Drug-induced expansion of infarct: morphologic and functional correlations

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ABSTRACT  It has been established that glucocorticoids and several nonsteroidal antiinflammatory drugs, when administered early after coronary occlusion, interfere with myocardial scar formation. To determine whether this action is associated with expansion of myocardial infarct during the first week of coronary occlusion and whether expansion affects ventricular function, the effects of indomethacin on the left ventricle in the early phase of infarction were studied. In a blinded randomized study, experimental myocardial infarction was produced in 17 open-chest dogs by ligation of the proximal left anterior descending coronary artery; the treated group (n = 8) received 10 mg/kg iv indomethacin at 15 min and 3 hr after occlusion, and the control group (n = 9) received saline. After 7 days, regional function expressed as percent change of area (%ΔA) of the left ventricular cavity was calculated from short-axis two-dimensional echocardiograms at the level of the infarct, the animals were killed, and their hearts were examined. The ratio of infarct thickness to noninfarcted wall thickness was 1.20 ± 0.08 (mean ± SEM) in the control group, and the ratio was lower in the indomethacin group, 0.96 ± 0.04 (p < .025). An expansion index of myocardial infarction was calculated as previously described and was 1.02 ± 0.04 in the control group vs 1.29 ± 0.06 in the indomethacin group (p < .005). In eight dogs (six control and two treated) without expansion (expansion index less than 1.09), regional function expressed as %ΔA was 46.8 ± 2.6% (SEM), and in nine dogs (six treated and three control) with expansion, %ΔA was significantly lower, 28.7 ± 4.0% (p < .005). In conclusion, indomethacin prevents the normal thickening of an infarct early in the healing phase; this interference with healing is associated with infarct expansion that in turn is associated with impaired regional function.


RECENTLY, it has been observed in our laboratory that treatment with indomethacin early after coronary occlusion causes marked thinning of myocardial scars when these are examined 6 weeks after experimental coronary occlusion.1 Similar effects were observed when methylprednisolone was administered,2 and similar, although less striking, changes were induced by ibuprofen administration.3 In all of these studies, the collagen content of the thinned scars determined 6 weeks after coronary occlusion was similar to that of untreated animals and the mechanisms responsible for scar thinning are unclear. One possible explanation is that these antiinflammatory drugs cause early postinfarction expansion (thinning and dilatation of the necrotic zone within the first week of infarction and before fibrosis), with eventual deposition of normal collagen into a thinned infarct. The present investigation was designed to determine whether indomethacin causes early infarct thinning, whether there is a relationship between thinning and expansion, and whether the latter alters left ventricular function.

Methods

Experimental preparation. Mongrel dogs of either sex with a mean weight of 14 ± 5 kg (SD) were sedated with acepromazine maleate (1.0 mg/kg sc), anesthetized with pentobarbital (30 mg/kg iv), and intubated and placed on a Harvard respirator (Ealing Co.; South Natick, MA).* A thoracotomy was performed 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

*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 Animals Resources, National Research Council (DHEW publication No. (NIH) 78-23, revised 1978).
the origin of the left anterior descending coronary artery immediately distal to the origin of the first diagonal branch. During a temporary 15 sec occlusion this always resulted in anteroseptal cyanosis.

**Experimental protocol.** After the administration of lidocaine (1.5 mg/kg iv), the left anterior descending coronary artery was permanently occluded with a silk ligature. A second similar dose of lidocaine was administered 5 min after coronary artery occlusion in all dogs. Group I consisted of control dogs, and these animals received 50 ml of saline infused over 5 min. Dogs in group II received indomethacin (Sigma Chemical Co.; St. Louis, MO) 10 mg/kg iv in 50 ml of normal saline infused over 5 min, at 15 min and 3 hr after occlusion. This dose of indomethacin caused marked scar thinning in a previous study. Each chest was closed, the air was evacuated from the thoracic cavity, and the animals were returned to the kennel. One dose of benzantine penicillin (1.2 million U) was injected intramuscularly in all animals. At the end of 7 days the animals were reanesthetized with pentobarbital (30 mg/kg iv), a thoracotomy was performed on each, and each heart was exposed and suspended in a pericardial cradle.

