Ibuprofen-Mediated Infarct Size Reduction: Effects on Regional Myocardial Function in Canine Myocardial Infarction

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Normal, marginal, and central ischemic regional myocardial function were evaluated in a canine model of myocardial infarction during 90 minute left circumflex coronary artery occlusion in 25 anesthetized dogs randomly assigned to intravenous ibuprofen infusion (n = 13, 5.36 mg/kg/h beginning 1 hour before occlusion) or vehicle solution as control (n = 12) and in 15 conscious, unsedated dogs 48 and 72 hours after 90 minute circumflex artery occlusion followed by reperfusion (ibuprofen, 5.36 mg/kg/h by intravenous infusion over 7 hours beginning 1 hour before occlusion, n = 7; or vehicle solution infusion as control, n = 8).

Miniature subendocardial sonomicrometer crystal pairs were used to calculate left ventricular regional end-diastolic segment length, end-systolic segment length, and regional percent systolic shortening. Infarct size was estimated in 72 hour animals by a postmortem dual perfusion technique using triphenyltetrazolium histochemical dye and Evan's blue dye for determination of infarct area, risk area, and area not at risk. Ibuprofen treatment significantly reduced infarct size expressed as percent of risk area (mean ± standard deviation of 44.6 ± 18 versus 64.4 ± 16% for control dogs, p <0.05) but it did not improve normal, marginal, or ischemic region end-diastolic length, end-systolic length or percent systolic shortening during coronary occlusion in anesthetized dogs or after reperfusion in conscious animals at 48 and 72 hours, and it did not enhance inotropic reserve at 72 hours in conscious animals. During 90 minute circumflex occlusion in anesthetized dogs, ibuprofen was associated with increases in systemic arterial pressure and worsened ischemic regional percent systolic shortening.

Thus, ibuprofen does not improve normal, marginal, or ischemic zone regional myocardial function during acute ischemia or 48 or 72 hours after myocardial reperfusion despite a significant reduction of infarct size.

Reducing the extent of ischemic injury has been proposed as a rational goal in the management of acute myocardial infarction.[1] Initial attempts to achieve this goal centered primarily on hemodynamic interventions which lessened myocardial oxygen consumption or improved blood flow or oxygen delivery to ischemic

myocardium; more recent investigations have evaluated a variety of agents whose primary effect is not hemodynamic but rather anti-inflammatory. The hypothesis supporting the use of these agents is that a significant determinant of ultimate ischemic injury is the inflammatory process itself. Among the many agents currently under evaluation is ibuprofen, a nonsteroidal antiinflammatory agent clinically employed for rheumatic disease.[2] Experimental studies have demonstrated a myocardial protective effect of ibuprofen in a variety of animal models,[3-7] presumably attributable to its antiinflammatory actions which affect arachidonic acid metabolism and consequent physiologic events.[8]

However, few studies on myocardial infarct size reduction involving either hemodynamic or antiinflammatory interventions have addressed the issue of the functional integrity of the salvaged myocardium or the effect of these agents on the function of myocardium removed from the area of ischemic injury. In order to assess the myocardial protective effect of ibuprofen on regional myocardial function, the following investigation was undertaken using implantable subendocardial

sonomicrometer crystals to measure segment length changes in normal, marginal and ischemic regions[9] in a canine model of left circumflex coronary artery occlusion and reperfusion. In this study, regional myocardial function was evaluated in open-chest, anesthetized dogs during coronary artery occlusion and in conscious, unsedated dogs 48 and 72 hours after circumflex occlusion and subsequent myocardial reperfusion.

Methods

Regional Myocardial Function During Coronary Artery Occlusion

Coronary occlusion: In order to assess the acute effects of ibuprofen on regional function during evolving myocardial injury due to coronary artery occlusion, 25 dogs were studied in the open-chest anesthetized state. Healthy male mongrel dogs weighing 10.2 to 18.9 kg (mean ± standard deviation 13.9 ± 1.9) were anesthetized with 30 mg/kg intravenous sodium pentobarbital and mechanically ventilated on room air by a Harvard animal respirator (Harvard Apparatus, Millis, Massachusetts) using an endotracheal tube. A left 5th intercostal space thorocotomy was performed and the pericardium incised and sutured to form a pericardial cradle. The left circumflex coronary artery was isolated distal to the left atrial appendage proximal to major branches. A zone of cyanosis was determined to be at least 9 cm^2 during transient (10 to 15 second) coronary occlusion. Three pairs of commercially manufactured miniature (3 mm) piezoelectric crystals (800 kHz frequency in radial mode with a resolution capability of 0.1 to 0.5 mm) connected to an oscilloscope sonomicrometer (Norland Instruments sonomicrometer crystals and NI-202 sonomicrometer) were implanted 1 to 2 cm apart in the left ventricular subendocardium (Fig. 1). One crystal pair (central ischemic zone) was implanted in the posterolateral left ventricular surface in the center of the cyanotic zone as determined by the transient circumflex occlusion, and a 2nd pair (marginal area) was implanted in the anterolateral left ven-

