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Preconditioning Effects of Dexmedetomidine on Myocardial Ischemia/Reperfusion Injury in Rats

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ABSTRACT

BACKGROUND: Preconditioning might protect the myocardium against ischemia/reperfusion injury by reducing infarct size and preventing arrhythmias. Dexmedetomidine (DEX) is a highly selective α_2-agonist used for sedoanalgesia in daily anesthetic practice. The cardioprotective effects of DEX on infarct size and on the incidence of arrhythmias observed after regional ischemia/reperfusion injury in vivo have not been reported.

OBJECTIVE: The aim of this study was to determine whether DEX exhibits a preconditioning effect and reduces infarct size and the incidence and duration of arrhythmias in a regional cardiac ischemia/reperfusion model in rats.

METHODS: Adult male Sprague-Dawley rats were anesthetized with sodium thiopental and mechanically ventilated (0.9 mL/100 g at 60 strokes/min) through a cannula inserted into the trachea after tracheotomy. Cardiac ischemia was then produced by ligating the left main coronary artery for 30 minutes, followed by a reperfusion period of 120 minutes. Blood pressure (BP) and heart rate (HR) were monitored and echocardiograms (ECGs) were performed. Arrhythmia was scored based on incidence and duration. The animals were randomly divided into 3 groups. The ischemic preconditioning (IPC) group underwent 5 minutes of ischemia followed by 5 minutes of reperfusion before the 30-minute ischemia/120-minute reperfusion period. In the DEX group, intraperitoneal (IP) DEX 1 mL (100 μg/kg) was administered 30 minutes before the ischemia/reperfusion period. In the control group, IP saline 1 mL was administered 30 minutes before the ischemia/reperfusion period. After reperfusion, the heart was excised, demarcated with saline and ethanol to identify the occluded and nonoccluded myocardium, and cut into slices ~2 mm thick, that were then stained and placed between 2 glass plates. The risk zone and the infarct zone were compared between groups. The investigator assessing the infarcts was blinded to the study group.

RESULTS: Twenty-one adult (aged 4–6 months) male Sprague-Dawley rats weighing 280 to 360 g were included in the study; 7 rats were assigned to each group. BP, HR, and ECG readings were not significantly different between groups and did not change during the study. Arrythmias occurred during ischemia and reperfusion in all

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groups. The duration of the arrhythmias was significantly shorter and the arrhythmia score was significantly lower in the IPC group (all, $P < 0.05$), compared with the control group; however, they were not significantly different in the DEX group. During the ischemic period, duration of ventricular tachycardia (VT) and ventricular premature contractions (VPC) in the DEX group was significantly longer than that observed in the IPC group (all, $P < 0.05$). The duration of VPC was also significantly shorter than that observed in the control group (both, $P < 0.05$). Duration of VT during the reperfusion period in the DEX group was significantly longer than that observed in both IPC and control groups (both, $P < 0.05$). The mean (SD) percentage of damage was significantly lower in the IPC group (44.1% [2.0%]) and the DEX group (26.7% [2.0%]) compared with the control group (69.0% [3.0%]; both, $P < 0.05$). The percentage of damage in the DEX group was also significantly lower compared with the IPC group ($P < 0.05$).

CONCLUSIONS: This small, experimental in vivo study found that DEX was associated with reduced infarct size in ischemia/reperfusion injury in regional ischemia in this rat model but had no effect on the incidence of arrhythmias. Future studies are needed to clarify these findings. (*Curr Ther Res Clin Exp.* 2008;69:150–158) © 2008 Excerpta Medica Inc.

KEY WORDS: dexmedetomidine, preconditioning, cardiac ischemia/reperfusion.

INTRODUCTION

Ischemia and subsequent reperfusion cause morphologic and functional damage that manifest as ventricular arrhythmias within minutes of the occlusion of related arteries. A short ischemic period, called *preconditioning*, before ischemia and reperfusion may protect the myocardium by reducing infarct size and preventing arrhythmias.[1–3] This preconditioning effect may also be achieved pharmacologically by perfusing certain drugs before and during ischemia. A number of drugs used in daily anesthetic practice (eg, volatile anesthetics,[4] nitrates,[5] opioids,[6,7] and levosimendan[8]) have been found to produce preconditioning effects. Dexmedetomidine (DEX) is a selective α_2-agonist indicated for sedoanalgesia in daily anesthetic practice, especially in intensive care units.[9] DEX has a broad spectrum of action in humans, including sedation, anesthetic-sparing effects, analgesia, and decreased heart rate and blood pressure.

