Dose-dependent effects of short-term methylprednisolone on myocardial infarct extent, scar formation, and ventricular function

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ABSTRACT  Treatment with methylprednisolone (MP) of patients with acute myocardial infarcts remains a controversial topic; although some studies have shown that MP reduces infarct size, others have shown that it alters the healing process. To determine whether short-term MP limits infarct size and whether it alters the healing process or function, we investigated effects of different doses of MP on infarct size, scar formation, and cardiac function. Experimental infarction was produced in 23 anesthetized open-chest dogs by ligation of the proximal left anterior descending coronary artery. Group 1 dogs (n = 8) received MP 50 mg/kg iv 15 min and 3, 24, and 48 hr after occlusion (high MP); group 2 (n = 7) received MP 30 mg/kg iv 15 min after occlusion (low MP). Group 3 (n = 8) received saline (control). After 6 weeks of coronary occlusion, two-dimensional (2D) echocardiograms were performed, dogs were killed, and their hearts were examined. Regional function, expressed as percent of change in the area of the left ventricular cavity calculated from short-axis 2D echocardiograms, was markedly reduced in the high MP group with the percent of change in the area of 23.3 ± 4.9% compared with 38.4 ± 3.9% in the control group (p < .05) and with 40.5 ± 4.4% in the low MP group (p < .05). A ratio obtained by dividing infarct thickness by noninfarcted wall thickness was lower in the high MP group (0.42 ± 0.04 mean ± SEM) vs both the low MP group (0.95 ± 0.05) and the control group (0.88 ± 0.07; p < .01). Infarct extent was smaller in the low MP group, with the percent of infarcted endocardial circumference of 19.0 ± 1.9% vs the high MP group (30.0 ± 3.5%; p < .05). The percent of infarcted endocardial circumference in the control group was 25.0 ± 1.8% (p = NS).

Scars in the treated and control groups were similar histologically and by hydroxyproline content. Therefore, high MP, but not low MP, caused marked scar thinning associated with reduction in regional function, without a change in collagen content.


THE POSSIBILITY that glucocorticosteroids may exert a beneficial effect on the ischemic myocardium has been investigated in both experimental models and in patients. The results of these experimental studies and clinical studies have been conflicting. In some studies, methylprednisolone (MP) has been shown to diminish loss of creatine kinase levels in tissue, to reduce epicardial ST segment elevation, and to limit the size of the infarct. However, not all studies have confirmed the favorable effects of MP in acute myocardial infarction. In some experimental models, MP failed to decrease infarct size or preserve enzyme activity measured 24 hr after coronary occlusion, and did not show any efficacious effect on lysosomal and microsomal enzymes after myocardial ischemia. In a clinical study, multiple doses of MP were shown to increase both infarct size and the incidence of malignant ventricular dysrhythmia. Other investigations have suggested that glucocorticosteroids impair healing after myocardial infarction. The present study was designed for a large animal model (dog) with a healing process more closely resembling the course in man than does that in the rat, in which earlier studies were carried out. The objective was to determine the late effects of different doses of MP administered over the short term on scar formation, its morphology, and

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its quality by means of collagen content and histologic analysis. We also investigated the effects of impairment in scar formation on the regional cardiac function.

Methods

Experimental preparation. Mongrel dogs of either sex, weighing between 11 and 19 kg, were sedated with acepromazine maleate (10.0 mg/kg sc), anesthetized with pentobarbital (30 mg/kg iv), 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 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, anteropetapalycyanosis always occurred.

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 was performed in all dogs. Dogs in group 1 received methylprednisolone sodium succinate (Upjohn, Kalamazoo, MI) 50 mg/kg iv at 15 min, 3, 24, and 48 hr after occlusion (high MP). Dogs in group 2 received methylprednisolone 30 mg/kg iv at 15 min after occlusion (low MP). Group 3 dogs served as controls and received saline infusion. Four dogs in the control group also served as controls in another study. In each dog, the chest was closed, the air was evacuated from the thoracic cavity, and the animals were returned to the kennel. One dose of benzathine penicillin of 1.2 million units was injected intramuscularly in all animals. After 6 weeks each dog was reanesthetized with pentobarbital (30 mg/ kg iv), a thoracotomy was performed, and each heart was exposed and suspended in a pericardial cradle.