FIGURE 1. Diagram of subendocardial sonomicrometer crystal placement in normal, marginal, and central ischemic left ventricular regions. LCX = left circumflex coronary artery. See text for discussion.

tricular wall to straddle the border between cyanotic and noncyanotic zones. The marginal area was defined in the present study as a region of intermediate function between normal and ischemic zones.[9] Normal zone sonomicrometer crystals were implanted on the anterior left ventricular wall in the region supplied by the left anterior descending coronary artery well removed from the ischemic area as determined by the zone of cyanosis. The crystals were implanted in a plane perpendicular to the base-apex cardiac axis and secured by an epicardial suture around the connecting wires as they emerged from the myocardium. Repeated calibrations at 1 μs intervals were obtained to eliminate variation due to signal drift.

In addition, a small left neck incision was made for insertion of a polyethylene carotid artery catheter for blood pressure measurement and an external jugular catheter for infusion of ibuprofen or control solution. The carotid artery catheter was connected to a Statham P23DC pressure transducer. Mean arterial pressure was calculated as diastolic pressure plus one third of the pulse pressure.

Ibuprofen infusion: Crystalline ibuprofen (sodium salt) was dissolved in 0.2 M sodium carbonate and adjusted to a pH of 8 by 1.0 N HCl. Solutions were mixed fresh before each experiment and diluted to 50 ml in physiologic saline solution. The ibuprofen dose employed was 5.36 mg/kg/h (7.14 ml/h of ibuprofen solution) by continuous intravenous infusion beginning 1 hour before occlusion and continuing throughout occlusion (90 minutes). This dose is similar to that used in a previous investigation[6] with the exception of initiation of the infusion before occlusion in the present study. Vehicle solution prepared in an identical manner without ibuprofen served as a control. A Harvard infusion pump was used to provide a constant infusion rate. Animals were randomly assigned to their treatment groups (13 ibuprofen, 12 control). A lead II electrocardiogram was monitored throughout the experiment.

After instrumentation, visible epicardial arterial anastomoses between the left anterior descending and circumflex coronary arteries were ligated to eliminate potentially large collateral routes of blood flow.[10] A subtotal left circumflex coronary artery stenosis was produced just distal to the left atrial appendage by tying 1-0 silk suture around both the artery and an 18 gauge needle followed by immediate needle removal; subsequent total circumflex artery occlusion was performed after 10 minutes with a total duration of occlusion of 90 minutes. Three mg/kg of intravenous lidocaine and 3 mg/kg of intramuscular lidocaine were administered at the time of coronary artery occlusion as antiarrhythmic therapy.[11] Approximately one fourth of the animals developed ventricular fibrillation during coronary occlusion, which was readily converted to sinus rhythm within 30 to 60 seconds by low energy (10 to 20 J) direct current cardioversion using epicardial paddles. No myocardial dysfunction has been reported at these energy levels.[12]

Regional myocardial segment length: Measurement of regional myocardial segment length was obtained by converting sonomicrometer crystal transit time in microseconds to distance in millimeters using a known value for the velocity of ultrasound in myocardium at the frequency employed: Distance (mm) = transit time (μs) $\times$ ultrasound velocity in myocardium (1.58 mm/μs).[13] Before occlusion, end-diastolic length was defined in each segment as the maximal crystal separation in millimeters before the onset of the carotid artery systolic pressure rise, and end-systolic length was defined as the minimal crystal separation in millimeters before the dicrotic notch of the carotid artery pressure tracing. Nonsimultaneous end-diastolic or end-systolic timing in marginal or
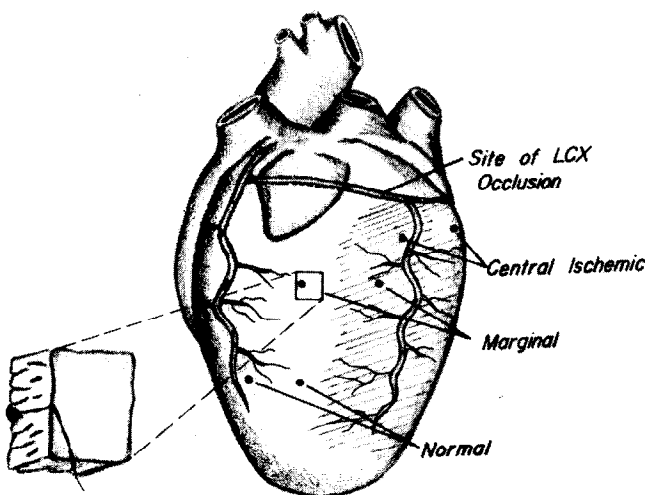
ischemic segments in relation to normal segments was noted before occlusion and used as a reference to determine end-diastole and end-systole in the presence of dyskinesia during coronary artery occlusion. Measurement of absolute end-diastolic and end-systolic length in millimeters were obtained and regional percent systolic shortening defined as end-diastolic minus end-systolic length divided by end-diastolic length ×100%. Subendocardial crystal placement was confirmed by postmortem examination.

