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Effects of the AT1 Receptor Blocker Candesartan on Myocardial Ischemia/Reperfusion in Isolated Rat Hearts

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ABSTRACT

Background: We sought to investigate the effects of the angiotension II receptor blocker candesartan on ischemia-reperfusion injury using a cardioplegia arrested isolated rat heart model.

Methods: Ischemia-reperfusion injury was induced in isolated rat hearts with 40 minutes of global ischemia followed by a 30-minute reperfusion protocol. Throughout the experiment, constant pressure perfusion was achieved using a Langendorff apparatus. Cardioplegic solution alone, and in combination with candesartan, was administered before ischemia and 20 minutes after ischemia. Post-ischemic recovery of contractile function, left ventricular developed pressure, left ventricular end-diastolic pressure and contraction and relaxation rates were evaluated.

Results: In the control group, left ventricular developed pressure, rate pressure product, contraction and relaxation rates and coronary flow significantly decreased but coronary resistance increased following reperfusion. With the administration of candesartan alone, parameters did not differ compared to controls. Contractile parameters improved in the group that received candesartan in combination with the cardioplegia compared to the group that received cardioplegia alone; however, the difference between these two groups was insignificant.

Conclusion: In this study, the addition of candesartan to a cardioplegic arrest protocol routinely performed during cardiac surgery did not provide a significant advantage in protection against ischemia-reperfusion injury compared with the administration of cardioplegic solution alone.

INTRODUCTION

Reperfusion injury to the heart after myocardial infarction (MI) starts on restoration of blood flow to the myocardium. Although reperfusion of the ischemic area is a prerequisite for rescue of myocardial cells from injury and improves cardiac function, it causes tissue damage in addition to that induced by ischemia. The cardiac renin-angiotensin system (RAS) plays an important role in coronary flow and in maintaining cardiac function in the normal heart and in pathologic conditions [Dzau 1994]. Both the cardiac and circulating RAS are activated in cardiovascular diseases such as hypertension and heart failure and during reconstruction (remodeling) after MI. Angiotensin II (ANG II), a key component of the RAS system, leads to the formation of reactive oxygen species (ROS) through the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Increased levels of ROS are involved in the pathogenesis of cardiac and vascular damage by inducing inflammatory responses through the activation or upregulation of mediators such as nuclear transcription factors, adhesion molecules, chemoattractant cytokines, and proinflammatory cytokines. These events begin the process of progressive structural cardiac remodeling [Muller 2000].

In addition to the free radical mechanisms, there is evidence to support the role of bradykinin in the protective effects of angiotensin-converting enzyme (ACE) inhibitors and ANG II receptor blockers in the ischemia-reperfusion (I/R) injury process. The protective effects of ACE inhibitors cause an accumulation of bradykinin and ANG II receptor blockers, leading to the release of bradykinin through the activation of the AT-2 receptors [Hartman 1993; Liu 1996; Jalowy 1998 Weidenbach 2000].

In this study we evaluated the selective nonpeptide ANG II receptor blocker candesartan cilexetil, which has a half-life of 9 hours. Candesartan cilexetil is a prodrug that transforms into its active metabolite, candesartan, during absorption, thereby binding proteins at a high rate [Ripley 2006]. In vitro studies show that the receptor affinity of candesartan is higher than that of other AT-1 receptor blockers. Furthermore, at higher concentrations of ANG II, the drug does not easily separate from its receptor. This firm and prolonged binding provides effective blockage and reduces the adverse cardiovascular effects of ANG II [De Rosa 2010]. Candesartan almost completely eliminates ANG II by reducing the maximum response. This inhibition cannot be eliminated by an increase in the concentration of ANG II [Vanderheyden 2000; Vauquelin 2000]. In this study we aimed to investigate the effects of candesartan on I/R injury, using a cardioplegia arrested isolated rat heart model.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (300-400 g) were used in this study. The animals were treated in compliance with the

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revised NIH Guide for the Care and Use of Laboratory Animals. G.Ü.ET-12050 code and 79-10831 ethics committee approval was granted by the Gazi University Experimental Animal Ethics Committee.

