The Heart Surgery Forum #2004-1074
7 (5), 2004 [Epub September 2004]
doi:10.1532/HSF98.20041074

Online address: www.hsforum.com/vol7/issue5/2004-1074.html

Effects on Reperfusion Injury of Adding Diltiazem to Tepid Blood Cardioplegia

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ABSTRACT

Background: Although the present techniques of myocardial preservation for limiting ischemia/reperfusion injury in open heart operations yield excellent results for most patients, certain subgroups of patients with advanced coronary artery disease present a challenge in terms of intraoperative safety.

Methods: In a prospective, randomized, controlled study, we assessed the myocardial protective effects of a total dose of 150 ± 150 = 300 μg/kg diltiazem added to induction and terminal (reperfusion) doses of tepid blood cardioplegia. We determined the myocardial morphological (ultrastructural) and enzymatic (serum assays for the cardiospecific isoenzyme of creatine kinase [CK-MB]) changes and functional recovery (atrioventricular [AV]-node recovery time and postoperative need for inotropic support) in patients undergoing elective coronary artery bypass operations. The determinations were made with respect to values for control patients, who received the same cardioplegia but without the addition of diltiazem.

Results: The mean isoenzyme CK-MB levels and semiquantitative ultrastructural score values of the diltiazem group were significantly less than those of the control group. Although AV-node recovery time was significantly prolonged ($P < .05$), this factor did not have major clinical impact.

Conclusions: We concluded that the addition of 150 ± 150 μg/kg diltiazem to the induction and terminal doses of tepid cardioplegia enhanced myocardial protection in elective aortocoronary bypass surgery in high-risk patients and presented no significant additional operative risk.

INTRODUCTION

Among patients who have an indication for coronary artery bypass surgery, the proportion of those at high operative risk is steadily on the rise [Christakis 1986, Cohen 1999]. On the other hand, postoperative low cardiac output syndrome, which carries significant risk of mortality and morbidity, is associated with insufficient myocardial protection during the operation. An essential part of the efforts to develop methods and remedies for better protection of the myocardium is focused on optimization of the composition, delivery method, and temperature of the cardioplegic solution [Cohen 1999, Chambers 2003]. Among various techniques, tepid blood cardioplegia, used in this study, is widely reported as an effective technique for myocardial protection [Choron 2000].

Various cardioplegic additives, including Ca^{2+} antagonists, are used to improve the protective effects of cardioplegic solutions. The logic behind using a Ca^{2+} antagonist during the ischemia and reperfusion periods is to restrict the uncontrolled Ca^{2+} influx that plays a major contributory role in formation of Ca^{2+} overload in the myocytes, because the overload leads to reperfusion injury [Allen 1986, Barner 1987]. The main drawback of Ca^{2+} antagonists is that they have dose-dependent negative inotropic and chronotropic effects. Results obtained in previous trials with Ca^{2+} antagonists, such as verapamil and nifedipine, have not been satisfactory. Diltiazem, an L-type Ca^{2+} antagonist, has been tested as an additive in both the induction and maintenance doses of cardioplegic solutions; that is, just before and during the ischemia period, diltiazem, among the pharmacological agents in this class, has been shown to provide optimal myocardial protection with the lowest cost in terms of cardiac function [Bourdillon 1980, Lathrop 1982, Yamamoto 1983, Grover 1989]. In a recent study performed with cold blood cardioplegia, 0.1 mol/L diltiazem added to "hot shot" reperfusion cardioplegia yielded optimal myocardial protection with minimal hemodynamic upset [Kröner 2002]. In this study, we determined the myocardial protective effects of a total dose of 150 ± 150 = 300 μg/kg diltiazem added to induction and terminal reperfusion doses of tepid blood cardioplegia. We assessed the myocardial morphological (ultrastructural) and enzymatic changes in serum cardiospecific isoenzyme of creatine kinase (CK-MB) assays and functional recovery (atrioventricular [AV]-node recovery time and postoperative need for inotropic support) in patients undergoing elective coronary artery bypass operations.