A Grass model 7 polygraph was used to record measurements at a paper speed of 100 mm/s. Determination of carotid artery pressure, heart rate, and regional segment lengths and percent systolic shortening were obtained before and at the end of 90 minutes of coronary occlusion. All measurements were obtained at end-expiration and when the animal was free of arrhythmias.

Regional Myocardial Function 48 and 72 Hours After Coronary Artery Occlusion-Reperfusion

The effect of ibuprofen on regional myocardial function and infarct size was evaluated in conscious, unsedated dogs after left circumflex coronary artery occlusion with subsequent myocardial reperfusion in the anesthetized state. Fifteen healthy male mongrel dogs weighing 13.6 ± 1.5 kg (range 10.2 to 15.8) were used. Instrumentation, baseline regional function measurements, and 90 minute circumflex occlusion followed by reperfusion were performed using aseptic technique in pentobarbital anesthetized, open-chest dogs as in the acute occlusion study. Animals were randomly assigned to either ibuprofen (n = 7) or vehicle as control (n = 8). Solutions were prepared as before and administered intravenously by Harvard infusion pump over 7 hours beginning 1 hour before occlusion with an ibuprofen dose of 5.36 mg/kg/h (7.14 ml/h of ibuprofen solution) or with an identical volume of vehicle as a control. Ischemic zone sonomicrometer crystals were associated with greater postoperative mortality in preliminary studies and therefore implanted in only 4 animals in each group. As in the acute occlusion study, a critical circumflex stenosis was placed 10 minutes before total occlusion using an 18 gauge needle and 1-0 silk suture. A Carolina Medical Electronics electromagnetic flow probe was placed around the circumflex artery proximal to the subtotal occlusion to document a critical stenosis (defined as a stenosis causing at least a 50% reduction in peak reactive hyperemic flow after 10 seconds of coronary occlusion without altering basal flow or sonomicrometer crystal segment length). This subtotal critical stenosis was left in place throughout the remainder of the experiment (until the animals were killed at 72 hours).

Coronary occlusion and reperfusion: Total left circumflex occlusion was performed using soft plastic tape for 90 minutes and the occlusion was slowly released over 5 to 10 minutes. Intravenous lidocaine, 3 mg/kg, was administered on reperfusion. Reperfusion occurred in all animals as documented by 3 or more of the following criteria: (1) the onset of ventricular arrhythmias within 15 to 30 minutes of occlusion release; (2) a decrease in marginal and ischemic region end-diastolic and end-systolic segment length on release of the occlusion; (3) at least 5 ml/min of circumflex artery flow on occlusion release documented by electromagnetic flow probe; and (4) the absence of visible coronary artery thrombosis at 72 hours when the animal was killed.

After reperfusion, the chest was closed and the animals were allowed to recover. Intramuscular ampicillin, 6 mg/kg, was administered twice on the day of surgery and daily thereafter. No recordings were obtained until 48 hours after surgery because of frequent ventricular arrhythmias. At 48 and 72 hours, recordings of sonomicrometer crystal segment

lengths were obtained in the conscious, unsedated state with the animal standing upright in a nylon sling. In order to assess residual ventricular contractile function, inotropic reserve was also tested at 72 hours after coronary occlusion-reperfusion, 5 minutes after administration of the beta-adrenergic stimulant prenalterol (30 μg/kg intravenous bolus).[14]

Determination of infarct size and risk area: Animals were then reanesthetized with sodium pentobarbital, the chest was reopened, and the hearts were electrically fibrillated and rapidly excised for immediate postmortem in vitro determinations of infarct size and risk area by a dual perfusion technique using 4% triphenyltetrazolium chloride mixed with phosphate buffer for histochemical staining of dehydrogenase enzyme and 0.5% Evan's blue flow-dependent staining as described in detail elsewhere.[7,15] Briefly, this method involves simultaneous selective left circumflex perfusion with triphenyltetrazolium and nonselective retrograde aortic perfusion with Evan's blue dye at physiologic pressure (100 mg Hg), with the resultant Evan's blue staining tissue identifying the area not at risk of ischemic injury and the remaining tissue representing the area at risk with red triphenyltetrazolium staining tissue identifying the viable, noninfarcted region, and the remaining tissue (unstained) representing the area of infarction. After staining, the heart was sliced from apex to base in 5 mm thick transverse sections and the atria and right ventricle were excised. Left ventricular weight was recorded and left ventricular slices were traced on transparent plastic sheets for hand planimeter determination of left ventricular area, area at risk, and area of infarction. Infarct size was measured as percent of area at risk and percent of total left ventricular area. The anatomic perfusion bed of an occluded coronary has been shown to correlate well with the area at risk of infarction in experimental canine coronary artery occlusion.[10] Subendocardial crystal placement was also confirmed at postmortem evaluation.

Light microscopic evaluation of hematoxylin and eosin stained left ventricular slices was performed in a blinded manner on 72 hour specimens from all animals in each group to assess the morphologic integrity of myocardial cells and the inflammatory response.

