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Effect of Verapamil on Infarct Size in Dogs Subjected to Coronary Artery Occlusion With Transient Reperfusion

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Reocclusion after successful coronary reperfusion occurs in 15 to 35% of patients receiving thrombolytic therapy for acute myocardial infarction. The present study was designed to simulate the clinical situation of reocclusion and determine whether verapamil might be effective in reducing myocardial necrosis and preserving high energy phosphates in this setting. Pentobarbital-anesthetized, open chest dogs underwent occlusion of the left anterior descending coronary artery for 2 hours followed by 1 hour of reperfusion and a further 4 hours of coronary artery occlusion. Treatment with verapamil (intravenous bolus dose of 0.2 mg/kg body weight followed by infusion of 0.56 ± 0.14 mg/kg per h) was begun 1 hour after occlusion and infusion was continued for the remainder of the experiment. The dose of verapamil was adjusted to lower mean arterial pressure to approximately 90 mm Hg. The area at risk was determined by intraatrial injection of monastral blue dye and the area of necrosis was assessed by triphenyltetrazolium chloride staining. In vivo myocardial needle biopsy for determination of adenosine triphosphate and creatine phosphate was performed at the end of the experiment.

The area of the left ventricle at risk was similar in both groups (control [n = 8], 20.2 ± 1.6% versus verapamil-treated [n = 9], 23.1 ± 2.9%; p = NS). The area of necrosis expressed as a percent of the area at risk was reduced in the verapamil-treated group compared with the control group (43.3 ± 5.0% versus 63.1 ± 6.8%, $p < 0.05$). At the termination of the experiment, the adenosine triphosphate and creatine phosphate were significantly higher in both the epicardium and endocardium of the ischemic area in the verapamil-treated group compared with the control group.

***In conclusion,* verapamil reduced or at least delayed the necrosis in an experimental model of reocclusion after an initial period of reperfusion and was associated with a preservation of high energy phosphate content.**

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Early reperfusion in dogs subjected to acute myocardial ischemia shows salvage of ischemic myocardium, an eventual reduction of myocardial dysfunction and improvement in myocardial high energy phosphate content (1–3). With the advent of thrombolytic therapy and emergency percutaneous transluminal coronary angioplasty, as well as the combination of these therapies, it is now possible to institute reperfusion within a few hours from the onset of ischemia in many patients with acute myocardial infarction (4). Clinical trials with thrombolytic agents have shown a reperfusion success rate ranging from 60 to 80% (5–9). However, thrombolytic therapy simply lyses the clot and does not affect the other factors that lead to occlusion of a coronary artery. Consequently, reocclusion of a coronary artery opened with a thrombolytic agent or by means of coronary angioplasty, or both, occurs in 15 to 35% of patients (10–12), and can negate the beneficial effects of the initial reperfusion.

The aim of the present study was to determine whether verapamil, a calcium channel blocking agent known to salvage ischemic myocardium, would be efficacious in an experiment in which reocclusion occurs after an initial period of ischemia and reperfusion.

Methods

Surgical procedures. Mongrel dogs of either sex, weighing between 17 and 30 kg, were anesthetized with sodium pentobarbital (30 mg/kg body weight intravenously), intubated and artifically ventilated with room air using a Harvard respiratory pump. Additional doses of 5 to

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10 mg/kg of sodium pentobarbital were administered intravenously as required to maintain adequate anesthesia throughout the experiment. Cannulas were positioned in the left carotid artery for monitoring arterial pressure and for withdrawal of reference blood samples to determine regional myocardial blood flow, and in the left jugular vein for administration of fluids and drugs. A thoracotomy was performed in the fifth left intercostal space, a pericardial cradle was constructed and the left anterior descending coronary artery was isolated proximal to the first major diagonal branch. A third cannula was placed into the left atrium for administration of radioactive microspheres to determine regional myocardial blood flow.