PATIENTS AND METHODS

After approval was obtained from the Ethical Committee of the Medical Faculty of Cukurova University, written

Presented at the 10th Annual CTT Meeting 2004, Miami Beach, Florida, USA, March 10-13, 2004.

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informed consent was obtained from all patients included in the study. Forty patients with multiple coronary vessel disease who were scheduled for elective coronary artery bypass grafting were included in the study. Patients excluded from the study were those with New York Heart Association class I functional status; patients with a history of preoperative congestive heart failure, marked arrhythmia, conduction defects, hepatic, renal, endocrine, or neurological disease, or previous cardiac surgery; and patients who had a left ventricular ejection fraction less than 0.40 and received a calcium channel blocker within 48 hours of the operation or experienced myocardial infarction within 90 days of the operation.

The patients were randomly assigned to 2 comparable groups of 20 individuals each (Table). Tepid blood cardioplegia was given only to patients in the control group. The patients in the diltiazem group received 150 ± 150 μg/kg diltiazem in the induction and terminal doses of tepid blood cardioplegia.

Cardioplegic Technique

After the institution of cardiopulmonary bypass, patients in both groups were cooled to 28°C, the cross-clamp was applied, and the patients' own blood (28°C) containing 25 to 30 mEq/L KCl was infused via the aortic root at a rate of 300 mL/min for 5 minutes (the induction dose). Once in every 20 minutes during cardiac arrest, the patients' own blood (28°C) containing 10 to 15 mEq/L KCl was infused via the aortic root at a rate of 150 mL/min for 3 minutes (the maintenance dose). Terminal cardioplegia solution (tepid injection of patients' own isothermic blood) containing 8 to 10 mEq/L KCl was infused at a rate of 200 mL/min for 3 minutes just before the cross-clamp was removed. In the diltiazem group, 150 ± 150 μg/kg diltiazem (supplied by Mustafa Nevzat Laboratories, Mecidiyekoy, Istanbul, Turkey) was added to the induction and terminal doses of tepid blood cardioplegia.

Perioperative Measures

Supportive cardiopulmonary bypass was continued for at least 30 minutes after the removal of the cross-clamp in all patients. Atrial or AV sequential pacing was planned for episodes of bradycardia (<50 beats/min) or asystole in this period and had to be instituted in 1 patient with bradycardia only (Table).

Variables

Age, sex, New York Heart Association functional class, history of myocardial infarction, preoperative ejection fraction, preoperative Ca^{2+} antagonist and β-blocker therapy, number of diseased vessels, number of bypassed vessels, cardiopulmonary bypass time, cross-clamp time, duration of supportive bypass, number of defibrillation attempts, AV-node recovery time, pacemaker requirement, duration of pacemaker use, and need for postoperative inotropic support were recorded (Table).

Myocardial isoenzyme CK-MB assays were performed by Sandwich technique (Elecys Systems; Roche Diagnostics, Indianapolis, IN, USA). Venous blood samples were collected before induction of anesthesia and 3, 6, 18, 24, 48, and 96 hours postoperatively (Figure 1).

Two myocardial transmural left ventricular biopsy specimens were obtained from the lower part of the left ventricular free wall with a Tru-Cut biopsy needle (Baxter, Chicago, IL, USA). The first specimen was sampled in the 20th minute

Preoperative, Operative, and Postoperative Data for Control and Diltiazem Groups*