Statistical methods: All results are expressed as mean ± 1 standard deviation. Statistical analysis was performed by a computerized statistical program of the University of Michigan Statistical Research Laboratory (Michigan Interactive Data Analysis System). Two factor analysis of variance for repeated measures (profile analysis)[16] was employed for the same variable measured more than twice, and paired or unpaired t test was performed as appropriate on data measured only twice. A probability value (p) less than 0.05 was considered significant.

Results

Regional myocardial function during coronary artery occlusion: In anesthetized, open-chest dogs 90 minute circumflex coronary artery occlusion resulted in a marked alteration of contractile function manifested by greatly diminished marginal and ischemic segment systolic shortening and increased end-diastolic and end-systolic segment lengths in all 3 regions, indicative of ischemia-associated ventricular dilation[9] (Fig. 2, Table I). Ibuprofen administration did not decrease these segment length increases or improve systolic shortening in any region during coronary artery occlusion. In the central ischemic zone, ibuprofen-treated animals developed more severe impairment of
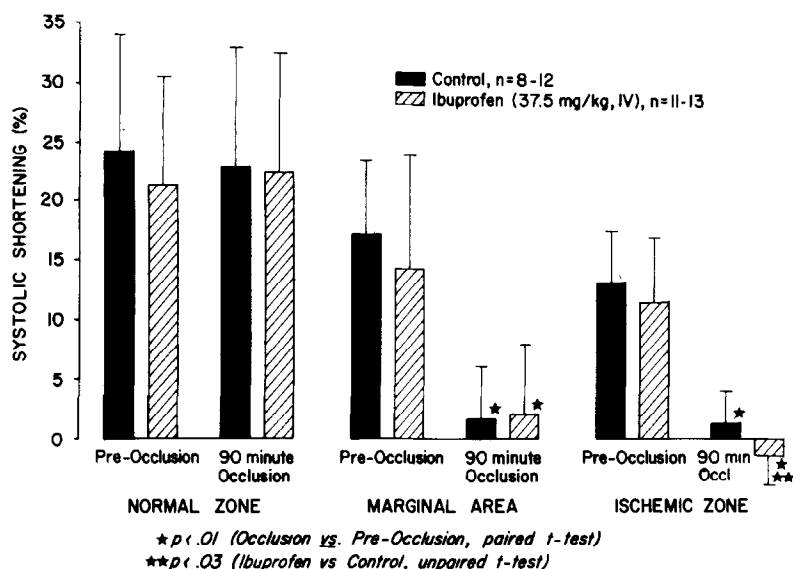
FIGURE 2. Effect of ibuprofen on regional myocardial function after 90 minute left circumflex coronary artery occlusion. Marginal and ischemic zone percent systolic shortening was reduced in both groups compared with preocclusion (paired t test), and ibuprofen animals had significantly worse systolic shortening than controls in the ischemic zone at the end of the 90 minute occlusion (unpaired t test)

systolic shortening with paradoxic systolic expansion (dyskinesia). Heart rate was not altered by ibuprofen during coronary occlusion; however, mean carotid artery pressure rose significantly with a rise in pressure-rate product (the product of mean arterial pressure and heart rate divided by 100) (Table I).

Regional myocardial function 48 and 72 hours after coronary artery occlusion-reperfusion: Serial changes in regional myocardial function in a control animal before, during, and up to 72 hours after coronary artery occlusion-reperfusion are shown in Figure 3. Similar baseline regional myocardial function was present in ibuprofen-treated and control animals in the anesthetized, open-chest state before coronary occlusion

(Table II, Fig. 4 to 6). In the conscious, unsedated state 48 and 72 hours after occlusion with myocardial reperfusion, both marginal and ischemic zone systolic shortening were impaired despite partial recovery, while normal zone systolic function remained relatively unaltered. Ibuprofen treatment did not improve systolic function or decrease ventricular end-diastolic or end-systolic segment lengths in normal, marginal, or ischemic regions at 48 hours or 72 hours before or after inotropic stimulation.

A significant reduction in the extent of ischemic injury as measured by percent of the area at risk infarcted was noted in ibuprofen-treated animals (44.6 ± 18%) compared with control animals (64.4 ± 16%, p

TABLE I   Effects of Ibuprofen During Coronary Occlusion

| | Heart Rate (beats/min) | | Mean Arterial Pressure (mm Hg) | | Pressure-Rate Product | | Normal Zone | | | | | |
| | | | | | | | End-Diastolic Length (mm) | | End-Systolic Length (mm) | | % Systolic Shortening | |
	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen
Baseline	167 ±25	159 ±17	111 ±14	120 ±20	187 ±46	194 ±50	12.6 ±3.1	14.7 ±2.4	9.7 ±3.2	11.5 ±2.3	24.3 ±9.7	21.4 ±9.2
90 minute coronary occlusion	171 ±20	174 ±18	106 ±14	137 ±15*	178 ±30	239 ±44*	14.6 ±4.0	17.1 ±3.0	11.4 ±3.9	13.3 ±3 1	23.0 ±9.9	22.5 ±10.0

