Annals of Nuclear Medicine Vol. 5, No. 3, 109–115, 1991

Evaluation of the protective effect of verapamil on reperfusion injury by ^{111}In anticardiac myosin antibody in canine myocardial infarction

June-Key CHUNG,* Sang Moo LIM,* Myung Chul LEE,* Chang-Soon KOH,* Munho LEE* and Jeong Wook SEO**

*Department of Internal Medicine, **Department of Pathology, College of Medicine, Seoul National University

We quantitated the protective effect of verapamil on reperfusion injury in canine myocardial infarct using ^{111}In-anticardiac myosin antibody and correlated to the electronmicroscopic findings. Experimental myocardial infarction was performed by one hour occlusion of the anterior descending coronary artery and followed by reperfusion. Saline or verapamil (0.6 mg/kg/hr) was started at 40 minutes after coronary artery occlusion and continued throughout the experiment. There was an inverse exponential relationship between anticardiac myosin uptake and regional coronary blood flow in both the control ($r=-0.86$) and the verapamil treated ($r=-0.71$) groups. Less uptake of ^{111}In-anticardiac myosin antibody was observed in the verapamil treated group than in the control group of the regions where blood flow was lower than 30% of normal. In the control group, the myocardium showed signs of the typical contraction band necrosis. In the verapamil treated group, however, the myocardium contained fewer electron dense granules and mild degree of contraction bands.

Key words: myocardial infarction, anticardiac myosin antibody, verapamil, contraction band necrosis

INTRODUCTION

PREVIOUS STUDIES have suggested that coagulation necrosis in myocardial infarction is caused by interruption of the blood flow to the myocardium, and contraction band necrosis develops during reperfusion.[1,2] When the ischemic myocardium is reperfused, myocardial cells gain a large amount of calcium ions from the blood which lead to inhibition of mitochondrial energy production and to cell death.[3,4]

Calcium antagonists have been shown to protect myocardial tissue from ischemic injury. Verapamil reduced epicardial ST segment elevation, histological necrosis,[5,6] and the release of cardiac enzyme.[7–9] Calcium antagonists improved various hemodynamic parameters.[10,11] However, deleterious effects such as no preservation of myocardial ATP,[9,12] no reduction in enzyme release,[13,14] and aggravation of histologic change[15,16] have also been reported.

We investigated the protective effect of calcium antagonist, verapamil, on myocardial damage using ^{111}In-anticardiac myosin antibody (ACM Ab) to quantify the severity of myocardial damage and correlated the result to electronmicroscopic findings.

MATERIALS AND METHODS

Animal Subjects

A total of 14 adult mongrel dogs (9–12 kg) were randomly selected for the saline control and the verapamil treated groups. Saline and verapamil were infused intravenously at 40 minutes after clamping the left anterior descending coronary artery (LAD) and continued throughout the experiment. 0.2 mg/kg of verapamil was loaded for 5 minutes and infused continuously at a rate of 0.6 mg/kg/hr.

Received March 29, 1991, revision accepted June 28, 1991.

For reprints contact: June-Key Chung, M.D., Department of Nuclear Medicine, Seoul National University Hospital, Yunkun-dong 28, Chongno-ku, Seoul 110–744, KOREA.

Experiment protocol

The animals were anesthetized with intravenous pentobarbital (30 mg/kg) and nitroxide inhalation through a respirator. Following left anterior thoracotomy, the LAD was carefully dissected free, and clamped at a level just distal to the first diagonal branch with a vascular clamp. Just before releasing the clamp, relative regional myocardial blood flow was measured with Strontium-85 labeled 16.5±0.1 u carbonized microspheres (New England Nuclear, Boston, MA), as described by Smith et al.[8] After 1 hour of occlusion, the clamp was released and reflow was confirmed by the development of reactive hyperemia in the previously cyanotic zone. At 30 minutes after reperfusion, 100 μCi (3.7 MBq) of ^{111}Indium anticardiac myosin antibody (Centocor Inc.) was infused through a 22 gauge needle into the proximal coronary artery. ACM Ab was Fab fragment of the monoclonal antibody and 0.25 mg of Fab was labeled with 1 mCi (37 MBq) of ^{111}Indium chloride. (Fig. 1)

Measurement of radionuclide uptake in the myocardium

Ninety minutes after LAD reperfusion, the animals were sacrificed by rapid injection of pentothal. The heart was excised en bloc and cut into parallel slices.[14] Transmural samples were sectioned from the slices. Each sample was subdivided into subepicardial and subendocardial layers of equal thickness. The samples were weighed and placed in glass scintillation tubes and the radioactivity was measured with correction of counts in each channel.[17] Relative uptakes of ^{85}Sr-microsphere and ^{111}In-ACM Ab were calculated as ratios of uptake in infarcted regions to the nonischemic posterior left ventricular myocardium.

