Role of polymorphonuclear leukocyte infiltration in the mechanism of anti-inflammatory effect of amiodarone

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Abstract: In many physiological bodily functions, and in the pathogenesis of inflammation, ions are exchanged between intracellular and extracellular areas. Amiodarone is a multiple ion channel (Ca\textsuperscript{2+}, Na\textsuperscript{+}, K\textsuperscript{+}) blocking drug, effective anti-arrhythmic drug, and phospholipase inhibitor. The aim of this study is to examine a role of polymorphonuclear leukocyte infiltration in amiodarone’s anti-inflammatory effect on experimental paw inflammation. After rats had been assigned to groups, their normal right hind paw volumes were measured using a plethysmometer. Amiodarone (25, 50 and 100 mg/kg) and distilled water were administrated to the experimental and control groups, respectively, by \textit{ip} route. Thirty minutes later, paw edema was induced in rats by subplantar injection of 0.1 ml of histamine (0.1%) to those paws. Subsequent volume readings for those paws were carried out at 30-min intervals. Results were expressed as percentages of change from the initial volumes. After the final measurements, the animals were killed by decapitation and their paw tissues were cut for pathological investigation. Amiodarone dose-dependently decreased the paw edema (25.05, 48.71 and 74.97%), and reduced polymorphonuclear leukocyte infiltration in the paw tissue (55.65, 69.76 and 84.58%). Our findings support the view that amiodarone dose-dependently exerts a powerful anti-inflammatory activity. This effect of amiodarone may be due to the activation of nitric oxide resulting from its calcium channel antagonistic effects, to the inhibition of phospholipase A2 and/or to a reduction in neutrophil movement and activation, which may reduce free radical production and proteolytic enzyme release.

Key words: amiodarone, histamine, inflammation, polymorphonuclear leukocytes, rat
Abbreviations: AIA – anti-inflammatory activity, CCB – calcium channel blockers, DHP – 1,4-dihydropyridine, PNL – polymorphonuclear leukocyte

Introduction

Inflammation is involved in the pathogenesis of various diseases, and its special components are hemodynamic changes, polymorphonuclear leukocyte (PNL) infiltration and secretion of inflammatory mediators [10]. In many physiological bodily functions, and in the pathogenesis of inflammation, ions are exchanged between intracellular and extracellular areas. It has been reported that increases in intracellular calcium ions stimulate inflammation events, and calcium channel blockers (CCBs) diminished these events [6–8, 17, 40]. Also, inflammation disturbs leukocyte functions and increases PNL infiltration [37], and calcium ions play an important role in the activation of PNLs [14, 21].

Amiodarone is a multiple ion channel (Ca++, Na+, K+) blocking drug, effective anti-arrhythmic medication used to treat a wide variety of ventricular and supraventricular tachyarrhythmias, and inhibits both inward calcium and sodium currents and outward potassium currents in cardiac cells. This drug also has been reported to have non-competitive anti-sympathetic effects, to modulate thyroid function and phospholipid metabolism [25], and to inhibit phospholipase A1, A2 and phospholipase C [43]. Phospholipase acts in the first step in the production of inflammatory mediators in the arachidonic acid metabolism.

Although amiodarone inhibits the phospholipase activity and blocks calcium channels, one literature data have been found related to its anti-inflammatory effect [18]. The aim of this study is to examine a role of polymorphonuclear leukocyte infiltration in amiodarone’s anti-inflammatory effect in the histamine-induced inflammation model.

Materials and Methods

Animals

Male Sprague-Dawley rats (175–200 g) which were obtained from the Atatürk University Pharmacology Laboratory and housed under normal conditions were used.

Chemicals

Amiodarone (Sanofi, Turkey), diclofenac sodium (Fako, Turkey) and histamine (Sigma, USA) were used for the experiments. All drugs were dissolved in saline solution.

Drug doses

We choose three different doses of amiodarone: 25, 50 and 100 mg/kg. These doses are very low treatment doses in humans, at which no side effects or toxicities occur [35].

