Level of SOD activity compared with CAT activity is a key factor for efficient SOD activity, and that the combined activity of CAT and SOD could potentially lead to either positive or negative effects on the antioxidant defense potential [25].

In an *in vitro* study, the activities of intracellular antioxidant enzymes, SOD and CAT, were increased in rats receiving Vit. E diet. It has been claimed that Vit. E increases the strength of endogenous antioxidant defense by this way. Vitamin E can indirectly effects the activity of several transcription factors involved in the transcriptional regulation of Mn-SOD. Furthermore, as ROS are potent inhibitors of SOD, vitamin E, through its superoxide scavenging activity, can up-regulate SOD activity [26].

The SOD enzyme has three different isoforms; cytoplasmic Cu/Zn-SOD (SOD1), extracellular EC-SOD (SOD3) and mitochondrial Mn-SOD (SOD2). *In vivo* studies showed that the cytoplasmic SOD activity did not change with different doses of Vit. E (10, 30 and 100 mg/kg BW); however, an increase was observed in mitochondrial SOD activity. In these studies, Vit. E directly increased the transcriptional activity of SOD gene by upregulating the mRNA activity of mitochondrial SOD. The over-expression of cytoplasmic SOD gene has been observed in increased ROS production and oxidative catabolism [26]. In the present study, SOD activity was performed in cell line lysate. The absence of any variation may be due to the lack of prooxidant effect at low Vit. E levels.

In a study performed by Hajiani et al.[26], no significant variation was determined in CAT activity with low-dose Vit. E administration. On the other hand, the CAT activity was significantly increased at the Vit. E doses of 30 and 100 mg/kg. An increase in high dose Vit. E administration was observed after a long time. In the present study, no significant variation was observed in 10 uM Vit. E administration; whereas, the CAT activity was increased in 50 uM Vit. E administration.

Another issue that has been emphasized in studies performed with Vit. E is the determination of required dose for the formation of antioxidant effect. It has been thought that low-dose Vit. E is unable to show adequate antioxidant effect; however, high-dose Vit. E, which is defined as mega dose, may cause synergic toxicity by interacting with other substances [27]. This is originated from dose-dependent antioxidant effect of Vit. E. In the present study, the CAT activity in HepG2 cells treated with 50 µM Vit. E was found to be higher in comparison to control group. On the other hands, it was lower in cells treated with 10 µM and 50 mM in comparison to control group. It is thought that Vit. E increases the strength of antioxidant defense by showing higher antioxidant effect at the dose of 50 mM in HepG2 cells.

While the GPx activities of HepG2 cells treated with 10 µM and 50 µM Vit. E were found to be decreased, a significant increase was determined in HepG2 cells treated with 50 mM Vit. E in comparison to the control group. In certain types of cancers, such as prostate, breast, skin and lung cancer, it has been reported that Vit. E may block cancer progression, and *in vivo* and *in vitro* progression of prostate tumor was slowed down by Vit. E in mice treated with different doses of chemotherapeutic agents [28,29]. The radical forms of Vit. E develop at high-doses of Vit. E (alpha-tocopherol) as a result of prooxidant effect. The radicals of Vit. E require glutathione (GSH) for regeneration [30]. The increase in the activity of GPx in cells treated with 50 nm Vit. E may be the result of an increase in GPx activit due to the induction of Vit. E radicals.

Pre-data study results of molecular studies investigating the mechanism of action of Vit. E on antioxidant enzymes have showed that Vit. E shows its dose-dependent activity by effecting intracellular enzyme activity in HepG2 cells.

It is required to measure the antioxidant enzyme activities and cellular oxidant levels in extensive studies involving long-term administration of Vit. E.

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