Relative uptake of ^{85}Sr-microsphere represented coronary blood flow to the myocardial sample, and uptake of ^{111}In-ACM Ab represented myocardial damage.

We did not make *in-vivo* or *ex-vivo* images of anticardiac myosin antibody in this study.

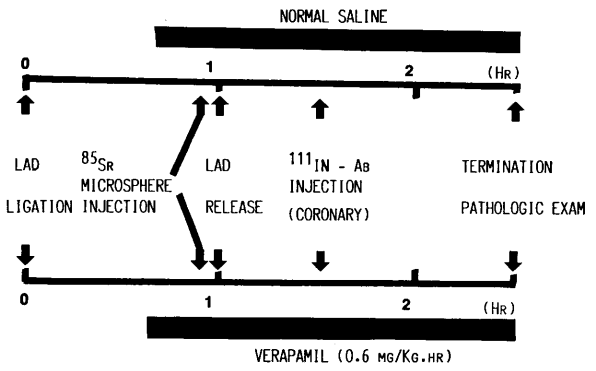

Fig. 1 Experiment protocol: experimental myocardial infarction was produced by occlusion of the left anterior descending coronary artery (LAD) for 1 hour followed by reperfusion.

Electronmicroscopic examination

Myocardial samples were obtained in the subendocardial layer of the central ischemic zone. A myocardial sample was placed in Karnovsky's fixative and processed by the usual method.[6]

Measurement of hemodynamic parameters

Systemic, pulmonary arterial and venous pressures were monitored from cannulae placed in a femoral artery and a vein with Honeywell VR-12 transducers. Cardiac output was calculated by the thermodilution method (American Edwards Laboratories). Two dogs developed ventricular fibrillation and were not included in these data.

Statistical analysis

The statistical significance was evaluated by Student-t test and Wilcoxon rank sum test. Variations from values were given as the standard error of the mean.

RESULTS

Hemodynamic changes

Data on systemic hemodynamic and stroke volume are shown in Table 1. The changes in heart rate and mean arterial pressure before and after coronary occlusion were not significantly different in the control and the verapamil treated groups. Verapamil infusion induced decreases in heart rate and arterial pressure. Pulmonary arterial pressure, pulmonary capillary wedge pressure, central venous pressure, and cardiac output were not altered by verapamil administration. In the control group, stroke volume was reduced from 17.6±0.6 m*l* to 15.3±0.6 m*l* ($p<0.05$) after 30 minutes of reperfusion, but was maintained in the verapamil treated group (20.9± 3.5 m*l* vs. 22.5±3.1 m*l*).

Measurement of ^{111}In-anticardiac myosin antibody in myocardium

We compared myocardial damage measured by ^{111}In-ACM Ab uptake to the coronary blood flow measured by ^{85}Sr-microsphere uptake. An inverse exponential relationship between this ACM Ab localization and the regional flow was evident with the correlation coefficients of −0.86 in the control group (Fig. 2) and −0.71 in the verapamil treated group (Fig. 3). The difference between these two groups was statistically significant ($p<0.05$).

In the myocardial infarct zone, where coronary flow was reduced to 0–10% of the normal flow, the

Table 1 Systemic hemodynamic data in control and verapamil-treated dogs (mean±standard error of the mean)

	Basal state	Occlusion			Reperfusion			
		5 min	40 min	60 min	5 min	30 min	60 min	90 min
Heart rate (min^{-1})								
Control	169±11	173±16	180±13	181±10	171±7	170±9	178±13	183±11
Verapamil-treated	166±12	167±10	173±9*	135±7*	129±7	131±10	131±12	129±11
Mean arterial pressure (mmHg)								
Control	103±2	102±8	97±6	92±4	101±7	107±14	101±11	114±2
Verapamil-treated	115±6	112±8	117±7*	92±8	84±7*	86±6	86±6	89±7
Stroke volume (m*l*)								
Control	19.0±1.9	17.9±3.1	19.3±3.8	16.0±1.7	19.0±0.7	17.6±0.6†	15.3±0.6†	14.2±0.9*
Verapamil-treated	20.8±1.9	19.8±1.7	21.2±1.9	22.0±3.5	20.9±3.5	22.5±3.1	21.5±2.7	20.4±3.5*

