Indomethacin-induced Scar Thinning
After Experimental Myocardial Infarction

HAIM HAMMERMANN, M.D., ROBERT A. KLONER, M.D., PH.D.,
FREDERICK J. SCHOFEN, M.D., PH.D., EDWARD J. BROWN, JR., M.D., SHARON HALE, B.S.,
AND EUGENE BRAUNWALD, M.D.

SUMMARY We investigated the effect of indomethacin, a widely used nonsteroidal antiinflammatory
drug, on the healing of myocardial infarction (MI). Experimental MI was produced in anesthetized, open-
chest dogs by occluding the left anterior descending coronary artery. Ten dogs received indomethacin, 10
mg/kg i.v., and 11 received saline, 15 minutes and 3 hours after occlusion. After 6 weeks, the dogs were
killed and their hearts were subjected to morphologic and biochemical analysis. The average thickness of
the transmurual scar and the noninfarcted left ventricular wall was measured at multiple sites in formalin-
fixed left ventricular slices and the ratio of the thickness of the transmural scar to the noninfarcted wall
determined. The average thickness of the noninfarcted wall was 8.80 ± 0.19 mm (mean ± SEM) in the
control group and 8.44 ± 0.26 mm in the indomethacin group (NS). The scar thickness was 7.24 ± 0.64
mm in the control group and 3.56 ± 0.40 mm in the indomethacin group (p < 0.001). The ratio of scar to
noninfarcted wall thickness was 0.83 ± 0.07 in the control group and 0.43 ± 0.04 in the indomethacin
group (p < 0.001). Scars in treated dogs did not differ from controls either by light microscopic histologic
analysis or by analysis of hydroxyproline content per unit weight. We conclude that indomethacin results in
marked scar thinning when given early after experimental MI.

ALTHOUGH interventions designed to reduce myo-
cardial infarction size have been studied,1,2 relatively
little is known about the effects of drugs on the for-
mation of the myocardial scar after myocardial infarction.
The healing phase has been viewed as a process that
follows a temporally predictable morphologic course.3,4 That this may not be true was demonstrated
when glucocorticosteroids were shown to retard the rate of myocardial infarction healing,5 cause scar thin-
ning after experimental infarction6 and possibly lead to
formation of myocardial aneurysms in humans.7 Ibuprofen, a nonsteroidal, antiinflammatory compound,
has been shown to cause scar thinning when adminis-
tered after experimental myocardial infarction, while
aspirin does not.8 We investigated the effects of indo-
methacin, which has a potent inhibitory effect on pro-
taglandin synthesis, on scar formation after myocardial
necrosis.

Methods

Experimental Preparation

Mongrel dogs of either sex that weighed 9–22 kg
were sedated with acepromazine maleate, 1.0 mg/kg
subcutaneously, anesthetized with pentobarbital, 30
mg/kg i.v., intubated and placed on a Harvard respira-
tor (Harvard Apparatus Co.). A thoracotomy was per-
formed in the fifth left intercostal space under sterile
conditions. The proximal portion of the left anterior
descending coronary artery was dissected free from
surrounding tissue and a silk ligature was placed
around the artery approximately 2 cm from the origin
of the left anterior descending coronary artery immedi-
ately distal to the origin of the first diagonal branch.
During a temporary 15-second occlusion, this always
resulted in anteroapical cyanosis.

Experimental Protocol

After lidocaine, 1.5 mg/kg i.v., was administered,
the left anterior descending coronary artery was per-
manently occluded with a silk ligature. A second,
similar dose of lidocaine was administered 5 minutes
after occlusion in all dogs. The first seven dogs served
as controls and received saline. (These dogs also
served as controls in another study.)9 The rest of the
dogs were randomized by drawing lots into two
groups, six saline and 13 indomethacin, and the inves-
tigators were blinded as to which agent the dog re-
ceived until all scar measurements had been complet-
ed. The control dogs received 50 ml of saline infused
over 5 minutes. The other 13 dogs received indometha-
cin (Sigma Chemical Company) (10 mg/kg i.v.) in 50
ml of normal saline infused over 5 minutes, at 15
minutes and 3 hours after occlusion (total dose of 20
mg/kg). This dose of indomethacin was used because it
was similar to that used by other investigators to ensure
complete inhibition of prostaglandin synthesis in the
cardiac vascular bed.9,10 All agents were infused
through a peripheral vein. The chest was closed, the air
was evacuated from the thoracic cavity and the dogs
were returned to the kennel. One dose of benzathine
penicillin, 1.2 million units, was given intramuscular-
ly to each dog.