As the outcome after ischemia and reperfusion is mainly affected by infarct size and is manifested as arrhythmias in the early period within minutes, most studies of preconditioning assess the occurrence of arrhythmias and infarct size.[1–3] Few studies of the cardioprotective effect of DEX in ischemia/reperfusion injury have been done.[10–12] One double-blind, randomized, dose-escalation trial[10] performed in 24 patients undergoing open-heart surgery found a beneficial effect—a decrease in the incidence of adverse cardiac events (eg, hemodynamic instability) of DEX (2.30–9.87 g/kg) on ischemic myocardium when used 1 hour preoperatively, perioperatively, and 48 hours postoperatively. An in vivo study[11] found the beneficial effect of sympatholysis produced by DEX during ischemia/reperfusion in dogs. An in vitro study[12] of the cardioprotective effects of DEX (1 and 10 nM) in isolated hearts of rats undergoing global ischemia/reperfusion

injury reported a significant reduction in coronary flow and significantly decreased infarct size.

The protective effects of DEX on infarct size and on the incidence of arrhythmias observed after regional ischemia/reperfusion using an in vivo model have not been reported. The aim of this study was to determine whether DEX* exhibits a preconditioning effect and reduces infarct size and the incidence and duration of arrhythmias in an experimental regional cardiac ischemia/reperfusion model.

MATERIALS AND METHODS

ANIMALS

Male Sprague-Dawley rats were used in the study. The rats were obtained from the Medical Laboratory Research Institute of Istanbul University, Istanbul, Turkey. The animals were housed in individual cages in an animal room (temperature 22°C, humidity 40%–70%) on a 12-hour light/dark cycle and were fed standard pellet food (210 kcal/100 g · d^{-1}) and water ad libitum after approval by the ethics committee of Abant Izzet Baysal University Medical School (Bolu, Turkey). The animals were kept and treated and the experiments were carried out in adherence with conditions delineated in the guidelines and recommendations of the World Medical Association.[13]

EXPERIMENTAL PROTOCOL

The animals were randomly divided into 3 groups (**Figure 1**). Randomization was achieved using a computer-based random number generation technique. The animals were anesthetized with intraperitoneal (IP) sodium thiopental 50 mg/kg and mechanically ventilated (0.9 mL/100 g at 60 strokes/min) through a cannula inserted into the trachea after tracheotomy. Blood pressure was continuously monitored through a cannula inserted into the left carotid artery. Needles inserted subcutaneously into the anterior thorax were used for the echocardiogram (ECG) (lead II) (AD Instruments, Oxfordshire, United Kingdom).

The heart was exposed after opening the chest at the fourth intercostal space, and a loose loop of atraumatic silk (5/0) was placed around the left main coronary artery ~2 mm distally from its origin for ligation during the ischemia period. In all 3 groups, cardiac ischemia was induced by ligation of the left main coronary artery with the previously placed silk loop for 30 minutes. The silk loop was then loosened to allow reperfusion for 120 minutes.

In the ischemic preconditioning (IPC) group, the rats underwent a 5-minute ischemia period followed by a 5-minute reperfusion period beginning 20 minutes after the anesthetic was administered. The animals in this group then underwent the 30-minute ischemia/120-minute reperfusion period. In the DEX group, 1 mL of a solution containing DEX 100 μg/kg was administered IP 30 minutes before the ischemia/reperfusion period. In the control group, IP saline 1 mL was administered 30 minutes before the ischemia/reperfusion period. After completion of the ischemia/reperfusion protocols, animals were heparinized and the heart was excised to assess infarction.

*Trademark: Precedex® (Abbott Laboratories, Abbott Park, Illinois).

