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Effects of captopril and angiotensin II receptor blockers (AT_1, AT_2) on myocardial ischemia–reperfusion induced infarct size

Hakan Parlakpinar [a,*], Mehmet Kaya Ozer [b], Ahmet Acet [a]

[a] Division of Pharmacology, Department of Medicine, University of Inonu, 44280 Malatya, Turkey
[b] Division of Pharmacology, Department of Medicine, University of Suleyman Demirel, Isparta, Turkey

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ABSTRACT

The renin–angiotensin system (RAS) plays a major role in regulating the cardiovascular system, and disorders of the RAS contribute largely to the cardiac pathophysiology, including myocardial ischemia–reperfusion (MI/R) injury. Two subtypes of angiotensin II (Ang II) receptors have been defined on the basis of their differential pharmacological properties. The current study was undertaken to address the question as to whether the inhibition of the angiotensin converting enzyme (ACE) by captopril and the AT_1 and AT_2 receptor blockers losartan and PD123319 modulate MI/R-induced infarct size in an *in vivo* rat model.

To produce necrosis, a branch of the descending left coronary artery was occluded for 30 min followed by two hours of reperfusion. ECG changes, blood pressure, and heart rate were measured during the experiment. Captopril (3 mg/kg), losartan (2 mg/kg), and PD123319 (20 μg/kg/min) were given in an IV 10 min before ischemia and were continued during the ischemic period. The infarcted area was measured by TTC staining. The volume of infarct and the risk zone was determined by planimetry.

Compared to the control group (55.62 ± 4.00%) both captopril and losartan significantly reduced the myocardial infarct size (30.50 ± 3.26% and 37.75 ± 4.44%), whereas neither PD123319 nor PD123319+losartan affected the infarct size volume (46.50 ± 3.72 and 54.62 ± 2.43).

Our data indicates that captopril and losartan exert cardioprotective activity after an MI/R injury. Also, infarct size reduction by losartan was halted by a blockade of the AT_2 receptor. Therefore, the activation of AT_2 receptors may be potentially protective and appear to oppose the effects mediated by the AT_1 receptors.

1. Introduction

Cardiovascular events are a leading cause of death worldwide and remain one of the major killers in modern society. These events can be initiated by multiple factors such as thrombolysis, percutaneous transluminal coronary angioplasty, and coronary bypass surgery. All of these can cause an myocardial ischemia–reperfusion (MI/R) injury, which is known to occur on the restoration of coronary flow after a period of myocardial ischemia [1,2]. Despite lifestyle modification and advances in pharmacotherapy, chronic and acute myocardial ischemia requires an intervention to salvage the viable myocardium at risk. Occlusion of coronary arteries induces myocardial necrosis. Although the restoration of blood flow is the only way to save the myocardium from eventual necrosis, reperfusion often exacerbates cardiac dysfunction, including arrhythmias, stunning, and microvascular damage, and cell death can be induced by reperfusion injury [3].

The renin–angiotensin system (RAS) is a coordinated hormonal cascade of crucial importance in cardiovascular and renal functions [4]. The RAS is activated during acute myocardial ischemia. Its major mediator, angiotensin II (Ang II), exerts a number of effects that exacerbate the consequences of myocardial ischemia. The magnitude of the heart damage contributed by each of these factors and the extent of their interactions are unresolved issues. Although angiotensin converting enzyme (ACE) inhibitors and Ang II type 1 (AT_1) receptor blockers (ARBs) are beneficial during MI/R, their effects on the myocardium remain controversial. The existence of at least two distinct Ang II receptor subtypes as AT_1 and AT_2 receptors have been defined. However, to date, less is known about the AT_2 receptor and many of the functions of these receptors are still an enigma. The AT_1 receptor is closely associated with the regulation of blood pressure, fluid, and electrolyte balance, while the role of the AT_2 receptor has been less clear due to the low level of AT_2 expression in healthy adults [5]. Moreover, AT_2 receptor expression is up-regulated in pathological conditions such as heart failure, renal failure, myocardial infarction, brain lesions, vascular injury, and wound healing [6].

* Corresponding author. Tel.: +90 422 341 0660x1227; fax: +90 422 341 0036.
E-mail address: hparlakpinar@inonu.edu.tr (H. Parlakpinar).

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Several studies with ARBs in large animal models of MI/R have shown evidence of AT_2 receptor activation and suggested that this contributed to the cardioprotective effects of ARBs. In fact, AT_1 antagonism increases plasma Ang II, which subsequently activates the AT_2 receptor, resulting from enhanced nitric oxide activity and prostaglandins production [7]. AT_2 receptors also suppress renin biosynthesis and secretion. Therefore, AT_2 receptors represent a potentially significant therapeutic target for preventing MI/R-induced RAS activation. It has been shown that the protective effect of an AT_1 receptor blockade by ARBs is attenuated in the presence of AT_2 receptor inhibition. The results suggest that the limitation of myocardial injury associated with the AT_1 receptor blockade, combined with upregulation of AT_2 receptor protein expression, contributes to the cardioprotective effects of ARBs during MI/R [8].

The therapeutic success of ACE inhibitors is related to their unique pharmacological profile involving both a reduction of plasma and tissue Ang II concentrations and potentiation of endogenous kinins. It is well established that ACE is also responsible for the degradation of kinins, and following ACE inhibition the concentration of bradykinin increases [9].