Histamine-induced paw edema [6]

In preliminary experiments, intraperitoneal (ip) amiodarone administrations produced no detectable edema. After the animals’ normal right hind paw volumes had been measured using a plethysmometer, amiodarone and diclofenac sodium (or distilled water to the control group) were administrated by ip route. Thirty minutes later, paw edema was induced in rats by subplantar injection of 0.1 ml of histamine (0.1%) to their right hind paw. Subsequent volume readings for those paws were carried out at 30-min intervals. Results were expressed as percentage differences versus initial volumes. The ratio of the drugs’ anti-inflammatory activity was calculated by the following equation:

\[
\text{Anti-inflammatory activity (AIA- %) } = \frac{1 - \left[ \frac{D}{C} \right]}{100}
\]

where: \(D\) represents the percentage difference in paw volume after the drug administration, and \(C\) represents the percentage difference in paw volume in the control group.

Pathological analysis of animals’ paw tissues

The animals were killed by decapitation 180 min after histamine administration, and their paw tissues were excised for pathological investigation. The specimens were fixed in 10% formalin and routinely processed for paraffin embedding. From each sample, 4 \(\mu\)m thick sections were obtained and stained with hematoxylin-eosin to evaluate acute inflammation. Polymorphonuclear leucocytes (PNLs) were counted...
in 10 separate microscopic fields (× 400) from two sections of each animal, and the mean was calculated. This procedure was applied to all animals in each group, and the final number of PNLs was expressed as the mean of the number counted in six animals per group.

**Statistical analysis**

Values reported are the mean ± SEM. Statistical analysis of data was performed by ANOVA and post-hoc Dunnett t-test. The significance level was accepted as p < 0.05.

**Results**

**Effects of amiodarone on histamine-induced paw edema**

In preliminary experiments, amiodarone as used in our study produced no detectable edema in the non-treated paw. As shown in Table 1 and Figure 1, after histamine injection, edema in rat paws was detected within 30 min (35.71 ± 6.08%) and 60 min (34.34 ± 4.78%). Amiodarone at 25 mg/kg decreased the inflammation at 60 min (AIA = 34.28%), but the decrease was not statistically significant. Amiodarone (50 mg/kg) decreased the inflammation at 60 min (AIA = 48.70%), and this decrease was statistically significant (p < 0.05). Amiodarone at 100 mg/kg decreased the inflammation at both 30 min (AIA = 51.58%) and 60 min (AIA = 74.97%), and both of these decreases were statistically significant (p < 0.05).

**Tab. 1.** The increases in paw edema after histamine injections are expressed as the mean ± SEM (%), for n = 6 rats

<table>
<thead>
<tr>
<th>Groups' names</th>
<th>Inflammation rates (%) in the paws</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td>35.71 ± 6.08</td>
</tr>
<tr>
<td>Amiodarone-25</td>
<td>34.19 ± 5.05 NS</td>
</tr>
<tr>
<td>Amiodarone-50</td>
<td>27.14 ± 1.69 NS</td>
</tr>
<tr>
<td>Amiodarone-100</td>
<td>17.29 ± 1.80**</td>
</tr>
<tr>
<td>Diclofenac-25</td>
<td>19.99 ± 2.40*</td>
</tr>
</tbody>
</table>

NS – not significant, * p < 0.05, ** p < 0.01 and *** p < 0.005 as compared to control group (Dunnett t-test)
Effects of amiodarone on PNL infiltration

Pathologically, PNL infiltration was observed in the damaged area. As shown in Figures 2 and 3, the number of PNLs was the highest (165.33 ± 5.19) in the control group. However, amiodarone reduced the number of PNLs in a dose-dependent manner (55.65, 69.76 and 84.58%; p = 0.000). Amiodarone’s maximal effect was noted at the 100 mg/kg dose (25.50 ± 2.26, p = 0.000). Diclofenac sodium also decreased the PNL number (46.00 ± 5.81, p = 0.000), but this decrease was smaller than that of 100 mg/kg amiodarone.

![Figure 3. Pathological localization of PNL in the rat paw.](image)

(A) Control group (only histamine), this group showed significant PNL infiltration; (B) amiodarone 25 mg/kg + histamine group; (C) amiodarone 50 mg/kg + histamine group; (D) amiodarone 100 mg/kg + histamine group; (E) diclofenac group. Large images are magnifications with H&E x 100, small images are magnifications with H&E x 400
Discussion

