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Amiodarone has anti-inflammatory and anti-oxidative properties: An experimental study in rats with carrageenan-induced paw edema

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Abstract

Amiodarone is a widely used anti-arrhythmic agent. We have investigated alterations in the glutathione (GSH) level and the activities of anti-oxidative enzymes (superoxide dismutase, catalase, glutathione *s*-transferase and glutathione reductase) and myeloperoxidase, as marker of acute inflammation, following oral administration of amiodarone and diclofenac in rats with carrageenan-induced paw edema. In the present study, we found that 1) Amiodarone reduced the development of carrageenan-induced paw edema, to a greater degree than diclofenac; 2) Amiodarone and diclofenac alleviated increases in the activities of catalase and glutathione *s*-transferase enzymes resulting from edema; 3) Amiodarone and diclofenac ameliorated depressions in the GSH level and the activities of superoxide dismutase and glutathione reductase enzymes caused by carrageenan injection; and 4) All doses of amiodarone and diclofenac caused an amplification in myeloperoxidase activity resulting from induced paw edema. These results suggest that the anti-inflammatory effect of amiodarone on carrageenan-induced acute inflammation can be attributed to its ameliorating effect on the oxidative damage.

Keywords: Amiodarone; Carrageenan; Acute inflammation; Myeloperoxidase; Anti-oxidant enzyme

1. Introduction

Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow (Ialenti et al., 1995). Several experimental models of paw edema have been described. Carrageenan-induced paw edema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation (Di Rosa and Willoughby, 1971), whereas prostaglandins are detectable in the late phase of inflammation (Salvemini et al., 1996a). It is well known that the acute inflammatory process, in which vascular permeability increases and leukocyte migration occurs, involves inflammation mediators including neutrophil-derived active oxygen species and free radicals, such as hydrogen peroxide, superoxide and the hydroxyl radical (Babior et al., 1973; Da Motta et al., 1994; Salvemini et al., 1996b) nitric oxide, prostaglandins and cytokines (Gualillo et al., 2001). Polymorphonuclear leukocytes (Jain et al., 2001) which are the first cells to arrive at the inflammatory site in the body, release free oxygen radicals (O_2^-) and free hydroxyl radicals (HO^-) (McCord and Roy, 1982). On the other hand, in a lot of physiological functions of the body and the pathogenesis of inflammation, ion exchanges between intracellular and extracellular areas have been identified. It was reported that increased intracellular calcium ion stimulates inflammation events and calcium channel blocker drugs diminishes these events (Briukhanov et al., 1994).

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Amiodarone {2-butyl-3-(3′;5′ diiodo-4′α-diethyl-amino-ethoxy-benzoyl)-benzofuran} is a multiple ion (Ca^{++}, Na^{+}, K^{+}) channel blocker drug and is also a non-competitive α- and β-blocker in cardiac cells. It is an effective anti-arrhythmic and is used to treat a wide variety of ventricular and supraventricular tachyarrhythmias (Mason, 1987) especially considered the gold standard for the pharmacological treatment of life-threatening ventricular tachyarrhythmias in high-risk patients (Gonzalez et al., 1998). This drug can also modulate thyroid function and phospholipid metabolism (Vrobel et al., 1989) and inhibits phospholipase A1, A2 and phospholipase C (Wilson et al., 1993). Phospholipase enzyme has played in the first step in production of inflammatory mediators in arachidonic acid metabolism.

Although amiodarone has inhibited the phospholipase enzyme and has blocked the calcium channels and also reported to exert anti-oxidant activity as another beneficial effect (Ide et al., 1999) the anti-inflammatory activity of amiodarone (Fig. 1) has not been elucidated to date. The purpose of the present study was to investigate the anti-inflammatory effect of amiodarone on carrageenan-induced paw edema in rats and to determine the relationship between its anti-inflammatory mechanism exerting its effects on the anti-oxidant system and myeloperoxidase activity in paw tissue.

2. Materials and methods

2.1. Animals

A total of 36 male, Sprague–Dawley rats, weighing 180–190 g, were provided by the Experimental Animal Laboratory of Ataturk University, Medicinal Faculty and the Department of Pharmacology. The animals were grouped before the experiments and kept under standard conditions (Bowd, 1998). Animals were assigned randomly to the control groups receiving either carrageenan (0.1 mL of 1% per animal) or water and experimental groups receiving carrageenan plus amiodarone (25, 50 and 100 mg/kg) or diclofenac (25 mg/kg). The experiments were conducted according to the ethical norms approved by the Ethic Committee of Experimental Animal Teaching and Researcher Center (No: B.30.2.ATA.0.70/71).