Experimental protocol. After obtaining baseline measurements of heart rate and arterial pressure on a Gould multi-channel pen recorder, a bolus of lidocaine (1.5 mg/kg intravenously) was injected and the left anterior descending coronary artery was occluded for 2 hours using an atraumatic Schwartz vascular clamp. A total of 81 dogs were entered into the study, of which 29 died of ventricular fibrillation within 30 minutes of coronary artery occlusion; the remaining 52 dogs were randomized 1 hour after occlusion. Of the randomized dogs, 17 were randomized to the protocol described herein (8 in the control group and 9 in the verapamil treated group) and 35 were randomized to a second study group (Campbell et al., unpublished data, 1986). The aim of the verapamil treatment was to reduce mean arterial pressure (electronically determined) to 90 mm Hg. This was achieved by administering an intravenous bolus of 0.2 mg/kg verapamil followed by a continuous infusion of verapamil utilizing a dose adjusted to produce the desired effect; the verapamil (or an equivalent volume of saline solution) was infused until the termination of the experiment. After 2 hours of coronary occlusion, reperfusion was instituted by abrupt removal of the Schwartz clamp. After 1 hour of reperfusion the left anterior descending coronary artery was reoccluded for 4 hours. At the end of the second occlusion, the in vivo area at risk was determined by injecting monastral blue dye (1 ml/kg) into the left atrium.

The dogs were killed with an intraatrial injection of potassium chloride (40 to 60 mEq) and the heart was excised. The atria and right ventricle were removed and the left ventricle was sectioned transversely from apex to base into 4 to 5 mm sections. The area of tissue unstained by monastral blue dye, representing the area at risk as previously described (13), was traced onto acetate sheets. The sections were then incubated in triphenyltetrazolium chloride at 35°C for 10 minutes and the area unstained by triphenyltetrazolium chloride, representing the area of necrosis, was traced onto acetate sheets. The size of the area at risk and area of necrosis was determined by the "cut and weigh" technique as previously described (13,14). Validation of the triphenyltetrazolium chloride technique to quantify the area of necrosis has been previously reported (15,16).

Regional myocardial blood flow determination. Samples of myocardium weighing approximately 1 g were cut from the epicardial, midmyocardial and endocardial regions in the centers of the necrotic and non-necrotic areas for determination of regional myocardial blood flow. The latter was determined before occlusion (before lidocaine), at 30 minutes after the first occlusion, and 30 minutes after the onset of reperfusion by the injection of 2.0×10^6 radioactive (scandium-46, tin-113, cesium-141) microspheres (10 ± 1 μm diameter) while an arterial reference blood sample was withdrawn at 15.3 ml/min using a Harvard withdrawal pump. Regional myocardial blood flow (RMBF) in the tissue sample (ml/min per g) was calculated using the formula: $RMBF = Cs \times (CB/CR)$, where Cs = counts in myocardial tissue sample corrected per gram; CB = rate of withdrawal of the reference blood sample and CR = total counts in reference blood sample (17).

Myocardial biopsy procedure. At the end of the experiment, that is, after 4 hours of reocclusion, and before the injection of monastral blue dye, single in vivo myocardial biopsy samples were obtained from the centers of the ischemic and nonischemic areas using a disposable biopsy needle (Tru-Cut, Travenol) as previously described (18,19). The biopsy samples were frozen within 5 seconds by immersion in liquid Freon 12 and stored at −65°C until analysis. Adenosine triphosphate and creatine phosphate were determined fluorimetrically (20).

The animals used in this study were maintained in accordance with guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. [NIH] 78-23, revised 1978).

Statistical analysis. Comparisons between the two groups were performed using Student's *t* tests, with the Bonferroni procedure where applicable for multiple hemodynamic measurements (21). A probability (p) level of less than 0.05 was considered statistically significant. All values are expressed as mean ± SEM.

