Effects of combined treatment of amiodarone and vitamin U on rat gingiva

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Amiodarone, the pharmaceutical drug used for the treatment of arrhythmias, is known to cause many side effects. S-Methylmethionine sulfonium chloride is a derivative of methionine and it is widely referred to as vitamin U (Vit U). In the peer-reviewed literature, there is no study reported on the protective effects of Vit U against amiodarone’s side effects on gingiva. In this study, we investigated the oxidative and inflammatory effect of amiodarone or putative protective role of Vit U on gingiva tissue. Rats were randomly divided into four groups as follows: control group: rats received corn oil; control+Vit U treated group (50 mg/kg/day); amiodarone group (100 mg/kg/day) and amiodarone+Vit U treated group (in same dose). Amiodarone and Vit U were given orally for 7 days. Vit U was given 1 h prior to the administration of amiodarone. Gingival tissues were taken from animals, homogenized in 0.9% NaCl. Lipid peroxidation and sialic acid levels were increased significantly, glutathione levels and the activity of superoxide dismutase following the oral administration of amiodarone. These results demonstrate that administration of Vit U may have protective effects on gingiva in amiodarone treatment by decreasing oxidative stress.

Keywords: Antiarrhythmic drug, Inflammation, S-methylmethionine sulfonium chloride, Oral tissue, Oxidative stress

Amiodarone is a widely used effective anti-arrhythmic drug. Despite its beneficial properties, amiodarone has several side effects including hepatotoxicity, steatosis, hepatitis, liver and pancreas fibrosis, and thyroid dysfunction¹-³, but its toxic mechanism has not been well studied yet. It has been suggested that oxidative stress might play a significant role in the development of such diseases due to tissue injury by free radical formation⁴. Formation of oxygen radicals leading to inflammatory process, involves inflammatory mediators including neutrophil-derived active oxygen species and free radicals; hydrogen peroxide, superoxide and the hydroxyl radicals. Oxidative stress and lipid peroxidation have been shown to be the main pathogenic mechanisms of amiodarone toxicity and tissue damage⁵.

Vitamin U (Vit U or S-methyl-methionine sulfonium chloride) is a vitamin-like active substance and fresh cabbage and cabbage juice are the richest sources of it. Vit U has beneficial power actions on gastric and intestinal functions, especially effective for treating peptic ulcers⁶. In previous studies, antiulcer properties, anti-inflammatory action, reduction of blood lipid, anti-depressant action and cytoprotective effects of Vit U have been demonstrated⁶-⁹.

There are conflicting reports regarding the role of amiodarone in the formation of free radical induced oxidative stress. Thus, in this study, we investigated the possible oxidative stress caused by amiodarone and the effect of Vit U on the protection of tissue damage due to free radical formation by amiodarone in gingiva tissue in a rat experimental model. For that purpose, we studied alterations in the lipid peroxidation, glutathione and sialic acid levels and the activity of superoxide dismutase following the oral administration of amiodarone and Vit U. To the best of our knowledge, this study describes for the first time, the protective effects of Vit U against amiodarone induced tissue damage and inflammation.

Materials and Methods

All experimental protocols were approved by the Marmara University Animal Care and Use Committee (No. 135.2013.mar). Thirty three Sprague Dawley male rats were randomly divided into four groups as follows: Control group received corn oil (n=6), control+Vit U treated group (50 mg/kg/day, n=7), amiodarone group (100 mg/kg/day, n=12) and amiodarone+Vit U treated group (in same dose, n=8). Amiodarone and Vit U were given orally for 7 days. Vit U was given 1 h prior to the administration of amiodarone. All the animals were fasted overnight and on the 8th day, they were sacrificed under anesthesia. Gingival tissues were taken from the animals, homogenized in 0.9% NaCl.

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and 10% (w/v) homogenates were prepared. Total protein, lipid peroxidation, glutathione, sialic acid levels and superoxide dismutase activities were determined in the gingiva homogenates. Experiments were carried out at the temperatures given in the procedures.