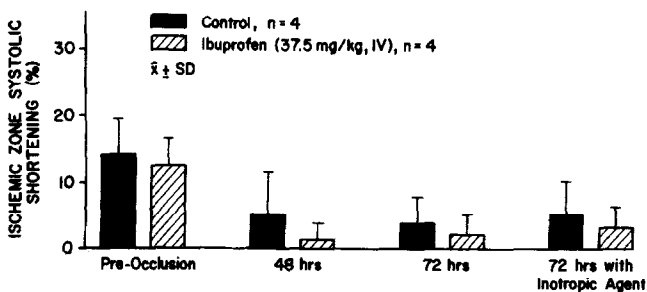| | Marginal Zone | | | | | | Ischemic Zone | | | | | |
| | End-Diastolic Length (mm) | | End-Systolic Length (mm) | | % Systolic Shortening | | End-Diastolic Length (mm) | | End-Systolic Length (mm) | | % Systolic Shortening | |
	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen
Baseline	15.0 ±2.9	13.9 ±5 2	12.5 ±3.0	12.1 ±5.3	17.2 ±6.3	14.3 ±9.7	15.6 ±4.6	17.1 ±4.6	13 6 ±4 2	15.3 ±4.6	13.3 ±4.2	11.6 ±5.5
90 minute coronary occlusion	18.1 ±2.8	17.2 ±6.0	17.8 ±3.1	16.8 ±5.9	1.7 ±4.4	2.1 ±5.9	18.4 ±5.4	19.7 ±5.2	18.1 ±4 9	20.0 ±5.1	1.3 ±2.7	−1.5 ±2 3†

* p <0.003; † p <0.03 (both unpaired t test).
Pressure rate product = mean arterial pressure X heart rate divided by 100. Values are mean ± 1 standard deviation
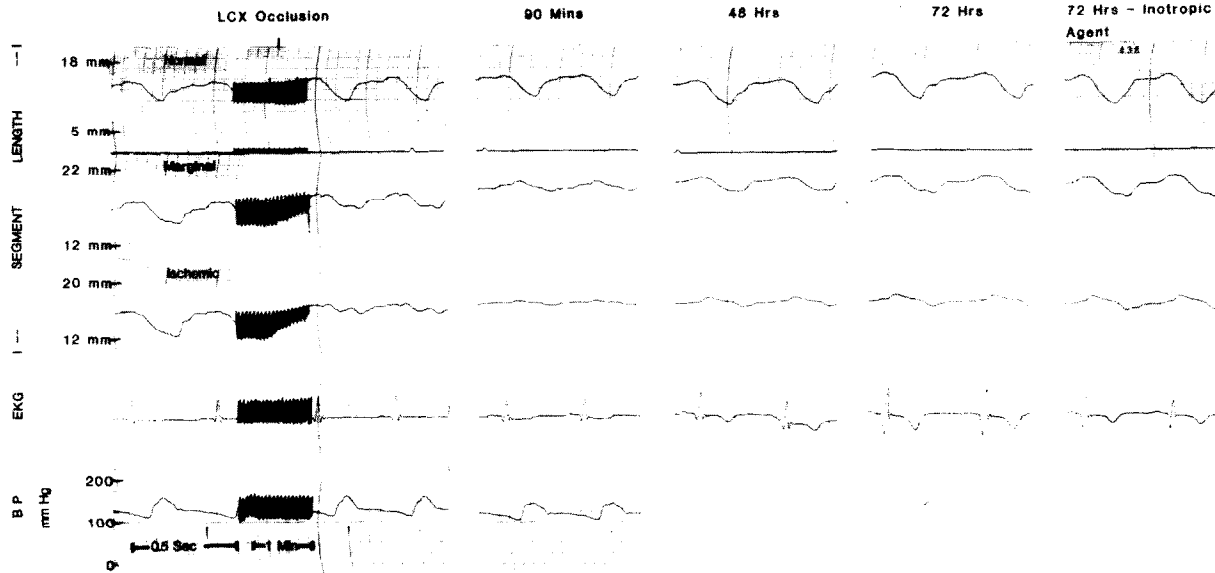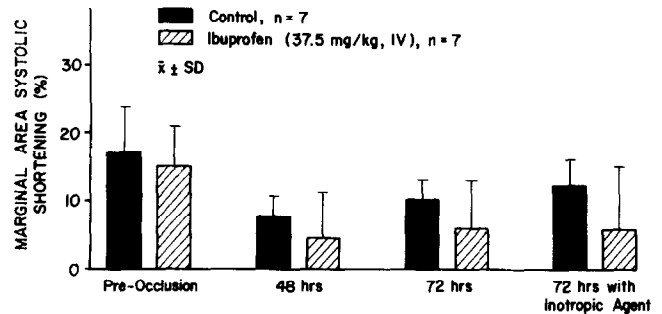
FIGURE 3. Serial changes in normal, marginal, and central ischemic segment length in a control animal before and at the end of 90 minute circumflex occlusion and 48 and 72 hours after reperfusion, and with inotropic stimulation (intravenous prenalterol, 30 μg/kg) at 72 hours. Acute effects of circumflex occlusion on regional myocardial function and evolutionary electrocardiographic changes of infarction are demonstrated. BP = blood pressure; EKG = lead II electrocardiogram; LCX = left circumflex coronary artery.
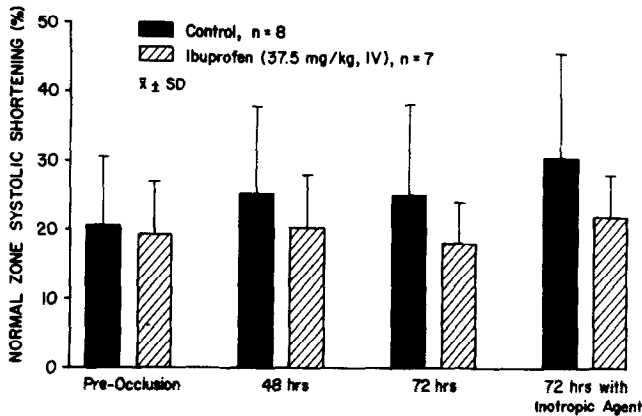

