Steroid Administration After Myocardial Infarction Promotes Early Infarct Expansion

A Study in the Rat

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Abstract

Whether steroids lead to thinner scars and larger aneurysms by delaying collagen deposition or worsening infarct expansion before significant collagen deposition begins is unknown. Rats underwent either transmural infarction by left coronary ligation or sham operation. Both infarct and sham rats were randomized to methylprednisolone 50 mg/kg i.p. × 4 or saline treatment within 24 h after operation. Sacrifice occurred before (3 d) or after (7 d) collagen deposition typically begins.

Despite similar infarct size, infarct wall thickness was 1.35±0.08 mm in the saline and 0.99±0.12 mm in the methylprednisolone group ($P < 0.001$) at 3 d. This decrease in wall thickness was explained by a decrease in the number of myocytes across the infarct wall ($r = 0.99$; $P < 0.001$), suggesting that steroids promote myocyte slippage. Furthermore, methlyprednisolone caused no further infarct thinning or cavity dilatation beyond 3 d.

Thus, high-dose methylprednisolone given within 24 h after transmural infarction worsens infarct expansion before collagen is laid down by promoting the slippage of necrotic myocytes.

Introduction

High-dose corticosteroids administered early after infarction cause thin scars, and aneurysms late after infarction (1–3). The mechanisms by which steroids promote scar thinning and aneurysm formation are unclear. There are at least three possibilities. Steroids might lessen or weaken the collagen laid down by fibroblasts creating a "weaker" scar, thereby allowing late scar stretch. Previous studies have shown, however, that the composition and amount of collagen in mature scars of steroid treated and control infarcts are no different (3). This makes late scar stretch a less likely mechanism of steroid-related aneurysmal dilatation. Because steroids are known to delay collagen deposition in healing infarcts (4), a second possibility is that steroids lead to a prolonged phase during which "soft" necrotic myocardium thins and dilates. The third possibility is that steroids act solely by promoting the early expansion of freshly necrotic myocardium before collagen deposition begins. Infarct expansion, the thinning and dilatation of freshly infarcted myocardium that begins within the first few hours after transmural infarction, has been shown to be the morphologic substrate for cardiac rupture (5) and late aneurysm formation (6). Because both of these adverse events have also been associated with steroid exposure (2), steroids might contribute to late scar thinning and aneurysmal dilatation by worsening early infarct expansion before collagen deposition begins. The cellular mechanism by which steroids might promote infarct expansion in unknown. There are several possible ways that infarct wall thinning could occur before the resorption of necrotic myocytes. First, necrotic myocytes may rupture or tear, leaving fewer intact myocytes across the wall resulting in wall thinning and cavity dilatation (5, 7). Another possibility is that necrotic myocytes are stretched so that cell lengthening and thinning account for the infarct wall thinning (8, 9). A final possibility is that wall thinning is due to a geometric rearrangement or a slippage of necrotic myocyte bundles resulting in fewer myocytes across the wall with little or no change in myocyte dimensions (9–11).

The purpose of this study was to examine whether high-dose methylprednisolone administered early after infarction worsens infarct expansion, and if so, to identify whether myocyte rupture, stretch, or slippage accounts for the increase in expansion. Because steroids also delay collagen deposition (4), we examined whether this delay promoted infarct thinning and cavity dilatation beyond the time that infarct expansion is known to plateau.

Methods

Infarct model. 128 Sprague-Dawley female rats, each weighing 200–250 g, were anesthetized with 35 mg/kg body weight intraperitoneal sodium methohexital. The rats were given intermittent positive pressure ventilation with 95% O_2 and 5% CO_2. The hearts were exposed through left intercostal thoracotomy, and pericardiotomy was performed. Rats were randomized to undergo either left coronary artery occlusion or sham operation. The chest was then closed and 100,000 U benzathine penicillin i.m. was given. The rats were awake within 30 min after surgery and subsequently maintained on standard rat chow and water ad lib. Both infarct and sham rats were further randomized to two subgroups: one to receive methylprednisolone 50 mg/kg i.p. at 5 min, 3 h, 6 h, and 24 h after coronary occlusion, the other to receive a saline placebo.