After 6 weeks, the dogs were reanesthetized with
pentobarbital, 30 mg/kg i.v., killed with an overdose

From the Cardiovascular Division, Department of Medicine, and the
Department of Pathology, Harvard Medical School, and Brigham and
Women's Hospital, Boston, Massachusetts.

Dr. Hammerman is a Stanley J. Sarnoff Society Fellow for Cardio-
vascular Research.

Dr. Brown's current address: Cardiology Division, State University
of New York, Stony Brook, New York.

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Address for correspondence: Robert A. Kloner, M.D., Ph.D., Har-
vard Medical School, 180 Longwood Avenue, Room 235, Boston,
Massachusetts 02115.

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of potassium chloride and the hearts were excised. Potassium chloride injection allowed all hearts to be arrested in diastole.

**Assessment of Wall Thickness and Expansion**

The left ventricle was dissected free from the right ventricular free wall and structures above the atrioventricular rings, loosely packed with gauze to prevent collapse of the left ventricular cavity, and allowed to fix in 10% neutral buffered formalin for 3 days. After fixation, the heart was sectioned from base to apex into 3–5-mm transverse sections parallel to the atrioventricular groove. Wall and scar thickness were measured on each section along radii that passed through the center of the ventricular cavity (fig. 1). The thicknesses of the normal wall and the scar in each slice were measured in three to five widely separated locations and the values for each set of measurements were averaged. To take into account variability in dog size, a ratio was determined of average scar thickness to average noninfarcted wall thickness in each slice. An average ratio in each dog was determined by averaging the ratios in each slice containing infarcted tissue. Measurements were performed in a blinded fashion.

Infarct size was assessed after 6 weeks by area planimetry and was expressed as a percentage of the left ventricle.

For quantification of the degree of expansion, an index was determined for each heart as previously described. The midpoint of the ventricular septum was identified by linear measurement and the perpendicular bisector of the left ventricular free wall was marked. Intersections of the septal bisector of the free wall divided each section into an anterior and posterior segment. The transverse section that contained the greatest percentage of infarcted tissue was selected for each heart, and the lengths of the endocardial margins for the anterior infarct segment and for the noninfarcted posterior segment were measured. An index of expansion was determined by dividing the length of the infarcted segment by the endocardial length of the noninfarcted segment. Expansion was considered if the expansion index was more than 1.09.

**Analysis of Hydroxyproline Content**

Tissue samples of 100–200 mg were obtained from the endocardial and epicardial myocardium of normal and infarcted areas, oven-dried to a constant weight and acid-hydrolyzed in 6N HCl at 110°C for 12 hours. After neutralization and decolorization of the hydrolysate, hydroxyproline concentration was assayed by the method of Newman and Logan.

**Histologic Analysis**

Representative formalin-fixed, transmural, transverse sections of noninfarcted and infarcted myocardium were conventionally processed and embedded in paraffin. Two to three such sections stained with hematoxylin-eosin and Masson’s trichrome were examined from each heart. Histologic features of necrosis and healing were assessed and graded from 0 (not present) to 4 (most prominent), as previously described, for necrosis, leukocytes, macrophages, neutrophils, vascular proliferation, fibroblasts and collagen. The histologic score for each feature for each dog was averaged over the several slices examined.

**Statistical Analysis**

Unpaired t tests were used to calculate the significance of differences of the wall thickness measurements, ratio of scar to noninfarcted wall thickness, infarct size and hydroxyproline content between the groups. Values listed in the text are the mean ± SEM.

**Results**

Twenty-six dogs entered the protocol. Three dogs developed ventricular fibrillation and died within 30 minutes of coronary occlusion. One dog died suddenly 3 days after coronary occlusion. One dog had subendocardial infarction at the time of examination. These five dogs were excluded from the study. Of the 21 dogs that survived for 6 weeks, 10 were in the indomethacin-treated group and 11 were in the control group.

**Measurements of Noninfarcted Wall, Scar Thickness and Expansion**

The mean noninfarcted wall thickness was 8.80 ± 0.19 mm in the control group and 8.44 ± 0.26 mm in the indomethacin group (NS).