Study Groups

Group 1 (control, Krebs–Henseleit bicarbonate buffer (KHB) n = 9): I/R with no treatment. Group 2 (cardioplegia, n = 8): I/R with St.Thomas cardioplegic solution (Plegisol). Group 3 (cardioplegia with candesartan, n = 9): A 10-4 M stock solution prepared by dissolving candesartan cilexetil (Abdi Ibrahim Pharmaceutical Industry M.W: 610.67) in dimethyl sulfoxide (DMSO) diluted to 10-7 M with the cardioplegic solution. Diluted solutions were freshly prepared prior to drug administration. Group 4 (KHB with candesartan, n = 4): The stock solution prepared with DMSO was diluted to 10-7M with KHB to evaluate the effects of DMSO on the results. Group 5 (cardioplegia with DMSO, n = 2): A 0.1% DMSO solution was administered in the cardioplegic solution to evaluate the effects of DMSO on the results.

Heart Preparation and Measurements

Rats were anesthetized using an intraperitoneal injection of ketamine hydrochloride (60 mg/kg) and xylazine (10 mg/kg). Under full anesthesia, hearts were removed and placed at 4°C in KHB with the following composition: NaCl 120, glucose 11, KCl 4.8, calcium $CaCl_2$ 2.5, $KH2PO_4$ 1.2, $MgSO_4$ 1.2, and $NaHCO_3$ 25 (all in mmol/L). After aortic cannulation, the hearts were flushed with KHB. The cannulated hearts were then connected to a Langendorff apparatus and perfused in a retrograde manner at a constant pressure of 100 cm H2O with the KHB solution (pH 7.4, 37°C, 95% O2 + 5% CO2) and allowed to equilibrate for 15 min. A distilled water filled polyvinyl chloride balloon was inserted into the left ventricular cavity. Left ventricular end-diastolic pressure (LVEDP) was adjusted to 5-10 mm/Hg. For measurement of intraventricular pressure (IVP), the balloon was connected to a pressure transducer and a data acquisition system (COMMAT Ltd., BSL Pro 36). LVEDP, left ventricular developed pressure (LVDP = peak pressure – LVEDP), and maximal rates of pressure increase (+dp/dt) and decline (-dp/dt) were calculated.

Experimental Protocol

A schematic representation of the experimental protocol is shown in Figure 1. The cannulated hearts were allowed to equilibrate for 15 minutes. Global ischemia was induced by discontinuing perfusion for 40 minutes, followed by reperfusion with the KHB solution for 30 mininutes. At the end of a 15-minute recovery period, KHB, cardioplegic solution, candesartan solution diluted with the cardioplegic solution, candesartan solution diluted with KHB, and DMSO solution in cardioplegic solution were administered to groups 1, 2, 3, 4, and 5, respectively, to create an ischemic period. St. Thomas cardioplegic solution containing 17.6 mg $CaCl_2$, 325.3 mg magnesium chloride hexahydrate (MgCl2•6H2O), 119.3 mg KCL, 643 mg NaCl, 2.4 mg mEq Ca, 32 mEq Mg, 16 mEq K, 110 mEq Na, and 160 mEq Cl per 100 mL was used. The temperature of the heart during ischemia was maintained at 37°C by placing it into a KHB-filled temperature-controlled recirculating chamber.

STATISTICAL ANALYSES

Results are expressed in the form of mean ± standard error of the mean (SEM). The difference between two groups in contractile parameters, which were measured at the end of reperfusion in terms of percentage of the initial values, was compared using one-way analysis of variance (ANOVA). Tukey's multiple comparison test was used to determine significant differences between groups. Differences between the measured value at the end of reperfusion and during stabilization were evaluated using a paired Student's t-test. *P* values of < .05 were considered to be statistically significant. GraphPad Prism 6 software (La Jolla, CA) was used for statistical analysis.

RESULTS

The data obtained from this study show no significant difference between hearts administered DMSO solution as a candesartan solvent in cardioplegia and cardioplegia alone in terms of the heart rate, LVEDP, LVDP, rate-pressure product (RPP), dp/dt max, dp/dt min, coronary flow, coronary resistance and perfusion pressure. Similarly, before and during ischemia,