Animals were sacrificed at two time periods. To assess the effect of methylprednisolone on infarct expansion we sacrificed animals at a time when the severity of infarct expansion peaks but before significant collagen deposition has begun. Infarct expansion peaks in severity at 3–5 d after transmural infarction in the rat (12). Fishbein et al. (13) has reported that little or no collagen is seen in the infarct zone until after the third day after infarction in the rat. Considering these findings we chose to sacrifice animals 3 d after operation to assess the effects of methylprednisolone on infarct expansion with little influence from the effect of methylprednisolone on collagen deposition. Masson trichrome staining for collagen was used to confirm the assumption that significant collagen deposition had not occurred at 3 d in either methylprednisolone-treated or saline-treated infarcts.

The second sacrifice time was chosen to examine the possible effect of steroid-induced delayed collagen deposition on infarct thinning and dilatation. For this purpose we chose to sacrifice animals 7 d after infarction. This is a time after the severity of infarct expansion plateaus

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(5 d) (12) and after significant collagen deposition has begun (4–5 d) (13). Methylprednisolone has also been shown to delay collagen in the infarct zone at 7 d (4). Again, Masson's trichrome staining for collagen was used to confirm in our model these previously reported findings.

Preparation of the hearts. At sacrifice the animals were heparinized and then anesthetized with intraperitoneal sodium methohexital. The hearts were rapidly excised and immediately submersed in ice-cold saline. Next the ascending aorta of each heart was cannulated with polyethylene tubing placed above the aortic valve and tied in place. The tubing was connected to a manifold and a gravity flow apparatus that permitted a constant perfusion pressure of 70 mmHg. The hearts were then vented with fenestrated polyethylene tubing placed into the left ventricle through the mitral valve via incision into the left atrial appendage. Next each heart was perfused retrograde from the aorta for 30 s with heparinized Krebs-Henseleit bicarbonate buffer solution at 37°C so that hearts would spontaneously beat under unloaded conditions. When the coronary sinus effluent was clear of blood, the hearts were next perfused with cold 30 mM KCl to achieve rapid and uniform diastolic arrest. Finally the hearts were perfused with 10% buffered formalin for 20 min. The hearts were kept in cold formalin for 24–48 h and then sliced by hand transversely parallel to the atrioventricular groove in four 2.0–2.5–mm sections from apex to base. The slices were embedded in paraffin, and two 5-μ–thick sections were prepared from each slice. One section was stained with hematoxylin-eosin, the other with Masson's trichrome stain to demonstrate collagen.

Data analysis. The histopathology of each section was assessed both qualitatively and quantitatively. The sections were numbered to keep the observer blinded with regard to the experimental group. For all infarct hearts, the infarcted and noninfarcted myocardium was examined for the presence of rupture. Rupture was defined as the presence of torn or disrupted myocytes across the wall. The fractional volume of the infarct zone containing inflammatory cell infiltrate was determined for each infarct heart by area-perimeter analysis (14). Collagen content of the infarct zone was semiquantitatively graded in six randomly selected hearts from each infarct group according to methods described by Kloner et al. (4): 0, no collagen deposition; 1+, occasional thin bundles (< 5 μ thick) of collagen; 2+, occasional thin plus occasional thick bundles of collagen; 3+, numerous thin plus occasional thick bundles of collagen; 4+, numerous thick bundles or wide sheets of collagen.

The degree of edema (intercellular space) in the infarct region was qualitatively assessed histologically for all 3-d infarcts. However, Fishbein et al. (13) report that edema peaks at 24–48 h after infarction in the rat. Three days might therefore be too late to assess differences in edema. Thus, we also chose to quantitatively assess the water content of 24-h infarcts by wet to dry weights in a separate group of methylprednisolone and saline-treated rats with infarcts. 12 Sprague-Dawley rats underwent left coronary ligation; six were treated with methylprednisolone 50 mg/kg i.p. at 5 min, 3 h, and 6 h after coronary occlusion; the rest were treated with saline. This is the same dose and dose schedule used in the other animals except for omission of the 24-h dose. Animals were sacrificed at 24 h; the hearts were excised and arrested by retrograde coronary perfusion with cold 30 mM KCl. The hearts were rapidly frozen at −70°C for 20 min and then sectioned into four transverse sections. The infarct region was identified by incubating the ventricular slices in triphenyl tetrazolium chloride (TTC)[1] for 20 min at 37°C. The infarct region (TTC negative) and the noninfarcted septum (TTC positive) were excised from each heart. The tissues were weighed fresh (wet weight) and again after 72 h of dessication (dry weight). Percent water weight was reported for the infarct region and the septum of each heart. Histologic sections were obtained to confirm that TTC positive region showed infarction.

