Calcium Antagonist Verapamil and Reperfusion Injury of the Heart

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Objective: **An experimental study to examine the effect of verapamil, given into a coronary artery, on reperfusion injury.**

Design: **The study was randomized but not blinded.**

Setting: **This study was conducted in the animal laboratory of the Department of Anesthesiology and Critical Care in an academic institution.**

Participants: **The study was performed in an anesthetized open-chest pig model.**

Interventions: **Left anterior coronary artery (LAD) occlusion for 15 minutes followed by 90 minutes of reperfusion. Verapamil or saline was given into the LAD artery either at the time the coronary artery was occluded (ie, during acute severe ischemia or during the reperfusion period).**

Measurements: **LAD artery blood flow, regional myocardial function, and metabolism were assessed by the end-systolic pressure length relationship, regional systolic shortening, postsystolic shortening, regional myocardial oxygen consumption, and local cardiac vein lactate.**

Conclusions: **Verapamil given during ischemia resulted in a shorter period of regional myocardial stunning when compared with saline or verapamil during reperfusion. The difference in the verapamil strategies (ie, verapamil administered during ischemia versus verapamil during the reperfusion period) can probably be explained by a difference in the effective dose of the drug present in the heart at the time reperfusion started rather than the period of administration per se.**

KEY WORDS: myocardial ischemia, reperfusion injury, calcium antagonist, verapamil

IT APPEARS AS if intracellular calcium overload is a recognized common pathway that can explain reperfusion injury.[1,2] A large body of data, generated in a number of human and animal models, has confirmed that a variety of calcium antagonists, administered at different times and doses, were more or less successful in limiting reperfusion injury.[3-10]

This study was undertaken to examine the effect of small doses of verapamil, administered directly into the coronary artery, on reperfusion injury. Specifically, the intention was to study the effect of the drug on reversible ischemic injury as opposed to prolonged ischemia and myocardial infarction. The hypothesis, based on the theory of reperfusion injury and the known pharmacology of verapamil, was that verapamil will decrease the extent and duration of the reperfusion injury.

METHODS

Permission for the study was obtained from the Ethics and Research Committee of the University of Stellenbosch. Animals were kept in a dedicated and supervised facility in accordance with institutional and Medical Research Council guidelines.

Forty-two pigs were used for the study. The general model has been described previously.[11] Premedication was with ketamine and anesthesia induced with pentobarbital, thiopental, and fentanyl. Anesthesia was maintained with a constant infusion of pentobarbital and fentanyl and ventilation facilitated with the use of pancuronium. Oxygen and nitrogen were used for ventilation, and arterial carbon dioxide, oxygen tension, and pH were maintained throughout the study. Animals received normal saline, and body temperature was maintained for the duration of the study.

An electrocardiogram was attached and lead I constantly displayed on the monitor (Datex Ohmeda S/5, Madison, WI). A neck dissection was performed and the carotid artery cannulated with a stiff catheter connected to a transducer (Medex Med Inc, Lancashire, UK). The catheter was advanced into the aortic arch for the measurement of aortic systolic and diastolic pressures. The mean arterial pressure was obtained by integration of the area under the intra-arterial pressure curve. The end-systolic pressure (Pes) was taken at the time the aortic valve closed (dicrotic notch).

A sternotomy and pericardiectomy were performed. A stiff cannula was sutured into the left ventricular (LV) apex and connected to a transducer that was used to transduce LV systolic and end-diastolic pressure. For both arterial and LV pressure, the midaxillary line was used as the zero reference point and the transducers were calibrated manually with reference to atmospheric pressure and 100 mmHg on a mercury manometer.

The left anterior descending coronary artery (LAD) was dissected free from the surrounding muscle, and a silk suture loop was placed around the artery. This was used to affect total LAD coronary artery occlusion as part of the protocol. A laser Doppler flow probe (Transonic Systems, Ithaca, NY) on the LAD, proximal to the snare, was used to measure coronary blood flow (CBF).

A 24-G cannula was inserted into the coronary vein draining the LV region supplied by the constricted artery. A 26-G catheter (Abbocat T, Abbott, Ireland) was inserted into the LAD artery just distal to the position of the LAD artery snare. Blood was sampled from the aorta and the coronary vein during the experimental steps. Immediately before the placement of the cannulae, the animal received 1 mg/kg of heparin (Heparin; Intramed, Port Elizabeth, South Africa) injected intravenously.