The results of the present study indicate that amiodarone shows an anti-inflammatory activity as an anti-inflammatory drug against development of histamine-induced acute inflammation. Amiodarone is a class III anti-arrhythmic drug. In addition, class I (Na+ channel blockade), II (non-competitive β-adrenergic blockade) and IV (Ca++ channel blockade) properties, as well as a reserpine-like sympatholytic action, have been reported [33]. The literature includes some studies related to amiodarone’s interaction with receptors for 1,4-dihydropyridine (DHP) in rat and rabbit myocardial membranes. These studies have revealed that amiodarone was able to displace DHP derived CCB₃ binding and displayed DHP-like effects [29, 34]. It has also been shown that amiodarone also inhibited T-type calcium channels [44], and T- and L-type CCB₃ have been known to suppress carrageenan- [40, 42], prostaglandin E1-, bradykinin-, serotonin- and histamine-induced inflammation [2, 6]. DHP’s anti-inflammatory activities have been examined by some researchers [2, 17, 42]; the anti-inflammatory activities of these group of CCB₃ have been determined to be more effective than those of the other groups of calcium channel blockers [2, 17]. Amiodarone has L- and T-type calcium channel blocker properties, and anti-inflammatory effects of CCBs are already known. This drug’s powerful anti-inflammatory effect may be related to its T- and L-type calcium channel blocking properties and/or its behavior as DHP-derived CCB₃.

In this study, acute local inflammation was induced with histamine in rats. Among the several models of acute inflammation, histamine-induced inflammation has been well established as a valid model to study polymorphonuclear leucocyte (PNL) infiltration in paw tissue after inflammatory states [6]. It is well known that the acute inflammatory process, in which vascular permeability increases and leucocyte migration occurs, involves several mediators including neutrophil-derived active oxygen species and free radicals, such as hydrogen peroxide, superoxide and the hydroxyl radical [4, 13, 39, 41]. Free radicals are toxic molecules and damage the molecules within cells. In recent years, free radicals and the L-arginine NO pathway have been proposed to play an important role in the inflammatory response [1, 12, 31, 32, 39]. Some studies have shown that amiodarone effectively stimulated the release and activation of nitric oxide [16, 36], and that it is a vasodilator drug, like other DHP-derived calcium channel blockers [11]. It has also been reported that CCB₃ reduce neutrophil movement and activation, which may reduce free radical production and proteolytic enzyme release [3, 38]. In the histamine-induced inflammation model, histamine causes the increase in vascular permeability and PNL infiltration [24]. PNL infiltration is a constant feature in acute inflammation. Histamine and histamine-activated PNL can trigger the production of oxygen-derived free radicals [5]. We did not directly investigate amiodarone’s antioxidant activity in this study, because we and some researchers already had described this activity [18, 23, 27]. We had observed its antioxidant activity in carrageenan-induced paw inflammation model. In this study, we examined amiodarone’s effect on PNL infiltration in histamine-induced inflammed paw tissues in rats. Our study found that amiodarone dose-dependently reduced both paw edema and PNL infiltration into paw tissue; It may induce the decrease in synovial damage and/or the inhibition of the inflammatory process. We have also thought that its anti-inflammatory effect is related with its antioxidant activity.

Another important fact related to amiodarone is its inhibitory effect on the lysosomal phospholipases A₁, A₂ and C₃ [19, 22, 26, 30, 43]. Lysosomal phospholipase A₂ is a converting enzyme in the synthesis of arachidonic acid from membrane phospholipids. This enzyme’s activation depends on Ca²⁺ ion and causes the release of an inflammatory mediator [9, 28], and its inhibition causes anti-inflammatory action (like steroidal anti-inflammatory drugs).

This is a beneficial effect and may be useful for patients with cardiovascular diseases. Many studies have reported that calcium channel blocking agents or oxygen free radical scavengers can be used to attenuate reperfusion injury; that administrations of drugs (such as amiodarone) prior to ischemic insult have helped in the recovery of myocardial contractile functions; and that their cellular protective effects are related to their roles in decreasing cytosolic calcium levels in the early phases of acute ischemia and in preventing membrane lipid peroxidation, and to their anti-inflammatory effects in the reperfusion period [15, 20]. Because amiodarone has these properties, especially in acute myocardial infarction, it may be useful for decreasing inflammation in the infarcted area during ischemia.
In conclusion, amiodarone either macroscopically or histopathologically presented protective effects in the histamine-induced inflammation model in rats. These effects of amiodarone may be due to the activation of nitric oxide resulting from its calcium channel antagonist effects, to the inhibition of phospholipase A2, and/or to a reduction in neutrophil movement and activation, which may reduce free radical production and proteolytic enzyme release.

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