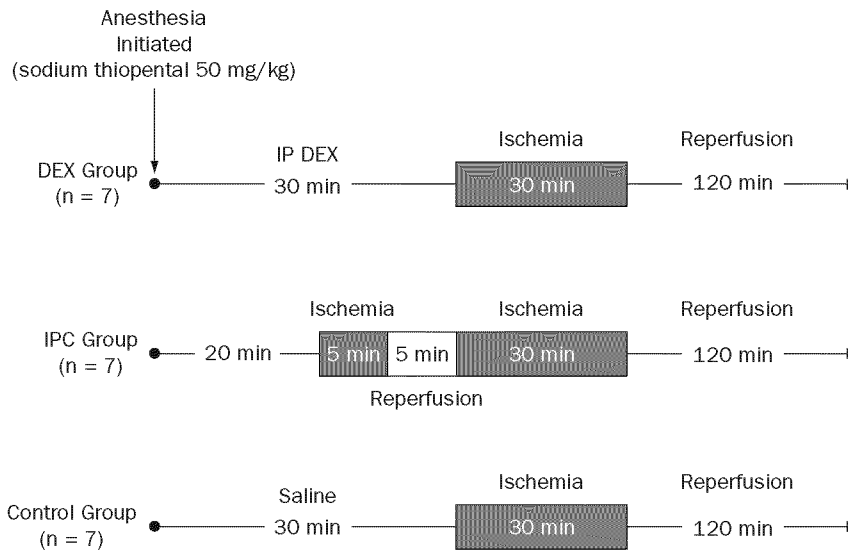

Figure 1. Study protocol. DEX = dexmedetomidine; IP = intraperitoneal; IPC = ischemic preconditioning.

ARRHYTHMIA ANALYSIS

Heart rate and the duration of arrhythmia were determined using ECG. The incidence of arrhythmia was analyzed (by one of the authors [O.B.] who was blinded to the group to which the animals were assigned) in accordance with the Lambeth Conventions[14] as ventricular premature contractions, ventricular fibrillation (VF), ventricular tachycardia, and other types of arrhythmias (extrasystole, bigeminy, and salvo). An arrhythmia score was determined using the following scale[15]: 0 = no arrhythmia; 1 = arrhythmia duration <10 seconds; 2 = duration 11 to 30 seconds; 3 = duration 31 to 90 seconds; 4 = duration 91 to 180 seconds or reversible VF for <10 seconds; 5 = duration >180 seconds or reversible VF >10 seconds; and 6 = irreversible VF or death. These scores were used to assess the arrhythmias and to make between-group comparisons.

DETERMINATION OF INFARCTED AREA

At the end of the experiment, the left coronary artery was retightened and the heart was retrogradely exposed to 10 mL of saline solution and then washed out with 2 mL of ethanol administered from the aorta to demarcate the occluded and nonoccluded myocardium.[16] Subsequently, hearts were cut into slices ~2 mm thick parallel to the atrioventricular groove. The slices were then stained with 0.1% nitroblue tetrazolium in phosphate buffer for 20 minutes and placed in formaldehyde solution for 20 minutes. The slices were placed between 2 glass plates. The risk zone and the infarct zone were traced on acetate sheets from these plates. These pictures were digitalized using a digital camera, and areas of infarction were measured using an image-editing software program (Adobe Photoshop, Adobe Systems Inc., San Jose, California) by an expert

blinded to the study group. The infarct area was divided by the risk zone to calculate the percentage of damage.

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS for Windows version 9.0 (SPSS Inc., Chicago, Illinois). Parameters were expressed as mean (SEM). The significance of the data obtained was evaluated using analysis of variance. The Mann-Whitney *U* test was used to compare the groups. The arrhythmia incidence and scores were compared using the χ^2 test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Twenty-one male Sprague-Dawley rats (aged 4–6 months; weight range, 280–360 g) were included in the study; 7 rats were assigned to each group.

Heart rate and blood pressure were similar in all 3 groups before ischemia/reperfusion. Heart rate did not change significantly during ischemia or reperfusion in any group. In all groups, arterial blood pressure decreased sharply after ligation and increased slowly during reperfusion; the changes were similar between the groups (Table I).