	Control Group (n = 20)	Diltiazem Group (n = 20)	*P*
Age, y	57.40 ± 6.02	58.00 ± 7.83	>.05
Sex, M/F	16/4	17/3	>.05
NYHA, I/II/III/IV	0/15/4/1	0/13/6/1	>.05
History of MI, n	4	5	>.05
EF, %	54.60 ± 6.32	52.60 ± 7.35	>.05
Ca^{2+} antagonist, n	6	8	>.05
β-Blocker, n	6	4	>.05
Number of diseased vessels, 2/3	2/18	4/16	>.05
CPB time, min	104.00 ± 10.67	98.25 ± 15.40	>.05
Cross-clamp time, min	61.80 ± 6.79	62.70 ± 8.04	>.05
Defibrillation attempts, n	7	3	<.05
Duration of defibrillation attempts, min	4.60 ± 2.40 (2.50-7.00)	6.00 ± 2.80 (4.20-7.30)	>.05
AV-node recovery time, min	13.25 ± 6.00 (7.30-21.00)	22.72 ± 7.40 (14.15-27.20)	<.05
Spontaneous recovery of AV-node functioning, n	13	20	<.05
PM requirement, n	—	1	
PM dependency, min	—	52	
Inotropic support, n	3	2	
Highest mean CK-MB, ng/mL	52.56 ± 22.90	35.11 ± 11.60	<.05

*NYHA indicates New York Heart Association functional classification; n, number of patients; MI, myocardial infarction; EF, ejection fraction, CPB, cardiopulmonary bypass; AV-node, atrioventricular node; PM, pacemaker; CK-MB, cardiospecific isoenzyme of creatine kinase.

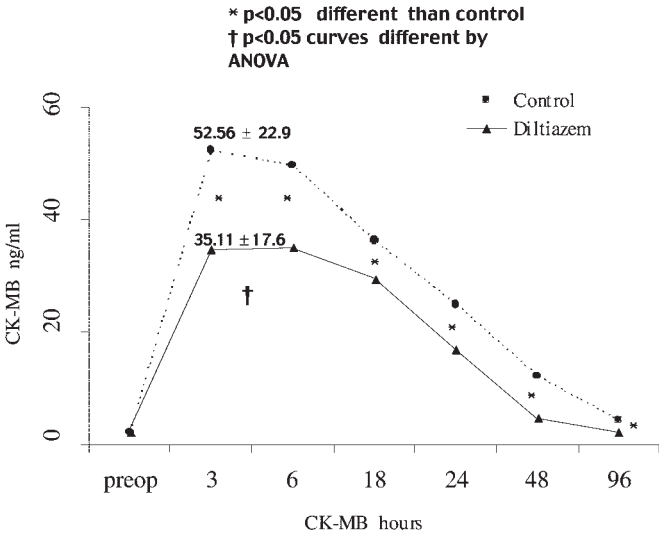

Figure 1. Cardiospecific isoenzyme of creatine kinase (CK-MB isoenzyme) concentration versus time curve.

of the ischemia period. The second specimen was sampled in the 30th minute of the reperfusion period. These samples were fixed in Karnovsky fixative solution for 1 hour, washed with 0.1M cacodylate buffer, treated with buffered osmium tetroxide solution, and fixed in epoxy after dehydration with ethanol. Tissue cross sections with a thickness of 1 μm were painted with toluidine blue and analyzed with light microscopy. Selected samples were sliced into cross sections with a thickness of 500 Å by use of an ultramicrotome (OMU3; Reichert, Vienna, Austria), placed into copper grids with 200 to 300 cells, and painted with uranyl acetate saturated in 70% ethyl alcohol and Reynolds lead citrate solution. The samples were subsequently analyzed under an electron microscope (EM 900; Carl Zeiss, Jena, Germany), and the micrographs were printed (Figures 2 and 3).

Ultrastructural changes in mitochondria were evaluated by means of a semiquantitative ultrastructural evaluation method [Schaper 1977, Flameng 1980]. For this purpose, 20 mitochondria were randomly selected in each micrograph, and ultrastructural changes in each mitochondrion were classified according to the following semiquantitative grading system on a scale of 0 to 4: score 0, normal mitochondrial ultrastructure; score 1, normal ultrastructure of the crests and matrix but absence of granular deposits; score 2, loss of matrix granules and clarification of the matrix without cleavage of crests; score 3, loss of matrix granules and uniform clarification of the matrix with disruption of crests (focal condensation also may be seen with clumps differing in size from ordinary mitochondrial granules to at least twice the usual size); score 4, loss of matrix granules, uniform disruption of crests, and loss of integrity of the mitochondrial membranes.

