The Effect of Ibuprofen on Accumulation of Indium-111-labeled Platelets and Leukocytes in Experimental Myocardial Infarction

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SUMMARY To assess the ability of ibuprofen to influence the extent of platelet aggregation and leukocyte infiltration during acute myocardial infarction, autologous indium-111 (111In)-labeled platelets or leukocytes were injected before 60 minutes of left circumflex coronary artery (LCx) occlusion, followed by 24 hours of reperfusion in the canine heart. Myocardial infarct size, as a percent of the area at risk, was reduced in the ibuprofen-treated group (12.5 mg/kg i.v. every 4 hours beginning 30 minutes before LCx occlusion) by 40%, from 48 ± 4% in control animals to 29 ± 4% in ibuprofen-treated dogs (p = 0.005). Quantification of the platelet-associated 111In radioactivity in irreversibly injured myocardium indicated that ibuprofen did not alter the accumulation of platelets in infarcted myocardium. In contrast, leukocyte accumulation in infarcted tissue was reduced significantly. In tissue samples with 0.41–0.60 gram infarct, the infarcted/normal ratio of leukocyte radioactivity was 12 ± 2 in control dogs and 4 ± 1 in ibuprofen-treated dogs, which represents a 67% reduction in leukocyte accumulation in ibuprofen-treated compared with control dogs. Similar reductions were found in other gram-infarct-weight categories. Although both platelets and leukocytes accumulate in infarcted canine myocardium, ibuprofen may exert its beneficial effect on ischemic myocardium by suppressing the inflammatory response associated with myocardial ischemia and infarction.

THE GOAL of much cardiovascular research has been to develop pharmacologic means of managing patients before or soon after an acute ischemic insult to minimize the extent of irreversible myocardial injury and subsequent loss of ventricular function. Several agents are effective in reducing the extent of myocardial infarction resulting from experimentally induced acute myocardial ischemia in a variety of animal models.1 One such agent, ibuprofen, is a nonsteroidal antiinflammatory compound that has been reported to exert cardioprotective effects by significantly reducing the extent of irreversible myocardial injury to experimental ischemia in the dog,2,3 the cat,4 and the rat.5 Ibuprofen renders its cardioprotective effects administrated orally,3 intravenously2 or intramuscularly.5 Several independent investigators have demonstrated protective effects in a variety of experimental models of myocardial ischemia and infarction, but little is known about ibuprofen’s mechanism of action. Apparently, the beneficial effects of ibuprofen do not derive from alteration of the balance of myocardial oxygen supply and demand in a favorable manner. Intravenous ibuprofen reportedly has negligible hemodynamic effects2,6 and almost no effect on the calculated rate-pressure product, an accepted approximation of myocardial oxygen consumption,7 during myocardial ischemia in the dog.6 Moreover, in the nonischemic, isolated, blood-perfused cat heart, an animal model that permits careful control of hemodynamic variables, ibuprofen did not alter myocardial oxygen consumption as measured by arteriovenous oxygen differences.6

The possibility that ibuprofen salvages ischemic myocardium by redistributing myocardial blood flow seems unlikely in light of the results of experiments using radioactive microspheres for determination of regional myocardial blood flow.2,6 Thus, the two conventional means of limiting myocardial infarct size — reduction in myocardial oxygen consumption and redistribution of myocardial blood flow — are not of particular relevance in delineating ibuprofen’s cardioprotective effects.

The experiments detailed in the present report were designed to evaluate two possible mechanisms by which ibuprofen salvages ischemic myocardium. First, the ability to suppress platelet aggregation by inhibition of cyclooxygenase8 may prevent platelet accumulation and obstruction of the myocardial microcirculation. Second, the antiinflammatory properties of ibuprofen may prevent tissue injury directly related to the accumulation of leukocytes in the injured myocardium.9,10 Autologous canine platelets or leukocytes were radiolabeled with indium-111 (111In) and reinjected into the dog before left circumflex coronary artery (LCx) occlusion for 60 minutes, followed by reperfusion. The technique permitted the quantification of the accumulation of these blood-formed elements in ischemic myocardium.
Methods