Arrhythmias occurred during ischemia and reperfusion in all groups. The mean total duration of arrhythmias was significantly shorter during ischemia and reperfusion and the arrhythmia scores were significantly lower in the IPC group compared with the control group (all, $P < 0.05$). During the ischemic period, the duration of ventricular tachycardia (VT) in the DEX group was significantly greater than that observed in the IPC group or the control group (both, $P < 0.05$). The duration of ventricular premature contractions during ischemia in the DEX group was significantly greater than that observed in the IPC group and less than that observed in the control group (both, $P < 0.05$). During the reperfusion period, duration of VT in the DEX group was greater than both the IPC and control groups (both, $P < 0.05$). The changes in the duration of arrhythmias in the DEX group were not significantly different compared with the control group. During ischemia, the arrhythmia score was significantly lower in the IPC group compared with the DEX group and the control group (0.33 [0.33] vs 1.66 [0.66] and 1.75 [0.47]; both, $P < 0.05$). During reperfusion, the arrhythmia score was also

Table I. Hemodynamic changes before ischemia and during ischemia and reperfusion in adult male Sprague-Dawley rats (N = 21).* Data are mean (SEM).

	Heart Rate, bpm			Blood Pressure, mm Hg		
Group	Before Ischemia	During Ischemia	During Reperfusion	Before Ischemia	During Ischemia	During Reperfusion
IPC	319 (9)	321 (7)	323 (10)	108 (6)	102 (4)	114 (7)
DEX	334 (12)	326 (8)	332 (12)	116 (5)	103 (5)	119 (8)
Control	338 (11)	311 (9)	341 (13)	104 (3)	96 (2)	110 (4)

IPC = ischemic preconditioning; DEX = dexmedetomidine.
*There were no significant between-group differences.

significantly lower in the IPC group compared with the DEX group and the control group (0.33 [0.33] vs 2.6 [1.33] and 2.55 [1.47]; both, $P < 0.05$) (**Table II**).

The mean size of the occluded area was similar in the IPC group (44.65 [3.3] mm^2), DEX group (46.47 [2.7] mm^2), and control group (37.42 [3.7] mm^2). The infarct area was significantly smaller in the DEX group compared with the IPC group and the control group (12.41 [2.5] mm^2 vs 19.71 [3.2] mm^2 and 25.83 [2.8] mm^2; both, $P < 0.05$). The mean (SD) percentage of damage was significantly lower in the IPC group (44.1% [2.0%]) and the DEX group (26.7% [2.0%]) compared with the control group (69.0% [3.0%]; both, $P < 0.05$). The percentage of damage in the DEX group was also significantly lower compared with the IPC group ($P < 0.05$) (**Figure 2**).

DISCUSSION

DEX is used for sedoanalgesia in intensive care units, in postoperative anesthetic care units, and in operating rooms during the perioperative period, especially for hemodynamic stabilization.[12,17] Myocardial ischemia may occur in all of these situations. Mangano et al[18] reported that myocardial ischemia is one of the most important risk factors for adverse cardiac outcome in surgical patients with coronary artery disease. This adverse outcome was reported to be reduced by perioperative infusion of DEX.[18] The mechanism by which DEX decreases myocardial ischemia was thought to be related to the sympatholytic effect of the drug.[11,19] Okada et al[12] also reported the cardioprotective effect of DEX to be mediated by α_2-adrenergic stimulation. Dahmani et al[17] investigated the possible mechanism of the preconditioning effect of DEX on cerebral focal ischemia and reported that increased phosphorylation of focal adhesion kinase via stimulation of α_2-receptors and decreased cleaved caspase-3 expression was significantly correlated with cell survival. DEX was also associated with significantly decreasing the infarct size in our study, but it did not decrease the incidence of arrhythmias. Similarly, hemodynamic changes did not support the differences in the percentage of damage, as no differences were found between groups in heart rate or blood pressure. We may speculate from these results that α_2-adrenergic stimulation using DEX may not totally block the factors that cause arrhythmia, although it may prevent ischemia/reperfusion injury. A decrease in adrenaline concentration caused by α_2-adrenergic stimulation might have been associated with the arrhythmia observed in the DEX group. This theory is supported by the study of Bozdogan et al,[15] in which the exogenous administration of adrenaline decreased the occurrence of arrhythmia in an ischemia/reperfusion model in rats. Therefore, the decrease in infarct size in the DEX group might be related to factors other than adrenaline. Hemodynamic stabilization during ischemia may also be related to the cardioprotective and sympatholytic effects of DEX that were observed via α_2-adrenergic stimulation.