2.2. Chemicals

All chemicals for laboratory experimentation were purchased from Sigma Chemical (Germany). Amiodarone was obtained from Sanofi-Turkey and diclofenac sodium from Fako-Turkey.

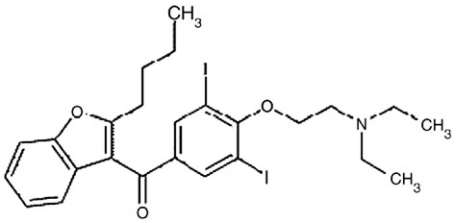

Fig. 1. Chemical structure of amiodarone.

2.3. Carrageenan-induced paw edema in rats

In these experiments, the effect of amiodarone and diclofenac on carrageenan-induced paw edema in rats was investigated (Moncada et al., 1973; Suleyman et al., 2003). Briefly, doses of amiodarone (25, 50 and 100 mg/kg) were administered orally to rats. The paw volumes of the animals were calculated plethysmometrically and 0.1 mL of 1% carrageenan was injected into the hind paw of each animal 1 h after the last dose. The change in paw volumes was determined by six replicate measurements carried out at 60 min intervals by plethysmometry. The anti-inflammatory potency of amiodarone was determined by comparing the results with those obtained from animals receiving equal volumes of diclofenac (25 mg/kg) and carrageenan (control).

2.4. Biochemical investigation of carrageenan-induced paw edema in rats

After the plethysmometric analyses, catalase, glutathione *s*-transferase, glutathione reductase, superoxide dismutase and myeloperoxidase enzyme activities and the GSH levels were determined in rats with paw edema. To prepare the tissue homogenates, paw edema tissues were ground with liquid nitrogen in a mortar. The ground tissues (0.5 g each) were then treated with 4.5 mL of appropriate buffer. The mixtures were homogenized on ice using an ultra-turrax homogenizer for 15 min. Homogenates were filtered and centrifuged using a refrigerated centrifuge at 4 °C and the supernatants used for the determination of enzyme activities. All assays were carried out at room temperature in triplicate.

2.5. Biochemical estimations

2.5.1. Superoxide dismutase activity

As outlined by Sun et al. (1988), superoxide dismutase estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitro blue tetrazolium to form formazan dye. Superoxide dismutase activity was then measured at 560 nm by the degree of inhibition of this reaction, and expressed as millimoles per minute per milligram tissue (mmol/min/mg tissue).

2.5.2. Catalase activity

Decomposition of H_2O_2 in the presence of catalase was measured at 240 nm (Aebi, 1984). Catalase activity was defined as the amount of enzyme required to decompose 1 nmol of H_2O_2 per minute, at 25 °C and pH 7.8. Results are expressed as millimoles per minute per milligram tissue (mmol/min/mg tissue).

2.5.3. Glutathione reductase activity

Glutathione reductase activity was determined spectrophotometrically by measuring the rate of NADPH oxidation at 340 nm (Carlberg and Mannervik, 1985). Results are expressed as the amount of enzyme that catalyzes the oxidation of 1 μmol of NADPH per minute per milligram tissue (μmol/min/mg tissue).

Table 1
Effects of amiodarone and diclofenac on carrageenan-induced paw edema in rats

Treatment	N	Dose mg/kg body wt.	Paw volume before inflammation (mL)	Difference between paw volumes (mL) at 4th hour	Anti-inflammatory effect. Inhibition %
Amiodarone	6	25	0.59	0.18±0.05[b]	65.3
Amiodarone	6	50	0.64	0.15±0.07[b]	71.2
Amiodarone	6	100	0.68	0.07±0.06[b]	86.5
Diclofenac	6	25	0.51	0.30±0.02[a]	42.3
Carrageenan (control)	6	–	0.55	0.52±0.05	–

Groups treated with three doses of amiodarone and one with diclofenac were compared with the carrageenan group. [a]Significant at $p<0.05$; [b]Significant at $p<0.01$.