*,† $P<0.05$ between them by Wilcoxon rank sum test

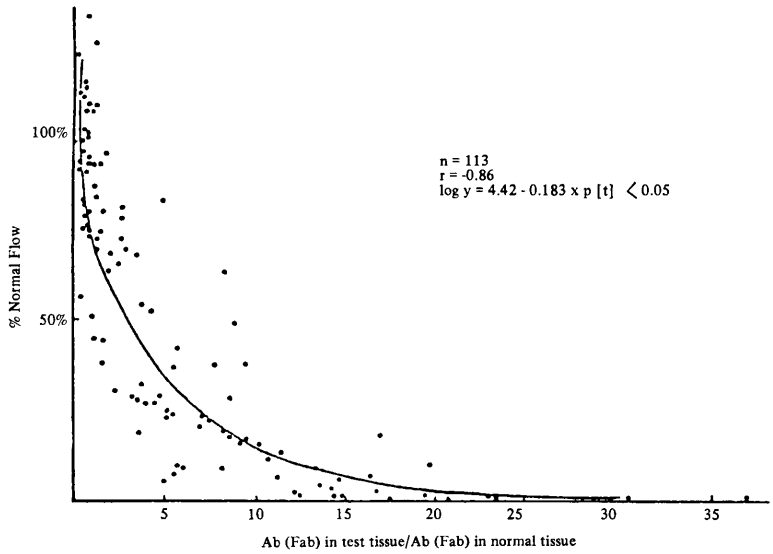

Fig. 2 Relationship between ^{111}In-anticardiac myosin antibody localization in myocardial samples and corresponding regional blood flow in the control group.

ratio of relative uptake of ^{111}In-ACM Ab uptake to normal myocardium was 16.3±1.4 in the control group, but it was reduced to 5.7±0.6 in the verapamil treated group ($p<0.001$). In region where coronary flow was 11–30% of normal, lesser uptake of ^{111}In-ACM Ab in the infarct zone was also observed in the verapamil treated group than in the control group (3.9±0.7 vs. 7.3±0.8) ($p<0.01$). In area where flow was only moderately (51–80%) or slightly (>81% of normal) reduced, ACM Ab uptake was not significantly different in the two groups (Fig. 4).

Electronmicroscopic findings

Cardiac sarcomeres showed wide I bands, loss of glycogens, swollen mitochondria, cytoplasmic vacuoles, intermyofibrillar edema, and sarcolemmal breaks. In the endocardial zone of the control group, the sarcomeres were contracted and the extreme contraction created hypercontraction bands. Three to eight sarcomeres had formed one hypercontraction band, with a width of 0.3–1.8 microns. Stretching of sarcomere was found at the intercalated disk (Fig. 5). In the verapamil treated group, in addition to the aforementioned contraction band, another type of spastic contraction was noted (Fig. 6). The latter showed regular homogenization of myosin filaments around the Z-line and was not surrounded by stretching zones. Contraction bands of this type were scattered at the margin of the classical contraction band area.

Electron dense granules were observed in the mitochondria. The size of these granules was about 0.1 micron. One to five granules were noted in each mitochondria. These granules were also found in the normal cells near the hypercontraction bands.