Two pairs of ultrasonic crystals were inserted into the LV endocardium. One pair was placed in the area supplied by the left circumflex artery and the other pair in the area supplied by the LAD artery (distal to where the snare was placed). Signals were transduced (TRX series 8; Sonometrics Corp, London, ON, Canada) and gave a continuous trace of regional LV lengthening and shortening. The distance between the crystals at the time when the aortic valve opened was termed "Lmax" (end-diastolic segment length), and the intercrystal distance at the time of aortic valve closure was termed "Lmin." The difference between Lmax and Lmin was regarded as the regional systolic shortening associated with ejection (dL). Any shortening that occurred after the aortic valve closed was termed "postsystolic shortening" (PSS) and was expressed as a percent of the total shortening (PSS% = PSS /[dL + PSS] × 100).[12] The ratio dL/Lmax (%) was termed "regional ejection fraction."

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Regional muscle length changes and LV pressure signals were combined on a computer to give a beat-by-beat pressure-length loop.[13,14] From the loops, the end-systolic elastance and regional stroke work were obtained. The extrapolated regional length when the LV pressure was 0 was termed "Lo" (in millimeters) and used as an index of LV end-systolic elastance.

Signals were viewed directly on the monitor screen and digitized at a sampling rate of 100 Hz for 20 seconds (after disconnection of the ventilator) onto a computer by using the software program ALAB (a hardware-software program created by Central Electronic Services of the university and the Department of Anesthesiology and Critical Care). The program recorded, stored, and displayed signals from monitoring equipment. The computer displayed signals in real time and allowed for postprocessing of data with a magnifying facility. After each step, data were reviewed for accuracy before going onto the next step.

Blood from the aorta and coronary vein was collected in heparinized syringes and immediately analyzed for blood gases (Medica, Bedfort, MA) and lactate concentration (Arkray Inc, Kyoto, Japan). After surgery was completed, animals were left for 20 minutes before the protocol was started (see protocol).

After completion of the experiment, the heart was arrested with an intravenous injection of KCl while the animal was maintained under anesthesia. The heart was removed, and the left mainstem coronary artery cannulated at the point in which the occlusion was applied. Black ink was injected slowly into the cannula under average live perfusion pressure and the demarcated area on the endocardium dissected. This was defined as the ischemic area, and the weight was recorded. This allowed for the normalization of some of the measurements or calculations (per gram tissue).

Arterial and coronary vein (cv) oxygen content (CaO_2 and $CcvO_2$ in mL of oxygen per 100 mL of blood) were calculated as (hemoglobin concentration $\times$ 1.39 $\times$ hemoglobin saturation) + (0.0031 $\times$ partial pressure of oxygen). Regional myocardial oxygen consumption was calculated from the Fick equation as follows: regional myocardial oxygen consumption = CBF ($CaO_2 - CcvO_2$).

Experimental Protocol

Animals were randomly assigned to 1 of the 6 groups by blind card draw.

1. No ischemia (n = 6): normal coronary artery (ie, no occlusion). Intracoronary verapamil administered as 8 μg/kg/min for 8 minutes (total dose 2 mg in a total volume of 8 mL [0.9% saline]). Recording was performed before; immediately after the infusion ended; and at 10, 30, 60, and 90 minutes thereafter.
2. The LAD artery was occluded for 15 minutes (n = 9). At 12 minutes' occlusion, 0.9% saline infusion (total volume 8 mL) was started at 1 mL/min for 8 minutes (ie, 3 minutes during occlusion and 5 minutes into reperfusion).
3. The LAD coronary artery was occluded for 15 minutes (n = 8). Recordings were made before occlusion; at 10 minutes of occlusion; and at 10, 30, 60, and 90 minutes after reperfusion was instituted. Three minutes before reperfusion (ie, at 12 minutes of occlusion), an intracoronary verapamil infusion distal to the occlusion was started at 2 μg/kg/min for 8 minutes (ie, 3 minutes during occlusion and 5 minutes into reperfusion). The total dose was 0.5 mg in a total volume of 8 mL (0.9 % saline).
4. The LAD coronary artery was occluded as in group 2 (n = 7). Three minutes before reperfusion, intracoronary verapamil infusion was started at 8 μg/kg/min for 8 minutes (ie, 3 minutes during occlusion and 5 minutes into reperfusion [total dose 2 mg in a total volume of 8 mL (0.9 % saline)]).
5. Intracoronary saline infusion was started at the onset of coronary artery occlusion (n = 6). The rate of infusion was 1 mL/min, and the total volume was 8 mL.
6. In this group, the intracoronary verapamil infusion into the ischemic segment was started at the onset of the LAD occlusion (n = 6). The dose was 8 μg/kg/min, and the total dose was 2 mg in 8 mL of 0.9% saline.