Echocardiographic analysis. We obtained 2D echocardiograms from the open-chest dogs with an ATL Mark III model with 850A real-time scan controller. Images were recorded on Scotch (3M) Videocassettes with a Panasonic NC-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 a stopped-frame image for five consecutive cardiac cycles. These tracings were taken at end-diastole and end-systole with the onset of the Qwave in lead II to define end-diastole and the peak of the Twave to define end-systole. At the time the images were traced, the investigator was unaware of the regimens the dogs had undergone. 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 area change, end-diastolic area was measured by planimetry from the maximum short-axis cross section at the end of diastole; end-systolic area was determined at the same location. Percent change of area (%AA) was calculated as follows:

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\%AA = \frac{EDA - ESA}{EDA}
\]

*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 (DH€W publication No. [NIH] 78-23, revised 1978).

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

After the 2D echocardiographic study, the animals were killed with an overdose of potassium chloride, and the hearts were arrested in diastole and were excised.

Assessment of wall thickness. After the heart was removed from each dog, 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 fixed 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 thickness of the normal wall in each slice was measured in three to five widely separated locations and values were averaged. A ratio of average scar thickness to average noninfarcted wall thickness, which was determined in each slice, was used to normalize variation in dog size. An average ratio in each dog was determined by averaging the ratios in each slice with infarcted tissue. The investigator performed measurements in a blinded fashion, without knowing to which group the heart belonged.

We assessed the extent of the infarct after the 6 weeks of occlusion by measuring the endocardial length of the infarcted region and expressing it as a percentage of the total endocardial circumference of all heart sections. All hearts were analyzed by two investigators for existence of left ventricular aneurysm according to criteria previously described by Cabin and Roberts23: (1) presence of a convex protrusion in the left ventricular free wall consisting of dense fibrous tissue with or without an occasional 'island' of myocardial fibers, (2) absence of fresh myocardial necrosis within the wall of the left ventricular aneurysm, and (3) diameter of the mouth of the left ventricular aneurysm larger than or similar to that of the aneurysm.

Analysis of hydroxyproline content. Tissue samples weighing 100 to 200 mg were obtained from the myocardium of normal and infarcted areas, oven-dried to a constant weight, and acid-hydrolyzed in 6N HCl at 110°C for 12 hr. After neutralization and decolorization of the hydrolysate, hydroxyproline concentration was assayed by the method of Newman and Logan22 and expressed as micrograms per milligram dry weight.

Histologic analysis. Representative transmural, transverse, Formalin-fixed sections of noninfarcted myocardium and of the infarcted zone were conventionally processed and embedded in paraffin. Sections stained with hematoxylin and eosin and Masson's trichrome were examined from each heart. The following histologic features of necrosis and healing were assessed and graded from 0 (not present) to 4 (most prominent) as previously described24,25: necrosis, leukocytes, macrophages, neutrophils, vascular proliferation, fibroblasts, and collagen. The histologic score for each feature for each animal was averaged over the several slices examined.

Statistical analysis. Analysis of variance and Tukey's method for multiple comparison were used to calculate the significance of differences of wall thickness measurements, ratios of infarcted to noninfarcted wall thickness, infarct size, hydroxyproline content, and echocardiographic percent of area change among the groups. Chi-square analysis was used to calculate the significance of difference for the existence of left ventricular aneurysm among groups.

Results

Thirty-three dogs were entered into the protocol. Three dogs developed ventricular fibrillation and died within 30 min after coronary occlusion. Four dogs died
within 3 days after coronary occlusion (three in the high MP group and one in the low MP group). In another three dogs there was no evidence of myocardial infarction at the time of examination (two in the high MP group, one in the low MP group). These differences in early death or absence of infarction were not statistically significant. These 10 dogs were excluded from the study.

Of the remaining 23 dogs that survived for 6 weeks, there were eight in the control group, eight in the high MP group, and seven in the low MP group.

Assessment of wall thickness and extent of infarct. The ratio of infarcted wall thickness to noninfarcted wall thickness was $0.88 \pm 0.07$ (mean $\pm$ SEM) in the control group and $0.95 \pm 0.05$ in the low MP group ($p = NS$). This ratio was significantly smaller ($0.42 \pm 0.04$) in the high MP group ($p < .01$ compared with both the control group and low MP group). In the control group all but one heart had a ratio exceeding 0.6; in the low MP group all hearts had a ratio greater than 0.6; in the high MP group all animals had ratios less than 0.6 (table 1, figures 1 and 2).

The percent of endocardial circumference that was infarcted was $25.0 \pm 1.8\%$ (± SEM) in the control group and $19.0 \pm 1.9\%$ in the low MP group ($p = NS$). In the high MP group, the percent of the circumference that was infarcted was significantly higher ($30.0 \pm 3.5\%$, $p < .05$) compared with the low MP group, but showed no statistically significant difference compared with the control group (table 1). In the high MP group, four out of eight hearts met pathologic criteria of left ventricular aneurysm, although none in the control or the low MP group met these criteria ($p < .025$) (figure 2).