The mean scar thickness was 7.24 ± 0.64 mm in the control group and was much thinner in the indomethacin-treated group, 3.56 ± 0.40 mm (p < 0.001) (fig. 2). The ratio of scar thickness to noninfarcted wall thickness was significantly higher in the control dogs, 0.83 ± 0.07, than that in the indomethacin-treated group, 0.43 ± 0.04 (p < 0.001). All but two hearts in the control group had a ratio exceeding 0.60; all but one heart in the treated group had a ratio of less than 0.60. (fig. 3). Representative transverse left ventricular slices from a control and an indomethacin-treated dog are shown in figure 4. Infarct size assessed by planimetry and expressed as a percentage of left ventri-
FIGURE 3. Ratio of scar thickness to noninfarcted wall thickness in control and indomethacin-treated dogs. Open circles and bars represent mean ± SEM for each group.

Hydroxyproline Assays

Noninfarcted epicardial and endocardial tissue contained small amounts of collagen, as reflected in hydroxyproline concentration range of 3.5–4.4 μg/mg dry weight in both control and treated groups. Larger quantities of collagen were contained within the epicardial and endocardial portions of the scar, 13.8–29.8 μg/mg. The hydroxyproline concentrations tended to be lower, but not significantly, in the scar tissue of the indomethacin-treated group. Thus, treatment with indomethacin did not affect the hydroxyproline concentration in the scar tissue or in the noninfarcted myocardium (table 1).

Histologic Analysis

The qualitative histologic appearances of the infarcted region were similar in the control and indomethacin-treated dogs. There was sharp demarcation between the evolving scar and normal myocardium. Islands of necrotic muscle and polymorphonuclear leukocytes were rare. Collagen was a prominent feature of these healing infarcts. There were prominent, thick-walled blood vessels and numerous fibroblasts and mononuclear inflammatory cells, some of which were pigmented. In most cases, the evolving scar was relatively homogeneous and essentially had the appearance of human 6-week-old myocardial infarcts. The similarity in the treated and control groups using the
Discussion

This study demonstrates marked thinning of myocardial scars 6 weeks after coronary ligation in dogs treated with indomethacin 15 minutes and 3 hours after coronary occlusion. In the treated group, nine of 10 dogs had evidence of severe scar thinning, defined as a ratio of the thickness of the scar to that of the noninfarcted wall of 0.60 or less; in contrast, in the control group, only two dogs had a scar thinning ratio of less than 0.60. Hydroxyproline concentration, which reflects the collagen content, and the histologic examination of the scars did not differ between control and the indomethacin-treated dogs. Thus, although the scars were thinner in the indomethacin group, the tissue was qualitatively similar in both groups. Indomethacin may have caused myocardial thinning or expansion early in the postinfarction course, which was followed by normal healing and collagen deposition on a thinned infarct. The larger expansion index in the indomethacin-treated dogs suggests that this might be the mechanism. In dogs, the infarct healing process is complete within 6 weeks. In smaller animals, such as rats, scar formation is complete by 3 weeks. We chose the dog model because it more closely resembles the time course of infarct healing in man.

In a recent study from our laboratory, ibuprofen, but not aspirin, administered after myocardial infarction resulted in post–myocardial infarct scar thinning.8 Indomethacin is a potent inhibitor of prostaglandin synthesis.14,15 Indomethacin inhibits cyclooxygenase activity and thereby diminishes generation of cyclic endoperoxides from arachidonic acid, blocking the ensuing cascade of prostaglandins and nonprostaglandin products.15 This accounts for the reduction in vasodilation and edema in inflammatory sites.15,16

In a model using carrageenin-induced inflammation in the rat, indomethacin in doses of 0.5–1.0 mg/kg reduced prostaglandin concentration by more than 90% from the control values and increased total leukocytes by approximately 65%. At doses greater than 2.0 mg/kg, however, leukocyte migration was reduced in a dose-dependent manner, while prostaglandin synthesis remained inhibited.16

The fact that indomethacin and other aspirin-like drugs have variable effects on leukocyte accumulation in inflammation and that inhibition of leukocyte migration is not correlated with cyclooxygenase activity suggests that additional mediators may play a role in inflammatory cell infiltration.16 The fatty acid precursors of the prostaglandins can be oxygenated by a lipoxygenase, which converts arachidonic acid to a hydroxy acid, 12-L hydroxyeicosatetraenoic acid (HETE). HETE is a potent chemotactic agent for polymorphonuclear leukocytes. Indomethacin and other aspirin-like drugs generally do not prevent the generation of HETE from arachidonic acid and may, in some cases, increase lipoxygenase products at low doses. This may explain their failure to prevent the accumulation of leukocytes in inflammation.17,18