Results

Hemodynamics (Fig. 1). Both groups maintained a similar mean arterial pressure for the first hour after occlusion of the left anterior descending coronary artery. At 1 hour after occlusion, the verapamil treatment commenced and the nine dogs receiving verapamil exhibited a mean arterial pressure of 90 mm Hg or less, whereas the eight dogs in the control (saline-treated) group maintained a stable mean arterial pressure throughout the 7 hour experimental period (Fig. 1A). The maintenance dose of verapamil required to maintain the mean arterial pressure at approximately 90 mm Hg was 0.56 ± 0.14 mg/kg per h, after a bolus injection of 0.2 mg/kg. The control group maintained a steady heart

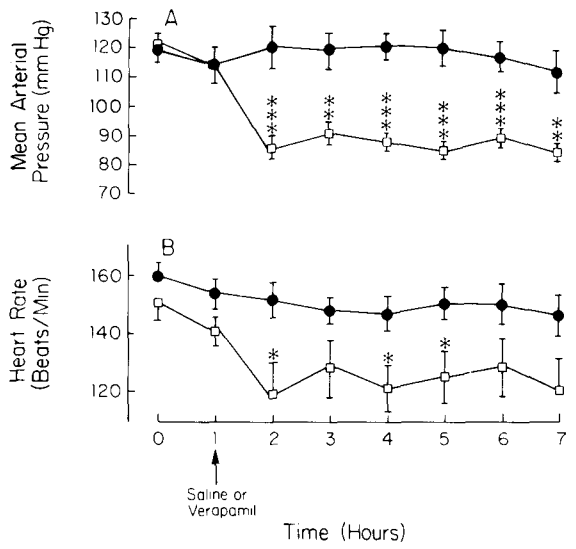

Figure 1. Mean arterial pressure (**A**) and heart rate (**B**) are shown before occlusion (0 hour) and at hourly intervals throughout the 7 hour observation period. Control animals (n = 8) are shown as **filled circles** and verapamil-treated animals (n = 9) as **open squares**. *p < 0.05; **p < 0.01; ***p < 0.001, control versus verapamil-treated group.

rate throughout the experimental period. In contrast, at 1 hour after occlusion in the treated group, the heart rate began to fall and was significantly lower than that of the control group at 2, 4 and 5 hours after occlusion (Fig. 1B).

Area at risk and area of necrosis. The area of myocardium at risk because of the occlusion of the left anterior descending coronary artery was similar in both the control and verapamil-treated groups (23.1 ± 1.9 versus 20.2 ± 1.4% of the left ventricle; p = NS). Verapamil treatment significantly reduced the area of necrosis expressed as a percent of the left ventricle, from 14.6 ± 1.9% in the control group to 8.7 ± 1.2% in the verapamil-treated group (p < 0.05). When the area of necrosis was expressed as a percent of the area at risk, the treated dogs exhibited a significant reduction compared with control dogs (63.3 ± 6.8 versus 43.3 ± 5.0%, p < 0.05).

High energy phosphate concentration (Table 1). In vivo myocardial biopsy specimens from ischemic and nonischemic areas were taken at the end of the experimental period. There were no differences between the verapamil and control groups in the concentration of adenosine triphosphate or creatine phosphate in the epicardium or endocardium of the nonischemic area. In contrast, in the ischemic area the concentrations of adenosine triphosphate and creatine phosphate in the epicardium and endocardium were significantly higher in the verapamil group than in the control group.

Regional myocardial blood flow (Table 2). The regional myocardial blood flow of the area supplied by the left anterior descending coronary artery was markedly and similarly reduced after coronary occlusion in the epicardium, midmyocardium and endocardium in the control and treated groups. The regional myocardial blood flow in the nonischemic area remained stable during the ischemic phase. There was a statistically insignificant trend for the regional myocardial blood flow measured 30 minutes after reperfusion to be higher in the verapamil-treated group than in the control group in all layers of the myocardium, both in normal and in previously ischemic areas.

