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*Research Article*

# The influence of vitamin U supplementation on liver injury of amiodarone-administered rats

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## Abstract

Amiodarone is a currently used drug with high efficacy for the treatment acute life-threatening arrhythmias. Although the use of this drug is widespread, it is associated with unwanted systemic effects. S-methylmethionine sulphonium, which is a derivative of the methionine amino acid, defined mostly as vitamin U and various beneficial effects of Vit U have been demonstrated. The present study was planned to determine whether the vitamin U exhibits preventive effects on amiodarone-induced liver toxicity. Male Sprague-Dawley rats were randomly divided into four groups. Group I; control animals receiving corn oil. Group II; control animals receiving Vit U (50 mg/kg) for 7 days orally. Group III; animals receiving 100 mg/kg amiodarone for 7 days orally. Group IV; animals receiving Vit U orally for 7 days (in the same dose and time) 1 h prior to the administration of amiodarone. Pretreatment with vitamin U particularly decreased degenerative morphological changes such as picnotic nucleus in hepatocytes, sinusoidal dilatation, hyperemia seen in amiodarone treated individuals. On the other hand liver aspartate transaminase, alanine transaminase and alkaline phosphatase activities were increased, catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase activities were decreased in amiodarone group. Administration of vitamin U reversed these effects in amiodarone group. In conclusion, it might be suggested that pretreatment with vitamin U have protective effects on liver injury induced with amiodarone through decreasing oxidative stress.

**Keywords**: Arrhythmia, amiodarone, liver injury, vitamin U.
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# Amiodaron verilen sıçanların karaciğer hasarı üzerine vitamin U desteğinin etkisi

## Özet

Amiodaron, yaşamı tehdit eden akut aritmilerin tedavisinde günümüzde kullanılmakta olan yüksek etkinliğe sahip bir ilaçtır. Bu ilaç kullanımı yaygın olmasına rağmen, istenmeyen sistemik etkiler ile ilişkilidir. Çoğunlukla U vitamini olarak tanımlanan ve metiyonin amino asidinin bir türevi olan S-metilmetiyonin sulfonyum'un çeşitli yararlı etkileri ortaya konmuştur. Bu çalışma, U vitaminin amiodaron ile indüklenen karaciğer toksisitesi üzerinde önleyici etki gösterip göstermediğini belirlemek amacı ile planlanmıştır. Erkek Sprague-Dawley sıçanlar rastgele dört gruba ayrılmıştır: 1. Grup; mısır yağı verilen kontrol hayvanlar. 2. Grup; 7 gün boyunca oral olarak U vitamini (50 mg/kg) verilen hayvanlar. 3. Grup; 7 gün boyunca oral olarak 100 mg/kg amiodaron verilen hayvanlar. 4. Grup; amiodaron uygulamasından 1 saat önce 7 gün boyunca oral olarak U vitamini (aynı doz ve zamanda) verilen hayvanlar. U vitaminin amiodaron uygulamasından önce verilmesi, amiodaron uygulanan hayvanlarda gözlenen hepatositlerdeki piknotik nukleus, sinüzoidal dilatasyon, hiperemi gibi dejeneratif morfolojik değişiklikleri kısmen azaltmıştır. Diğer taraftan, amiodaron grubunda karaciğer aspartat transaminaz, alanine transaminaz ve alkalin fosfataz aktiviteleri artmış, katalaz, süperoksit dismutaz, glutatyon peroksidaz, glutatyon-S- transferaz aktiviteleri ise azalmıştır. U vitaminin uygulanması amiodaron grubundaki bu etkileri tersine çevirmiştir. Sonuç olarak, U vitamininin önceden uygulanmasının, amiodaron ile indüklenen karaciğer hasarı üzerinde oksidatif stresi azaltarak koruyucu etkilere sahip olduğu öne sürülebilir.