Echocardiographic measurements. Echocardiographic

![Graph](image.png)

**FIGURE 1.** Ratio of infarct thickness to normal wall thickness in control, low MP, and high MP groups. Closed circles, values for individual dogs; open circles and bars, mean ± SEM for each group of animals ($p < .01$ for high MP vs control and vs low MP).

measurements were available for seven out of eight control animals and for all the animals in the groups treated with MP.

Short-axis area change calculated from images at the center of the infarct was $38.4 \pm 3.9\%$ (± SEM) in the control group and $40.5 \pm 4.1\%$ in the low MP group; this difference was not statistically significant. Significantly reduced regional function was found in the high MP group, with a percent area change of $23.3\% \pm 4.9\%$ (± SEM), as compared to control and low MP groups ($p < .05$) (figure 3).

A poor correlation was found between regional function (expressed as percent area change) and the ratio of infarct thickness to normal wall thickness for all of the dogs in all groups. However, a good correlation ($r = .77$) was found between these two variables in the 10 dogs in which the percent of infarcted circumference exceeded 25%. No correlation was found in 12 dogs with smaller infarcts (circumference equal to or less than 25%) (figure 4).

Hydroxyproline assay. Noninfarcted myocardial tissue contained small amounts of collagen, as reflected in the hydroxyproline concentration of $3.6 \pm 0.3$ ($\mu g/mg$ ± SEM) in the control group, $3.7 \pm 0.3$ in the low MP group, and $4.5 \pm 0.5$ in the high MP group ($p = NS$). Larger quantities of hydroxyproline concentration were contained within the scar tissue—$17.0 \pm 2.1$ in the control group, $17.0 \pm 2.4$ in the low MP group, and $22.7 \pm 2.2$ in the high MP group ($p = NS$). Thus MP treatment did not affect the hydroxyproline concentration either in the infarcted tissue or normal myocardium.

Histologic analysis. The qualitative histologic appearance of the infarcted tissue was similar in the control animals and animals receiving MP at 6 weeks after
myocardial infarction. Necrotic tissue and polymorphonuclear leukocytes were rare. Collagen, thick-walled blood vessels, and numerous fibroblasts and mononuclear inflammatory cells were prominent features of these infarcts in the healing process. In most instances the evolving scar was relatively homogeneous in appearance and essentially had the appearance of myocardial infarcts 6 weeks old in humans. The similarity in the treated and control groups by the semi-quantitative grading of histologic features is summarized in table 2.

Discussion
This study demonstrates dose-dependent effects of MP on scar formation, infarct extent, and regional ventricular function 6 weeks after myocardial infarction in dogs. Repeated high doses of MP started immediately after coronary occlusion caused significant thinning of the myocardial scar, a finding consistent with investigations in smaller animals.\textsuperscript{16} However, an important and new finding in this investigation was that this scar thinning was associated with impairment in left ventricular function. In contrast, a single dose of MP did not cause any deleterious effect on scar formation or on ventricular function. Collagen content, as reflected by hydroxyproline concentration and histologic examination, did not differ among the control, low MP, or high MP groups.

In dogs, scar formation after infarction is well advanced after 6 weeks.\textsuperscript{25} Assessment of ventricular performance at 6 weeks, once scar formation is completed, represents regional function after the rapid changes that occur during the early phases of the healing process have subsided.

The possibility that glucocorticosteroids may exert a beneficial effect on the ischemic myocardium and limit
infarct size has been extensively investigated in both experimental models and man. However, the results have been conflicting. Several investigators have suggested that the beneficial effects of glucocorticosteroids in general and MP in particular are secondary to their lysosomal and membrane-stabilizing actions. MP has also been shown to increase myocardial collateral blood flow, decrease preload and afterload, and reduce myocardial oxygen consumption.

The suggestion that glucocorticosteroids have deleterious effects on infarct healing comes from studies which demonstrated that multiple doses of MP after infarction delayed removal of necrotic myocardium with reduced phagocytosis, which results in large sheets of dead but architecturally preserved cells (mummification) and in scar thinning.

Similarly, there is a suggestion from a single case report that this deleterious effect also occurs in patients. In a patient at autopsy after treatment for Dressler’s syndrome with multiple doses of steroids, histologic examination of infarcted myocardium 63 days after infarction revealed persistent necrosis, inflammatory cells, and small foci of loose fibrous tissue; these findings would be expected to occur 10 to 14 days after myocardial infarct.