FIGURE 4. Effect of ibuprofen on normal zone regional myocardial function before circumflex occlusion and in conscious, unsedated animals at 48 and 72 hours and with inotropic stimulation at 72 hours. No significant differences were present between groups (analysis of variance for repeated measures). IV = intravenous; SD = standard deviation.

FIGURE 5. Effect of ibuprofen on marginal area regional function (as in Figure 4). No significant differences between groups were present (analysis of variance for repeated measures).

FIGURE 6. Effect of ibuprofen on central ischemic zone regional function (as in Figure 4). No significant differences between groups were present (analysis of variance for repeated measures).
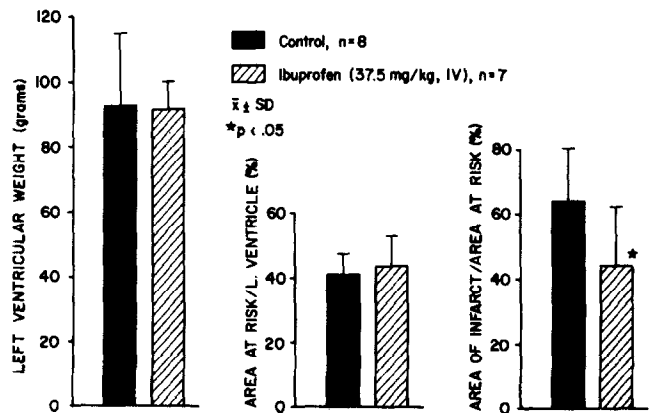
FIGURE 7. Effect of ibuprofen on infarct size. No differences were present between ibuprofen and control animals in left (L.) ventricular weight or area at risk; ibuprofen significantly reduced infarct size expressed as percentage of area at risk (unpaired t test).

TABLE II Effects of Ibuprofen on Regional Myocardial Function After Reperfusion

	Normal Zone						Marginal Zone						Ischemic Zone					
	End-Diastolic Length (mm)		End-Systolic Length (mm)		% Systolic Shortening		End-Diastolic Length (mm)		End-Systolic Length (mm)		% Systolic Shortening		End-Diastolic Length (mm)		End-Systolic Length (mm)		% Systolic Shortening	
	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen	Control	Ibuprofen
Baseline	13.3 ±2.6	15.2 ±2.7	10.6 ±2.9	12.2 ±2.4	20.7 ±9.8	19.4 ±7.9	14.9 ±3.1	15.8 ±5.0	12.5 ±3.4	13.5 ±4.8	17.3 ±6.6	15.2 ±5.8	15.5 ±4.1	17.6 ±2.2	13.4 ±4.1	15.4 ±2.6	14.1 ±5.4	12.6 ±4.1
48 hours after reperfusion	15.3 ±2.8	17.0 ±3.5	11.5 ±3.7	13.6 ±3.3	25.5 ±12.7	20.3 ±7.9	17.7 ±3.0	19.1 ±5.2	16.3 ±2.8	18.4 ±5.7	7.8 ±3.0	4.5 ±6.8	17.6 ±3.7	18.5 ±2.4	16.6 ±3.8	18.3 ±2.7	5.3 ±6.4	1.5 ±2.5
72 hours after reperfusion	15.1 ±2.4	16.7 ±3.8	11.4 ±3.4	13.7 ±3.9	25.3 ±13.0	18.3 ±6.0	17.9 ±3.4	19.0 ±5.7	16.1 ±3.1	18.1 ±6.5	10.0 ±3.1	6.0 ±7.1	17.2 ±4.2	18.9 ±3.2	16.5 ±3.9	18.5 ±3.5	4.0 ±4.1	2.3 ±2.8
72 hours with inotropic agent	14.5 ±2.3	15.4 ±3.8	10.2 ±3.4	12.1 ±3.7	30.8 ±1.5	22.3 ±6.1	17.4 ±3.2	18.2 ±5.6	15.3 ±3.1	17.3 ±6.4	12.5 ±3.8	6.2 ±9.0	17.1 ±4.3	18.7 ±3.3	16.2 ±3.9	18.1 ±3.7	5.2 ±5.1	3.5 ±3.0

Values are mean ± 1 standard deviation. Differences between ibuprofen and control groups were not significant (analysis of variance for repeated measures).