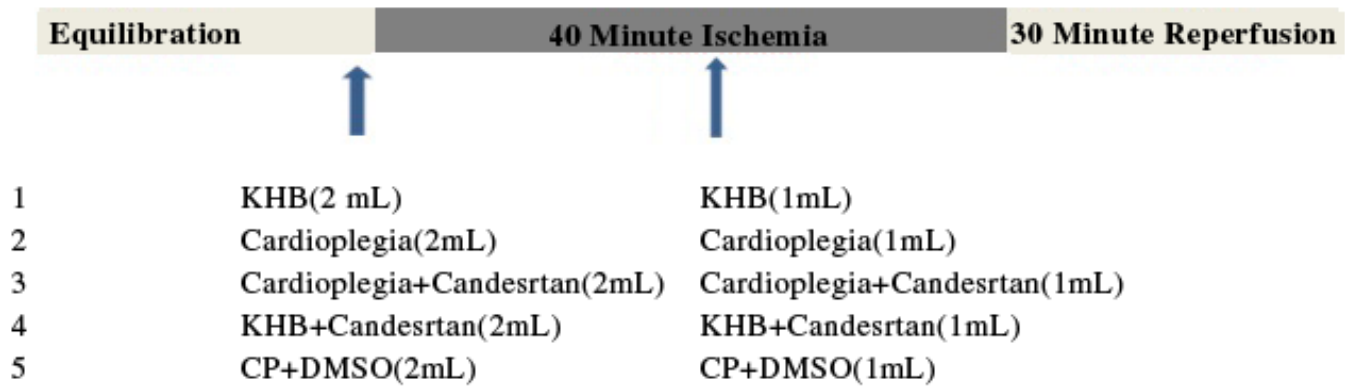

Figure 1. A schematic view of the experimental protocol.

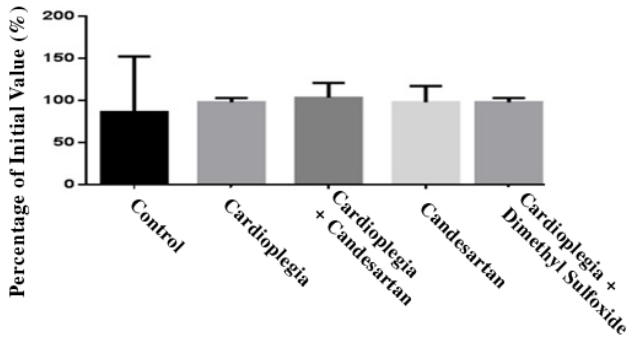

Figure 2. The recorded heart rates after 15 minute equilibration period.

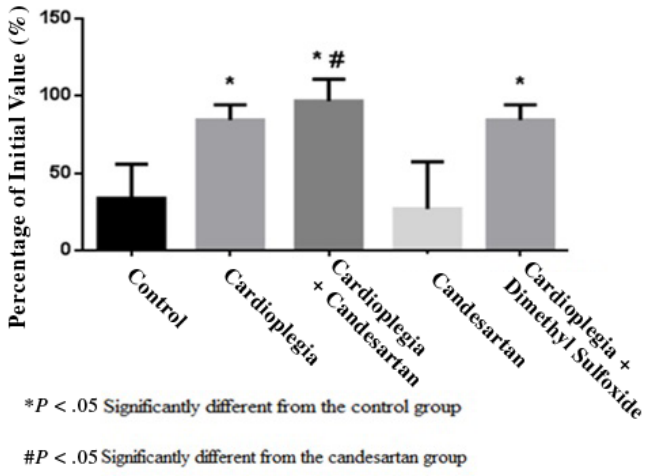

Figure 3. After 15 minute equilibration period, measured values of LVEDP.

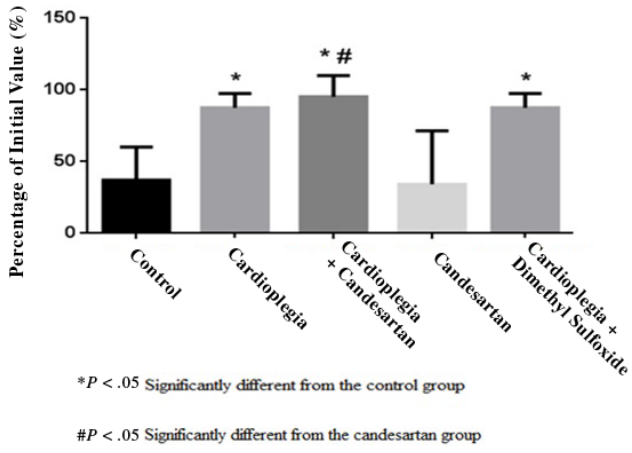

Figure 4. After 15 minute equilibration period measured rate-pressure product (HBI) values.

cardioplegic solution, candesartan + cardioplegic solution, and candesartan alone did not cause any change in coronary flow or coronary resistance at the end of reperfusion compared with that of the onset of reperfusion (Tables 1 and 2).