Occlusion-Reperfusion Model of Irreversible Ischemic Myocardial Injury

Induction of Ischemic Injury and Myocardial Infarction

Ischemic myocardial injury was produced in dogs by 60 minutes of LCx coronary occlusion followed by reperfusion using a technique previously described. Male mongrel dogs that weighed 10–15 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.), intubated and ventilated with room air by a Harvard respirator. Aseptic techniques were used for all surgical procedures. Catheters were implanted in the left jugular vein and common carotid artery, then exteriorized at the back of the neck. A left thoracotomy was performed at the fifth intercostal space and the LCx isolated near its origin under the left atrium, distal to its atrial branch and proximal to any major ventricular branches (fig. 1B). A basal LCx coronary blood flow measurement was obtained with an electromagnetic flow probe affixed to the artery. The LCx was partially constricted with a ligature tied around the artery and a 20-gauge needle. The degree of partial constriction was considered adequate when the hyperemic response to a 10-second occlusion was abolished without altering basal LCx coronary blood flow. After 20 minutes of partial constriction, the LCx was occluded completely with a second ligature. Total occlusion was maintained for 60 minutes, followed by reperfusion with the partial constriction remaining in place. This critical stenosis limits the reperfusion hyperemia, thus reducing the severity of reperfusion arrhythmias and the incidence of hemorrhagic myocardial infarcts.

A. Ex Vivo Platelet or Leukocyte Labelling

Whole Blood → Reinject Platelets or Leukocytes

B. 60 Minutes LCx Occlusion/Reperfusion

C. Delineation of Area at Risk and Infarcted Myocardium

D. Tissue Sampling

Figure 1. The experimental protocol. (A) Ex vivo labeling of platelets or leukocytes isolated from whole blood. The indium-111 (111In)-labeled blood elements are reinfused into the dog before left circumflex coronary artery (LCx) occlusion. (B) The site of LCx occlusion. (C) Perfusion system for delineation of area at risk and infarct size. The LCx vascular bed is perfused with 1.5% triphenyltetrazolium (TPT) and the aorta is perfused in a retrograde manner with 0.5% Evans blue. The Evans blue delineates the nonrisk region and the TPT distinguishes viable myocardium from infarcted tissue within the area at risk. Both the TPT and Evans blue reservoirs are maintained at a constant pressure of 100 mm Hg by the zinc bromide column. (D) Tissue sampling for determination of 111In radioactivity in infarcted and nonischemic myocardium by gamma spectrometry. Transmural sections are taken from the area at risk and normal zone. Each tissue section from the area at risk is carefully traced onto clear sheets, noting the regions of infarcted myocardium. PRP = platelet-rich plasma; LRP = leukocyte-rich plasma; LAD = left anterior descending coronary artery.
Dogs that developed postocclusion ventricular fibrillation or did not survive for 24 hours after LCx occlusion were excluded from the data analysis.

One hour before complete LCx occlusion, 300–450 μCi of the autologous 111In-labeled platelets or 111In-labeled leukocytes were injected in volumes of 4.5–9.0 ml into the left jugular vein (fig. 1A). Dogs were assigned to the ibuprofen or control groups with the aid of a random numbers table by a technician not directly involved in the experiments. The primary investigator was not aware of the treatment group to which each dog was assigned until after the experiment was completed. Ibuprofen (12.5 mg/kg) or drug vehicle was administered i.v. every 4 hours beginning 30 minutes before the initiation of complete LCx occlusion. No antiarrhythmic agents or antibiotics were administered because they can alter leukocyte or platelet function. The thoracotomy incision was closed in layers and the dog was allowed to recover from the surgical procedure.

Twenty-four hours after complete LCx occlusion/reperfusion, the dogs were reanesthetized and the original thoracotomy incision reopened to expose the heart. The heart was fibrillated electrically and removed rapidly for postmortem quantification of infarct size.

Two modifications of the occlusion/reperfusion protocol were made to gain a better understanding of the processes involved in the accumulation of platelets in infarcted myocardium. In four dogs, 111In platelets were administered 30 minutes before LCx occlusion. In these dogs, however, occlusion of the LCx was maintained for the duration of the 24-hour experiment (permanent occlusion group). In a second group of four dogs, LCx occlusion/reperfusion was carried out according to the standard protocol detailed above; however, the 111In platelets were administered 72 hours after, rather than before, the ischemic insult. These dogs were killed 24 hours after the administration of the radiolabeled platelets (chronic infarct group).