**Anahtar Kelimeler:** Aritmi, amiodaron, karaciğer hasarı, U vitamini.

## Introduction

Amiodarone (AMD; 2-butyl-3-[3',5'-diiodo-4'α-diethylaminoethoxybenzoyl]-benzofuran) is an iodinated amphiphilic and a class III anti-arrhythmic drug and has been widely used for treatment supraventricular and ventricular tachyarrhythmias and atrial fibrillation (Waldhauser et al. 2006; Goldschlager et al. 2007). It has been well documented that AMD causes side effects in the skin, thyroid, lung, liver, cornea, peripheral nervous system and muscle during long-term therapy (Harris, 1983; Vassallo and Trohman 2007) and AMD and its metabolite desethylamiodarone accumulate in several tissues such as liver, lung, pancreas, thyroid gland, kidney, brain, heart (Brien et al. 1987).

The liver is the central organ for drug metabolism and removal (Pandit et al. 2012) and any imbalance in the activity of drug metabolizing enzymes leads to a free radical generation which is harmful to macromolecules and causes liver toxicity (Singh et al. 2016). AMD causes idiosyncratic, drug-induced liver injury in human especially after long-term oral intake and acute intravenous administration (Rotmensch et al. 1984; Lewis et al. 1989; Rätz Bravo et al. 2005). It has been reported that AMD and its major metabolite desethylamiodarone accumulate in the highly-perfused liver due to their lipophilic characters (Lewis et al. 1989) and the relationship between amiodarone and its hepatic toxicity based on AMD elimination by CYP3A4, one of the isoforms of cytochrome P450 (Shayeganpour et al. 2006; Waldhauser et al. 2006; Zahno et al. 2011).

S-Methylmethionine sulfonium is a derivative of the amino acid methionine and referred as vitamin U (Vit U) due to its effects in the treatment gastrointestinal ulcers (Salim et al. 1993a,b; Kopinski et al. 2007). The raw cabbage has anti-ulcer properties and is rich in Vit U (Roche-Vitec, 1990). In addition to anti-ulcer properties, Vit U has anti-inflammatory, antidepressant, wound-healing, reduction of blood lipid, cytoprotective, and adipocyte differentiation effects (Urazaeva 1976; Stoliarov and Mys'ko 1981; Watanabe et

al. 1996; Kim et al. 2010; Lee et al. 2012). It has also been shown that Vit U has protective effects on liver and renal injury induced by valproic acid, an anti-epileptic drug (Sokmen et al. 2012; Gezginci-Oktayoglu et al. 2016).

The studies related to possible protective effects of vitamins and flavonoid-type antioxidants against AMD-induced toxicity remained limited with vitamin E, silibinin and silymarin (Kachel et al. 1990; Kannan et al. 1990; Vereckei et al. 1993; Honegger et al. 1995; Agoston et al. 2003). According to our knowledge, this is the first study that shows protective effects of Vit U on liver injury induced by amiodarone (AMD) in rats.

## Materials and methods

*Animals*

The experimental procedures were approved by the local Animal Care and Use Committee of Istanbul University, with the certification on the Application for the Use of Animals dated September 27, 2012 (approval ID: 2012 / 127). In this study, 3.5-4 months aged male Sprague-Dawley rats (Istanbul University Experimental Medical Research and Application Institute, DETAE) were used. Their diet consisted of standard animal pellet food and tap water *ad libitum*. Application of AMD dose and time were determined as Reasor et al. (1996). Vit U dose were administered according to Sokmen et al. (2012).

*Experimental design*

A total of twenty nine rats were divided into 4 groups as follows. The groups include: Group I, control animals receiving corn oil for 7 days (n=6); Group II, animals receiving Vit U (50 mg/kg) for 7 days (n=7); Group III; animals receiving AMD (100 mg/kg) for 7 days (n=8); and Group IV, animals receiving Vit U (50 mg/kg) for 7 days 1 h prior to the administration of AMD (100 mg/kg) (n=8). AMD and Vit U were administered to rats by gavage. On the 8$^{th}$ day, all the animals fasted overnight were sacrificed.