2.5.4. Myeloperoxidase activity

Myeloperoxidase activity was measured according to the modified method of Bradley et al. (1982). The homogenized samples were frozen and thawed three times, and centrifuged at 1500 *g* for 10 min at 4 °C. Myeloperoxidase activity in the supernatant was determined by adding 100 mL of the supernatant to 1.9 mL of 10 mmol/L phosphate buffer (pH 6.0) and 1 mL of 1.5 mmol/L *o*-dianisidine hydrochloride containing 0.0005% (wt/vol) hydrogen peroxide. The changes in absorbance at 450 nm of each sample were recorded on a UV–vis spectrophotometer. Myeloperoxidase activity in tissues was expressed as micromoles per minute per milligram tissue (μmol/min/mg tissue).

2.5.5. Glutathione s-transferase activity

Total glutathione *s*-transferase activity was determined as described by Habig and Jakoby (1981). Briefly, the enzyme activity was assayed spectrophotometrically at 340 nm in a 4 ml cuvette containing 0.1 M PBS (pH 6.5), 30 mM glutathione, 30 mM 1-chloro-2,6-dinitrobenzene and tissue homogenate. Enzyme activity was expressed as nanomoles per minute per milligram tissue (nmol/min/mg tissue).

2.5.6. Total glutathione (GSH) determination

The amount of GSH in the paw edema was measured according to the method of Sedlak and Lindsay (1968). The paw edema was collected by scraping, weighed, and homogenized in 2 mL of 50 mM Tris–HCl buffer containing 20 mM EDTA and 0.2 M sucrose, pH 7.5. The homogenate was centrifuged. After centrifugation at 4200 rpm for 40 min at 4 °C, the supernatant was used to determine GSH using 5,5-dithiobis(2-nitrobenzoic acid). Absorbance was measured at 412 nm using a spectrophotometer. The results of the GSH level in the rat paws were expressed as nanomoles per milligram tissue (nmol/min/mg tissue).

2.6. Statistical analyses

Data of enzyme activity and inflammation score were subjected to one-way ANOVA, with the presence of negative and positive controls, by using SPSS 11.0 software. Differences between groups were attained using LSD option and significance was declared at $P<0.05$ and $P<0.01$.

3. Results

3.1. Carrageenan-induced paw edema

Intraplantar injection of carrageenan in rats led to a quadratic and time-dependent increase in paw volume (Table 1 and Fig. 2). The increase in paw volume was observed at 1 h and was maximal (1.07 mL) at 4 h after administration. However, carrageenan-induced paw edema was significantly reduced in a dose-dependent manner by treatment with amiodarone at 1, 2, 3, 4 and 5 h after injection of carrageenan (Fig. 2). 25, 50 and 100 mg/kg doses of amiodarone significantly ($P<0.01$) reduced carrageenan-induced paw edema by 65.3, 71.2 and 86.5%, respectively, at the fourth hour (Table 1). Diclofenac (25 mg/kg) also significantly reduced the edema by 42.3%. The paw volume increased by 0.52 mL in the control group relative to the baseline values, while increases of 0.18 mL, 0.15 mL, 0.07 mL and 0.30 mL were observed in the groups given amiodarone 25, 50, and 100 mg/kg and diclofenac, respectively. The present results show that diclofenac and all doses of amiodarone (Fig. 2) had a significant anti-inflammatory effect on the paw edema caused by carrageenan. The anti-inflammatory effect of amiodarone was also stronger than that of diclofenac, which inhibits prostaglandin synthesis by cyclooxygenase, non-selectively inhibiting both cyclooxygenase-1 and cyclooxygenase-2 isoforms (Shield, 1998).

3.2. Comparison of enzymatic activities and glutathione level in rat paw tissues

In order to explore the effects of anti-oxidant defenses on the inflammation process in all paw tissues, the anti-oxidant levels (superoxide dismutase, catalase, glutathione reductase and GSH) were evaluated. The results are presented in Tables 2 and 3. Table 2 shows that superoxide dismutase for carrageenan-injected groups was lower and catalase level was higher than those for the healthy (intact) rat group. However, compared with catalase and superoxide dismutase levels in amiodarone and diclofenac-administrated paw tissues, the opposite results were

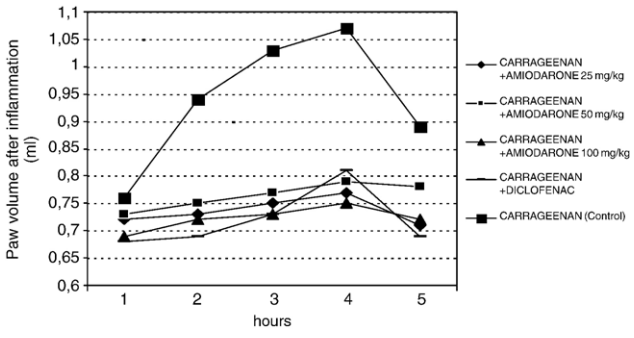

Fig. 2. Effects of different doses of amiodarone and diclofenac on carrageenan-induced edema of rat paw as measured by volume at various time intervals after treatment.

