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Protecting effect of vitamin U against amiodarone-induced hepatic damage via its antioxidative activity

I. B. Turkyilmaz*, R. Yanardag

Department of Chemistry, Faculty of Engineering, Istanbul University-Cerrahpasa , 34320, Avcilar-Istanbul, Turkey

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In this study, we aimed to investigate the protective effect of Vitamin U (Vit U) on amiodarone (AMD)-induced hepatotoxicity. Male Sprague-Dawley rats were grouped as control, Vit U given control (50 mg/kg, by gavage), AMD (100 mg/kg, by gavage) and Vit U given AMD (in same dose and time). AMD and Vit U were given for 7 days. On the 8th day, all animals were sacrificed. Serum aspartate and alanine aminotransferase, alkaline phosphatase activities and total lipid and total bilirubin levels, liver lipid peroxidation and protein carbonyl levels, lactate dehydrogenase, myeloperoxidase, xanthine oxidase, arginase, prolidase, DT-diaphorase activities were found to be increased and liver glutathione levels, paraoxonase and Na^+/K^+-ATPase activities were found to be decreased in AMD group. Administration of Vit U reversed these effects. Based on our results, we may suggest that Vit U has a protective effect on AMD-induced hepatotoxicity in rats.

Keywords: Vitamin U; amiodarone; liver; toxicity; rat

INTRODUCTION

Amiodarone (AMD), 2-butyl-3-(3',5'-diiodo-4-α-diethyl aminoethoxybenzoyl) benzofuran, is a class III antiarrhythmic drug, effective myocardial infarction or congestive heart failure treatment, which has been widely used in medicine [1]. This drug is effective in preventing ventricular and supraventricular tachyarrhythmias [2]. Despite having effective properties on cardiac arrhythmias, AMD is highly lipophilic in nature, has poor availability and also has a long half-life [3]. So these properties make this drug tend to accumulate in many organs and side effects and toxicity occur during therapy. Organs like the liver, lung, kidneys, gastrointestinal and neuromuscular systems make up the list of tissues adversely affected by AMD [4]. AMD-induced hepatotoxicity is characterized by steatosis, enlarged hepatocytes, inflammation, fibrosis and phospholipidosis [5]. In in vivo and in vitro studies, AMD has been shown to generate free radicals that may be involved in the pathogenesis of its toxicity [6].

Vitamin U (Vit U), S-methyl methionine sulphonium chloride, is a methionine derivative. It is reported that Vit U is present in the largest quantity in species belonging to the Brassicaceae family [7]. Vit U is found in cabbage and other green vegetables. This vitamin has a beneficial power action on gastric and intestinal functions [8]. Vit U is reported to have hypolipidemic effect, hepatoprotective, cytoprotective, anti-inflammatory and antidepressant actions, adipocyte differentiating and wound-healing properties [9].

In this study, we aimed to investigate the protective effect of Vit U on AMD-induced hepatotoxicity.

EXPERIMENTAL

Animals and experimental design

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 were used. Application of AMD dose and time were determined as Reasor *et al.* [10]. Vit U (Fluka 64382) dose were administered according to Sokmen *et al.* [9]. 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 administration of AMD (100 mg/kg) (n=8). AMD and Vit U were administered to rats by gavage. On the 8th day, blood samples were taken before sacrification and then all the animals were sacrificed.

* To whom all correspondence should be sent:
E-mail: burchemistry@gmail.com

Biochemical assays

Experiments were made in serum and liver tissues of all groups. Aspartate (AST) and alanine aminotransferase (ALT) activities [11], alkaline phosphatase (ALP) [12], total lipid levels [13], total bilirubin [14] were determined in serum. Liver samples were homogenized. Homogenates were centrifuged. Glutathione (GSH) [15], lipid peroxidation (LPO) [16], protein carbonyl (PC) levels [17], lactate dehydrogenase (LDH) [18], myeloperoxidase (MPO) [19], paraoxonase (PON1) [20], xanthine oxidase (XO) [21], arginase [22], Na^+/K^+-ATPase [23], prolidase [24], DT-diaphorase (DTD) activities [25], and protein content [26] were determined in the supernatant.

Statistical analysis

Biochemical analysis was performed by one-way ANOVA followed by Duncan's Newman-Keuls multiple comparison test. The values are expressed as the mean ± standard deviation (SD). *P* values less than 0.05 were considered to be significant.