Statistical Analysis

The preoperative, intraoperative, and postoperative data were entered in a database and analyzed. All calculations were performed with a commercially available statistical package (SPSS 9.0 for Windows). Differences between the preoperative and intraoperative variables for the 2 groups were evaluated with the unpaired 2-tailed Student *t* test, and the intragroup differences were analyzed by 2-way repeated-measures analysis of variance (ANOVA). All values were expressed as mean ± SD. $P < .05$ was considered statistically significant.

The electron microscopy scores were analyzed by nonparametric analysis according to the method of Schaper [1977]. The average score of 20 mitochondria per specimen was then calculated. All micrographs were examined in a

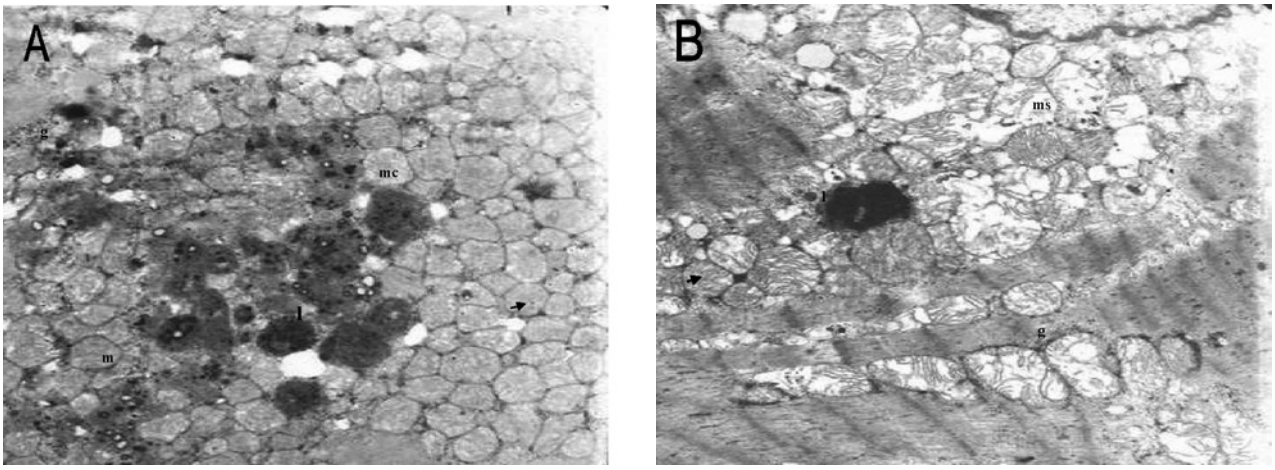

Figure 2. Ultrastructural changes in mitochondria in the control group. A, The mitochondrial changes are milder, and the damage is not homogeneous. Some mitochondria exhibit matrix clearing. The number of mitochondrial granules (arrowhead) has decreased, but the number of glycogen particles is unchanged (score 2). However, there is abundant lipofuscin accumulation B, Mitochondrial swelling with disorganized and disrupted cristae. Mitochondrial membranes are well preserved, the number of mitochondrial granules (arrowhead) has decreased, the number of glycogen particles has increased, and lipofuscin pigment is present in large amounts (score 3). A, Original magnification ×14,000; B, original magnification ×24,000; m indicates normal mitochondria; g, glycogen particles; mc, matrix clearing; l, lipofuscin pigment; ms, mitochondrial swelling.