Quantification of Area at Risk and Infarct Size

An in vitro dual perfusion technique was developed so that the mass of tissue undergoing irreversible ischemic injury and the size of the involved coronary vascular bed could be quantified simultaneously (fig. 1C). The aorta was cannulated above the coronary ostia. The coronary vasculature was perfused with 500 ml of warm (37°C) saline (0.9% NaCl) to assure that radioactive blood was not nonspecifically trapped within the coronary vasculature. Several times, the first few milliliters of coronary sinus effluent were collected and analyzed by gamma spectrometry. Insignificant amounts of radioactivity were present in these effluent samples. A cannula was then inserted into the LCx immediately distal to the site at which the LCx was occluded. The LCx coronary bed was perfused with 1.5% triphenyltetrazolium (TPT) chloride in 20 mM potassium phosphate buffer (pH 7.4, 37°C). The aorta was perfused in a retrograde manner with 0.5% Evans blue. Both solutions were perfused through their respective cannulas at a constant pressure of 100 mm Hg for 5 minutes. The heart was sliced into 1.0-cm-thick sections perpendicular to the apex-base axis. The area of left ventricle at risk due to its anatomic dependence on the LCx for blood flow was identified by the lack of Evans blue in this region. The regions of infarcted myocardium within the area at risk were demarcated by the lack of staining of the tissue perfused with TPT. The area at risk, in addition to the regions of infarcted myocardium, were carefully traced onto clear plastic sheets for analysis of infarct size by planimetry. Comparison of data quantitating myocardial infarct size by planimetric vs gravimetric analysis indicated an excellent correlation (r = 0.96, p < 0.01, n = 18) between the two methods (data not shown). The technician doing the planimetry was unaware of the treatment group to which each heart belonged. This technique allows the extent of infarction to be expressed both as a percentage of the total left ventricle and as a percentage of the area of myocardium at risk of infarction because of its dependence on the LCx for coronary blood flow.

Tissue Analysis of Radioactivity

The area at risk and the normal zone of the myocardial rings were divided into transmural tissue samples that weighed approximately 1 g each (fig. 1D) to determine radioactivity by gamma spectrometry. Care was taken to be certain that the tissue samples from the area at risk did not contain tissue that was part of the nonischemic zone. These tissue sections were weighed and then traced onto clear plastic sheets, carefully marking the borders between infarcted and noninfarcted tissue. The percent of infarcted tissue in each section was determined by planimetry. Knowing the weight of each transmural tissue sample, we could calculate the number of grams of infarcted tissue in each section. The tissue sections were then placed in a Packard model 5320 gamma spectrometer to determine the amount of 111In radioactivity present in each piece of tissue.

Preparation of Radiolabeled Platelets

Autologous 111In-labeled platelets were prepared by first drawing 34 ml of venous blood from the dog into a syringe that contained 6 ml of acid citrate dextrose. The whole blood sample was then centrifuged at 180 g for 3 minutes to prepare the platelet-rich plasma (PRP). A platelet pellet was formed by centrifuging the PRP fraction for 10 minutes at 1000 g. The platelets were resuspended in modified Tyrode solution14 and incubated with 500 μCi of 111In oxine for 10 minutes at 37°C. The radiolabeled platelets were pelleted by centrifugation at 1000 g for 5 minutes and then resuspended in platelet-poor plasma (PPP). Labeling efficiency, defined as

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\frac{111\text{In bound to platelets}}{111\text{In bound to platelets} + \text{unbound } 111\text{In activity}} \times 100
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was 86 ± 2% (range 67–93%). The viability of the platelets after radiolabeling was assessed in vitro and in vivo. In vitro platelet function was determined, before administration of the 111In-labeled platelets, by
established spectrophotometric methods using a PAP-3 platelet aggregometer (Bio/Data Corp.). After adjusting the platelet count of the PRP to 200,000/mm³, aggregation was initiated by the addition of 50 µl of collagen suspension to 450 µl of diluted PRP to yield final collagen concentrations of 312 or 625 µg/ml. All values are expressed as percentage of light transmission standardized to the PRP and PPP samples yielding zero and 100% light transmission, respectively.

In vivo platelet viability was assessed by calculating the percent of administered radioactivity present in the circulation shortly after injection. Thirty minutes after the radiolabeled platelets were administered through the surgically implanted jugular cannula, a 1-ml blood sample was obtained from a peripheral vein. The percent recovery of radiolabel was calculated by standard methods. No attempt was made to adjust in vivo recoveries for residual activity in the syringe after injection.