The intracoronary administration was performed with a syringe driver at the rates as indicated earlier. The total volume of the infusate was similar for the groups. If more than 3 ventricular ectopic beats/min occurred or if ectopic beats were multifocal, 1 mg/kg of lidocaine was administered intravenously. The dose was repeated if necessary, and the total dose of the lidocaine was recorded. Ventricular tachycardia or fibrillation was treated with lidocaine as for ectopic beats, and direct defibrillation was done with 5, 5, 10, 10, and 15 J sequentially. The total joules were recorded.

Data were directly stored on disk and summarized by means and standard deviation of the mean (Sigma Stat and Sigma Plot; SPSS, Chicago, IL). To examine data for differences within groups, 1-way analysis of variance was used. Data were tested for normality and equal variance. If the test passed the normality test, specific differences between various steps was sought with multiple comparisons (Tukey test). If normality failed, repeated measures analysis of variance based on ranks were performed.

Each step was compared with its own control (the before intervention step). The time at which any specific parameter recovered to the initial control (before intervention step) was noted. For comparison between the protocols, the times at recovery (to its own control) were compared. For comparison of the incidence of arrhythmias and defibrillation requirements, the chi-square test was applied. The probability of <0.05 was accepted as indicative of statistically significant differences between steps.

RESULTS

The control segment, area supplied by the left circumflex artery, was not affected by any of the interventions.

Evidence for Myocardial Ischemia

Constriction of the LAD coronary artery resulted in a reduction in CBF from 27.1 $\pm$ 7.8 to 1.5 $\pm$ 5.6 mL/100 g/min. This was associated with an increase in LADmax from 19.1 $\pm$ 0.1 to 21.0 $\pm$ 0.3, and Lo from 10.6 $\pm$ 1.6 mm to 16.6 $\pm$ 0.9 mm. Regional shortening was reduced from 4.8 $\pm$ 0.8 to 1.62 $\pm$1.2 mm, and PSS increased from 2.1% $\pm$ 0% to 52.7% $\pm$ 25%.

Evidence of tissue hypoxia and ischemia in the LAD supply area confirmed significant ischemia. Venous PO_2 decreased from 3.4 $\pm$ 0.3 to 2.8 $\pm$ 0.8 kPa, PCO_2 increased from 7.8 $\pm$ 2.7 to 9.8 $\pm$ 1.8 kPa, lactate increased from 0.5 $\pm$ 0.7 to 3.0 $\pm$ 0.2 mmol/L, and the regional VO_2 decreased from 6.6 $\pm$ 2.7 to 0.4 $\pm$ 1.2 mL/100 g/min. All of these differences were significant at the 0.001 (paired *t* test) level.

Effect of 2 mg of Verapamil on the Normal Myocardium and CBF

Verapamil resulted in a significant increase in CBF from 26.3 $\pm$ 10.8 to 75.5 $\pm$ 20.7 mL/min in Group 1 (Table 1). The CBF remained above the control value until the 90-minute measurement was done when it still was different from the control (initial) value. After 30 minutes, the heart rate decreased and remained lower than the control for the rest of the experiment. The mean arterial pressure decreased immediately after the verapamil infusion was terminated and returned to control values by 30 minutes.

In the LAD region (where verapamil was infused), Lmax and

Table 1. Verapamil, 8 μg/kg/min (Total Dose 2 mg in 8 mL of Saline), Infused Over 8 Minutes Into the Normal LAD Coronary Artery