An attempt was made in our study to answer the following crucial questions:

1. What are the final changes and effects in an animal model (dog) in which the healing process more closely resembles the process in man than does that in the rat, in which earlier studies were carried out?

2. Are the morphologic changes in scar formation related to biochemical or histologic changes?

3. What is the functional significance of scar thinning?

To our knowledge previous studies have not addressed these important questions. Our data indicate...

FIGURE 3. Echocardiographic percent area change (%ΔA) of short-axis views in control, low MP and high MP groups. Closed circles, values for individual dogs, open circles and bars, mean ± SEM for each group of animals (p < .05 for high MP vs control and low MP groups).

FIGURE 4. Linear regression analysis of echocardiographic percent area change (%ΔA) vs ratio of infarct thickness divided by noninfarcted wall thickness for animals in all groups. A, Hearts with infarct endocardial circumference exceeding 25% exhibit good correlation (r = .77). B, In contrast, hearts with infarct endocardial circumference of 25% and less exhibit lack of correlation between two variables. Open circles, values for individual control animals; closed circles, values for individual animals in the low MP groups, and triangles, values for dogs in the high MP group.
that a single dose of MP (low MP group) administered soon after coronary occlusion does not impair the healing process and results in a scar without thinning or deleterious topographic alterations compared with those in the control group. Repeated pharmacologic doses administered up to 48 hr after coronary occlusion (high MP group) result in significant scar thinning, either because of the high dose or its prolonged administration. At the time of examination these abnormally thin scars had developed into ventricular aneurysms in some hearts. The increased percent of infarcted endocardial circumference in the high MP group as compared with the low MP group may be due to a combination of two processes: expansion of the infarcted segment in the high MP group and a trend toward decreased infarct size in the low MP group.27, 28

Infarcts in humans have been shown to be well healed by 6 weeks in many studies.23, 24 The course of infarct healing in dogs resembles that in man. Analysis of collagen content is important because many studies have shown glucocorticoid inhibition of collagen synthesis, both in vivo as well as in cell culture studies.29, 30 It also appears that collagen degradation is enhanced in the presence of steroids.31 Most studies, however, have shown that only massive long-term doses of glucocorticosteroids reduced collagen synthesis; large, intermittent doses of corticosteroids did not alter collagen synthesis.32 On the other hand, glucocorticosteroids have been shown to inhibit neutrophil and macrophage accumulation in injured tissues and to reduce the number of new capillaries that are important for normal healing.33 In this study, we found similar collagen content and histologic appearance at 6 weeks in the control group as well as in the treated groups of dogs. Thus, it appears that the mechanism of scar thinning cannot be related to collagen content or histologic appearance. A possible explanation for our findings is that high doses of MP during the early phases caused a transient delay in the healing process perhaps through inhibition of macrophage infiltration and neovascularization, or possibly through "mummification" and retardation of necrotic tissue disintegration. It is possible that these weakened infarcts underwent expansion and thinning before the deposition of normal collagen. Hence, at 6 weeks, manifestations of altered healing were thinned scars with normal histologic appearance and collagen content.

Previous studies from our laboratory indicated that various nonsteroidal anti-inflammatory drugs (ibuprofen and indomethacin but not aspirin) administered soon after coronary occlusion caused scar thinning after myocardial infarction,20, 34 but the functional significance of this thinning had been unclear. To the best of our knowledge, this is the first study showing that infarct scar thinning induced by an anti-inflammatory drug is associated with impaired regional function. The present study demonstrates the functional significance of scar thinning after myocardial infarction. The functional impairment correlated with the extent of scar thinning in dogs with relatively large infarcts, that is, in which the percentage of endocardial circumference infarcted exceeded 25%. This finding was not unexpected, since, if the infarct is small, any dysfunction caused by thinning may not be evident. In contrast, a large thinned infarct might be expected to interfere with ventricular contraction.

The objective of this study was to elucidate factors that affect the healing process; we did not attempt to mimic the clinical situation in which patients might be receiving long-term lower doses of steroids. Caution should be used before applying these results from the experimental model to humans with myocardial infarction. However, we did note that a single dose given shortly after infarction does not seem to cause any detrimental effect. This is of clinical importance in view of reports of patients treated with a single dose of corticosteroids before coronary reperfusion with streptokinase.35

In conclusion, multiple high doses of methylprednisolone started immediately after coronary occlusion caused significant thinning of the infarct scar associated with impairment of regional function 6 weeks later. This deleterious effect is dose dependent, since a single dose of methylprednisolone does not cause scar thinning or regional functional deterioration.

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