Preparation of Radiolabeled Leukocytes

The 111In autologous leukocytes were prepared by drawing 50 ml of venous blood into 330 IU of heparin (USP). To this blood sample, 3.0 ml of hydroxethyl starch (6% VOLEX, McGaw Laboratories, Inc.) and 3.0 ml of normal saline (USP) were added. The blood sample was allowed to incubate at room temperature for 60 minutes to allow the separation of the leukocyte-rich plasma (LRP). The leukocytes were pelleted by centrifugation of the LRP for 5 minutes at 450 g. The leukocyte pellet was washed twice by centrifugation and resuspended in normal saline. The washed leukocytes were then incubated with 500 µCi of 111In oxine for 15 minutes at room temperature. The labeled leukocytes were then pelleted and resuspended in autologous leukocyte-free plasma. Leukocyte labeling efficiency was 86 ± 2% (range 64–97%). Leukocyte viability after 111In labeling was assessed by in vivo recovery of radioactivity 30 minutes after administration.

Calculation of 111In Accumulation and Statistics

The accumulation of 111In-labeled platelets or leukocytes is expressed as the increase in 111In radioactivity in infarcted myocardium over 111In radioactivity in nonischemic myocardium, subsequently referred to as the platelet or leukocyte accumulation ratio. Ibuprofen reduces the extent of irreversible myocardial injury. To control for this cardioprotective property, and thereby focus on whether ibuprofen exerts its effect by preventing accumulation of platelets or leukocytes in injured myocardium, tissue sections from control and drug-treated dogs with equal amounts of tissue injury (i.e., grams of infarcted tissue) were compared. To accomplish this, the raw radioactive counts of each tissue section were divided by the tissue wet weight to yield counts per minute per gram (cpm/g) of tissue. By dividing the activity of each tissue section taken from the area at risk (cpm/g) by the mean activity of nonischemic tissue sections (cpm/g), the increase in 111In radioactivity in ischemic zone tissue sections was calculated, yielding the platelet or leukocyte accumulation ratio. For each dog, the values for the increases in radioactivity in the eight to 12 ischemic zone tissue sections were distributed into 0.2-g-infarct intervals, based on the amount of infarcted tissue present in each tissue section. The values that fell within each of the 0.2-g-infarct intervals (i.e., 0.01–0.2-g infarct) were averaged and the mean was used to represent the increase in 111In radioactivity in that 0.2-g interval for each particular animal. Gram-infarct intervals that did not have more than one tissue section were not represented in that particular animal. Therefore, each accumulation ratio calculated for an animal represents the mean of the values from two or more tissue sections in each 0.2-g-infarct division.

The means ± SEM for the accumulation ratios from the various animals were computed separately for each 0.2-g-infarct division in the control and ibuprofen-treated groups. Statistical evaluation of the hypothesis that the mean accumulation ratio for ibuprofen-treated dogs was equal to that of control dogs was carried out for each of the respective 0.2-g divisions using a t test. These tests were statistically dependent, inasmuch as they all relate to the same set of dogs. However, the purpose of these analyses, to assess whether mean differences exist between ibuprofen-treated and control animals with comparable degrees of tissue injury, is still statistically valid (i.e., individual p values are correct, but lack experimental α error protection). Data are expressed as the mean ± SEM.

Drug Administration

Ibuprofen (Upjohn Company) was prepared for i.v. administration by dissolving the crystalline material in 0.2 M Na2CO3. The pH was adjusted to 7.5–8.0 with 1.0 N HCl. Ibuprofen was administered intravenously at a dose of 12.5 mg/kg, 30 minutes before LCx occlusion. Subsequent doses of 12.5 mg/kg were given every 4 hours so that a total dose of 75 mg/kg was administered over 24 hours. Control dogs received drug vehicle in equal volume to that given to ibuprofen-treated dogs.

Results

Forty-seven dogs were subjected to 60 minutes of LCx occlusion, followed by 24 hours of reperfusion as part of the protocol evaluating the effect of ibuprofen on the accumulation of platelets and leukocytes in infarcted canine myocardium. Eight dogs were excluded from data analysis because they did not survive the 24-hour protocol and four were excluded because they required DC cardioversion due to ventricular fibrillation during occlusion/reperfusion. Of the 35 dogs that successfully completed the experimental protocol, 19 received 111In-labeled platelets and were distributed among four groups: drug vehicle (n = 5), ibuprofen-treated (n = 6), LCx occlusion without reperfusion (permanent occlusion group) (n = 4), and chronic infarct (111In-labeled platelet administration 72 hours after LCx occlusion/reperfusion) (n = 4). Sixteen
dogs received autologous $^{111}$In-labeled leukocytes and were distributed in two groups: drug vehicle (n = 9) and ibuprofen-treated (n = 7).