*Histological evaluation of liver tissues*

The liver tissues were fixed in Bouin's solution for 24 h and washed with 70% ethanol to remove picric acid. The tissues were dehydrated in a series of graded ethanol, cleared in xylene and embedded in paraffin. Sections at 5 µm thickness were taken at rotary microtome (Leica, RT) and stained with Hematoxylin-Eosin and Masson's trichrome. Sections were evaluated histologically using a light microscope (Olympus, CX23) and photomicrographs were taken using a photomicroscope (Olympus, CX41) at a magnification of x200.

*Biochemical assays*

For biochemical assays, the liver tissues were taken from animals under anesthesia. Tissue samples were washed with physiological saline (0.9 % NaCl) and kept frozen until the day of the experiments. On the day of the experiments, liver samples were homogenized in cold saline with a glass homogenizer to make up to a 10 % (w/v) homogenate. The homogenates were centrifuged, and the clear supernatant fraction was removed for biochemical analysis. Liver aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed according to Reitman and Frankel (1957), alkaline phosphatase (ALP) activity was determined by Two Point Method (Walter and Schutt 1974). The catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities were assessed according to the following methods, respectively: Aebi (1984), Mylorie et al. (1986), Wendel (1981), Habig and Jacoby (1981). Protein levels of liver homogenizates were determined according to Lowry method (Lowry et al. 1951).

*Statistical analysis*

The biochemical results were evaluated using an unpaired t-test and ANOVA variance analysis using the NCSS statistical computer software. Data were expressed as mean±standard deviation (SD). $p<0.05$ was considered as significant.

## Results

*Histopathological results*

The control group and Vit U given control group have the normal liver microstructure (Fig.1). AMD caused mild degenerative

morphological changes such as picnotic nucleus in hepatocytes, sinusoidal dilatation, hyperemia, rupturings in endothelium of central vein seen in amiodarone treated rats (Fig.1). Pretreatment with Vit U particularly regressed these degenerative changes (Fig.1).

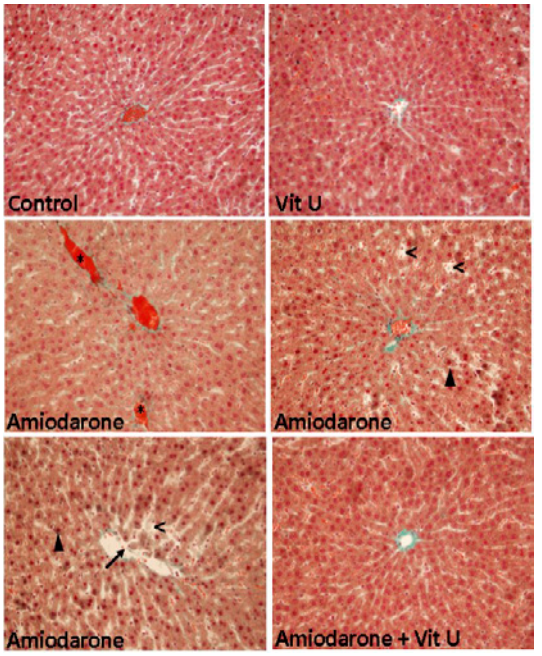


**Figure 1.** Endothelial rupture in central vein (→), sinusoidal dilatation (<), hyperemia (*) and picnotic nucleus ▲) were observed in amiodarone treated group. Vit U prevented these degenerative changes.

*Biochemical results*

Liver AST, ALT and ALP activities of all groups are shown in Table 1. According to the results, all of the enzyme activities were significantly increased in AMD group as compared to control group, respectively ($p<0.05$, $p<0.01$, $p<0.0001$). Administration of Vit U reversed these enzyme activities of AMD group in a significant manner, respectively ($p<0.0001$, $p<0.01$, $p<0.05$).

In Table 2, liver CAT and SOD activities are given. Both the enzyme activities were found to be significantly decreased in AMD group when compared to control group, respectively ($p<0.05$, $p< 0.01$). In Vit U given AMD group, CAT and SOD activities were increased significantly ($p<0.05$).

Liver GPx and GST activities are shown in Table 3. GST activity in Vit U group showed a significant decrease when compared to control group ($p<0.0001$). In AMD group, both GPx and GST activities were found to be decreased significantly as compared to control group, respectively ($p<0.0001$, $p<0.01$). Administration of Vit U reversed these activities significantly in AMD group ($p < 0.05$, $p< 0.01$).