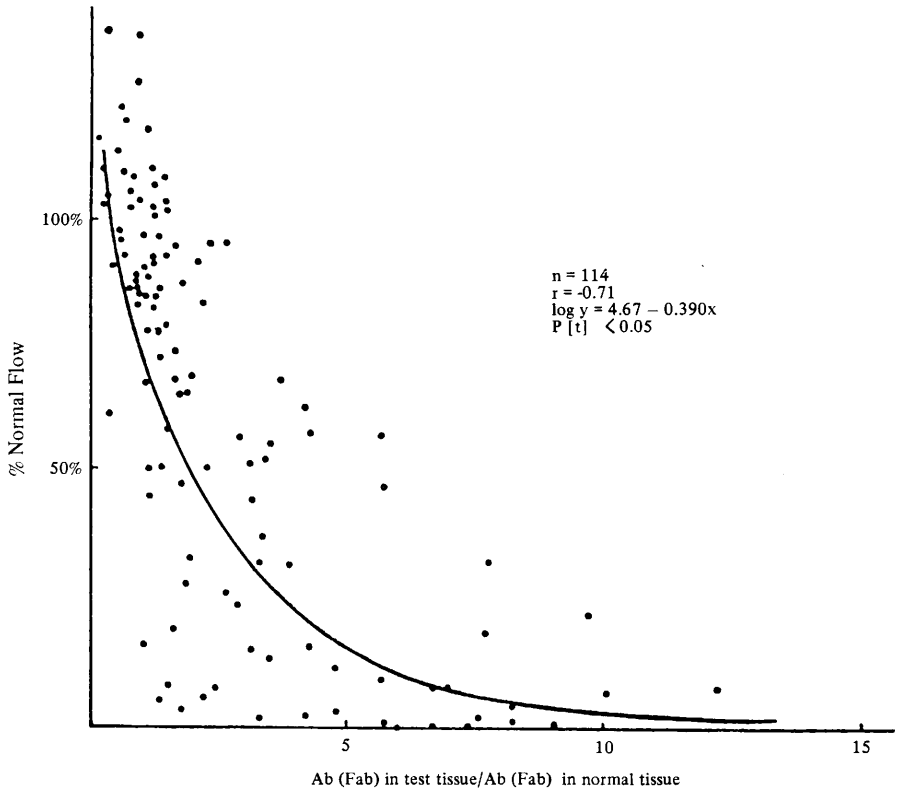

Fig. 3 Relationship between ^{111}In-anticardiac myosin antibody localization in myocardial samples and corresponding regional blood flow in the verapamil treated group.

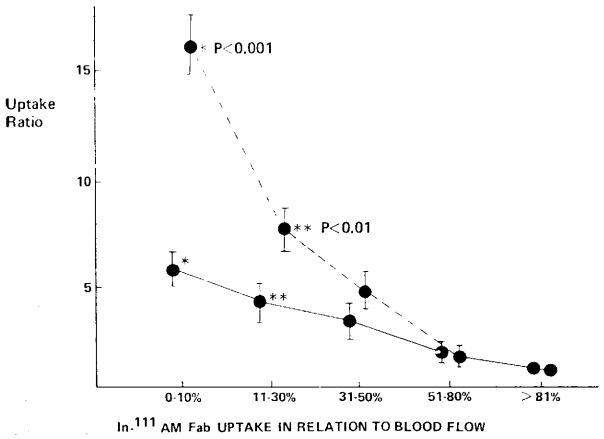

Fig. 4 Comparison of myocardial uptake of ^{111}In-anticardiac myosin antibody in the verapamil-treated group (———) and the control group (------). The x-axis represents coronary blood flow to the myocardial sample and the y-axis represents relative uptake of ^{111}In-anticardiac myosin antibody of the sample to normal myocardium. Each point represents the uptake ratio (mean±standard error) in designated regions of graded flow diminution (*$p<0.001$, **$p<0.01$ by Wilcoxon rank sum test)

DISCUSSION

Myocardial reperfusion is now an important method used in treating myocardial infarction produced by coronary occlusion. Both mechanical and thrombolytic therapy for restoring adequate perfusion following myocardial infarction have been mainstays of treatment to prevent or limit myocardial necrosis.[18]

However, during the period of recovery, myocardial cells may be vulnerable to stress produced by reperfusion.[11,14,19] The reperfusion injury has been suggested to be related in part to calcium overloading of intracellular organelles. It has been shown that reperfusion of irreversibly injured ischemic myocardium is followed by massive influx of calcium ion and intramitochondrial deposition of calcium phosphate.[20,21] Translocation of calcium into cells could produce mitochondrial damage and lead to cell injury.[1] Also intracellular calcium increase could contribute to the formation of a contraction band. In the experimental infarction, Frame et al[14] reported that uptake of ACM Ab increased 170% during the 45 minutes of reperfusion compared to the beginning of coronary reflow.