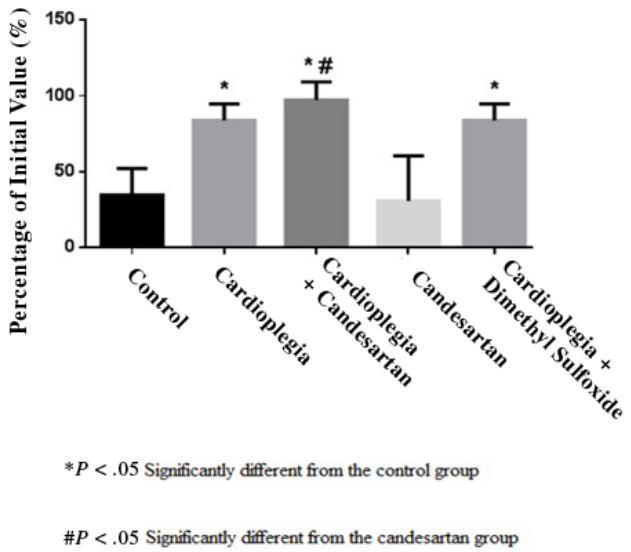

Figure 5. After 15 min equilibration period the rate of contraction (dP/dt max) values.

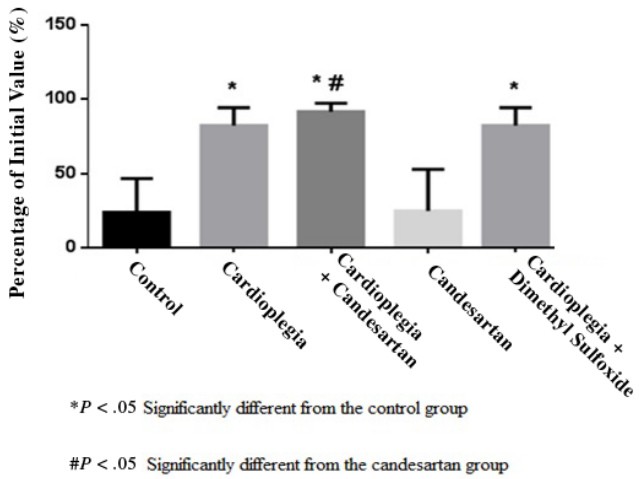

Figure 6. After 15 minute equilibration period relaxation rates (dP/dt min).

Control Hearts (Group 1)

In the control group at the end of reperfusion, HR did not significantly change with respect to baseline (Figure 2); however, severe arrhythmias were observed during reperfusion. LVEDP significantly decreased at the end of reperfusion, and compared to baseline values, contractile function was 33.7% ± 7.4% preserved (Figure 3). In parallel with this finding, RPP, +dp/dt, and -dp/dt decreased at the end of reperfusion and were preserved at a rate of 23.8% ± 8.1%, 36.9% ± 8.2%, and 34.8% ± 6.1%, respectively (Figures 4, 5 and 6). Baseline values before ischemia and at 30-minute reperfusion are shown in Tables 1 and 2.

As a result of the relaxation response of the heart after I/R injury, a significant increase in LVEDP was observed at the

Table 1. Baseline Values After 15-Minute Equilibration Period

BASELINE	Control	CP	CP+CAN	CAN	CP+DMSO
Left Ventricular End Diastolic Pressure	3.9 ± 0.8	5.7 ± 0.5	3.4 ± 0.7	5.5 ± 1.5	8.9 ± 1.05
Left Ventricular Developed Pressure	84.6 ± 8.5	82.2 ± 5.8	82.4 ± 4.5	88.5 ± 6.7	68.6 ± 7.4
Heart Rate	217.4 ± 22.8	213.5 ± 10.1	191.3 ± 7.6	238.8 ± 13.8	226.0 ± 38
Rate-pressure Product	17182 ± 896	17624 ± 1582	16439 ± 939	18871 ± 413.6	15785 ± 4268
Relaxation rate, dp/dt min	-1324 ± 94.5	-1118 ± 83.7	-1156 ± 87.7	-1581 ± 162.7	-948.9 ± 197.1
Construction rate, dp/dt max	1423 ± 103	1317 ± 114.6	1320 ± 93.6	1627.0 ± 6.4	1010 ± 193.3
Coronary Flow	18.5 ± 1.7	15.1 ± 1.8	16.0 ± 2.2	20.3 ± 4.2	21.2 ± 7.8
Perfusion Pressure	72.5 ± 0.3	74.1 ± 0.3	73.7 ± 0.4	73.4 ± 0.4	74.6 ± 0.4
Coronary Resistance	4.1 ± 0.4	4.6 ± 0.4	4.4 ± 0.6	3.8 ± 0.8	4.1 ± 1.5

end of reperfusion with respect to baseline in control hearts, as expected (Tables 1 and 2).