The effect of ibuprofen on the extent of myocardial injury in the LCX occlusion/reperfusion model is summarized in figure 2. The percent of the total left ventricle susceptible to infarction (area at risk) as a result of LCX occlusion in the ibuprofen-treated group was not significantly different from that in the control group (40 ± 1% vs 39 ± 1%) (fig. 2). The extent of irreversible injury, expressed as a percent of the area at risk of infarction or as a percent of the total left ventricle, was significantly less in the ibuprofen-treated group. When the extent of irreversible injury is expressed as a percent of the area at risk of infarction, the ibuprofen-treated group of dogs had an average infarct mass that was 40% less than the infarct mass developed in the control dogs (29 ± 4% vs 48 ± 4%, p = 0.005). When the average infarct mass is expressed as a percent of the total left ventricle, ibuprofen reduced the extent of irreversible injury by 37% compared with the control group (12 ± 2% vs 19 ± 2%, respectively, p = 0.017). Thus, whether the data are expressed on the basis of area at risk or total left ventricle, i.e. ibuprofen (12.5 mg/kg every 4 hours) exerts a beneficial effect in limiting the extent of irreversible myocardial injury. In another series of experiments, oral ibuprofen (12.5 mg/kg every 4 hours) under similar experimental conditions resulted in a 35% and a 39% reduction in myocardial infarct size when expressed as a percent of area at risk or as a percent of total left ventricle, respectively.

$^{111}$In Platelet Accumulation in Infarcted Myocardium

The fragility of the blood platelets demands that particular care be taken in handling during ex vivo labeling with $^{111}$In-oxine. To verify that platelet viability was not altered during labeling, two independent tests of platelet function were performed. Figure 3 is a summary of the data from the in vitro aggregation of platelets in response to collagen. It shows typical platelet-
aggregation curves in response to two concentrations of collagen. Increases in percent light transmission indicate a greater degree of platelet aggregation. These aggregation curves reveal rapid, irreversible aggregation to both collagen concentrations. In addition, there appears to be little qualitative difference in the aggregation curves between the unlabeled and the $^{111}$In-labeled platelets. Figure 3C is a summary of the data from the in vitro assessment of platelet function. At both concentrations of collagen, the maximum percent light transmission is nearly identical for the unlabeled and $^{111}$In-labeled platelets (e.g., for collagen 1:160, unlabeled platelets 33 ± 9% vs $^{111}$In-labeled platelets 37 ± 7% light transmission [max]).

Another suggested method for assessing platelet viability hinges on determining the amount of platelet-associated radioactivity present in the circulating blood pool after a specified period. This method assumes that $^{111}$In-labeled platelets that were injured in the labeling process are sequestered by the spleen and liver within 30 minutes of administration into the circulatory system. By determining the amount of radioactivity in a peripheral blood sample, and knowing the approximate blood volume of the animal, one can determine the fraction of administered radioactivity present in the bloodstream after 30 minutes. In these experiments, an average of 56 ± 3% (range 35–69%) of total administered $^{111}$In activity was present in the circulation after 30 minutes. These in vitro and in vivo data indicate that the ex vivo labeling of platelets with $^{111}$In does not substantially alter normal platelet function.

Quantitative assessment of platelet accumulation in infarcted myocardium was obtained by calculating the increase in radioactivity present in infarcted tissue compared to the $^{111}$In activity present in nonischemic tissue (i.e., platelet accumulation ratio). When these values are grouped according to the amount of infarcted myocardium in each tissue section, a trend of substantial increases in $^{111}$In activity with increasing amounts of tissue damage becomes apparent (fig. 4). In tissue sections taken from dogs that received drug vehicle only, the platelet accumulation ratio in samples with 0.01–0.20 g of infarcted tissue was $6.3 ± 0.1$, whereas those samples with 0.81–1.0 g of infarcted tissue had a mean platelet accumulation ratio of $19.6 ± 3.2$. Thus, in the control dogs there was a 211% increase in the platelet accumulation ratio when comparing the smallest (0.01–0.20 g) with the largest (0.81–1.0 g) infarct-size intervals. While the platelet accumulation ratio in control dogs was directly related to the amount of infarct in each tissue section, myocardial samples from dogs subjected to permanent LCx occlusion had platelet accumulation ratios that did not substantially rise with increases in infarcted tissue. The platelet accumulation ratio is markedly less in each infarct interval of permanent occlusion dogs compared with control dogs (fig. 4). These data suggest that reperfusion, after LCx occlusion that has produced irreversible myocardial injury, is necessary for substantial platelet accumulation to occur.