Discussion

In this study, a total of 6 hours of left anterior descending coronary artery occlusion, which was interrupted by 1 hour of reperfusion, resulted in an area at risk that proceeded to a level of necrosis averaging 63%, which is lower than that

Table 1. Intramyocardial Adenosine Triphosphate and Creatine Phosphate Concentrations (nmol/mg cardiac protein) in the Epicardium and Endocardium of Ischemic and Nonischemic Myocardium

	Ischemic		Nonischemic	
	Epicardium	Endocardium	Epicardium	Endocardium
ATP				
Control (n = 8)	12.03 ± 2.59	3.68 ± 0.72	33.12 ± 2.03	33.63 ± 1.53
Verapamil (n = 9)	22.08 ± 2.39	8.41 ± 0.67	31.96 ± 1.03	35.40 ± 1.97
p Value	<0.05	<0.01	NS	NS
CP				
Control (n = 8)	37.88 ± 8.40	15.80 ± 3.18	54.42 ± 4.83	53.10 ± 3.57
Verapamil (n = 9)	64.47 ± 6.09	43.53 ± 7.19	62.06 ± 2.37	58.21 ± 2.63
p Value	<0.05	<0.001	NS	NS

ATP = adenosine triphosphate; CP = creatine phosphate; NS = not significant.

Table 2. Regional Myocardial Blood Flow (ml/min per g) Determined Before Occlusion (Pre), 30 Minutes After Occlusion (Post) and 30 Minutes After reperfusion (Rep) in Control (n = 8) and Verapamil-Treated (n = 9) Groups

	Ischemic			Nonischemic		
	Control	Verapamil	p Value	Control	Verapamil	p Value
Pre						
Epi	1.20 ± 0.09	1.11 ± 0.19	NS	1.10 ± 0.09	0.94 ± 0.19	NS
Mid	1.27 ± 0.11	1.11 ± 0.14	NS	1.36 ± 0.09	1.17 ± 0.18	NS
Endo	1.23 ± 0.10	1.08 ± 0.13	NS	1.44 ± 0.13	1.33 ± 0.14	NS
Post						
Epi	0.28 ± 0.09	0.33 ± 0.08	NS	1.04 ± 0.11	0.85 ± 0.10	NS
Mid	0.11 ± 0.04	0.13 ± 0.05	NS	1.32 ± 0.13	1.27 ± 0.12	NS
Endo	0.05 ± 0.02	0.05 ± 0.03	NS	1.43 ± 0.15	1.43 ± 0.09	NS
Rep						
Epi	1.42 ± 0.18	1.55 ± 0.40	NS	1.02 ± 0.20	1.73 ± 0.77	NS
Mid	1.31 ± 0.20	1.57 ± 0.49	NS	1.08 ± 0.09	1.60 ± 0.59	NS
Endo	1.38 ± 0.21	2.23 ± 0.45	NS	1.12 ± 0.16	1.26 ± 0.31	NS

Endo = endocardium; Epi = epicardium; Mid = midmyocardium; NS = not significant.

in models of permanent coronary occlusion of 6 hours' duration (18). The effect of the verapamil treatment was to reduce or at least delay the progress of the wave front of necrosis (22), to produce an area at risk that proceeded to necrosis of 43.3 ± 5.0%. Associated with the reduction in infarct size, verapamil preserved high energy phosphates in the area at risk. These phenomena occurred while arterial blood pressure was intentionally lowered.

High energy phosphates. During the initial phase of ischemia, before the development of irreversible damage and cell death, the stores of adenosine triphosphate and creatine phosphate rapidly become depleted (19). Adenosine triphosphate is metabolized to adenosine, inosine and hypoxanthine. Because the cell membrane is permeable to these metabolites, they are washed out during reperfusion. The repletion of adenosine triphosphate must occur by de novo synthesis of purines, a slow process. In the present study there was preservation of the subepicardial rim of tissue which had been subjected to ischemia but did not progress to necrosis, both in the control and in the verapamil-treated group. The calcium channel blocker not only reduced the extent of necrosis but also protected the reperfused, salvaged myocardium from further reduction in high energy phosphate content.