RESULTS

AST, ALT and ALP activities ($P < 0.0001$, $P < 0.005$), lipid ($P < 0.005$) and bilirubin levels ($P < 0.05$) were significantly increased in AMD group when we compared to control group. Administration of Vit U reversed these effects in AMD group significantly, respectively ($P < 0.05$, $P < 0.0001$, $P < 0.005$) (Table 1).

Table 1. Serum AST, ALT and ALP activities, total lipid and total bilirubin levels of all groups.

Groups	AST (U/L)*	ALT (U/L)*	ALP (U/L)*	Total Lipid (mg/dL)*	Total Bilirubin (mg/dL)*
Control	21.68 ± 6.15	21.41 ± 8.35	58.08 ± 9.73	135.10 ± 39.69	0.281 ± 0.011
Control + Vit U	38.07 ± 16.76[a]	14.83 ± 8.12[a]	62.63 ± 20.61[a]	149.45 ± 9.57[a]	0.366 ± 0.103[a]
AMD	69.17 ± 14.23[b]	55.63 ± 21.16[d]	216.71 ± 11.13[b]	208.89 ± 24.75[d]	0.358 ± 0.039[f]
AMD + Vit U	38.12 ± 19.72[c]	31.72 ± 10.87[c]	155.15 ± 0.30[e]	159.60 ± 16.98[c]	0.260 ± 0.034[g]

*Mean ± SD, [a]p >0.05 *versus* control group, [b]$p < 0.0001$ *versus* control group, [c]$p < 0.05$ *versus* AMD group, [d]$p < 0.005$ *versus* control group, [e]$p < 0.0001$ *versus* AMD group, [f]$p < 0.05$ *versus* control group, [g]$p < 0.005$ *versus* AMD group.

Table 2. Liver GSH, LPO and PC levels of all groups.

Groups	GSH (nmol GSH/mg protein)*	LPO (nmol MDA/mg protein)*	PC (nmol carbonyl/mg protein)*
Control	14.13 ± 1.39	3.35 ± 0.32	8.34 ± 0.36
Control + Vit U	11.67 ± 2.10[a]	2.83 ± 0.58[a]	8.53 ± 1.85[a]
AMD	6.14 ± 1.79[b]	4.08 ± 0.49[a]	16.69 ± 5.21[e]
AMD + Vit U	8.60 ± 0.67[c]	2.88 ± 0.58[d]	10.39 ± 1.41[d]

*Mean ± SD, [a]p >0.05 versus control group, [b]$p < 0.0001$ versus control group, [c]p >0.05 versus AMD group, [d]$p < 0.05$ versus AMD group, [e]$p < 0.05$ versus control group

Table 3. Liver LDH, MPO, PON1 and XO activities of all groups

Groups	LDH (U/mg protein)*	MPO (U/g tissue)*	PON1 (U/g protein)*	XO (U/g protein)*
Control	13.08 ± 4.01	1.89 ± 0.68	10.83 ± 0.46	0.45 ± 0.06
Control + Vit U	23.08 ± 12.01[a]	2.00 ± 0.56[a]	7.91 ± 2.11[a]	0.34 ± 0.32[a]
AMD	61.52 ± 14.70[b]	3.04 ± 0.60[d]	5.92 ± 2.03[d]	1.24 ± 0.31[d]
AMD + Vit U	37.25 ± 5.55[c]	1.31 ± 0.42[e]	9.25 ± 2.37[c]	0.23 ± 0.10[c]

*Mean ± SD, [a]p >0.05 versus control group, [b]$p < 0.005$ versus control group, [c]$p < 0.05$ versus AMD group, [d]$p < 0.05$ versus control group, [e]$p < 0.005$ versus AMD group

GSH levels were found to be decreased significantly ($P < 0.0001$) and PC levels were found to be increased significantly in AMD group as compared to control group ($P < 0.05$). However, the increasing of LPO levels were not in a significant manner in AMD group when compared to control group ($P > 0.05$). Besides, administration of Vit U decreased LPO and PC levels in a significant manner in AMD group ($P < 0.05$). Decreased GSH levels were found to be increased insignificantly in AMD + Vit U as we compared to AMD group ($P > 0.05$) (Table 2).