The aim of this study was to examine the effects of ACE inhibitor captopril, AT_1 and AT_2 receptor blockers losartan and PD123319 on ischemia–reperfusion-induced myocardial infarct size in an *in vivo* rat model. Also, we focused on whether or not exogenously applied PD123319 aggravates myocardial infarct size.

2. Materials and methods

2.1. Animals and groups

For this study, 40 male Wistar albino rats, ages 10–12 weeks and weighing 250–300 g, were obtained from the Inonu University Laboratory Animals Research Center and placed in a temperature (21 ± 2 °C) and humidity (60 ± 5%) controlled room in which a 12:12 h light:dark cycle was maintained. The rats were randomly assigned to five groups with $n = 8$ each as follows: (1) control (MI/R), (2) captopril 3 mg/kg, (3) losartan 2 mg/kg, (4) PD123319 20 μg/kg/min, and (5) losartan+PD123319. All groups were fed a standard chow pellet diet with tap water *ad libitum*. All experiments in this study were performed in accordance with the Guidelines for Animal Research from the National Institutes of Health and were approved by the Committee on Animal Research (numbered with 2004/7) at Inonu University, Malatya, Turkey.

2.2. Ischemia–reperfusion procedure

The rats were anesthetized with urethane 1.2 g/kg administered intraperitoneally (i.p.). The right jugular vein and the trachea were cannulated for the drug administration and artificial respiration when required. The chest was opened by a left thoracotomy, followed by sectioning the fourth and fifth ribs, about 2 mm to the left of the sternum. Positive-pressure artificial respiration was started immediately with room air, using a volume of 1.5 ml/100 g body weights at a rate of 60 strokes/min to maintain normal PCO_2, PO_2, and pH parameters. After the pericardium was incised, a gentle pressure on the right side of the rib cage exteriorized the heart. A 6/0 silk suture attached to a 10-mm micropoint reverse-cutting needle was quickly placed under the left main coronary artery. The heart was then carefully replaced in the chest, and the animal was allowed to recover for 20 min. Any animal in which this procedure caused a sustained decrease in mean arterial blood pressure (MAP) to less than 70 mm Hg was discarded. A small plastic snare was threaded through the ligature and placed in contact with the heart. Applying tension to the ligature could then occlude the artery, and reperfusion was achieved by releasing the tension. The left coronary artery was occluded for 30 min and then reperfused for 120 min more before the experiment was terminated. The duration of MI/R and the number of rats were chosen on the bases of previous related studies [10–12].

2.3. Drug administration

After the rats were anesthetized, the right jugular vein was catheterized for the drug and vehicle administrations. All pharmacological agent treatments began at 10 min before coronary artery occlusion and continued throughout the ischemic period (30 min) by infusion pump for a total of 40 min as the infusion time. To achieve the same experimental protocol conditions in all groups, we infused a comparable volume of the vehicle in the control and MI/R groups. In the current study, we used a rat model of *in vivo* MI/R similar to the one used in our previous studies [13].

2.4. Evaluation of hemodynamic parameters

Systemic blood pressure (BP) was monitored from the carotid artery by a Harvard model 50-8952 transducer and displayed on a Harvard Universal penrecorder along with a standard lead-1 electrocardiogram (ECG). ECG changes, MAP, and heart rate (HR) were measured at baseline (before the administration of the drugs or vehicle), at the end of the 30 min period of ischemia, and after 30, 60, and 120 min of reperfusion. HR and MAP were analyzed at selected times to determine the hemodynamic effects associated with coronary occlusion and with the drug treatment according to our previous study [14], which used the same experimental protocol.

2.5. Evaluation of tissue death

At the end of the each *in vivo* study, the heart was quickly removed and mounted on a Langendorff apparatus where it was flushed with saline at room temperature for 60 s. The coronary branch was then re-occluded and zinc–cadmium fluorescent particles (1–10 μm in diameter from Duke Scientific Corb, Palo Alto, CA, USA) were infused into the perfusate to mark the risk zone, which is recognized as the area perfused with the blood of left coronary artery. The heart was then frozen and weighed and a total of four transverse slices, 2 mm in size, from each heart were cut starting from the apex. The slices were incubated in 1% triphenyl tetrazolium chloride (TTC) in pH 7.4 buffer at 37 °C for 20 min. TTC stains living tissue a deep-red[1] color, while necrotic tissue is TTC negative and appears as tan color (Fig. 4B). The formazan precipitate resulting from the reaction of lactate dehydrogenase in normal and ischemic regions delineated the area at risk from the infarcted tissue. In this procedure, the slices were examined under UV light to visualize the normal area, which appeared bright because of the presence of the fluorescent particles (Fig. 4A). After we traced this area, each infarcted portion within the risk region was traced onto the same acetate sheet. These acetate tracings were then photocopied and enlarged 100-fold. The volume of infarct and the risk zone were determined by computerized-planimetry of each tracing and multiplying by the slice thickness. The infarct size was normalized by expressing it as a percentage of the area at risk. The studies were carried out in a blinded fashion, so that the investigator conducting the infarct analysis was unaware of the treatment.

[1] For interpretation of color in Figs. 1, 2, 4, the reader is referred to the web version of this article.