<0.05, unpaired t test) despite a similar area at risk and left ventricular weight (Fig. 7). The percent of total left ventricle infarcted was substantially reduced by ibuprofen (19.7 ± 9.1% versus 26.0 ± 5.5% for controls), but this did not reach significance (p = 0.12, unpaired t test). By visual inspection of stained ventricular slices, the predominant area of tissue salvage in ibuprofen-treated animals was at the epicardial and lateral margins of the infarct.

Light microscopic analysis of hematoxylin and eosin stained left ventricular slices revealed no evident differences in the extent of inflammatory infiltrate or hemorrhage or in the appearance of necrotic myocardial cells. The appearance of contraction band lesions and of fibroblastic proliferation was comparable in both groups.

Visual inspection of the left circumflex coronary artery after death demonstrated no detectable thrombus in ibuprofen-treated animals and visible thrombus in 1 of 8 control animals.

Discussion

Rationale for this model: Coronary artery occlusion with myocardial reperfusion through a critical stenosis as employed in this model may more closely simulate the evolution of human myocardial infarction than permanent coronary artery occlusion. DeWood et al.[17] demonstrated a progressive decline in the prevalence of total coronary occlusion over 24 hours in patients with acute myocardial infarction, a finding consistent with coronary artery spasm superimposed on fixed obstructive disease[18] or with partial resolution of a coronary artery thrombosis,[17] or both. In addition, experimental animal data indicate that reperfusion may enhance survival[19] and improve the likelihood of myocardial functional recovery.[20] Thus, a rationale exists for the coronary artery occlusion-reperfusion model of myocardial infarction.

Possible mechanisms of ibuprofen's myocardial protective effect: The present study demonstrates a myocardial protective effect of ibuprofen during experimental myocardial infarction without improvement of normal, marginal, or ischemic regional myocardial function during coronary artery occlusion or 48 or 72 hours after reperfusion or of inotropic reserve at 72 hours. The reduction in infarct size noted with ibuprofen in this study is consistent with previous investigations[3-7] employing a variety of dosage schedules and routes of administration. While the mechanisms of infarct size reduction mediated by ibuprofen have not been established, several possibilities have been evaluated.

First, it is unlikely that ibuprofen exerts a hemodynamic effect through diminished myocardial oxygen consumption or increased myocardial blood flow. Jugdutt et al.[6] and Romson et al.[7] noted no significant effect of ibuprofen on blood pressure or heart rate during myocardial ischemia, and the drug has only a minimal effect on ventricular contractile force.[4] In our present study, continuous ibuprofen infusion beginning 1 hour before ischemia resulted in significant elevations of systemic arterial pressure (Table I) without an effect

on heart rate after 90 minutes of coronary occlusion. This result, not found in previous investigations employing different models of ischemia and different drug administration schedules, would increase rather than decrease myocardial oxygen consumption and thus tend to worsen ischemic injury.[21] In addition, directly measured myocardial oxygen consumption is not altered by ibuprofen administration during 60 minutes of normothermic global ischemia in the isolated, blood-perfused feline heart.[15]

Increased blood flow to ischemic tissue by direct coronary artery vasodilation or enhanced collateral blood flow is also an unlikely mechanism of ibuprofen's beneficial effect. Tracer microsphere studies in experimental myocardial infarction have failed to demonstrate increased blood flow to ischemic tissue up to 6 hours[6] or 24 hours[15] after coronary occlusion in ibuprofen-treated experimental animals.

An antithrombotic effect of ibuprofen is a 2nd possible mechanism by which ibuprofen may reduce infarct size.[7] In the present study, however, coronary artery reperfusion occurred in all animals and only 1 of 8 control animals developed visible thrombus at 72 hour postmortem examination. Also, the antithrombotic agent sulfinpyrazone does not appear to reduce infarct size.[22] Thus, it is improbable that an antithrombotic effect of ibuprofen played a role in the present study. The potentially related phenomenon of platelet deposition at the site of ischemic injury also occurs in myocardial infarction and is diminished by aspirin.[23] Ibuprofen, however, does not alter indium-111–labeled platelet accumulation in the ischemic myocardium.[24]