Liver LDH, MPO and XO activities were increased and PON1 activity was decreased in AMD group in a significant manner as compared to control group ($P < 0.005$ and $P < 0.05$).

Table 4. The liver arginase, Na^+/K^+-ATPase, prolidase and DT-Diaphorase activities of all groups

Groups	Arginase (μmol urea/mg protein)*	Na^+/K^+- ATPase (nmol Pi/ mgproteinxh)*	Prolidase (U/g protein)*	DT-Diaphorase (μmol/min mg protein)*
Control	469.33 ± 20.81	2.50 ± 0.56	968.18 ± 374.77	179.75 ± 43.54
Control + Vit U	489.08 ± 290.05[a]	1.69 ± 0.43[a]	973.67 ± 571.86[a]	329.02 ± 71.55[c]
AMD	529.00 ± 134.56[a]	1.30 ± 0.52[c]	1884.06 ± 670.39[c]	240.86 ± 14.21[a]
AMD + Vit U	178.56 ± 98.51[b]	4.30 ± 1.05[d]	937.14 ± 480.42[d]	145.13 ± 20.92[d]

*Mean ± SD, [a]p >0.05 *versus* control group, [b]$p < 0.005$ *versus* AMD group, [c]$p < 0.05$ *versus* control group, [d]$p < 0.05$ *versus* AMD group; Na^+/K^+- ATPase: sodium potassium ATPase

When we applied Vit U to AMD group, we determined decreased activities for LDH, MPO, XO and increased activity for PON1 when we compared to AMD group ($P < 0.05$ and $P < 0.005$) (Table 3). The arginase and DTD activities were found to be increased insignificantly (P>0.05) while the increasing activity of prolidase was in a significant manner in AMD group as we compared to control group ($P < 0.05$). In addition to this, Na^+/K^+-ATPase activity was also found to be significantly decreased in AMD group as compared to control group ($P < 0.05$). Administration of Vit U reversed these activities in AMD group in a significant manner ($P < 0.005$ and $P < 0.05$) (Table 4).

DISCUSSION

The liver is a vital organ that regulates various biochemical processes and plays an important role in the metabolism of carbohydrates, lipids and proteins [27]. It also participates actively in the elimination and detoxification of drugs and in metabolic homeostasis during which a number of reactive oxygen species (ROS) generating reactions are involved [28]. Thus, its metabolic role and importance make the liver more vulnerable. Finding a solution for drug and chemical induced hepatotoxicity by using safer therapeutic agents became crucial.

Serum aminotransferases and phosphatases are both markers and gold standard enzymes which are usually used for determining the liver injury [29]. Elevated levels of the activities of these enzymes indicate cellular leakage and function loss of cell membrane. Various researchers reported elevated AST, ALT and ALP activities in patients who are treated with AMD [30]. In accordance with these results, we found elevated activities of AST, ALT and ALP in the AMD group. Administration of Vit U to the AMD group significantly decreased these activities. Vit U may have used its cellular repair function while decreasing these activities [7]. We may conclude that Vit U protects the liver against AMD induced injury.

AMD is a phospholipase inhibitor and causes lipid accumulation in the liver. The increased levels of serum lipid profile in AMD treated rats have been shown in literature [31]. In our study, we recorded increased levels of total lipid in AMD group. Besides, Sokmen *et al.* showed that Vit U has a hypolipidemic effect on valproic acid induced hepatotoxicity [9]. Seri *et al.* reported that Vit U has a hypolipidemic metabolism because of initiating an acceleration of fetal excretion of lipid molecules and its acidic metabolites [32]. In the light of these reports, we found decreasing levels of total lipid in AMD+Vit U group.

In this study, AMD treatment produced significantly increase in bilirubin as well as ALP activity. These results demonstrate that the toxin included insult to the liver could also precipitate biliary obstruction resulting in mild hyperbilirubinemia in addition to hepatocellular necrosis. But, administration of Vit U reversed this increase in the AMD group. This reverse effect of Vit U may be associated with protective effect on hepatocellular damage.