All histologic sections of the 3- and 7-d infarct groups were reviewed for the presence and extent of infarct expansion. Expansion was defined as the presence of disproportionate thinning and dilatation of the infarct zone: 0, no thinning or dilatation; 1+, mild thinning; 2+, mild thinning and dilatation; 3+, moderate thinning and dilatation; 4+, marked thinning and dilatation. We have shown that this semiquantitative method correlates well with quantitative measurements of infarct expansion including infarct wall thickness and left ventricular cavity volume (15). All hearts were graded independently and blindly by three observers. Interobserver agreement was good with complete concordance in assessing the presence and severity of expansion. For each heart, total left ventricular volume, left ventricular cavity volume, and infarct volume were determined by serial reconstruction of integrated cross-sectional areas of histologic sections. This was done by projecting the heart sections at 7× magnification onto a digitizing tablet interfaced with a Videoplan image analysis microcomputer (Carl Zeiss, Inc., Thornwood, NY). The left ventricular epicardial and endocardial contours were traced, the infarct region, if present, was delineated, and the specified cross-sectional areas and volumes were calculated. Infarct size was then expressed as a percentage of total left ventricular volume.

Further detailed structural evaluation was performed on the transverse section second down from the base of the heart. This section always contained the infarct region. Left ventricular and right ventricular centers of mass were determined for each section by continuously tracing the left ventricular and right ventricular endocardial contours respectively onto a digitizing tablet (Fig. 1). The center of mass was calculated by computer algorithm as the mean x and y coordinate of all points within the digitized contour (16). A radian connecting the right ventricular and left ventricular centers of gravity defined the mid-septum. A radian was drawn from the left ventricular center of gravity to the epicardial surface of the thinnest locus of the infarct region wall. Measurements of infarct wall thickness were made along the course of this radian. For sham-operated animals, the equivalent infarct region wall thickness was measured along a radian drawn from the left ventricular center of gravity at a rotational angle from the midseptal radian comparable with the average location of the thinnest locus of the infarct region in infarct hearts.

We used the following method to distinguish whether steroids affect infarct thinning by promoting myocyte slippage (a decrease in the number of cells across the wall), or by promoting cell stretch (thinning and elongation of individual myocytes). The number of myocytes across the wall of the infarct region was counted in randomly selected 3-d hearts (four from sham-operated animals, six from steroid-treated, and six from saline-treated infarct animals). Myocytes were counted along the radian that crossed the area of greatest infarct wall thinning. At this locus a region was selected that contained no inflammatory cells and no myocyte drop-out. In this region, all infarcted myocytes were still intact and distinct without visible sarcolemmal disruption. This allowed for easy and reproducible counting of necrotic myocytes. The number of myocytes across the full thickness of left ventricular wall were counted using a light microscope interfaced with a digitizing tablet via a camera lucida. The wall thickness at the site of the cell count was also measured. All counting was performed at a magnification of 400×. A grid reticle eyepiece was used to divide the wall thickness into arbitrary unit lengths of 100 μ. The tablet's LED cursor was tracked transmurally from epicardial to endocardial surface. Each time the cursor light crossed a myocyte, an event counter was triggered. The counts were tabulated such that the number of myocytes per unit width of wall thickness (myocyte density)

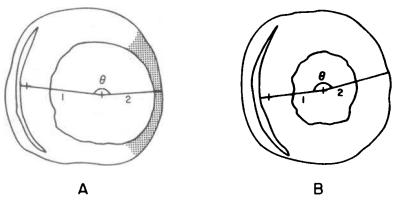

Figure 1. (*A*) Diagram of a typical infarct heart. Radian 1 connects left ventricular and right ventricular centers of mass. Radian 2 connects left ventricular center with thinnest locus of the infarct region. θ is the rotational angle between radians. Stippled area is the infarct region. (*B*) Diagram of sham heart. The mean rotational angle θ is determined from the infarct group. Radian 2, which defines the equivalent thinnest infarct region for sham hearts, is drawn at this angle from radian 1.

1. *Abbreviation used in this paper:* TTC, triphenyl tetrazolium chloride.

as well as the total number of myocytes across the wall were determined. Because myocytes gradually change in orientation from the cross-sectional plane in the epicardium to the longitudinal plane in the middle layer and then again to the cross-sectional plane in the endocardium, we recognized that myocyte density may not be uniform across the infarct wall. The cell number per 100 μ wall thickness also allowed us to assess possible regional changes in cell density across the wall due to changes in fiber orientation. The counts were performed twice for each region. The cell counts did not differ by > 3%. The mean values for myocyte density and total myocytes across the wall were used for each heart. Myocyte stretch was defined as follows. When compared with noninfarcted control myocardium, the total number of myocytes across the wall was similar but average myocyte density was increased. Myocyte slippage was defined as decreased transmural myocyte count with no change in myocyte density (Fig. 2).