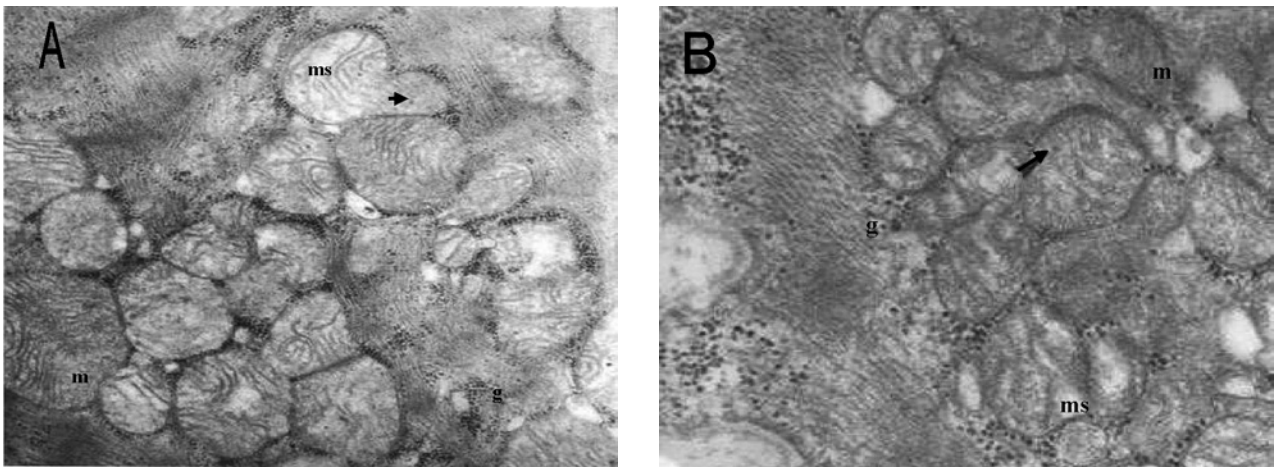

Figure 3. Ultrastructural changes in mitochondria in the diltiazem group. A, Gross ultrastructure is normal; mitochondria are well preserved. However, the number of mitochondrial granules (arrowhead) has decreased. There is slight intracellular edema, and mitochondrial swelling with focally clear matrix is evident. The number of glycogen particles is unchanged (score 1-2). B, Gross ultrastructure is normal, and mitochondria are well preserved. There is slight swelling, the number of mitochondrial granules (arrowhead) has decreased, and the number of glycogen particles has increased (score 1-2). A, Original magnification ×40,000; B, original magnification ×60,000; m indicates normal mitochondria; ms, mitochondrial swelling; g, glycogen particles.

blinded manner. The results were then analyzed by 2-way repeated-measures ANOVA.

RESULTS

There were no significant differences in preoperative and postoperative data between the diltiazem and control groups except for spontaneous AV-node recovery time and mean plasma CK-MB levels.

The important intraoperative and early postoperative outcomes considered in this study are AV-node recovery time and postoperative need for a pacemaker. Seven patients in the control group needed defibrillation 2.50 to 7.00 minutes (mean, 4.60 ± 2.40 minutes) after the cross-clamp was removed, but all recovered sinus rhythm after defibrillation. In this group, spontaneous AV-node function was regained within 7.30 to 21.00 minutes (mean, 13.25 ± 6.00 minutes). In the diltiazem group, 3 patients went into ventricular fibrillation 4.20 to 7.30 minutes (mean, 6.00 ± 2.80 minutes) after the cross-clamp was removed. Fibrillation terminated in asystole and spontaneous ventricular rhythm after defibrillation. In all 20 patients in this group, complete AV block lasted for 14.15 to 27.20 minutes (mean AV-node recovery time, 22.72 ± 7.40 minutes). One of these patients needed ventricular pacing for 52 minutes because of bradycardia during supportive cardiopulmonary bypass. The difference in the mean AV-node recovery time for the diltiazem group was statistically significant with respect to that of the control group ($P < .05$). Nevertheless, none of the patients in the diltiazem group needed supportive cardiopulmonary bypass for more than 30 minutes owing to cardiac asystole as a result of prolonged AV-node recovery time (Table). Three patients in the control group and 2 patients in the diltiazem group needed postoperative inotropic support. Although we did not assess left ventricular function by means of hemodynamic measurements in the immediately postoperative period, the differences between the diltiazem and control groups regarding the need for inotropic support were not significant (Table).