**Echoangiographic analysis.** Two-dimensional echocardiograms were recorded with an ATL Mark III model with an 850A real-time scan controller from the open-chest dogs 7 days after coronary occlusion. Images were recorded on Scotch (3M) Videocassettes with a Panasonic NV-8200 recorder. A saline-filled glove was placed between the epicardium and the transducer to place the epicardial surface within the focal zone. Short-axis echocardiographic images were traced directly from the video display (ATL 315A) from stop-frame images for three consecutive cardiac cycles. These tracings were taken at end-diastole and end-systole, with the onset of the Q wave in lead II defining end-diastole and the peak of the T wave defining end-systole. The images were traced by an investigator who was blinded as to the treatment group of each dog. Short-axis images for calculation were taken from the center of the infarcted zone, which was clearly visible in each open-chest dog, at the level of the papillary muscles. For studies of short-axis change, end-diastolic area was measured by planimetry from the maximum short-axis cross section at end-diastole; end-systolic area was determined at the same location. Percent change of area (%ΔA) was calculated as follows:

\[
\%\Delta A = \frac{(EDA - ESA)}{EDA}
\]

where EDA = end-diastolic area and ESA = end-systolic area.

After the two-dimensional echocardiographic study, the animals were killed with an overdose of KCl and their hearts were arrested in diastole and excised.

**Assessment of wall thickness and expansion.** After removal, the ventricles were dissected free from the structures above the atrioventricular ring, and the left ventricle was loosely packed with gauze to prevent collapse of the left ventricular cavity and was allowed to fix in 10% neutral buffered formalin for 3 days. After fixation, the hearts were sectioned from base to apex into 5 mm transverse sections parallel to the atrioventricular groove. Measurements of wall and scar thickness were made on each section along radii that passed through the center of the ventricular cavity. The thicknesses of the normal wall and of the scar in each slice were measured in three widely separated locations, and the values were averaged. A "thinning" ratio of average scar thickness to average noninfarcted wall thickness in each slice was determined (figure 1). An average ratio for each dog was determined by averaging the ratios in each slice containing infarcted tissue. Measurements were performed by an investigator who did not know treatment assignment.

Infarct size was assessed at the end of 7 days by planimetry and was expressed as a percentage of the left ventricle.

For quantification of the degree of expansion, the method described by Eaton and Bulkeley was used. 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 containing the greatest percentage of infarcted tissue was selected for each heart, and the lengths of the endocardial margins for the anterior segment containing the infarct as well as for the noninfarcted posterior segment were measured. An index of expansion was determined by dividing the endocardial length of the segment containing the infarct by the endocardial length of the noninfarcted segment. Expansion was considered if the index was more than 1.09 (figure 1). Measurements of wall thickness and the equivalent of expansion index (length of anterior endocardial circumference divided by length of posterior endocardial circumference) were obtained for noninfarcted hearts from a separate group of five dogs from previous studies that had been subjected to coronary occlusion but did not develop myocardial infarction.

**Analysis of hydroxyproline content.** Tissue samples weighing 100 to 200 mg were obtained from the endocardial and epicardial myocardium of nonischemic areas and from the center of the infarcted areas, the samples were oven-dried to a constant weight and were acid hydrolyzed in 6N HCl at 110° C for 12 hr. The center of the infarct was chosen to avoid contami-

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**FIGURE 1.** The method of quantification of infarct expansion. A midventricular transverse section of a heart with an anterior infarction (stippled area). Horizontal line marks the course of the septal bisector determined as described in text. Index is calculated as the ratio between the length of the infarct containing segment (A) and the noninfarcted segment length (B) (adopted from Eaton and Bulkeley, ref. 4). Thinning index is calculated as the ratio of infarct thickness (a) to noninfarcted wall thickness (b). See text for further details.
nation with noninfarcted tissue. After neutralization and decolorization of the hydrolysate, hydroxyproline concentration was assayed by the method of Newman and Logan3 and expressed as micrograms per milligram dry weight.