The cardioprotective effect of DEX was reported to be related to a decrease in myocardial oxygen demand, which was attributed to hemodynamic effects (eg, reduction in heart rate and preservation of blood flow in ischemic myocardium).[11] The preservation of blood flow in ischemic myocardium by α_2-agonists is thought to be caused by local metabolic stimuli that prevent adrenergic vasoconstriction during ischemia.[11] DEX use has been associated with several simultaneous hemodynamic effects—a central sym-

Table II. Duration of arrhythmia during ischemia and reperfusion and arrhythmia score in adult male Sprague-Dawley rats (N = 21). Data are mean (SEM).

		Duration of Arrhythmia During Ischemia, min				Duration of Arrhythmia During Reperfusion, min				Arrhythmia Score*	
Group	No.	VPC	VT	VF	Total	VPC	VT	VF	Total	Ischemia	Reperfusion
IPC	7	1.2 (0.12)†	2.25 (3.9)‡	0	2.3 (2.3)†	0.2 (0.2)†	0‡	0	0.2 (0.2)†	0.33 (0.33)†	0.33 (0.33)†
DEX	7	4.6 (0.53)§	8.2 (0.82)§	0	12.8 (07.4)	4.36 (0.54)	1.26 (1.26)§	0	5.62 (0.69)	1.66 (0.66)	2.6 (1.33)
Control	7	8.35 (3.02)	2.52 (0.52)	0	10.88 (3.0)	3.33 (2.81)	0.16 (0.16)	0	3.48 (2.96)	1.75 (0.47)	2.55 (1.47)

VPC = ventricular premature contractions; VT = ventricular tachycardia; VF = ventricular fibrillation; IPC = ischemic preconditioning; DEX = dexmedetomidine.

*Scale[15]: 0 = no arrhythmia; 1 = arrhythmia duration <10 seconds; 2 = duration 11 to 30 seconds; 3 = duration 31 to 90 seconds; 4 = duration 91 to 180 seconds or reversible VF for <10 seconds; 5 = duration >180 seconds or reversible VF >10 seconds; and 6 = irreversible VF or death.

†$P < 0.05$ versus the DEX group and the control group.

‡$P < 0.05$ versus the DEX group.

§$P < 0.05$ versus the IPC group and the control group.

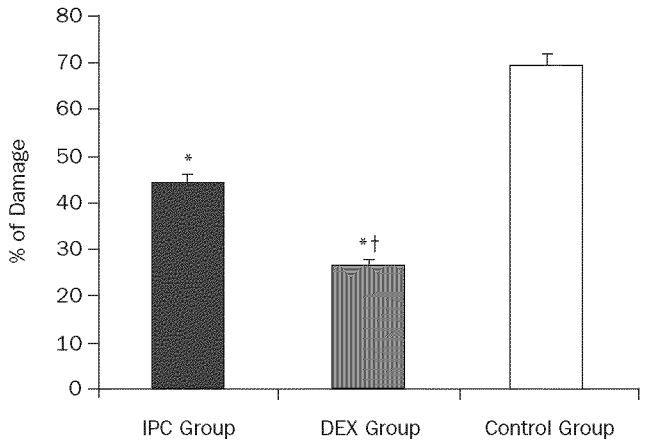

Figure 2. Mean (SEM) percentage of infarction by group in male Sprague-Dawley rats undergoing ischemia and reperfusion (N = 21). IPC = ischemic preconditioning; DEX = dexmedetomidine. *$P < 0.05$ versus the control group; †$P < 0.05$ versus the IPC group.

patholytic effect that might reduce blood pressure and a peripheral vasoconstrictive effect that might increase blood pressure. Okada et al[12] used an isolated heart model to investigate the local effects of DEX on the heart. We used an in vivo model to attempt to avoid underestimating the systemic effects of DEX on the cardiovascular system.

LIMITATIONS

One of the limitations of this study was not measuring adrenaline concentration, a mediator of ischemic preconditioning. We could not clearly explain the conflicting data between the occurrence of arrhythmias and the decrease in infarct size in this rat model. Although it is clear that DEX was associated with reduced infarct size and protection of the heart against ischemia/reperfusion injury, we expected these effects to be supported by a decrease in arrhythmias. Further studies are needed to examine these conflicting data and to investigate the applicability of these results to humans.

CONCLUSIONS

This small, experimental in vivo study found that DEX was associated with reduced infarct size in ischemia/reperfusion injury in regional ischemia in this rat model but had no effect on the incidence of arrhythmias. Future studies are needed to clarify these findings.

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The authors have nothing to disclose.

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