Mechanisms of action. The mechanisms of action of calcium channel blockers are complex and involve the antagonism of transmembrane calcium fluxes, vasodilation of coronary and peripheral blood vessels and reductions in cardiac contractility and automaticity (23,24). Because verapamil treatment lowered heart rate and arterial pressure, it is probable that it reduced global oxygen demand, which may have contributed to the reduction in infarct size. Other interventions that reduce global oxygen demand have been shown to reduce infarct size (25). However, because the contractility of the myocardium is drastically reduced during ischemia (26), the relation between global oxygen requirements and the oxygen requirements of the ischemic myocardium is unclear. It is also uncertain whether calcium channel blockers have any effect on the collateral circulation; some studies show an increase (27), whereas others show no effect (28). Verapamil can also inhibit platelet aggregation (29) and possibly thrombus formation in the coronary vasculature, thereby reducing the obstruction to coronary blood flow and the degree of ischemia. Finally, verapamil may have had a direct effect on calcium flux across the sarcolemmal membrane or within intracellular compartments which could have resulted in a protective action on reversibly injured myocytes.

The time at which verapamil began its beneficial effect in this study is unclear. It may have commenced during the first hour of its administration, that is, during the second hour of the initial occlusion, because verapamil has been shown to be protective when administered during ischemia (13,30). It seems unlikely that the major protective effect occurred during reperfusion, because in other experiments in our laboratory, it was observed that intracoronary verapamil had no effect on infarct size when its administration was begun at the time of reperfusion 3 hours after coronary occlusion (13). One major effect of reperfusion in the present experiments may have been to transport verapamil into the previously ischemic area. Therefore, because verapamil had been delivered to the ischemic area during the reperfusion phase, it is possible that its major protective effect occurred during the second occlusion.

Comparison with previous studies. The limitation of infarct size in dogs by verapamil has been previously reported (30–32). These studies have varied both the duration of coronary artery occlusion (from 40 minutes to 24 hours) and the start of verapamil treatment (from 15 minutes before occlusion up to 1 hour after occlusion). Reimer and Jennings (32) reported that infusion of verapamil beginning before occlusion and lasting for the duration of the occlusion re-

duced infarct size in animals subjected to 40 minutes of occlusion plus 4 days of reperfusion; however, no such protection was observed when the verapamil infusion was begun 15 minutes after occlusion in a protocol of 3 hours of occlusion plus 4 days of reperfusion. The discrepancy between these two protocols has two possible explanations: 1) protection occurred when verapamil was given before occlusion, thus allowing verapamil into the area soon to become ischemic, or 2) verapamil delayed the onset of necrosis but not the ultimate infarct size. In our present study, although verapamil treatment began 1 hour after occlusion, at 2 hours after occlusion reperfusion was instituted, thereby allowing verapamil free access to the previously ischemic area before the second occlusion.

In summary, the protocol used in this study was designed to simulate the clinical situation in which a coronary artery is reoccluded after an initial phase of severe myocardial ischemia followed by successful reperfusion. Infusion of verapamil, at a dose that causes only modest lowering of arterial pressure, commencing during the initial ischemic period before reperfusion, was found to reduce or at least delay the ultimate size of the infarct and was associated with the preservation of adenosine triphosphate and creatine phosphate levels within the ischemic area.

Clinical implications. Reperfusion, either by thrombolytic therapy or coronary angioplasty, or both, is increasingly employed in the treatment of acute myocardial infarction (4). The majority of patients who survive the initial phase of acute myocardial infarction can receive thrombolytic therapy, by either the intravenous or the intracoronary route, within a few hours of the onset of symptoms. Unfortunately, the reocclusion rate after successful recanalization ranges from 15 to 35% (10–12). The extrapolation of experimental data to the human situation requires caution; in particular, a heart rate at rest of 150 beats/min during acute myocardial infarction is uncommon in humans. Also, it is unlikely that maneuvers aimed at reducing infarct size could be initiated within 1 hour of clinical symptoms of acute myocardial infarction. However, these experiments suggest that if verapamil is delivered to the myocardium before reperfusion, it might delay cell death should reocclusion occur and provide time for the reinstitution of thrombolytic therapy or for proceeding with mechanical means of restoring myocardial perfusion.

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