	MAP (mmHg)	HR (beats/min)	CBF (mL/min)	Lmax (mm)	Lo (mm)	dL (mm)	PSS (%)	Lactate (mmol/L)	VO_2 (mL/min/100 g)
Control	83.0 (18.3)	101.0 (14.3)	26.3 (10.9)	19.2 (3.7)	10.6 (2.9)	4.4 (0.7)	0.5 (11.9)	0.6 (0.6)	6.0 (3.8)
0 minutes	66.3 (10.5)	100 (14.3)	75.5 (20.6)	20.8 (3.3)	15.0 (2.3)	2.7 (1.1)	32.5 (23.5)	0.6 (0.7)	3.1 (1.5)
10 minutes	69.5 (11.7)	95.8 (17.4)	54.2 (18.2)	20.1 (3.4)	**12.7 (2.4)**	**3.3 (1.1)**	19.4 (16.9)	0.6 (0.6)	**4.1 (1.3)**
30 minutes	**79.8 (13.4)**	88.0 (16.9)	**31.0 (6.5)**	**17.9 (4.8)**	11.0 (2.4)	3.2 (1.6)	**2.9 (7.2)**	0.5 (0.5)	5.0 (1.3)
60 minutes	86.8 (11.5)	87.3 (16.4)	32.5 (5.5)	18.9 (3.2)	9.8 (3.4)	4.6 (0.8)	0.0 (0.0)	0.6 (0.8)	5.2 (1.7)
90 minutes	91.0 (16.6)	87.0 (14.7)*	31.5 (5.0)	18.9 (3.2)	10.5 (2.8)	4.4 (0.9)	1.3 (3.2)	0.7 (0.7) NC	6.4 (2.6)

NOTE. Values are mean ± standard deviation; n = 6, LAD segment data. Boldface indicates time values returned to control.

Contol refers to values before any intervention; 0 to 90 minutes refer to data obtained immediately after verapamil infusion was completed (0); and 10 to 90 minutes refer to data collected at these times (in minutes) after completion of the infusion of the verapamil.

Abbreviations: NC, no change; MAP, mean arterial pressure; HR, heart rate; Lmax, end-diastolic regional length; Lo, extrapolated regional length when LV presssure is 0; PSS, postsystolic shortening; VO_2, regional oxygen consumption.

*Heart rate decreased below control and remained below control.

Lo increased after verapamil infusion and returned to control by 30 and 10 minutes. Regional shortening (dL) decreased after verapamil and returned to control by 30 minutes. The PSS increased after verapamil and returned to control by 30 minutes. The venous oxygen tension increased significantly after verapamil and regional oxygen consumption decreased after verapamil to return to control values at 30 minutes. In summary, verapamil resulted in a temporary depression of regional myocardial metabolism and function and caused a significant increase in CBF.

Saline Infusion Started 3 Minutes Before Reperfusion and Continued During Reperfusion

Occlusion of the LAD artery resulted in severe ischemic changes as gauged from the Lmax, Lo, dL, venous lactate, and oxygen consumption (Table 2). This was similar in all the LAD occlusion experiments. The heart rate and arterial blood pressure did not change in any of the LAD occlusion experiments.

On reperfusion in Group 2, CBF normalized by 30 minutes, and the Lmax returned to control by 60 minutes; Lo was

Table 2. Saline and Verapamil Injected Into the LAD Artery After 12 Minutes of Occlusion and Continued for 5 minutes Into the Reperfusion Period