The platelet accumulation ratios from animals in the chronic infarct group are presented in figure 4. Administration of $^{111}$In-labeled platelets 72 hours after LCx occlusion/reperfusion resulted in minimal accumulation of platelets in infarcted myocardium, and platelet accumulation ratios in these dogs appear to be independent of the amount of infarcted tissue in each tissue

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Quantitative assessment of the accumulation of indium-$^{111}$ ($^{111}$In)-labeled platelets in infarcted myocardium was accomplished by calculating the platelet accumulation ratio (PAR), defined as the increase in radioactivity present in infarcted myocardium compared with the $^{111}$In activity present in nonischemic tissue. The PAR was calculated for each transmural myocardial tissue section taken from the area at risk. These values were then grouped according to the number of grams of infarcted tissue in each transmural section (see Methods). In dogs that underwent 60 minutes of LCC occlusion followed by 24 hours of reperfusion (control), there was a marked increase in PAR with greater amounts of infarcted tissue. In contrast, the PAR did not increase substantially with greater amounts of infarcted myocardium in dogs subjected to permanent occlusion or those receiving the $^{111}$In-labeled platelets 72 hours after LCx occlusion/reperfusion (chronic infarct group). Thus, reperfusion and administration of the $^{111}$In-labeled platelets soon after the ischemic insult are apparently necessary for marked platelet accumulation in infarcted myocardium.
myocardial infarction. As with the ex vivo $^{111}$In labeling of platelets, it was important to ascertain the viability of the leukocytes after they were subjected to the $^{111}$In labeling protocol. Using the in vivo radioactivity recovery technique used to assess platelet viability, 30 ± 4% (range 16–74%) of the leukocyte-associated $^{111}$In activity originally administered was present in the circulation 30 minutes after injection. This value is comparable to that reported by Weiblen et al.18

The time course of the infiltration of leukocytes into infarcted myocardium has been well characterized. In animal models of permanent coronary artery occlusion, leukocytes migrate to regions of myocardial injury within 24 hours, reaching a peak within 4–5 days.19 However, Sommers and Jennings20 reported that reestablishing myocardial blood flow after temporary coronary artery occlusion accelerated the infiltration of inflammatory cells into injured myocardium. Therefore, in the present study in which reperfusion was initiated after 60 minutes of LCx occlusion, the accumulation of leukocytes in infarcted myocardium was assessed 24 hours after the ischemic insult.

Figure 6 is an assessment of the effect of ibuprofen on leukocyte accumulation. The leukocyte accumulation ratio is defined as the increase in $^{111}$In radioactivity of tissue sections from the area at risk compared with the radioactivity present in nonischemic tissue samples. In the control dogs, a marked increase in the leukocyte accumulation ratio is seen with greater amounts of infarcted myocardium. These values range from 3.6 ± 0.3 in the 0.01–0.20-g-infarct interval to 17.9 ± 3.0 in the 1.01+ g-infarct interval, an increase in the leukocyte accumulation ratio of 397%. Treatment with ibuprofen (12.5 mg/kg i.v. every 4 hours) markedly reduced the leukocyte accumulation ratio so that only a 76% increase was noted, from 3.7 ± 0.6 in the 0.01–0.20-g-infarct interval, to 6.5 ± 1.2 in the 1.01 + g-infarct interval. The percent reduction in leukocyte accumulation ratio by ibuprofen treatment and the level of statistical significance between control and ibuprofen-treated groups are indicated at the bottom of figure 6. The maximum difference between control and ibuprofen-treated groups is in the 1.01 + g-infarct interval, where ibuprofen administration reduced leukocyte accumulation by 64% compared with the control group (ibuprofen 6.5 ± 1.2; control 17.9 ± 3.0) ($p = 0.028$).

Discussion

The introduction of $^{111}$In oxine as a means of specifically labeling any major blood-formed element, without altering its biologic function, has opened new avenues to investigate the role these blood-borne particles play in a variety of pathologic states.