**Table 1.** Liver aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities of control and experimental groups.

| Groups | AST (U.g protein$^{-1}$)* | ALT (U.g protein$^{-1}$)* | ALP (U.g protein$^{-1}$)* |
|---|---|---|---|
| Control | 6.89 ± 1.90 | 6.87 ± 1.15 | 0.92 ± 0.01 |
| Vit U | 8.45 ± 2.07 | 5.59 ± 2.11 | 0.94 ± 0.11 |
| AMD | 10.78 ± 1.85[a] | 12.53 ± 2.89[c] | 1.44 ± 0.23[e] |
| AMD+Vit U | 1.86 ± 1.18[b] | 5.44 ± 1.87[d] | 0.81 ± 0.24[f] |
| $P_{ANOVA}$ | 0.001 | 0.0001 | 0.007 |

*Mean ± SD

[a]$p<0.05$ versus control group, [b]$p<0.0001$ versus AMD group, [c]$p<0.01$ versus control group, [d]$p< 0.01$ versus AMD group, [e]$p< 0.0001$ versus control group, [f]$p< 0.05$ versus AMD group

**Table 2.** Liver catalase (CAT) and superoxide dismutase (SOD) activites of control and experimental groups.

| Groups | CAT (U.mg protein$^{-1}$)* | SOD (U.g protein$^{-1}$)* |
|---|---|---|
| Control | 81.75 ± 18.35 | 7.50 ± 0.98 |
| Vit U | 87.94 ± 22.53 | 8.70 ± 0.74 |
| AMD | 57.09 ± 17.67[a] | 5.03 ± 0.25[c] |
| AMD+Vit U | 80.50 ± 11.32[b] | 5.69 ± 0.32[b] |
| $P_{ANOVA}$ | 0.045 | 0.0001 |

*Mean ± SD

[a]p<0.05 versus control group, [b]p< 0.05 versus AMD group, [c]p< 0.01 versus control group

**Table 3.** Liver glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities of control and experimental groups.

| Groups | GPx (U.g protein$^{-1}$)* | GST (mU.mg protein$^{-1}$)* |
|---|---|---|
| Control | 3.31 ± 0.68 | 501.01 ± 66.79 |
| Vit U | 3.31 ± 1.06 | 352.14 ± 19.22[a] |
| AMD | 1.87 ± 0.13[a] | 350.99 ± 39.23[c] |
| AMD+Vit U | 3.79 ± 0.89[b] | 469.77 ± 25.70[d] |
| $P_{ANOVA}$ | 0.071 | 0.010 |

*Mean± SD

[a]p<0.0001 versus control group, [b]p< 0.05 versus AMD group, [c]p< 0.01 versus control group, [d]p< 0.01 versus AMD group

## Discussion

Drug-induced liver toxicity is one of the most common adverse effects of medicines, and the protective compounds gain attention to mitigate the drug-induced liver injury. AMD is a commonly used drug for treatment cardiac arrhythmias, but the use of AMD is often restricted by its side effects. Although the most toxic effect of AMD is seen in lungs, it has also been reported that the level of serum aminotransferases, which show liver damage, of more than 20% of patients was increased (Lewis et al. 1989).

AMD has an amphipathic nature and a weak solubility in water. This situation gives a high affinity for membranes to AMD. Besides, the drug has a very long half-life and is known to be stored in the liver (Harris et al. 1983). AMD induces hepatitis through its toxic effects to mitochondria, uncoupling of oxidative phosphorylation, inhibiting of the electron transport chain and b-oxidation of fatty acids (Fromenty et al. 1990a,b; Berson et al. 1998; Spaniol et al. 2001). Moreover, it has been shown by *in vitro* studies, that AMD increases reactive oxygen species concentration and lipid peroxidation (Waldhauser et al. 2006; Ouazzani-Chahdi et al. 2007). Furthermore, AMD interacts with membrane phospholipids and leads to modifications in membrane phospholipid composition of both tissues and organelles, such as lysosomes and mitochondria (Rabkin 2006; Serviddio et al. 2011). The interactions between AMD and lysosomal membrane produce drug-lipid complexes which are seen as lamellar lysosomal bodies (Poucell et al. 1984). In respect of studies with isolated mitochondria, AMD inhibited complex I activity and uncoupled oxidative phosphorylation, which leads to a decrease in hepatic ATP production (Serviddio et al. 2011).