The Effects of Cardioplegic Solution (Group 2)

In hearts administered cardioplegic solution, LVEDP improved at a rate of 84.4% ± 3.4% and this improvement was statistically significant compared with the control group. HR did not significantly change compared with baseline at the end of reperfusion (Figure 3). However, RPP, +dp/dt, and -dp/dt rates were maintained at 82.4% ± 4.6%, 87.5% ± 4.0% and 83.8% ± 4.4%, respectively (Figures 4, 5, and 6). In these parameters, the recovery of contractile function was better than that in the control group. Contractile parameters in hearts administered cardioplegic solution are shown in Tables 1 and 2. Compared with baseline, implementation of cardioplegia did not significantly change LVEDP at the end of reperfusion (Tables 1 and 2). At the end of reperfusion, LVEDP was significantly lower in the control group.

The Effects of Cardioplegia with Candesartan (Group 3)

In the group treated with candesartan plus cardioplegia, HR did not change compared to baseline at the end of reperfusion. In control hearts, arrhythmias were not observed at the end of reperfusion in both the cardioplegia group and the cardioplegia with candesartan group. In addition, HR during reperfusion was found to be more stable in the controls. Compared with initial values, in candesartan treated hearts, LVEDP, RPP, dp/dt max, and dp/dt min improved by 96.8% ± 5.3%, 91.8% ± 1.2%, 95.03% ± 4.9%, and 97.4% ± 4.2%, respectively. The improvements in these parameters were higher than those in the group that had cardioplegia alone, but the difference was not statistically significant. However, compared with the control group, the administration of candesartan in cardioplegia showed a statistically significant improvement in contractile function (Figures 3, 4, 5, and 6). The administration of candesartan in cardioplegia solution did not change LVEDP at the end of reperfusion compared with initial value (Tables 1 and 2). LVEDP at the end of reperfusion was significantly lower than that in the control group, and this value was similar to that in the group that had cardioplegia alone.

The Effects of KHB with Candesartan (Group 4)

In the I/R protocol, before and during ischemia, the effects of 100 nM candesartan in KHB [Fukumoto 2012] on the contractile function were similar to control I/R hearts. Candesartan administered alone before and during ischemia did not create a cardioprotective effect. The effects of candesartan alone on LVDP, RPP, and ± dp/dt are shown in Figures 3, 4, 5, and 6.

LVEDP significantly increased at the end of reperfusion in the candesartan in KHB group compared to baseline (Tables 1 and 2). LVEDP at the end of reperfusion was similar to that in the control group.

The Effects of Cardioplegia with DMSO (Group 5)

In the group treated with cardioplegia plus 0.1% DMSO an improvement of 81.9% ± 0.9 and 2.5 ± 0.3%, respectively was shown in LVEDP and RPP. The impact on ± dp/dt parelleled the effects on LVDP and RPP. The effects of 0.1% DMSO in cardioplegia on cardiac parameters were similar to the cardioplegia group (Figures 3, 4, 5, and 6).

Using 0.1% DMSO in cardioplegic solution did not change the LVEDP at the end of reperfusion compared to the initial value (Tables 1 and 2). LVEDP at the end of reperfusion was significantly lower than in the control group, and were similar to the cardioplegia only group.