Cell culture hepatotoxicity studies done with AMD revealed that AMD decreases GSH levels and increases LPO levels [33]. These levels in AMD toxicity can be explained with this approach: AMD is also called as a cationic amphiphilic drug because of its nature. This nature provides it an elevation of substrates for LPO and also another elevation for ROS that oxidize these substrates. In addition to that GSH protects the membrane lipids from peroxidation and an increase in LPO levels means that the oxidation of reduced GSH by LPO products. In our study, we observed decreased GSH and increased LPO levels in AMD group compared to the control group in parallel to this approach. Administration of Vit U reversed these effects. The effect of Vit U on these levels may be directly elevation of GSH levels by the reversible effect on the sulfhydryl compounds. So elevation of GSH levels by Vit U indicate decreased LPO levels indirectly.

Curtis *et al.* reported that some liver pathologies like alcoholic liver disease, nonalcoholic fatty liver disease and steatohepatitis may involve PC formation [34]. In our study, we observed elevated

PC levels in AMD group. Administration of Vit U reversed this effect in AMD+Vit U group. The reversing effect of Vit U may be explained by this approach. A hydrophilic nature provides an easy interaction of sulfhydryl groups with injured protein structure [35]. Vit U can be dissolved in aqueous media which means it has a hydrophilic nature. By having this advantage, sulfhydryl group of Vit U shows a protective effect by repairing tissue damage.

An increase in serum and tissue LDH activities in AMD toxicity was reported in literature [36]. In our study, an increase in LDH activity was observed in the AMD group. Administration of Vit U decreased these effects in the AMD group. We may suggest that decreasing LDH activity and in turn decreasing levels of ROS are associated with antioxidant property of Vit U.

Increased levels of free radicals activate neutrophils and then activated neutrophils secrete MPO more than ever in the region of injured tissue [37]. PON1 has a thiol group at its active site and Navab *et al.* reported that PON1 synthesis is reduced under in vivo and in vitro oxidative stress conditions [38]. Increased activities of XO mean there is an injury in purine metabolism which may be due to ROS. We measured increased MPO and XO activities and decreased PON1 activity in AMD group. Administration of Vit U reversed these enzyme activities in AMD group. Salim reported that the substances which have sulfhydryl groups bind oxyradicals and this binding can enhance the tissue healing process by removing the harmful agents [39]. So as being a sulphur containing substance, Vit U may have shown its antioxidant effect by reversing MPO, PON1 and XO levels in AMD treated groups.

Abdel-Azeem *et al.* reported that arginase activity increases in chemical-induced hepatotoxicity model [40]. In the present study, we observed elevated arginase activity in the AMD group. In the AMD+Vit U group, we observed that the activity of this enzyme decreased compared to the AMD group. This reductive effect may be associated with antioxidant properties of Vit U.

Decreased Na^+/K^+-ATPase was reported in AMD induced small intestinal toxicity in rats [41]. In this study, a significant decrease was observed in the AMD group. Vit U increased this enzyme activity in AMD group. The reversing effect of Vit U may result due to two reasons. One of them is a probability of indirectly providing thiol group because Vit U is converted to methionine, later cysteine through many steps in vivo [42]. Second reason may be that Vit U fixes the membrane stability property [7].

An increase in prolidase activity was associated with many diseases in various studies including liver diseases [43]. In the present study, a significant increase was observed in the AMD group. However, a significant decrease was determined in the AMD+Vit U group compared to AMD group.

DTD activity was found to be increased in chemical-induced hepatotoxicity models [44]. In the present study, we observed a significant increase of DTD activity in the AMD group. In the AMD+Vit U group, DTD activity was found to be significantly decreased. This may be related to radical scavenging activity of Vit U.

Our results indicate that AMD-induced liver damage is associated with increased oxidative stress. Our results have important implications for the treatment of AMD-induced hepatotoxicity. Sulfhydryl compounds have been described to possess antioxidant, anticancer, antihepatotoxic and neurotropic properties [45]. Sulfur-methyl-methionine (Vit U) is one of the sulfhydryl compounds that have been reported to provide various biological functions including inhibition of free radical production, gastric motility disorder, vasolateral pressure and direct cellular damage [46].

In conclusion, our present results demonstrated the amendatory potential of Vit U against AMD-induced hepatocellular changes, cholestasis, hyperlipidemia and oxidative stress induced by AMD due to its antioxidant, free radical scavenger and membrane-stabilizing properties. The protective effect of Vit U may be due to its sulfhydryl group. This is the first study showing a protective effect of Vit U against AMD – induced hepatotoxicity. In heart patients receiving AMD therapy, our findings may be helpful for the prevention of these side effects of AMD before treatment or other conditions.

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