Determination of proteins
Total protein levels of gingival tissues were estimated by the method of Lowry. Briefly, proteins are reacted with copper ions in alkali medium and reduced by Folin reaction. The absorbance of the blue colored product was evaluated at a wavelength of 500 nm. Bovine serum albumin was used as a standard and total protein levels of gingival tissues were used to express the results of the parameters per protein.

Determination of lipid peroxidation
Lipid peroxidation (LPO) in gingival tissues were evaluated by the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA), according to the method described by Yagi. LPO was expressed in terms of MDA equivalents using the extinction coefficient of 1.56×10^5 M^-1cm^-1. The results are expressed as nmol MDA/mg protein.

Determination of glutathione
Glutathione (GSH) levels were determined in gingival tissues according to the method of Beutler. Metaphosphoric acid was used for the protein precipitation and 5-5-dithiobis-2-nitro benzoic acid for colour development. The extinction coefficient of 1.36×10^4 M^-1cm^-1 was used for the calculation of GSH levels. The results are expressed in mg GSH/g protein.

Determination of sialic acid
Sialic acid (SA) levels of gingival tissues were determined by the method of Warren. The gingiva samples were incubated at 80°C for 1 h in diluted sulfuric acid to liberate the bound SA. This method consisted of oxidizing SA with periodate, terminated by the addition of arsenite and then adding thiobarbituric acid. This reaction resulted in the formation of a red-colored substance extracted in cyclohexanone. The absorbance of the sample was read by spectrophotometer at 549 nm. The results are expressed in mg SA/g protein.

Determination of superoxide dismutase activity
Superoxide dismutase (SOD) activities of gingival tissues were determined by the method which is based on the ability to increase the effect of riboflavin-sensitized photo-oxidation of o-dianisidine. The activity of SOD is generated by illuminating the reaction mixture, which contains o-dianisidine 2-HCl and riboflavin by light from a fluorescent lamp. The oxidation of o-dianisidine, which is sensitized by riboflavin, is enhanced by SOD and the increase is linearly dependent on the SOD concentration. The absorbance of the colored product is quantified spectrophotometrically at a wavelength of 460 nm. Absorbances of gingiva samples were measured at 0 and 8 min of illumination, the net absorbance was calculated, and the results were expressed in SOD U/mg protein.

Statistics
All the statistical analyses were performed with the Graphpad Prism 5.0 (Graphpad Software, San Diego, California, USA) and the data are presented as the mean ± standard deviation. The differences between the values of the groups were tested with Kruskal-Wallis test followed by Mann Whitney U test. Values of P < 0.05 were regarded as significant.

Results and Discussion
The LPO levels of the experimental groups and controls are given in Fig 1A. The LPO levels in gingival tissues of amiodarone group increased significantly (P < 0.001) as compared with the controls and also increased significantly (P < 0.001) as compared with control+Vit U group. A significant decrease (P < 0.001) was detected in amiodarone+Vit U group as compared with amiodarone group (Fig 1A).

As shown in Fig 1B, a significant decrease was observed in the gingival GSH levels of amiodarone group as compared with the controls (P < 0.01) and compared with control+Vit U group (P < 0.05). Also, the GSH levels increased significantly (P < 0.05) in amiodarone+Vit U group as compared with amiodarone group (Fig 1B).

The SA levels of amiodarone group increased significantly (P<0.001) as compared with controls and also increased significantly (P < 0.001) as compared with control+Vit U group (Fig 1C). A significant decrease was observed in the SA levels (P < 0.001) of amiodarone+Vit U group as compared with amiodarone group (Fig 1C).

As depicted in Fig 1D, the SOD activity of amiodarone group decreased significantly (P < 0.01) as compared with the controls and also decreased significantly (P < 0.001) as compared with control+Vit U group. Also, a significant decrease was observed in amiodarone+Vit U group compared with control+...
Values are given as mean ± standard deviation. MDA: malondialdehyde, A: amiodarone, U: vitamin U, C: control group, C+U: rats received vitamin U only, A: rats received A only, A+U: rats received A and U. ** P <0.01, *** P <0.001 significantly different from group C; * P <0.05, ** P <0.01, *** P <0.001 significantly different from group C+U; and ^P < 0.05, △△△P <0.001 significantly different from group A]

Vit U group (P <0.001). A significant increase in the SOD activity was observed in amiodarone+Vit U group as compared with amiodarone group (P <0.05, Fig 1D).