**Histologic analysis.** Representative formalin-fixed transmural transverse sections of noninfarcted myocardium and of the infarcted zone were conventionally processed and embedded in paraffin, were cut at 6 \( \mu m \) thickness, and were stained with hematoxylin and eosin and Masson’s trichrome. The following histologic features of necrosis and healing were assessed and graded from 0 (not present) to 4 (most prominent) as previously described6,7: necrosis, hemorrhage, edema, neutrophils, macrophages, vascular proliferation, fibroblasts, and collagen. The histologic score for each feature for each animal was averaged over the several slices examined.

**Statistical analysis.** Unpaired Student’s t tests were used to calculate the significance of differences of ratios of infarct to noninfarcted wall thickness, expansion index, infarct size, and hydroxyproline content, and echocardiographic measurements.

**Results**

Twenty-three animals were entered into the protocol. Three dogs died of ventricular fibrillation within 3 hr after coronary occlusion. One dog died 24 hr after coronary occlusion. In one dog there was no evidence of infarction at the time of the examination. In one dog treated with indomethacin, the infarct involved mainly the septal wall and the expansion index could not be determined. These six dogs were excluded from the study. Of the remaining 17 dogs, there were nine in the control group and eight in the indomethacin group.

In the five dogs from previous studies that did not develop myocardial infarction despite coronary occlusion, the ratio of the thickness of the anterior to the posterior wall was 1.00 ± 0.04 (mean ± SD) and the expansion index or ratio of anterior to posterior endocardial circumference was 0.97 ± 0.03 (mean ± SD). On the basis of these measurements, and as previously described by Eaton and Bulkleys,4 expansion was considered if the expansion index was greater than 1.09.

The ratio of infarct thickness to the noninfarcted wall thickness was 1.20 ± 0.08 (mean ± SEM) in the control group, and 0.96 ± 0.04 (mean ± SEM) in the treated group (p < .025); in the control group all but one dog had a ratio exceeding 1.0, and in the indomethacin group all but two had ratios less than 1.0 (figure 2). Thus, at 7 days the infarcted wall in the control animals was thicker compared with the noninfarcted wall. Average wall thickness containing scar was 11 ± 1 mm in control dogs (range 9 to 14), and it was 9 ± 1 mm in the treated group (range 8 to 12, .05 < p < .1). The expansion index was 1.02 ± 0.04 (mean ± SEM) in the control group and was significantly higher in the indomethacin-treated animals, 1.29 ± 0.06 (mean ± SEM) (p < .005) (figures 3 and 4). Infarct size, determined by planimetry as percent of

**FIGURE 2.** Ratio of infarct thickness to the noninfarcted wall thickness in control dogs and indomethacin-treated dogs. Open circles, Individual values for control dogs; closed circles, indomethacin-treated animals. Circles and bar, Mean ± SEM for each group (p < .025 control vs indomethacin-treated).

**FIGURE 3.** Expansion index in control and indomethacin-treated dogs. Circles and bars, Mean ± SEM for each group. Dashed line separates values of more or less than 1.09 (p < .005 control vs indomethacin-treated).
FIGURE 4. Midventricular section the seventh day after infarction. A, Control. The infarct is thicker compared with noninfarcted wall, without evidence of expansion of the infarcted segment. B, Indomethacin. The infarcted wall is not as thick as in the control and has almost the same width as the noninfarcted wall. There is evidence of expansion of the segment containing the infarct (segment above arrows).

3.6 (TR) + 1.2 (TR^2), where TR = thinning ratio and EI = expansion index; r^2 = .735, p < .05. There was no significant correlation in treated animals. In the 10 dogs with thinning ratios greater than 1.0 (eight control and two treated), the expansion index averaged 1.07 ± 0.05, a value significantly less (p < .05) than that in the seven dogs (one control and six treated) with abnormal thinning ratios (<1.0) in whom the expansion index averaged 1.25 ± 0.07.

Echocardiographic measurements. The change in the area of the short axis calculated from images at the center of the infarct (%AA) was 42.2 ± 4.5% in the control group and was lower, 31.6 ± 4.2% (mean ± SEM), though not significantly so (p > .10), in the indomethacin-treated group. However, a significant inverse correlation was found between %ΔA and the expansion index (%ΔA = -52.0 EI + 96.7; r = .77, p < .001) (figure 6) when all of the dogs were considered, regardless of treatment. When all dogs were divided into a group with expansion (expansion index greater than 1.09) and a group without expansion (expansion index less than 1.09), regardless of whether or not they were treated, then in nine dogs (six treated, and three control) with expansion, %ΔA was significantly lower (28.7 ± 4.0%) compared with that of eight dogs (six control and two treated) without expansion (%ΔA = 46.8 ± 2.6%, p < .005).