The present study revealed that the verapamil inhibited myocardial damage induced by reperfusion. The mechanism of action by which verapamil reduces

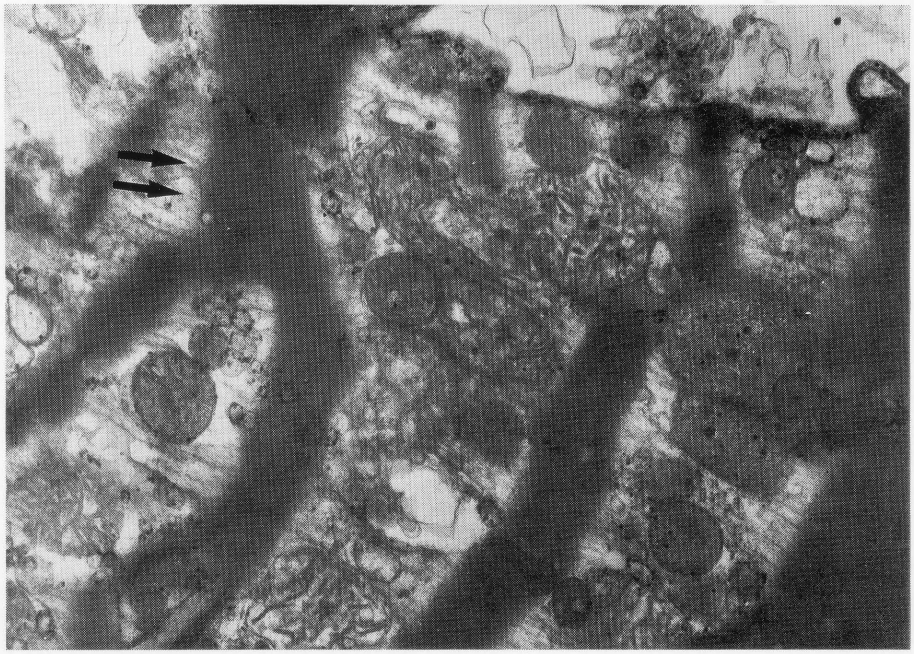

Fig. 5 Electron micrograph of a saline control dog. The cell was swollen with disruption of the cellular architecture and a large hypercontraction band (arrow). Stretching of the sarcomere was noted. There were separations of mitochondrial cristae and electron dense granules within the mitochondria ($\times$ 6,000).

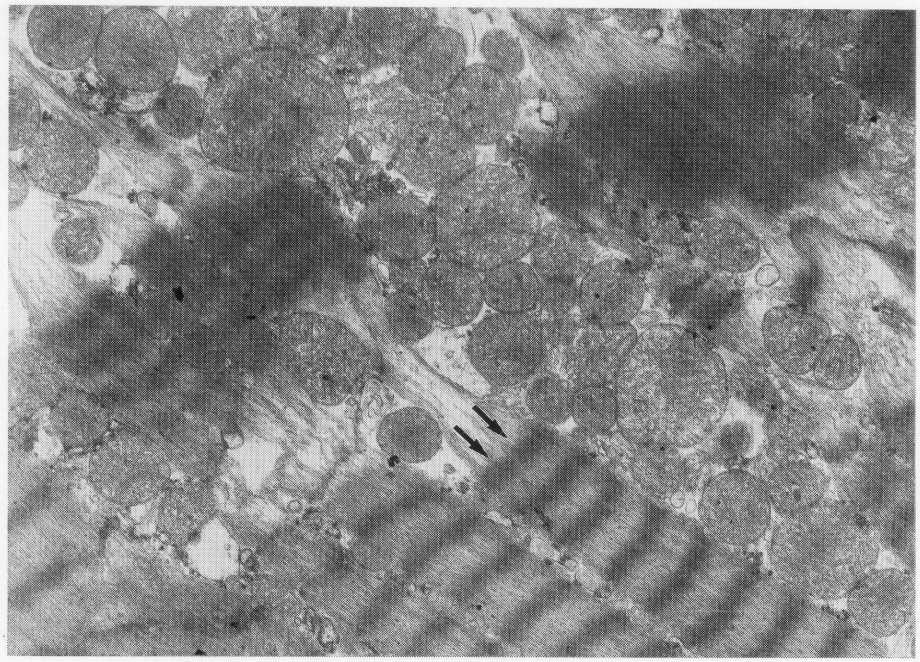

Fig. 6 Electron micrograph of a verapamil treated dog showing transition area between regular contraction injury (arrow) and hypercontraction band ($\times$ 6,000).

ischemic injury is not entirely clear but some hypotheses have been postulated,[22] i.e. the reduction of transmembrane calcium influx and in afterload, preservation of mitochondrial function, reduction in heart rate, and improvement in coronary collateral flow to the ischemic region.