Ibuprofen has been shown to reduce the extent of irreversible myocardial injury in a variety of animal models of myocardial ischemia and infarction.24 In the present study, ibuprofen administration (12.5 mg/kg every 4 hours) reduced the extent of irreversible myocardial injury by 40%, from 48 ± 4% of the area at risk of infarction in control animals, to 29 ± 4% of the area at risk in ibuprofen-treated animals (fig. 2). The
possible pathologic involvement of circulating platelets or leukocytes in evolving myocardial infarcts has not been adequately considered in attempting to explain ibuprofen's cardioprotective effects.

In the present study, $^{111}$In-labeled platelets and leukocytes were used to ascertain the importance of blood-formed elements in the pathophysiology of experimentally induced acute myocardial infarction as well as to gain insight into how ibuprofen exerts its beneficial effects during myocardial ischemia.

**Platelet Accumulation in Infarcted Myocardium**

The accumulation of $^{111}$In-labeled platelets in infarcted myocardium was not significantly altered by ibuprofen (fig. 5), but considerable insight into the relationship between platelets and myocardial infarction was gained. By modifying the standard occlusion/reperfusion protocol, we gained an understanding of some of the factors important in the accumulation of $^{111}$In-labeled platelets in infarcted myocardium. By permanent ligation of the LCx, we could reduce the platelet accumulation ratio by as much as 82%, from 20.2 ± 1.4 in control dogs (0.61–0.80-g-infarct interval) to 3.6 ± 0.2 in permanent occlusion dogs (0.61–0.80-g-infarct interval) (fig. 4). The platelet accumulation ratio for the permanent occlusion group remained nearly constant, at 3.5–3.6, over the entire range of gram-infarct intervals. These data clearly indicate the importance of reperfusion for the accumulation of platelets in infarcted myocardium. It is unlikely that the pronounced accumulation of platelets in reperfused animals (fig. 4) is the result of hemorrhage occurring during reperfusion. The presence of a critical stenosis limits the extent of hemorrhagic infarction in this model of regional myocardial ischemia. In addition, each myocardial section was examined carefully to verify that significant hemorrhagic regions were not present. Thus, reperfusion appears to be critical to substantial platelet accumulation in infarcted myocardium. As further evidence of the importance of reperfusion to platelet accumulation in infarcted myocardium, Moschos et al.\textsuperscript{21} and Ruf et al.\textsuperscript{22} reported that in animal models of permanent coronary artery occlusion, radiolabeled platelet accumulation was one- to twofold higher in ischemic myocardium than in nonischemic myocardium. These values are comparable to those in figure 4 for the permanent occlusion group of dogs. These data suggest that reperfusion delivers platelets to regions of the myocardium where microvascular injury has resulted during the ischemic insult\textsuperscript{23} and
the early phase of reperfusion. The pronounced microvascular damage and the accumulation of vasoactive and metabolic products during ischemia are potent stimuli for platelets to coalesce within injured myocardium.

In the chronic infarct experiments, in which the 111In-labeled platelets were administered 72 hours after the ischemic insult, further insight was gained into the factors leading to the marked platelet accumulation seen in control dogs. Delayed administration of the 111In-labeled platelets in the chronic infarct group resulted in relatively small platelet accumulation ratios of approximately 6 over the entire range of gram-infarct intervals (fig. 4). Thus, the greatest degree of platelet accumulation must occur early in the evolution of the myocardial infarct. Laws et al.24 reported that under experimental conditions similar to those in the present study, maximum 111In-labeled platelet accumulation occurred 24 hours after the initiation of reperfusion. In addition, reperfusion was necessary for marked platelet accumulation to occur.

Ibuprofen significantly reduced the extent of irreversible myocardial injury (fig. 2) and is a platelet aggregation inhibitor,25 but did not impede the accumulation of platelets in infarcted myocardium (fig. 5). These data suggest that the protective effect of ibuprofen on ischemic myocardium does not result from the prevention of platelet aggregation within, and presumably, occlusion of, the myocardial microvasculature. This is substantiated by studies using radioactive microspheres for determining regional myocardial blood flow. If ibuprofen could improve myocardial perfusion by preventing platelet occlusion of the microvasculature, enhanced regional myocardial blood flows should be observed in the drug-treated dogs. However, ibuprofen did not significantly alter regional blood flow compared with control dogs.2, 6