Amiodarone is one of the most effective and frequently used anti-arrhythmic drugs. There are conflicting reports about the side effects of amiodarone, and the toxicity mechanism is not clear yet. The administration of amiodarone is limited by its toxic side effects. It was reported that amiodarone has a highly lipophilic compound and suggested a high affinity of this drug to the plasma membranes causing interaction of amiodarone with the membrane lipids and effects on the free radical chain reactions. Also, it was reported in previous experimental studies, that amiodarone increases non-esterified fatty acids and low- and high- density lipoprotein cholesterol levels in mice. These reactions may be the reason for increased tendency of tissue toxicity and injury in amiodarone treatment.

The present study was conducted to investigate the deleterious and inflammatory effects of amiodarone and the protectivity of Vit U by the experimental determination of some key biochemical parameters such as LPO, GSH, SA, and SOD on rat gingival tissues. MDA, the end product of LPO, was examined as a biomarker of oxidative stress and cellular damage, in the present study. Significantly increased levels of MDA were observed in amiodarone group, which means amiodarone causes elevated oxidative stress in the cells.

SA, acylated derivatives of neuraminic acid, is considered to be a marker for several pathologic conditions and increased SA levels have been reported during inflammatory processes, likely resulting from the increased levels of sialylated acute-phase glycoproteins. Earlier studies suggest that oxidative stress causes induction of mucin synthesis and it was reported that SA is a potential target for superoxide and other related reactive oxygen species by its glycosidic linkage.

Oxygen derived free radicals are highly reactive and have a toxic potential for living organisms. Their formation is not completely deleterious to the cells. Enzymatic and non-enzymatic antioxidants react on these oxygen species and they are neutralized by the endogenous enzymes and free radical scavengers. The antioxidant enzyme, SOD catalyzes the dismutation of superoxide anions to hydrogen peroxide and the GSH-related enzymes preserve GSH status and play critical role(s) in the anti-oxidant defense system by ensuring the degradation of these species to less harmful compounds.

In the present study, we established that amiodarone decreases SOD activity and GSH level. Our results are in accordance with the previous studies showing the deleterious effect of amiodarone is oxidative damage. The increase in hydrogen peroxide due excessive free radical production may be the reason of diminished GSH level and SOD activity in the cells.

Although amiodarone has clearly demonstrated the adverse toxic effects, there are some reports suggesting anti-inflammatory and antioxidative properties or lack of effect on the activity of antioxidant enzymes. The varying doses in the administration of amiodarone may be the potential reasons of different results in the previous studies. For example, amiodarone was administered at doses ranging from 12 to 600 mg/kg body weight in some experimental studies, whereas the recommended dosages for the long-term treatment in human range from 200 to 400 mg per day. Also, different experimental conditions which do not clearly reflect the in vivo exposure profiles and the findings resulted from in vitro experiments or intraperitoneal administration of a short-term
high dose of amiodarone can be the cause of these varying results26.

To the best of our knowledge, this study describes for the first time, the protective effects of Vit U against amiodarone induced gingiva tissue damage. The decreased LPO level and increased GSH activity were observed in our amiodarone group treated with Vit U. Also, we found decreased SA level in amiodarone group treated with Vit U, which may be a reason of suppressed inflammation. Based on these results, we suggest that Vit U protects the gingiva tissue against oxidative stress mediated injury by scavenging oxygen free radicals. In view of the increasing evidence that free radical-mediated tissue injury is implicated in the pathogenesis of various disorders and Vit U may be useful for preventing tissue damage during the treatment. These results may influence the clinical applications and toxicity management of amiodarone in the treatment of patients with gingiva.

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