The contraction band necrosis developed mainly within 20 minutes after coronary reperfusion.[20] There should be an adequate amount of verapamil in the myocardial tissue at the moment of reperfusion. Keefe and Kates[23] indicated that distribution and equilibrium between the plasma and the myocardium

was achieved within 10 minutes after the administration of verapamil in dogs. Verapamil accumulated in other tissues within 22 minutes. Therefore we started verapamil infusion at 20 minutes before reperfusion.

Cardiac myosin is the high molecular, major intracellular protein of the myocardial cell and has unique structural and antigenic features not shared by skeletal and smooth muscle myosins.[24] Monoclonal antibody for cardiac myosin binds specifically to cardiac myosin when cell membrane is disrupted.[22,25] Khaw et al.[26] have demonstrated with the scanning electronmicroscope that ACM Ab is a marker of membrane damage, i.e. cell death. They also showed that the amount of ACM Ab found in the tissue provided a quantitative method to evaluate cell damage.[27,28] In this study, we found an inverse relationship between ^{111}In-ACM Ab and coronary blood flow. ACM Ab uptake can be used as a valuable method to quantitate tissue damage.

Specific localization of anticardiac myosin antibody was enhanced by using Fab fragmants that are one third of the molecular weight of intact antibodies, but retain valency for antibody combining sites. Fab fragments can enter damaged cells easily and remaining antibodies in the circulation are eliminated by the kidney.[29]

The massive conglomeration of sarcomeres and passive stretching of the adjacent area may correspond with the ultrastructural finding of an irregular transverse hypercontraction band observed in light microscopy. A second type of myofibrillar contraction damage without a stretched zone was found by electronmicroscopy. The transitional zone from the classical hypercontraction band to regular contraction damage was noted in the verapamil treated group. These findings suggested that a regular contraction band might be a precursor of a hypercontraction band or a mild form of myofibrillar contraction damage. In addition to the morphological similarities between the two lesions, the low incidence of mitochondria containing matrix granules in the regular contraction band area (23.5% compared to 64.9% of the classical contraction band area) would be one item of evidence indicating a mild form of injury. This type of contraction band appeared to be a prominent type of injury to the endocardium of the verapamil treated group. However, we actually examined only a very small part of the lesions, and it was possible that the ultrastructural findings were not the same in all the lesions. To prove the presence and transition of the second type of myofibrillar contraction damage, we suggest a further detailed study with massive electronmicroscopic mapping.

The protective effect of verapamil appeared in an ischemic zone which had a coronary flow less than 30% of the normal. This finding is consistent with those of Frame et al.[14] and Ashraf et al.[4] who reported the development of contraction band necrosis in the subendocardium within 1 hour of occlusion and reperfusion. It has been postulated that in reperfusion, contraction band necrosis develops when the structure and function of the cell membrane have been altered by ischemia. Verapamil prevented tissue damage in the ischemic zone following reperfusion.

ACKNOWLEDGMENT

The authors appreciate the critical review of the manuscript by Dr. B. William Choi, Cardiology Division, Department of Medicine, George Washington University Medical Center, U.S.A.

REFERENCES

1. Whalen DA, Hamilton DG, Ganote CE, et al: Effect of a transient period of ischemia on myocardial cells: I. Effects on cell volume regulation. *Am J Pathol* 74: 381–397, 1974
2. Jennings RB, Ganote CE, Whalen DA: Recent advances in studies on cardiac structure and metabolism. Baltimore, University Park Press, pp 405–413, 1975
3. Bouchardy B, Majno G: Histopathology of early myocardial infarcts. *Am J Pathol* 74: 301–330, 1974
4. Ashraf M, White F, Bloor CM: Ultrastructural influences of reperfusing dog myocardium with calcium-free blood after coronary artery occlusion. *Am J Pathol* 90: 423–434, 1978
5. Reimer KA, Lowe JE, Jennings RB: Effect of the calcium antagonist verapamil on necrosis following temporal coronary artery occlusion in dogs. *Circulation* 55: 581–587, 1977
6. Kloner RA, Deboer JE, Carlson, et al: The effect of verapamil on myocardial ultrastructure during and following release of coronary artery occlusion. *Exp Mol Pathol* 36: 277–286, 1982
7. Wende W, Bleifeld W, Meyer J, et al: Reduction of the size of acute experimental myocardial infarction by verapamil. *Basic Res Cardiol* 70: 198–208, 1975
8. Smith HJ, Singh BN, Norris RM, et al: The effect of verapamil on experimental myocardial ischemia with particular reference to regional myocardial blood flow and metabolism. *Aust NZ J Med* 7: 114–121, 1977
9. Lefer AM, Polansky EW, Bianchi CP: Influence of verapamil on cellular integrity and electrolyte concentration of ischemic myocardial tissue in the cat. *Basic Res Cardiol* 74: 555–567, 1979
10. Magee PG, Flaherty JT, Bixler TJ, et al: Comparison of myocardial protection with nifedipine and potassium. *Circulation* 60 (suppl I): 151–157, 1979
11. Clark RE, Christlieb IY, Henry PD, et al: Nifedifine: A myocardial protective agent. *Am J Cardiol* 44: 825–831, 1979