A 3rd possible mechanism by which ibuprofen may exert a myocardial protective effect is its antiinflammatory action, related to inhibition of the arachidonic acid pathway.[8] Cyclooxygenase enzyme inhibition could have a beneficial effect in ischemic tissue through a reduction in the synthesis of thromboxane, a potent vasoconstrictor. However, other nonsteroidal cyclooxygenase inhibitors do not reduce the extent of experimental ischemic injury.[22,25,26] An alternative hypothesis also related to the arachidonic acid cascade which may in part explain ibuprofen's beneficial effect in myocardial ischemia is inhibition of the lipoxygenase pathway. This branch of the arachidonic acid cascade is capable of producing powerful chemotactic substances (hydroxyeicosatetraenoic acid [HETE] derivatives)[27] and leukotrienes, which are additional mediators of the inflammatory response.[27] Ibuprofen has been shown to inhibit the synthesis of 12-HETE, catalyzed by a putative peroxidase enzyme.[28] Lipoxygenase pathway inhibition may in part account for ibuprofen's ability to inhibit the migration of polymorphonuclear leukocytes,[29] an effect noted with ibuprofen in experimental myocardial ischemia.[24]

A 4th mechanism of potential significance in explaining ibuprofen's myocardial protective action is inflammatory cell lysosomal membrane stabilization, an effect suggested by data from noncardiac in vitro preparations[30-32] and by indirect evidence in canine cardiac ischemia.[4] In addition, ibuprofen affects kinin and histamine release[2] and superoxide radical forma-

tion,[33,34] which may in part account for its anti-inflammatory effect.

The use of lidocaine in our study could also reduce the severity of myocardial ischemic injury.[35] Our data, however, indicate extensive infarction despite lidocaine administration and an additional beneficial effect of ibuprofen if lidocaine myocardial preservation occurred.

Discordance between infarct size reduction and functional recovery: The discrepancy between myocardial infarct size and functional recovery in this investigation may be due to several factors. Improvement in regional myocardial function may occur at a time not evaluated in this study. A functionally beneficial effect of ibuprofen between the onset of reperfusion and 48 hours cannot be excluded; in the present study a functional assessment was not performed during that time because of marked beat-to-beat segment length changes associated with frequent reperfusion arrhythmias. Alternatively, improvement in myocardial function with ibuprofen could occur at a time later than 72 hours. This possibility is supported by sonomicrometer crystal data in conscious dogs studied up to 4 weeks after 2 hour coronary artery occlusion.[20] Substantial recovery of marginal and ischemic segment systolic shortening occurred 1 to 4 weeks after coronary artery occlusion-reperfusion. Further studies will be necessary to determine whether ibuprofen treatment alters regional myocardial function more than 3 days after myocardial infarction.

Improvement in indexes of myocardial function other than subendocardial segment length changes is another possible explanation of the discordance between infarct size reduction and functional recovery in this investigation. Roan et al.[36] noted spontaneous improvement in ischemic zone ventricular wall paradoxic systolic thinning between 24 hours and 1 week after coronary artery occlusion. Other measures of regional function which were not evaluated in this study include mid-myocardial and subepicardial segment length changes, which may have been altered more than subendocardial functional measurements in view of predominant subepicardial tissue salvage by gross inspection.

A persistent inability of ibuprofen to significantly improve marginal or ischemic region myocardial function despite infarct size reduction as determined by histochemical staining is also possible. Glucocorticoids have been implicated in "mummification" of ischemic myocardium (loss of nuclei with intact myofibril striations and sarcolemma).[37] Such tissue may retain the ability to stain dehydrogenase with triphenyltetrazolium despite the loss of functional integrity. The absence of such a "mummification" effect on light microscopic evaluation in this investigation provides evidence against that possibility.

A recent publication by Kloner et al.[38] demonstrated that a 15 minute period of coronary occlusion followed by reperfusion, which is not associated with the development of necrosis, nonetheless results in prolonged biochemical, functional, and ultrastructural abnormalities. Wall motion, as determined by ultrasonic

crystal measurements, was still reduced at 3 days of reperfusion, whereas creatine phosphate levels and regional myocardial blood flow recovered rapidly and had returned to normal after 90 minutes of coronary reperfusion. Brief periods (15 minutes) of regional ischemia followed by full reperfusion, therefore, can be associated with abnormalities in cardiac contractile function which persist for a relatively long period. Bush et al.[39] showed that the biochemical abnormalities associated with regional ischemia after reperfusion also extend to the normally perfused myocardium. Thus, a regional ischemic event may produce an influence on a remote region of myocardium which may persist for several days after the restoration of coronary blood flow.[38,39]

In the present investigation, coronary artery occlusion was maintained for 90 minutes and was followed by reperfusion in the presence of a critical stenosis. It is conceivable that the failure of the salvaged myocardial segment to regain contractile function could be related to the relatively persistent derangement in segment length shortening which is seen even in the absence of cellular necrosis. Future attempts to assess the potential benefits of pharmacologic interventions in protecting ischemic myocardium should consider the functional recovery of the "salvaged" myocardium days to weeks after the ischemic episode.

Implications: Intravenous ibuprofen infusion reduces myocardial infarct size without improving normal, marginal, or ischemic region myocardial function during coronary artery occlusion or 48 or 72 hours after myocardial reperfusion, or inotropic reserve at 72 hours. Further studies are necessary to assess ibuprofen's effect on regional myocardial function at intervals greater than 72 hours after myocardial infarction and to further elucidate the mechanisms by which ibuprofen exerts a protective effect on ischemic myocardial tissue.

Acknowledgment

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