	CBF m (mL/min)	Lmax (mm)	Lo (mm)	dL (mm)	PSS (%)	Lactate (mmol/L)	VO_2 (mL/100 g/ Min)
Saline (8 mL) (n = 9)—Group 2							
Control	24.2 (3.8)	19.7 (3.9)	11.1 (3.7)	5.0 (0.8)	0 (0)	0.9 (0.7)	7.7 (3.2)
Ischemia	1.4 (3.0)	21.4 (3.2)	14.9 (3.2)	2.2 (1.1)	31.3 (14.6)	3.1 (1.6)	0.5 (1.0)
10 minutes	41.3 (18.2)	21,4 (4.3)	**12.0 (2.7)**	3.8 (1.8)	12.5 (9.9)	**1.7 (0.3)**	**5.6 (1.8)**
30 minutes	**26.1 (14.8)**	21.2 (4.1)	12.9 (2.7)	3.4 (1.7)	13.8 (8.4)	1.4 (0.8)	5.2 (2.2)
60 minutes	23.2 (9.8)	**20.6 (3.9)**	12.4 (2.8)	3.6 (1.4)	8.4 (10.1)	1.2 (0.7)	5.4 (2.2)
90 minutes	24.8 (11.7)	20.5 (3.8)	10.5 (2.4)	3.6 (1.8) NR	**6.7 (9.9)**	1.4 (0.6)	6.2 (2.6)
Verapamil, 2 μg/kg/min (total dose = 0.5 mg) (n = 8)—Group 3							
Control	33.8 (8.4)	20.8 (0.7)	12.7 (1.9)	5.1 (1.0)	3.7 (9.1)	0.2 (0.3)	8.6 (2.3)
Ischemia	2.7 (3.0)	22.9 (1.5)	18.9 (1.8)	1.3 (1.0)	61.9 (26.5)	4.3 (2.8)	0.6 (0.7)
10 minutes	**64.5 (22.3)**	22.4 (1.6)	16.1 (3.1)	2.8 (1.1)	26.7 (24.8)	**1.2 (1.9)**	5.2 (1.7)
30 minutes	34.7 (12.6)	21.9 (1.7)	**13.7 (2.4)**	3.2 (0.9)	**19.4 (10.1)**	0.9 (0.9)	6.1 (1.9)
60 minutes	35.0 (12.3)	**20.8 (1.4)**	12.6 (2.8)	3.3 (0.9)	8.1 (10.4)	0.9 (0.9)	**6.6 (1.9)**
90 minutes	32.7 (10.3)	20.5 (1.6)	11.2 (3.2)	3.2 (1.1) NR	9.4 (8.7)	0.7 (0.7)	6.9 (1.5)
Verapamil, 8 μg/kg/min (total dose = 2.0 mg) (n = 7)—Group 4							
Control	22.1 (13.3)	18.8 (4.5)	8.5 (3.4)	5.5 (2.2)	0 (0)	0.6 (0.6)	4.2 (1.6)
Ischemia	0.6 (1.0)	21.1 (5.6)	16.9 (4.6)	1.8 (1.4)	54.5 (26.6)	1.9 (1.0)	0.1 (0.2)
10 minutes	43.9 (9.4)	20.8 (5.2)	14.8 (4.5)	3.5 (1.8)	**21.0 (15.4)**	**1.1 (0.8)**	**3.3 (1.0)**
30 minutes	**20.1 (3.3)**	**20.1 (4.9)**	**10.4 (2.2)**	**4.4 (2.1)**	7.7 (18.7)	0.6 (0.7)	3.0 (1.3)
60 minutes	21.7 (4.1)	19.6 (4.7)	10.8 (2.5)	4.5 (1.6)	2.1 (5.6)	0.7 (0.7)	3.7 (0.5)
90 minutes	20.3 (5.3)	19.1 (4.9)	11.2 (3.1)	4.3 (1.7)	1.8 (4.8)	0.7 (0.6)	3.9 (1.1)

NOTE. Values are mean ± standard error of the mean. Boldface indicates the time the particular value returned to control.

Control refers to values before occlusion of the LAD artery; ischemia reflects values taken 10 minutes after occlusion, and 10 to 90 minutes are values registered after reperfusion was established.

Abbreviations: NR, did not return to control values; Lo, extrapolated segment length when LV pressure is 0; dL, regional systolic shortening; PSS, postsystolic shortening; VO_2, regional oxygen consumption.

Table 3. Saline and Verapamil Infused Into the LAD Artery from the Onset of Ischemia and Continued for 8 Minutes