12. Watte JA, Koch CD, LaNoue KF: Effect of Ca antagonism on energy metabolism: Ca and heart function after ischemia. *Am J Physiol* 238: H909–H916, 1980
13. Nayer WG, Ferrari R, Williams A: Protective effect of pretreatment on mitochondrial function in ischemic and reperfused myocardium. *Am J Cardiol* 46: 242–248, 1980
14. Frame LH, Lopez JA, Khaw BA, et al: Early membrane damage during coronary reperfusion in dogs. Detection by radiolabeled anticardiac myosin $F(ab)'_2$. *J Clin Invest* 71: 535–544, 1983
15. Karlsberg RP, Henry PD, Ahmed SA, et al: Lack of protection of ischemic myocardium by verapamil in conscious dog. *Eur J Pharmacol* 42: 339–346, 1977
16. Lo H-M, Kloner RA, Braunwald E: Effect of intracoronary verapamil on infarct size in the ischemic, reperfused canine heart: Critical importance of the timing of treatment. *Am J Cardiol* 56: 672–677, 1985
17. Harbert J, da Rocha AFG. Textbook of nuclear medicine. 2nd ed. Philadelphia: Lea & Febiger; 59–60, 1984
18. Braunwald E: Symposium on modern thrombolytic therapy. *J Am Coll Cardiol* 10 (suppl B): 1B–104B, 1987
19. Puri PS: Contractile and biochemical effects of coronary reperfusion after extended periods of coronary occlusion. *Am J Cardiol* 36: 244–251, 1975
20. Kloner RA, Ganote CE, Whalen DA Jr, et al: Effect of a transient period of ischemia on myocardial cells. II. Fine structure during the first few minutes of a reflow. *Am J Pathol* 74: 399–422, 1974
21. Jennings RB, Sommers HM, Kaltenbach JP, et al: Electolyte alterations in acute myocardial ischemic injury. *Circ Res* 14: 260–269, 1964
22. Lang T-W, Corday E, Gold H, et al: Consequences of reperfusion after coronary occlusion. Effect of hemodynamic and regional myocardial metabolic function. *Am J Cardiol* 33: 69–81, 1974
23. Keete DL, Kates RE: Myocardial deposition and cardiac pharmacodynamics of verapamil in the dog. *J Pharmocol Exp Ther* 220: 91–96, 1982
24. Festisova TV, Zabolotnyuk IP: Changes in heart muscle and liver proteins in experimental myocardial infarction. *Fed Proc* 24: T960–T962, 1965
25. Khaw BA, Beller GA, Haber E, et al: Localization of cardiac myosin specific antibody in myocardial infarction. *J Clin Invest* 58: 439–446, 1976
26. Khaw BA, Scott J, Fellon JT, et al: Quantitation by cell sorting initiated with antimyosin fluorescent sphere. *Science* 217: 1050–1053, 1982
27. Khaw BA, Beller GA, Habe E: Experimental myocardial infarction imaging following intravenous administration of 131-I labeled antibody $(Fab)'_2$ fragment specific for cardiac myosin. *Circulation* 57: 743–750, 1978
28. Khaw BA, Gold HK, Leinbach RC, et al: Early imaging of experimental myocardial infarction by intracoronary administration of ^{131}I-labeled anticardiac myosin $(Fab)'_2$ fragment. *Circulation* 58: 1137–1142, 1978
29. Senekowitsch R, Kriegel H, Baum RP, et al: Biokinetic studies and scintigraphic imaging of human carcinoma xenografts with radiolabeled monoclonal antibodies. In Srivastava SC(ed): *Radiolabeled monoclonal antibodies for imaging and therapy*. New York, Plenum, pp 323–341, 1988