Table 2
Effects of amiodarone and diclofenac on certain enzyme activities in carrageenan-induced edema in paws (5th hour) of rats

Treatment	N	Dose mg/kg body wt.	Catalase (mmol/min/mg tissue)	Superoxide dismutase (mmol/min/mg tissue)
Amiodarone	6	25	93.9±1.5[a]	125.2±0.3[a]
Amiodarone	6	50	85.7±1.0[a]	126.3±0.4[a]
Amiodarone	6	100	80.4±0.4[a]	130.9±0.4[a]
Diclofenac	6	25	82.4±0.3[a]	127.4±0.2[a]
Carrageenan (control)	6	–	105.8±0.8[a]	120.5±0.4[a]
Healthy (intact)	6	–	87.8±0.9	133.6±0.6

The measurements were calculated from 3 replicates. Three doses of amiodarone and a diclofenac-treated group were compared with the carrageenan group. Carrageenan group was compared with the healthy group. [a]Significant at $p<0.05$.

found for the levels of catalase and superoxide dismutase activities in carrageenan-injected tissues. In contrast to carrageenan-injected paws, all doses of amiodarone and diclofenac increased superoxide dismutase activity ($P<0.05$) and decreased catalase activity ($P<0.05$).

Table 3 shows that the activity of glutathione *s*-transferase was increased by carrageenan. Glutathione *s*-transferase activity decreased on administration of 50 and 100 mg amiodarone per kg ($P<0.05$), while the activity of glutathione reductase elevated by carrageenan was ameliorated more by 100 mg amiodarone per kg ($P<0.05$). Likewise, diclofenac and all doses of amiodarone had an increasing effect ($P<0.05$) on level of GSH alleviated by carrageenan (Table 3).

In the present study, the changes of myeloperoxidase activity in paw tissues, an index of neutrophil infiltration into inflammation tissues, were also determined (Fig. 3). As can be seen from this figure, the injection of carrageenan increased myeloperoxidase activity in comparison to healthy (intact) rat tissues. The administration of diclofenac and all doses of amiodarone significantly increased the level of myeloperoxidase activity even further ($P<0.05$).

Table 3
Effects of amiodarone and diclofenac on changes in glutathione level and glutathione reductase enzyme in carrageenan-induced paw edema (5th hour) in rats

Treatment	N	Dose mg/kg body wt.	Glutathione *s*-transferase (μmol/min/mg tissue)	Glutathione reductase (μmol/min/mg tissue)	GSH (nmol/mg tissue)
Amiodarone	6	25	13.77±0.31	20.4±0.5	3.12±0.02
Amiodarone	6	50	12.77±0.15[a]	21.4±0.7	3.34±0.1[a]
Amiodarone	6	100	11.50±0.60[a]	23.2±0.3[a]	3.82±0.1[a]
Diclofenac	6	25	13.87±0.40	18.8±0.4[a]	3.34±0.04[a]
Carrageenan (control)	6	–	14.43±0.86[a]	21.4±0.4[a]	3.02±0.05[a]
Healthy (intact)	6	–	13.30±0.46	19.5±0.5	3.46±0.02

The measurements were calculated from 3 replicates. Three doses of amiodarone and diclofenac-treated groups were compared with the carrageenan group. Carrageenan group was compared with the healthy group. [a]Significant at $p<0.05$.