Serum CK-MB values for both groups peaked between 3 and 18 hours postoperatively, and they were significantly higher than the preoperative values ($P < .05$). The highest recorded CK-MB value in the control group was 52.56 ± 22.9 ng/mL, against 35.11 ± 11.6 ng/mL in the diltiazem group (Table and Figure 1). The entire CK-MB coenzyme concentration versus time curve was significantly lower in the diltiazem group ($P < .05$) (Figure 1).

There were significant differences between the ultrastructural changes in the biopsy specimens for both groups 20 minutes after application of the cross-clamp and 30 minutes after removal of the cross-clamp, corresponding with the ischemia and reperfusion periods, respectively. For the control group, the mean semiquantitative mitochondrial ultrastructural scores for the ischemia and reperfusion periods were, respectively, 1.97 ± 0.43 (range, 1.00-2.60) and 2.31 ± 0.24 (range, 2.00-2.70), and the difference between these values was significant ($P < .05$) (Figure 4, a-b). For the diltiazem group, on the other hand, the mean semiquantitative mitochondrial ultrastructural scores for the ischemia and reperfusion periods were, respectively, 1.56 ± 0.31 (range, 0.90-2.00) and 1.40 ± 0.49 (range, 0.65-2.00). The difference between these values was not statistically significant ($P > .05$) (Figure 4, c-d). The mean semiquantitative mitochondrial ultrastructural scores for both the ischemia and the reperfusion periods for the diltiazem group were significantly lower than those of the control group ($P < .05$) (Figure 4, a-c and b-d).

DISCUSSION

In previous studies, as described previously in the literature, diltiazem was added to induction or maintenance doses

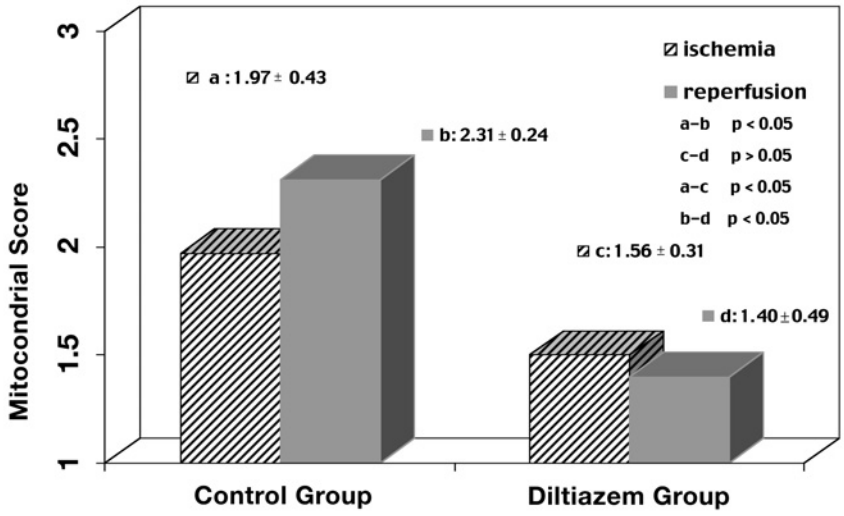

Figure 4. Semiquantitative mitochondrial ultrastructure score for control and diltiazem groups during ischemia and reperfusion. a indicates control group ischemia period; b, control group reperfusion period; c, diltiazem group ischemia period; d, diltiazem group reperfusion period.

of cardioplegic solutions during cardiopulmonary bypass to limit ischemic injury. In this study, diltiazem was added to the cardioplegic dose in 2 stages: first, immediately after application of the cross-clamp (induction dose) to achieve cardioplegic arrest and to improve myocardial tolerance of ischemia and, second, immediately before the cross-clamp was released (terminal dose) to restrict reperfusion injury.