There is little doubt that platelet accumulation occurs within regions of myocardial infarcts, as documented by experimental26 and autopsy27 studies. In the present series of experiments, autoradiography of transverse myocardial tissue sections indicated that 111In-labeled platelets accumulate specifically within infarcted myocardium in as much as the borders of the intensely radioactive area coincide precisely with the borders of infarcted myocardium. The present report demonstrates that pronounced platelet accumulation occurs when the platelets are delivered to injured myocardium by reperfusion, and this accumulation occurs primarily during the early stages of infarction, when myocardial cells are still undergoing biochemical transformations leading to cell death. It is unlikely that the marked accumulation of platelets seen in the control animals (fig. 4) results from nonspecific platelet trapping by infarcted myocardium. If this were so, similar degrees of platelet accumulation would have been found in myocardial infarcts that had passed the state of dynamic extension which occurs within 24 hours of the ischemic insult. Clearly, the experiments in which 111In-labeled platelets were injected 72 hours after L CX occlusion/reperfusion and the work reported by Laws et al.24 indicate that platelet accumulation is associated with the early stages of developing myocardial infarcts. What is not clear from the data is the role of platelet accumulation in the evolution of the myocardial infarct. Based on ibuprofen's inability to alter the distribution of 111In-labeled platelets, while effectively reducing the extent of myocardial injury, we hypothesize that the accumulation of platelets in infarcted myocardium is secondary to the microvascular injury and myocardial damage of severe ischemia. This view is further supported by the work of Kloner et al.,28 which demonstrated that microvascular injury, presumably a major stimulus for platelet accumulation in infarcted myocardium, occurs after ischemia induced myocardial cellular injury. Thus, microvascular injury and subsequent platelet accumulation appear to result from, and not to be responsible for, myocardial ischemia and infarction.

Leukocyte Accumulation in Infarcted Myocardium

That ibuprofen significantly limits 111In-labeled leukocyte accumulation in infarcted myocardium is our most noteworthy observation. Autoradiography of transverse myocardial tissue sections indicated that 111In-labeled leukocyte accumulation occurred primarily within the boundaries of infarcted myocardium. Gamma spectrometry of myocardial tissue sections permitted quantification of 111In-labeled leukocyte radioactivity and the calculation of leukocyte accumulation ratios. Ibuprofen lessened the accumulation of leukocytes in infarcted myocardium (fig. 6). While the leukocyte accumulation ratios for control dogs were 3.6 ± 0.3 (0.01–0.20-g-infarct interval) to 17.9 ± 3.0 (1.01 + g -infarct interval), ibuprofen-treated dogs had average leukocyte accumulation ratios of 3.7 ± 0.6 (0.01–0.20-g-infarct interval) to 6.5 ± 1.2 (1.01 + g -infarct interval). Averaged over all six gram-infarct intervals, ibuprofen treatment reduced leukocyte accumulation ratios by 46% compared with control dogs.

The infiltration of polymorphonuclear leukocytes into myocardium irreversibly injured by regional ischemia has been well documented.19 With the growing understanding of the involvement of leukocytes in the "reparative" process of transforming necrotic myocardium into scar tissue has come the realization that massive accumulation of inflammatory cells may actually injure viable myocardium. Stimulated neutrophils transform molecular oxygen into highly reactive and cytotoxic free radical superoxide anion, hydroxyl radical, hydrogen peroxide and, perhaps, singlet oxygen. When not limited to phagocytic vacuoles, these oxygen radicals degrade extracellular macromolecules, attack membrane phospholipids and promote cell injury or death.9 In addition, release of the enzymatic contents of neutrophil granules into the extracellular space may result in proteolytic attack on viable tissue.10 The influx of leukocytes into regions of myocardial necrosis in numbers far greater than necessary for the repair and resolution of injured myocardium may exacerbate the ischemic insult. Thus, the anti-inflammatory prop-
erties of ibuprofen may be an important mechanism by which the drug exerts its cardioprotective effects during myocardial ischemia.

Certain limitations of the present study must be considered. There is clearly a correlation between the suppression of leukocyte accumulation after ibuprofen administration and a reduction in myocardial infarct size, but this does not prove that the two phenomena are causally related. It is also important to caution against the extrapolation of data obtained in an animal model of regional myocardial ischemia to the treatment of acute myocardial infarction in man. When put into the proper perspective, however, the results of the present study provide useful insight into the involvement of platelets and leukocytes in ischemic myocardial injury.

The results of these experiments tend to negate the importance of the platelet in explaining ibuprofen's protective effects in ischemic myocardium. Instead, further investigation into ibuprofen's mechanism of action must be directed toward the pathophysiologic role of leukocytes in evolving myocardial infarcts.

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