Antiinflammatory corticosteroids reduce prostaglandin concentrations by mechanisms other than cyclooxygenase inhibition.17 Corticosteroids also prevent the release of fatty acids from phospholipids, resulting in a reduction of both cyclooxygenase and lipoxygenase products. Thus, if HETE is an important mediator of chemotaxis in vivo, a reduction of lipoxygenase products by antiinflammatory steroids may explain why steroids strongly suppress cell migration. This dual inhibition of both arachidonate cyclooxygenase (prostaglandin synthetase) and lipoxygenase may explain the more potent antiinflammatory activity of the steroids relative to indomethacin and other aspirin-like drugs. Perhaps the reduced leukocyte migration observed at high doses of the nonsteroidal antiinflammatory agents is due to dual inhibition of cyclooxygenase and lipoxygenase.16,19 Whether the relationship of these metabolic pathways and their differential inhibition by glucocorticosteroids, indomethacin, ibuprofen and aspirin are responsible for their different effects on scar formation after myocardial infarction is not known.

Indomethacin, in addition to its antiinflammatory activity, may inhibit collagen synthesis in some situations. For example, it reduces collagen concentration in granulation tissue induced by cotton pellets20 and inhibits experimental pulmonary fibrosis induced by bleomycin in rats.21 However, in our study, neither the histologic appearance nor collagen concentrations differed between hearts of indomethacin-treated and control dogs, so it is unlikely that inhibition of collagen synthesis by indomethacin is responsible for the scar thinning demonstrated in this study.

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<th>Table 2. Summary of Histologic Findings</th>
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<td>Control</td>
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<td>No. of dogs with necrosis</td>
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Scores are mean ± sd.
The possibility that prostaglandins and thromboxanes play a role in myocardial ischemic events has been studied.22, 23 Much evidence has accumulated linking prostaglandins to coronary vascular regulation. Recent studies have shown a temporal relation between acute ischemic events and a transient increase in thromboxane A2 released into the coronary venous effluent, and it has been suggested that vascular integrity depends on a balance between thromboxane and prostacyclin.24 Alexander et al.6 inhibited prostaglandin synthesis with indomethacin and demonstrated a reduction in reactive hyperemia after transient coronary occlusion.25 Friedman et al.26 showed that indomethacin exerts a coronary vasoconstrictor effect in patients with ischemic heart disease.

Jugdutt et al.10 demonstrated that indomethacin increased the absolute size of myocardial infarcts as well as the ratio of infarct size to the risk region in the conscious dog. This effect may result from coronary vasoconstriction or from the inhibition of metabolic and cellular effects of prostaglandins. Ogletree and Lefer27 found that E-prostaglandins protect ischemic myocardium, presumably by stabilizing cardiac lysosomal membranes. Inhibition of prostaglandin synthesis by indomethacin might therefore be expected to result in lysosomal disruption in the ischemic region. We found no difference in infarct size between control and treated dogs 6 weeks after infarction.

Healing of myocardial infarction may not always follow a predictable course; studies have shown deleterious effects of glucocorticoids on infarct healing. Multiple doses of methylprednisolone after infarction delayed removal of necrotic myocardium with reduced phagocytosis, resulting in large sheets of dead but architecturally preserved cells (mummification) and in scar thinning.6, 28 However, one or two doses of steroids early in the course of infarction do not appear to have this effect.6 In a patient who came to autopsy after treatment with multiple doses of steroids for Dressler’s syndrome, histologic study of the infarcted myocardium 63 days after infarction revealed persistent necrosis, inflammatory cells and small foci of loose fibrous tissue, findings expected 10–14 days after infarction. An association between the development of left ventricular aneurysms and steroid therapy after infarction has also been noted.27

Hutchins and Bulkley29 developed the concept of infarct expansion, which refers to thinning and dilatation of the infarcted myocardial wall.29 This process, which also has been shown to alter the course of myocardial healing, is associated with large transmural infarcts, and was shown to develop as early as 3 days after acute infarction. Infarct expansion has occurred in animals with experimental myocardial infarction as well as in humans.11, 30, 31

Reports on the deleterious effects of multiple doses of steroids, of ibuprofen-induced scar thinning, and the observation presented herein that indomethacin causes marked scar thinning after infarction suggest that the healing of myocardial infarction can be altered by certain antiinflammatory drugs administered early in the course of infarction. The functional significance of scar thinning induced by indomethacin remains to be determined, but it may well cause infarct expansion. Whether scar thinning predisposes to late aneurysm formation and deterioration of left ventricular function is unclear.

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Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication (NIH) 78-23, revised 1978).

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