CK-MB, which was used in this study to determine the extent of myocardial injury, is a sensitive biochemical marker. The serum concentration peaks 3 to 8 hours after the onset of myocardial injury and returns to normal values in 48 to 96 hours [Adams 1993]. That postoperative peak CK-MB activity and the entire enzyme concentration versus time curve for the diltiazem group were significantly lower than those for the control group ($P < .05$) demonstrated that ischemic myocardial injury was limited by diltiazem (Table and Figure 1). This result is supported by results of several other studies [Grondin 1983, Barner 1987].

It has been reported that mitochondria are the first cellular organs affected by ischemia and that although the ultrastructural changes during the reperfusion period are essentially reversible [Zografos 1990], these changes are intimately related to the functional and metabolic derangement of myocardial cells caused by ischemia. In this study, we used a semiquantitative grading system for classification of ultrastructural changes in the mitochondria. Our results show that diltiazem added to warm blood cardioplegia significantly limits mitochondrial ultrastructural injury in the ischemia and reperfusion periods with respect to control values ($P < .05$) (Figure 4, a-c and b-d) and improves myocardial recovery (Figures 2 and 3). Furthermore, in the diltiazem group, there was no significant difference in mean mitochondrial ultrastructural scores for the ischemia and reperfusion periods ($P > .05$) (Figure 4, c-d). This finding implied that myocardial protection was improved by the addition of diltiazem to the terminal cardioplegic dose (Figure 3). These cytochemical and ultrastructural findings indicated that the myocardial injury in both the ischemia and the reperfusion periods was restricted by diltiazem.

The profound and prolonged electromechanical arrest achieved with the addition of diltiazem to the terminal cardioplegia dose prior to reperfusion limits unnecessary energy expenditure during the early reperfusion period. Thus when aerobic metabolism recommences with the reperfusion of warm oxygenated blood, metabolic recovery of the myocardial cells is further facilitated by allocation of this saved oxygen and energy to cellular recovery and repair [Teoh 1986, Cohen 1999, Kröner 2002].

The optimal dose of diltiazem used as an adjunct in cardioplegia should be both safe and effective. It should be cardioprotective (should provide profound cardioplegic arrest), and at the same time it should improve metabolic recovery. Electromechanical arrest should be short-lived. In addition to its contribution to myocardial protection from ischemic injury and postischemia myocardial recovery, diltiazem has a dose-dependent cardiodepressive effect and presents the risk of causing temporary AV block [Christakis 1986, Barner 1987, Grover 1989].

Results of a similar study using 75±75 μg/kg (total 150 μg/kg) diltiazem in the induction and terminal doses of cold crystalloid cardioplegia in elective coronary bypass operations confirmed that the use of diltiazem reduced ischemic injury and improved postoperative recovery and the metabolic function of the myocardium. Nevertheless, in that study postoperative systolic function was depressed for 6 hours, and AV-node dysfunction lasted for 82 minutes [Christakis 1986]. On the other hand, several studies with higher doses of diltiazem added to blood cardioplegia showed that improved myocardial protection was achieved without postoperative systolic dysfunction or unacceptable AV-node dysfunction. This variability in the myocardial effects of diltiazem likely is due to the differences between crystalloid and blood used as a cardioplegic medium. Because diltiazem bonds to plasma

proteins, the pharmacological equivalence of diltiazem in blood cardioplegia is lower than when crystalloid cardioplegia is used [Barner 1987, Kröner 2002]. The results obtained in our study with a total dose of 300 μg/kg diltiazem also support these findings. After removal of the cross-clamp, the mean AV-node recovery time of the diltiazem group was significantly longer than that of the control group (22.72 ± 7.40 minutes and 13.25 ± 6.00 minutes, respectively) ($P < .05$). Spontaneous sinus rhythm was restored within the first 30 minutes after removal of the cross-clamp in all but 1 patient, who needed pacing for 52 minutes because of bradycardia (Table).

CONCLUSION

This prospective, randomized trial showed that addition of 150 ± 150 μg/kg diltiazem to the induction and terminal doses of tepid blood cardioplegia enhanced myocardial protection in elective aortocoronary bypass surgery in high-risk patients with a left ventricular ejection fraction greater than 40% without significant additional operative risk.

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