	CBF m (mL/min)	Lmax (mm)	Lo (mm)	dL (mm)	PSS (%)	Lactate (mmol/L)	VO_2 (mL/100 g/min)
Saline (8 mL) (n = 6)—Group 5							
Control	24.3 (3.8)	19.6 (3.9)	11.1 (3.8)	5.0 (0.8)	0 (0)	0.8 (0.6)	7.8 (3.2)
Ischemia	1.4 (3.1)	21.3 (4.3)	14.9 (3.2)	2.2 (1.0)	31.3 (14.6)	3.1 (1.6)	0.5 (1.0)
10 minutes	41.3 (18.2)	21.4 (4.3)	**12.1 (2.7)**	3.8 (1.8)	12.5 (9.8)	**1.7 (0.3)**	5.7 (1.8)
30 minutes	**26.1 (14.8)**	21.2 (4.2)	12.9 (2.7)	3.4 (1.7)	13.8 (8.4)	1.4 (0.8)	5.2 (2.2)
60 minutes	23.1 (9.8)	**20.6 (3.9)**	12.4 (2.8)	3.5 (1.5)	**8.4 (10.1)**	1.2 (0.7)	5.5 (2.2)
90 minutes	24.8 (11.8)	20.8 (3.8)	10.5 (2.4)	3.6 (1.8)	6.8 (9.9)	1.4 (0.6)	**6.2 (2.6)**
				NR			
Verapamil, 8 μg/kg/min (total dose = 2.0 mg) (n = 6)—Group 6							
Control	25.6 (11.9)	16.9 (2.8)	10.5 (2.2)	3.9 (1.1)	0 (0)	0.9 (0.7)	6.3 (2.6)
Ischemia	0.8 (1.6)	18.7 (3.4)	15.0 (3.8)	1.1 (0.6)	68.7 (20.3)	2.4 (1.5)	0.2 (0.5)
10 minutes	49.0 (11.8)	**17.9 (3.4)**	**11.7 (3.1)**	**3.4 (0.9)**	**12.3 (12.4)**	**1.5 (1.0)**	**4.8 (1.5)**
30 minutes	**29.8 (8.1)**	17.1 (3.4)	10.6 (2.7)	3.5 (1.2)	13.2 (23.3)	1.4 (0.7)	5.1 (2.1)
60 minutes	33.7 (10.7)	16.6 (2.9)	9.9 (2.7)	3.4 (0.9)	5.4 (8.9)	1.1 (0.8)	6.2 (1.6)
90 minutes	32.3 (11.6)	16.3 (2.7)	9.6 (3.2)	3.4 (0.7)	2.0 (4.9)	1.0 (0.8)	6.1 (1.7)

NOTE. Values are mean ± standard error of the mean. Boldface indicates the time the particular value returned to control.

Control refers to values before occlusion of the LAD artery; ischemia reflects values taken 10 minutes after occlusion; and 10 to 90 minutes are values registered after reperfusion was established.

Abbreviations: NR, did not return to control values; Lo, extrapolated segment length when LV pressure is 0; dL, regional systolic shortening; PSS, postsystolic shortening; VO_2, regional oxygen consumption.

normalized by 10 minutes and dL did not reach control values by the end of the experiment. PSS returned to control by 90 minutes. Coronary venous lactate and oxygen consumption normalized by 10 minutes of reperfusion.

Verapamil (Total Dose of 0.5 mg) Started 3 Minutes Before and Into the Reperfusion Period

Lmax returned to control value at 60 minutes after reperfusion was instituted in Group 3 (Table 2). Lo normalized by 30 minutes reperfusion, and dL remained less than control for the duration of the experiment and PSS returned to control by 30 minutes. Oxygen consumption returned to control by 60 minutes, and the lactate normalized by 10 minutes reperfusion.

Verapamil (2 mg Total Dose) Administered From 3 Minutes Before and into the Reperfusion Period

On reperfusion in Group 4, the CBF was higher than the control and returned to control at 30 minutes (Table 2). Lmax, Lo, and dL returned to control by 30 minutes while PSS normalized by 10 minutes. Oxygen consumption returned to control by 10 minutes.

Saline Infusion Started From the Onset of Ischemia

Lmax increased during ischemia and only returned to control at 60 minutes; whereas the Lo, which increased during ischemia, returned to control by 10 minutes after reperfusion was established in Group 5. dL decreased during ischemia and remained less than the control until the end of the experiment, whereas PSS returned to control by 60 minutes. Coronary venous lactate increased during ischemia but returned to control by 10 minutes reperfusion, whereas the oxygen consumption returned to normal values at 90 minutes after reperfusion (Table 3).

Verapamil (2 mg) From the Onset of Ischemia

On reperfusion in Group 6, the Lmax, Lo, dL, and PSS venous lactate and oxygen consumption returned to control by 10 minutes (Table 3).

Reperfusion Arrhythmias

More lidocaine and defibrillation were required in experiments in which verapamil was not used (Table 4). Similarly, animals receiving verapamil had fewer episodes of ventricular fibrillation.

DISCUSSION

The aim of this experiment was to establish whether verapamil will attenuate the reperfusion injury in the myocardium subjected to significant but reversible ischemia if (1) administered in a timely fashion, (2) directly into the coronary artery supplying the ischemic area, and (3) in a dose sufficient to be effective locally but without systemic effects.