Numerous studies have shown that long-term or acute AMD use causes steatosis, fibrosis in portal and sinusoidal area, neutrophil infiltration, centrilobular necrosis and ballonning degeneration and Mallory bodies in hepatocytes of liver in humans and animals models (Poucellet al. 1984; Lewis et al. 1989; Chang et al. 1999; Gluck et al. 2011; Vitins et al. 2014). In addition, treatment of cultured mouse hepatocytes with AMD results in swollen hepatocytes and accumulation of lipids which is seen as clear cytoplasmic droplets (Ouazzani-Chahdi et al. 2007). Furthermore, it has been shown by ultrastructural studies that AMD induces the formation of lysosomal lamellar phospholipid inclusions and lipid droplets (Chang et al. 1999; Agoston et al. 2003). The degenerative changes in our animal models were moderate, and we have observed hyperemia, sinusoidal dilatation, rupturings in the endothelium of central vein, the picnotic nucleus in hepatocytes. This discrepancy could be related to dose and duration of the treatment with AMD. On the other hand, we could not observe any lysosomal lamellar bodies due to the fact that our histopathological examination was at light microscopic level. However, Vit U decreased the mild degenerative changes formed by AMD administration, and the tissue morphology was similar to the control groups.

Serum transaminases AST and ALT are the most common used parameters to indicate the liver injury because of their relatively long half-life (Sakka 2007). They are mostly found in the liver as well as in other tissues. When cellular membrane damage occurs during the liver injury, AST and ALT activities rise (Borlak et al. 2014). We have got elevated AST and ALT activities in liver tissue of AMD group as compared to control group. In parallel to our results, Merz and Fuller (2007) and Ng et al. (2012) have found elevated AST and ALT activities in patients who were treated with AMD. Administration of Vit U decreased these enzyme levels *via* its cellular repair function (Racz et al. 2008). In addition to this, phosphatases are also good marker enzymes for determining liver injuries. They hydrolyze different phosphate esters at specific pH levels and liberate phosphate groups of the stored substrates of hepatocytes in various conditions like hepatic injuries (Vijayavel et al. 2013). In our study, AMD caused an elevation in ALP activities. Administration of Vit U reversed this activity in AMD group.

The free radical-induced oxidative stress results from the excess production of reactive oxygen species or diminished antioxidant levels of cells. This situation is accepted as a former mechanism for AMD-induced cytotoxicity. AMD has a tendency for producing free radicals. Because of its cationic amphiphilic nature, AMD accumulates in cells. Meanwhile it inhibits electron transport chain which is related to free radical production. In addition to that, either AMD or a metabolite can start this harmful production. Therefore, generation of ROS becomes at higher amounts. In this point, cellular antioxidants play an important role for detoxifying these substances. CAT becomes important when $H_2O_2$ levels are low and when $H_2O_2$ levels are at higher concentrations, GPx plays a role for quenching this substance. SOD is also important because of the very short life of superoxide anion. GST is a marker enzyme as being phase II detoxifying enzyme for liver (Kaufmann et al. 2005; Ozer et al. 2008; Durukan et al. 2012; Kalyanaraman 2013). In the current study, CAT, SOD, GPx and GST activities are found to be decreased in AMD group as compared to control group. These diminished activities may be explained by the excess free radical production caused by AMD. Administration of Vit U increased these activities in AMD group. This protective effect of Vit U may be related its antioxidative effects which is reported by Sokmen et al. (2012).

In conclusion, our morphological and biochemical results indicate that Vit U prevents liver toxicity due to the usage of amiodarone through anti-oxidative mechanisms.

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