Hydroxyproline analysis. Noninfarcted epicardial and endocardial tissue contained small amounts of collagen, as reflected in hydroxyproline concentration, in the range of 3.4 to 3.8 µg/mg dry weight. Larger quantities of collagen were contained in the epicardial and endocardial portions of the infarct, but there were no differences between control and treated animals. Thus, treatment with indomethacin did not affect hydroxyproline concentration (table 1).

Histologic analysis. All infarcts were transmural. The infarcted tissue was extremely heterogeneous in all

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dogs and consisted of an active zone of repair surrounding a central core of necrotic, wavy, thinned myocardium devoid of inflammation, but with variable multifocal hemorrhage and mild diffuse intercellular edema. Healing proceeded inward from the endocardial and epicardial surfaces and from the lateral borders. The most advanced healing consisted of myocardial replacement by a dense infiltrate of macrophages and proliferation of fibroblasts with a prominent fine network of capillary-sized neovascularity and early formation of collagen. Neutrophils were most numerous at the junction of regions of necrosis and repair. There were no detectable microscopic differences between the myocardium from indomethacin-treated animals and control animals, either qualitatively or by semiquantitative grading of the various parameters used to assess inflammation and healing (table 2). Furthermore, no differences were noted when the myocardium from hearts with expansion was compared with that from nonexpanded hearts by semiquantitative ratings.

**Discussion**

A major finding in this study is that indomethacin induced early expansion of myocardial infarcts. Collagen content and histologic examination of the infarct tissue did not differ between the control animals and indomethacin-treated animals. In a previous study from our laboratory, marked thinning of myocardial scars 6 weeks after coronary ligation was observed in animals treated with indomethacin 15 min and 3 hr after coronary artery occlusion. Similar effects were induced by methylprednisolone and by another nonsteroidal drug, ibuprofen, in a similar preparation. The finding that a short-term intervention during the acute phase of myocardial infarction affects late scar formation raised the question as to whether this change might be related to an early alteration in left ventricular topography. In the present study we investigated the early effects of indomethacin on the morphologic char-

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**Figure 5.** Thinning ratio vs expansion index. Open circles, Control animals; solid circles, indomethacin-treated animals. Average values for the two groups are shown as open and closed circles with standard error bars. See text for further explanation ($r^2 = .735$, p < .05).

**Figure 6.** Correlation between echocardiographic %ΔA and expansion index for all animals regardless of therapy. Open circles, Control dogs; closed circles, indomethacin-treated dogs.
TABLE 1

<table>
<thead>
<tr>
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<th>Infarcted zone</th>
<th>Normal zone</th>
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<tbody>
<tr>
<td></td>
<td>Epicardium</td>
<td>Endocardium</td>
</tr>
<tr>
<td>Control</td>
<td>6.5 ± 0.8</td>
<td>5.2 ± 0.8</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>6.9 ± 0.7</td>
<td>5.7 ± 0.7</td>
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Furthermore, the effects of the topographic changes on the regional ventricular function were studied. The studies were carried out 7 days after coronary occlusion for two reasons: (1) if expansion exists it should manifest itself by this time and (2) since collagen deposition has already begun, any early changes in collagen formation can be detected.

Hutchins and Bulkley\(^6\) developed the concept of infarct expansion, which refers to thinning and dilatation of the infarcted myocardial wall. Such changes have been observed to commence between 6 and 24 hr after infarction and to progress with time.\(^4\) This process may be important in the development of cardiac rupture\(^5\) and late formation of aneurysms.\(^10,11\)

A clinical study has shown a significantly greater 8-week mortality for patients with regional expansion after acute myocardial infarction, suggesting that regional cardiac dilatation may be an early lethal consequence of transmural infarcts.\(^12\)

Clinical as well as experimental studies have suggested that certain morphologic features, including transmural necrosis and infarct size, are determinants of expansion.\(^4,12\)

It was observed in the present study that indomethacin caused myocardial infarct expansion within the first week of coronary occlusion.