DISCUSSION

Various agents have been added to cardioplegia solutions and tested in both clinical trials and animal studies. The inhibition of cardiac RAS is one strategy for prevention of I/R injury. Studies have shown that the addition of ACE inhibitors to cardioplegia during cardiac surgery has positive effects on myocardial function and can prevent I/R injury, including changes in creatine kinase, lactate dehydrogenase, and troponin-T, arrhythmias and infarcts [Gurevitch 1997;

Table 2. Thirty-minute Reperfusion Values After 15-Minute Equilibration Period

REP 30 min	Control	CP	CP+CAN	CAN	CP+DMSO
Left Ventricular End Diastolic Pressure	24.0 ± 8.2 Δ	4.2 ± 1.1	4.1 ± 1.0#	37.2 ± 16.4	4.5 ± 1.8
Left Ventricular Developed Pressure	28.6 ± 7.2 Δ	65.8 ± 5.4* Δ	79.9 ± 6.8*#	23 ± 13.4 Δ	56.3 ± 6.6
Heart Rate	184.8 ± 39.4	216.0 ± 7.5	187.8 ± 15.1	232.3 ± 23.0	227.5 ± 36.5
Rate-pressure Product	3902 ± 1118 Δ	14493 ± 1489* Δ	15009 ± 1079*† Δ	4664 ± 2476 Δ	13038 ± 3562
Relaxation Rate, dp/dt min	-453 ± 85.4 Δ	-980.6 ± 98.5* Δ	-1121 ± 107.8*†	-482 ± 238.7 Δ	-823.1 ± 173.7
Construction rate, dp/dt max	520.5 ± 126.1 Δ	1159 ± 113.6* Δ	1255 ± 116.3*†	554.5 ± 355 Δ	921.7 ± 155.5
Coronary Flow	14.2 ± 3.0	13.8 ± 1.5	15.3 ± 1.8	14.0 ± 4.0	16.8 ± 7.3
Perfusion Pressure	73.8 ± 0.4	73.8 ± 0.6	73.4 ± 0.3	73.1 ± 1.4	73.0 ± 0.6
Coronary Resistance	5.9 ± 1.6	5.7 ± 0.7	4.9 ± 0.7	5.0 ± 1.5	5.4 ± 2.4

*$P < .05$ significantly different compared to the control group, †$P < .05$ candesartan (CAN) group significantly

Paz 1998; Leva 2006; Lucchese 2011]. Although there are a limited number of studies which show that the addition of ARBs to cardioplegia can be protective, there are no specific studies using candesartan, one of the ARBs, in cardioplegia.

In our study, cardioplegia alone and cardioplegia plus candesartan significantly reduced myocardial injury compared to controls, and consistent with the findings of other studies, the cardioplegia solution maintained LVDP, RPP and contraction and relaxation rates [Gurevitch 1997; Leva 2006]. However, in the candesartan + cardioplegia group, markers of contractile function (LVDP, RPP, ± dp/dt) did not significantly change at the end of reperfusion. Although improvement in myocardial contraction was better in the candesartan + cardioplegia group than in the group getting cardioplegia alone, there was no significant difference in terms of % change in contractility between these two treatment groups at the end of reperfusion compared to initial values. The reason the addition of candesartan to cardioplegia did not provide additional protection may be due to the time and frequency of administration of the drug. We administered candesartan in a single dose before and during ischemia and investigated changes in cardioprotective effects during reperfusion.

In addition to the time of application, lack of a screening dose was another limiting factor in this study. In studies where ACE inhibitors were added to the cardioplegic solution, the administration of these agents before and during ischemia did not provide additional protection. Better cardiac protection was achieved after administration of zofenopril for one week orally or the administration of captopril both before and during reperfusion [Gurevitch 1997; Leva 2006]. In another study, the application of high-dose losartan, an ARB blocker, in cardioplegia provided better protection than cardioplegic arrest [Lucchese 2011]. The results of these studies support our claim that candesartan plus cardioplegia does not provide additional myocardial protection.

This finding is similar to those in a study by Paz et al. [Paz 1998] which showed that the addition of losartan in a cardioplegic arrest protocol protects the heart against I/R, whereas low concentrations of losartan in KHB did not result in improvement in contractile parameters compared with the control. Furthermore, in a study that investigated the effects of candesartan on ischemia-induced release of norepinephrine in isolated rat hearts, Fukumoto et al. showed that candesartan did not improve contractile function in hearts exposed to a 40-minute period of global ischemia, similar to our findings. The study found that, depending on the duration of ischemia, norepinephrine was released through exocytosis or through a carrier, and that candesartan was only protective against short-term ischemia (20 minutes) by inhibiting the release of exocytosis-mediated norepinephrine [Fukumoto 2012]. In our study, this may be the reason why candesartan was not protective against 40 minutes of global ischemia. Additional studies are needed for further elucidation of this.

CONCLUSION

The addition of candesartan to the cardioplegia routinely used during cardiac surgery to protect against I/R injury was not shown to be superior to the administration of cardioplegic solution alone.

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