Data from the present experiment showed the following:

1. The higher dose of verapamil administered during ischemia resulted in faster regional myocardial recovery during reperfusion. Functional recovery was completed at 10 minutes of reperfusion compared with saline where regional systolic shortening did not recover for the duration of the experiment despite normal coronary

Table 4. Reperfusion Arrhythmias

	n	VF (%)	VES (%)	Joules	Lidocaine (mg)
Verapamil, 2 mg	13.0	0.0	0.0	0.0	14.6
Verapamil, 0.5 mg	8.0	4.0	3.0	17.6	54.0
Saline	15.0	6.0	8.0	12.5	43.1

NOTE. Verapamil, 2 mg, the protocols in which a total of 2 mg of verapamil were given (ie, during ischemia and during reperfusion) were combined. Similar for saline.

Abbreviations: VF%, percentage of animals developing ventricular fibrillation; VES%, percentage of animals who had ventricular extra systoles; Joules, total amount of joules per animal; lidocaine, total amount of lidocaine per animal.

blood flow and metabolic markers for ischemia being absent in the latter group.
2. Verapamil given during the reperfusion period resulted in better recovery than saline. Recovery was complete at 30 minutes reperfusion. Saline did not result in recovery of the reperfused segment for the duration of the experiments.
3. Recovery of regional function was more rapid in the experiments in which the verapamil was given during ischemia compared with verapamil given during reperfusion.

The present data also suggested a dose-related effect for verapamil. The lower dose of verapamil (total dose 0.5 mg) did not result in similar reperfusion recovery as the 2-mg dose. The higher dose (2 mg) was chosen after a pilot study showed that doses in excess of this resulted in a significant decrease in arterial pressure that the authors aimed to avoid with the small dose of verapamil admistered into the coronary artery.

The authors' method of initiating the administration of verapamil 3 minutes before the reperfusion was started and continuing into the reperfusion period requires clarification. In effect, verapamil was started at 12 minutes occlusion of the LAD, and the ischemic metabolic and functional effects of ischemia were already well established. The 3 minutes before reperfusion is somewhat arbitrary, but the initial calculation showed this to be the time required (at the infusion rate used) to fill the coronary bed distally to the occlusion. The aim was to ensure that a sufficient dose was in place at the time reperfusion was initiated. The authors believe that this section of the experiments should be viewed as an experiment in which the calcium antagonist was given from very early on in the reperfusion process.

It has previously been shown that the administration of verapamil must occur early in the reperfusion process and the dose must be sufficient.[4] The authors speculate that the present results, which showed improved recovery if verapamil was given during ischemia (compared with verapamil during reperfusion), can be explained by the larger dose achieved before reperfusion. In addition, because there was very little if any coronary blood flow, it was not washed out of the coronary artery before the release of the LAD occlusion. A large dose was bound to the receptor and protected the myocardium against reperfusion. In the group in which the verapamil was started immediately before the release of the coronary occlusion, a much smaller dose was delivered at the time reperfusion was initiated. In addition, because of the significant hyperemic coronary blood flow (after ischemia) and the direct effect of the verapamil per se on coronary blood flow, there probably was significant washout of the drug.

The proposed methods by which verapamil protects the heart during ischemia and reperfusion are either its effect on the L-type calcium channels or by an adenosine triphosphate (ATP)-sparing effect. The first theory relates to the role of calcium in the ischemic and reperfusion injury sequence. Myocardial ischemia is characterized by a rapid reduction in the ATP production, intracellular lactate accumulation, and acidosis.[15,16] The Na^+/H^+ exchanger extrudes hydrogen ions in exchange for sodium[17] and the Na^+/HCO_3^- symporter transports sodium and bicarbonate into the cell. The result is an increase in cytosolic sodium.[18] The Na^+/K^+ adenosine triphosphatase (ATPase) is inhibited by the low concentration of ATP and acidosis, and cells fail to extrude the excess cytosolic sodium.[19] The intracellular sodium accumulation is furthered by the inward current of sodium through the sodium fast channel during the upstroke of the action potential (which is not affected in the first 10 to 20 minutes of ischemia).[20]

In the final analysis, the cytosol is sodium overloaded during ischemia.[21] This causes the Na^+/Ca^{2+} exchanger to operate in reverse mode leading to an absolute increase in intracellular calcium.[22] The sarcolemmal and sarcoplasmic reticulum (SR) calcium ATPase fail (because of ischemic ATP depletion) and result in the failure to extrude the intracellular calcium.[23] During reperfusion, the extracellular fluid is replaced with perfusate of normal pH, and this rapidly leads to reactivation of the Na^+/K^+ ATPase. The restoration of mitochondrial oxidative phosphorylation, recovery from the acidosis, and washout of ischemic metabolites, together with the increased cytosolic sodium, accelerate the Na^+/K^+ ATPase function.[21] The restoration of ATP leads to recovery of contractile function and the recovery of Ca^{2+} and Na^+/K^+ ATPase before the excess intracellular calcium is extruded.[24] The result is that the intracellular to extracellular gradient for sodium is restored, and this is one of the major drivers for the efflux of calcium via the Na^+/Ca^{2+}exchanger, which switches to the forward mode for the extrusion of calcium in exchange for sodium. However, this process takes some time.