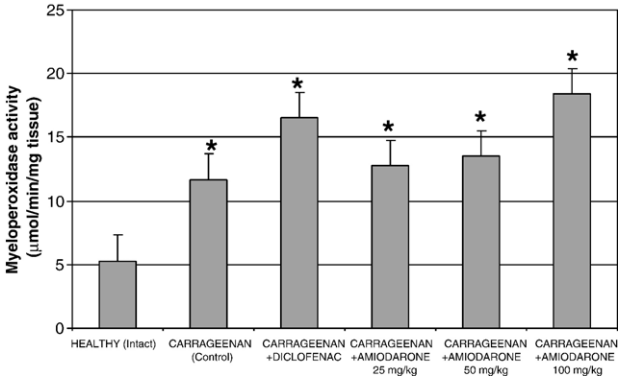

Fig. 3. Effects of amiodarone and diclofenac on changes in activity of myeloperoxidase enzyme in carrageenan-induced paw edema (5th hour) in rats. The measurements were calculated from 3 replicates. Three doses of amiodarone and diclofenac-treated groups were compared with the carrageenan group. Carrageenan group was compared with the healthy (intact) group. *Significant at $p<0.05$.

4. Discussion

Inflammatory processes are the physiological response of the organism to different stimuli such as trauma, infections or immunological mechanisms. The arachidonic acid cascade is highly activated during inflammation, resulting in the formation of eicosanoids, and it is mediated by cyclooxygenase and 5-lipoxygenase enzymes (Heller et al., 1998). These complex inflammatory reactions involve the release of a wide variety of inflammatory mediators *i.e.* prostaglandins, thromboxanes and leukotrienes. Although drugs with a central mechanism of antipyretic and anti-inflammatory action are known to down-regulate fever and inflammation, the role of anti-oxidant mechanism pathway in mediating the action of such agents has not yet been elucidated. The inflammation consists of two phases. It appears that the early phase is related to the production of histamine, 5-hydroxytryptamin, bradykinin and cyclooxygenase products, while the delayed phase has been linked to neutrophil infiltration, as well as to the continuing production of arachidonic metabolites (Salvemini et al., 1996b).

In the presented work, the anti-inflammatory effects of amiodarone, a highly effective anti-arrhythmic agent, were investigated. The effect of amiodarone on the acute phase of inflammation was observed in the carrageenan-induced paw edema test and its anti-inflammatory potency was compared to that of the cyclooxygenase-1 and cyclooxygenase-2 non-selective anti-inflammatory drug, diclofenac (Shield, 1998).

In our experiment, the mean paw volume of the control group reached its peak at the fourth hour. Carrageenan-induced rat paw edema is a suitable experimental animal model for evaluating the anti-inflammatory effect of chemical products and it is believed to be biphasic (Winter et al., 1962).,The first phase (1 h) involves the release of histamine and serotonin and the second phase (over 1 h) is due to the release of prostaglandin-like substances. Based on this, the second phase

may be explained by an inhibition of cyclooxygenase or exerting of anti-oxidative properties (Boughton-Smith et al., 1999; Salvemini et al., 1996b). In order to explore the effects of anti-oxidant defenses on the acute inflammation process, in all paw tissues, the anti-oxidant levels (glutathione *s*-transferase, glutathione reductase, catalase, myeloperoxidase and glutathione level) were evaluated. In accordance to these data, the mean paw volume in groups receiving carrageenan started to decrease at the fourth hour (the peak inflammatory response). 25, 50 and 100 mg/kg doses of amiodarone showed anti-inflammatory effects at all hours after carrageenan injection. But the most significant anti-inflammatory response occurred at the fourth hour: reduction in paw edema by 65.3, 71.2 and 86.5%, respectively, for 25, 50 and 100 mg/kg doses, at the fourth hour (Table 1). Diclofenac, which significantly reduced the increase in paw volume by 42.3%, showed a smaller effect in this model of inflammation.

The glutathione *s*-transferases (E.C. 2.5.1.18) are a multigene family of isoenzymes responsible for detoxification of xenobiotics in aerobic organisms. Endogenous substrates include the toxic products of tissue damage, including the hydroperoxide products of oxidative damage (including lipid peroxides), and aromatic xenobiotics. Glutathione *s*-transferases are catalysts of reactions in which GSH (reduced glutathione) acts as a nucleophile, conjugating to and facilitating removal or reduction of the second substrate. In all organisms, glutathione *s*-transferase activity (multiple forms of the enzyme which may or may not be tissue-specific) has been discovered (Halliwell, 1994). In the present study it was determined that glutathione *s*-transferase activity was inhibited by amiodarone in carrageenan-induced inflammatory-paw tissue in which the level of glutathione *s*-transferase significantly increased (Table 3). These results correlate well with the decreased cellular content that we found and are suggestive of a down-regulation of oxidant activity in paw after amiodarone treatment. Sirajudeen et al. reported that glutathione *s*-transferase activity was decreased in amiodarone-induced pulmonary- and hepato-toxicity (Sirajudeen et al., 1998). However, amiodarone did not affect glutathione *s*-transferase activity in human embryonic lung L132 cells or hepatocyte carcinoma Hep 3B cells (Rrivier et al., 1997).