Indomethacin, a widely used nonsteroidal antiinflammatory drug, is a potent inhibitor of prostaglandin synthesis.\(^13,14\)

This drug inhibits cyclooxygenase activity and thereby diminishes the generation of cyclic endoperoxides from arachidonic acid, thus blocking the ensuing cascade of prostaglandin and nonprostaglandin products. This accounts for reduction in vasodilation and edema in inflammation sites. In addition, a high dose of indomethacin (more than 2.0 mg/kg) reduces the migration of leukocytes to inflammatory regions.\(^15\)

The mechanism by which indomethacin induces expansion is unclear. Although indomethacin was previously shown to increase infarct size,\(^16\) this could not have been the explanation in our study since infarct size was similar in both groups. Indomethacin has been shown to inhibit collagen synthesis in some forms of inflammation;\(^17\) however, since hydroxyproline concentrations did not differ even in the early phases of collagen deposition between control animals and treated animals, it is unlikely that this is responsible for expansion.

A possible mechanism by which indomethacin caused infarct expansion is that by reduction of edema and tissue swelling it weakened the infarcted wall. In the present study, the ratio of infarct thickness to noninfarcted thickness was 1.20 ± 0.08 in the control animals and 0.96 ± 0.04 in the indomethacin-treated animals; i.e., in the hearts of control dogs, infarcts were thicker than the opposing noninfarcted wall and were thicker than the infarcts in indomethacin-treated animals. These findings are also consistent with the observations of Reimer and Jennings,\(^18\) who reported that in the early stages of myocardial infarction, the infarcted region gains weight by the addition of edema fluid and cellular elements. This may cause an overestimation of infarct size, which in their study (at 4 days) averaged 41% of the left ventricle.

By contrast, 28 days after coronary ligation, necrotic myocardium was largely replaced by dense scar tissue occupying only 11% of the left ventricle.\(^18\)

We suggest that the antiinflammatory action of indomethacin prevented the initial gain of volume by the infarcted tissue and caused a thin scar, which in turn was responsible for the expansion of the infarct. Although histologic analysis did not reveal significant differences in the amount of interstitial edema between groups, quantitating degree of edema between cells by light microscopy can be difficult. Routine histologic examination may not have been a sensitive enough technique to detect differences in interstitial edema between groups. Also, we cannot rule out the possibility that intracellular edema may have been greater in the control animals. A second major finding of the present study was the demonstration of the functional significance of infarct expansion, irrespective of whether or not the animals were treated.

TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>Indomethacin (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis</td>
<td>3.9 ± 0.3</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>Intercellular edema</td>
<td>1.7 ± 0.5</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1.8 ± 1.1</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>Vascular proliferation</td>
<td>2.2 ± 0.4</td>
<td>2.0 ± 0.7</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.3 ± 1.0</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Macrophages</td>
<td>2.8 ± 0.4</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>2.2 ± 0.7</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>Collagen</td>
<td>1.6 ± 0.5</td>
<td>1.2 ± 0.4</td>
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\(^4\)Includes specimen with septal expansion, which was omitted from topographic calculations.
with indomethacin. Significant impairment of function, as reflected in systolic reduction of ventricular cross-sectional areas, was observed in animals with evidence of expansion compared with that of animals without expansion, and a significant inverse correlation was found between function and expansion.

In conclusion, in this study it was observed that (1) indomethacin administered immediately after coronary ligation prevents the thickening of the infarct normally observed 1 week after infarction; (2) this failure of normal thickening is associated with early expansion of the infarcted segment, and the indomethacin-induced expansion may be related to the late thinning of the myocardial scar that we have previously reported'; (3) infarct expansion, regardless of whether it occurs spontaneously or after indomethacin treatment, is associated with impaired regional function, and this impairment of function is dependent on the degree of expansion. These observations point to the potentially adverse effects of interventions that prevent the normal early weight gain and thickening of infarcted tissue and the deleterious functional consequences of infarct expansion.

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References