The restoration of the ion pump function will allow the SR to accumulate calcium (because of the high cytosolic calcium). Once full, the SR starts releasing calcium leading to an oscillatory calcium concentration elevation in the cytosol, and this spontaneous release of calcium will induce calcium release from adjacent SRs, resulting in a propagated wave of calcium that leads to contracture.[25,26]

In addition, the extracellular calcium cycle (ie, calcium entering the cell through the L-type calcium channel) will result in the intracellular calcium cycle (ie, the release of excessive amounts of calcium from the SR [calcium-induced calcium release]).[27] This leads to hypercontracture.[28]

The second theory refers to studies that have shown that verapamil results in reduced myocardial contractility and therefore less oxygen consumption. This leads to an improved maintenance of ATP during ischemia resulting in less reperfusion injury (improved ATP conservation).[29] However, another study suggested a reduction in myocardial oxygen consumption (and an associated sparing of ATP) were not related to the contractility reduction caused by verapamil.[30]

Although the authors have no objective data to support any one of the possible explanations, it is speculated that the ATP-sparing effect of verapamil is unlikely to explain the present findings. Once the LAD was occluded, regional function was abolished or reduced to insignificant levels within a few beats. This was the result of severe acute ischemia and reduction in available ATP. In the animals in which the authors administered verapamil from the onset of ischemia, the dose that was present at the time function was largely abolished, was small (given the infusion rate), and was unlikely to have significant protective effects. The authors' observations and calculation suggest that the volume of infusate present at the time of the observed ischemic dysfunction did not even fill the coronary vasculature distal to the occlusion. In the group in which the verapamil was only given toward the end of ischemia and into

reperfusion, the ischemic dysfunction had been present for some time and again an ATP-sparing effect is an unlikely explanation.

The authors' choice of 15-minute acute and severe normothermic occlusion was based on data showing that cardiomyocytes remain viable for up to approximately 20 minutes after normothermic severe ischemia.[31] Restoration of flow after 18 to 20 minutes of ischemia should result in complete recovery after the reperfusion injury has subsided. The authors' data on ischemic regional mechanical function and myocardial metabolism objectively confirm that the model was subjected to significant ischemia.[32-38] In addition, the authors' saline experiments showed the typical reperfusion injury. Although coronary blood flow and aerobic oxygen consumption normalized once reperfusion was established, regional systolic and diastolic function did not show complete recovery.

Verapamil infused into the normal coronary artery resulted in a significant increase in coronary blood flow, reduction in regional systolic function (approximately 40%), and increased systolic and diastolic compliance. These data support previous findings.[39,40] In addition, this small dose of verapamil resulted in blood pressure reduction, which was sufficient to have influenced regional myocardial work and oxygen consumption.[41] Regional cardiac function and general hemodynamics returned to normal by 30 minutes except for the regional oxygen consumption, which normalized by 10 minutes. The recovery of oxygen consumption before recovery of systemic blood pressure (and hence cardiac work) and regional function suggests that the effects of verapamil on myocardial oxygen consumption are only partially explained by the effect of the drug on general hemodynamics and direct myocardial depression.

This study was primarily undertaken in view of the cardiac surgery-induced myocardial ischemia. The latter is either global (probably incomplete) ischemia (on cardiopulmonary bypass with aortic cross-clamping) or more regional (off-pump coronary artery bypass procedures). It was speculated that a small dose of verapamil could be beneficial and that this benefit could be achieved without the general hemodynamic upset often associated with an effective intravenous dose of the drug. The inherent negative effects of verapamil on systolic and diastolic function in the normal heart should not be confused with its beneficial effects during ischemia and reperfusion. According to the present experimental results, verapamil did afford significant protection to the heart subjected to ischemia and reperfusion.

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