Although oxygen intermediates are highly reactive and potentially cytotoxic, their formation is not typically deleterious to the cells, because these reactive oxygen species are neutralized by endogenous enzymes and free radical scavengers. First, superoxide dismutase catalyzing the dismutation of superoxide anions ($O_2^{-\cdot}$) to H_2O_2 and glutathione-related enzymes preserving glutathione status play an important role in the anti-oxidant defense system by ensuring the degradation of these species to less harmful compounds (Winterbourn, 1993).

In the present study, we established that amiodarone increased superoxide dismutase activity, which was inhibited by carrageenan in rat paw tissues (Wu et al., 2006). In many laboratory models and in a few clinical trials, superoxide dismutase has proven therapeutically useful in protecting injured tissues (ischemia, inflammation, hyperoxia, *etc.*) from one of these active oxygen species, the superoxide radical (McCord, 1992). Superoxide dismutase destroys the highly reactive radical superoxide ($O_2^{-\cdot}$) by converting it into the less reactive peroxide (H_2O_2) that can be destroyed by catalase reaction.

Catalase is a highly reactive enzyme, reacting with H_2O_2 to form water and molecular oxygen and can form methanol, ethanol, formic acid or phenols by donating hydrogen (Matés and Sánchez-Jiménez, 1999). In the present study, we established that all doses of amiodarone and diclofenac decreased catalase activity (Table 2), which was increased by carrageenan in rat paw tissues (Sirajudeen et al., 1998). On the other hand, Ribeiro et al. (1997) reported that amiodarone has no effect on the activity of the catalase enzyme but has a protective effect against lipid peroxidation in mitochondrial membranes.

During normal body conditions, a balance exists between free radicals and the natural scavengers of the body, but in a traumatic state the balance diminishes and reactive oxygen metabolites increase dramatically in number. At this stage, therapeutic application of enzymatic and non-enzymatic antioxidants becomes essential. Increased generation of oxygen free radicals in the extracellular space is seen in the inflammatory state in which the relatively low concentrations of superoxide dismutase and catalase increase the susceptibility of extracellular components to oxygen radical injury and may stimulate chemotaxis for other inflammatory cells (Del Maestro et al., 1980). In contrast to this, our findings show that catalase activity was increased by carrageenan injection while superoxide dismutase activity was inhibited. Chen et al. (1998) suggested that catalase stimulated the expression of mRNA and protein for cyclooxygenase-2 in rat aortic smooth muscle cells, although it did not affect the expression of either mRNA or protein for cyclooxygenase-1. In other words, catalase exerted a biphasic effect on prostaglandin synthesis and enhanced prostaglandin production at low concentrations. Chen et al. suggested that at low concentrations, increased catalase activity may cause inflammation as reflected by increased cyclooxygenase-2 activity. One of the factors causing formation of the carrageenan-induced acute inflammation process is possibly an augmentation of catalase activity, which was ascertained in the results of the present experiment.

GSH has pleiotropic roles, which include the maintenance of cells in a reduced state, serving as an electron donor for certain anti-oxidative enzymes (glutathione peroxidase, *e.g.*), and the formation of conjugates with some harmful endogenous and xenobiotic compounds *via* catalysis of glutathione *s*-transferase. GSH levels are maintained by two systems. One is *de novo* synthesis from building blocks, glutamate, cysteine, and glycine, *via* two ATP-consuming steps involving c-glutamylcysteine synthetase and glutathione synthetase. The other constitutes a recycling system involving glutathione reductase (NADPH: oxidized glutathione oxidoreductase, EC 1.6.4.2) which is a flavoprotein and reduces oxidized glutathione (GSSG) back to GSH in an NADPH-dependent manner (Meister and Anderson, 1983). Thus, glutathione reductase indirectly participates in the protection of cells against oxidative

stress. The enzymatic activities of glutathione reductase have been investigated in various tissues under physiological and pathological conditions (Shacter et al., 1991).

The anti-inflammatory effects of the amiodarone can be supported by GSH levels and activity of glutathione reductase in carrageenan-induced rat paw tissues. Furthermore, treatment with amiodarone enhanced GSH levels and glutathione reductase activities which were decreased by carrageenan in the rat paws. Amiodarone enhanced GSH levels at doses of 50 mg/kg and 100 mg/kg (Table 3), but glutathione reductase activity only at a dose of 100 mg/kg. This effect was also noted by Leeder et al. (1996) who showed that amiodarone increased glutathione reductase in pulmonary toxicity *in vivo* and *in vitro* and by Sin et al. (1997) who determined that carrageenan decreased the GSH level for up to 12 h in mice. In the present study, amiodarone alleviated the decreases in GSH level and glutathione reductase activity in carrageenan-induced acute inflammation. In other studies, it was shown that amiodarone decreased GSH levels in Hep 3B cells (Rrivier et al., 1997) and the activity of glutathione reductase in pulmonary- and hepatotoxicity (Sirajudeen et al., 1998).

Myeloperoxidase is an enzyme found primarily in azurophilic granules of neutrophils, which is used as a marker for tissue neutrophil content and its inhibition implies the presence of anti-inflammatory activity (Bradley et al., 1982). Tissue myeloperoxidase activity is a sensitive and specific marker for acute inflammation and reflects polymorphonuclear cell infiltration of the parenchyma. At 5 h after carrageenan administration, paws were examined for tissue damage for myeloperoxidase activity. In accordance with the literature (Saleh et al., 1999; Jain et al., 2001; Paino et al., 2005; Rocha et al., 2006), myeloperoxidase activity significantly ($P<0.05$) increased in the paw at 5 h after carrageenan injection when compared to healthy (intact) rats (Fig. 3). Moreover, myeloperoxidase activity was elevated by 50 and 100 mg/kg doses of amiodarone and diclofenac more than carrageenan.

It has been reported that release of myeloperoxidase from inflammatory cells is another indicator for evaluating the degree of inflammation (Saleh et al., 1999; Jain et al., 2001; Paino et al., 2005; Rocha et al., 2006) and anti-inflammatory drugs may also exert their effects *via* inhibition of myeloperoxidase pathways (Shacter et al., 1991). A variety of anti-inflammatory drugs (diclofenac, indomethacin, naproxen, piroxicam and tenoxicam) depresses the increases in myeloperoxidase activity in case of inflammation (Lee et al., 2002; Paino et al., 2005). Our data show that amiodarone and diclofenac increased myeloperoxidase activity further (Fig. 3). The effect of diclofenac on myeloperoxidase activity is inconsistent (Matsui et al., 2001) because of variations in different tissues. Tissue myeloperoxidase activity is a sensitive and specific marker for acute inflammation and reflects polymorphonuclear cell infiltration of the parenchyma. Nevertheless, amiodarone may also have a different mechanism of action. Each molecule of amiodarone contains two atoms of iodine (Fig. 1) and it has been recognized that myeloperoxidase (Welinder, 1992) and other mammalian peroxidases constitute a separate peroxidase super family (Kimura and Ikeda-Saito, 1988) which displays catalytic activity in the presence of iodide. Because of the high iodine content, amiodarone can activate myeloperoxidase activity in rat paws. As opposed to other anti-inflammatory drugs, which decrease myeloperoxidase activity, increase in myeloperoxidase activity in response to amiodarone could be linked to the iodine present in its chemical structure.

In conclusion, this experiment showed that carrageenan successfully induced edema in paws. Both tested drugs (amiodarone and diclofenac) reduced paw volume, the response to amiodarone being greater than that to diclofenac. The level of anti-oxidant system enzymes (superoxide dismutase, glutathione reductase, glutathione *s*-transferase, catalase) and GSH level were adversely affected by edema induction. Both drugs alleviated the adverse effects of edema on these enzymes. The anti-inflammatory properties of amiodarone could be related to its positive effects on the anti-oxidant system in rats with paw edema. Since amiodarone have anti-inflammatory property, especially in acute myocardial infarction (gold standard for the pharmacological treatment of life-threatening ventricular tachyarrhythmias after acute myocardial infarction) it may decreased the inflammation occurred during ischemia in infarct area. It may be the second beneficial indication after patients with myocardial infarction?

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