Because myocytes frequently branch, failure to recognize branching myocytes could affect the accuracy of the cell counts. For this analysis, when a myocyte was seen to branch near the radian where counting was done, the branch cell was counted as a single cell. In the longitudinal plane (mid-myocardium) branching is easily recognized (Fig. 3 *A*). However in the cross-sectional plane (epicardium and endocardium) branching is difficult to recognize. To identify branch cells in cross-section we took advantage of the finding that branched myocyte fibers have smaller diameters than unbranched myocytes and that these branched fibers were adjacent to unbranched fibers of normal diameter. Thus if a cluster of small fibers was seen in cross-section along the counting radian, it was counted as a single cell if an unbranched cell was seen adjacent to the branched cluster (Fig. 3 *B*). To validate this counting method we assumed that branching was the same in all layers of the myocardial wall. We calculated the frequency of branching myocytes seen along the counting radian in the longitudinal plane (mid-myocardium) vs. the frequency of branching myocytes seen along the counting radian in the cross-sectional plane (epicardium and endocardium). Branching myocytes were 12.0±2.0% (SD) of the total in the cross-sectional plane vs. 10.9 ± 2.4% in the longitudinal plane. Because branching was not significantly different in either plane we felt that this method adequately corrected for branching myocytes.

Statistical analysis. Both exploratory graphical methods and confirmatory analyses were employed to analyze the data (17). Graphical displays of the data, using empirical quantile–quantile plots are shown to convey information about differences between data distributions (18). For confirmatory testing procedures, the study was viewed as a two-way analysis of variance (19) with a two-level drug intervention factor (steroid/no steroid) and a two-level infarction factor (infarct/no infarct). Covariates such as infarct size, extent of transmurality, and time after infarction were included in the design model. All hypotheses were tested by analysis of the main effects and interaction effects. Regression analysis was used to assess the relationship of stretch and slippage with regional wall thickness changes using a general linear model. A P value of < 0.05 was considered significant. When significant differences were found, comparison between individual groups were made using the Neuman-Keuls multiple range test. Prevalence of expansion between saline-treated and steroid-treated infarcts was assessed by Chi-square analysis (19). The results are reported as the mean±standard error of the mean for values within individual groups. For between-group comparisons, we report the mean values and overall standard deviation.

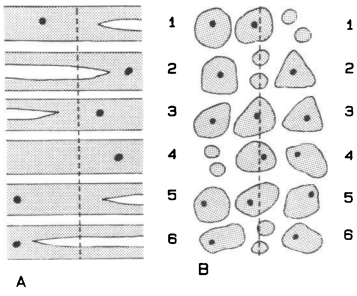

Figure 3. Schematic diagram showing how the counting method identifies branching myocytes. (*A*) In the longitudinal plane, branching fibers crossing the counting radian (*dashed line*) can easily be detected. (*B*) In cross-section, branched myocyte fibers are of smaller caliber than unbranched myocytes. When small caliber fibers cross the counting radian, the size of the myocytes adjacent to these small fibers are used to guide the myocyte count.

Results

106 of 128 rats that underwent surgery survived to the sacrifice date, 30 sham-operated and 76 coronary ligation. None of the sham animals died. All mortality among infarcted animals occurred within the first 6 h after surgery. Mortality was 21.7% in saline-treated and 23.1% in methylprednisolone-treated animals ($P = 0.71$). 70 of 76 rats had successful ligation of the left coronary artery producing myocardial infarction; 58 of these had transmural infarcts. The remaining 12 (5 methylprednisolone-treated, 7 saline-treated) had small (< 13%) nontransmural infarcts and were not included in the statistical analysis. No difference in infarct size or the transmurality of infarcts between steroid-treated and saline-treated infarcts was seen. Prevalence of expansion was no different between infarct groups at either 3 or 7 d (Table I). No hearts sustained rupture.

Histopathologic findings. Table II summarizes the quantitative histologic findings. In saline-treated animals, histologic evaluation at 3 d showed the usual changes of infarction including hypereosinophilia of myocytes with intense inflammatory infiltration and myocyte degeneration from the margins toward the center of the infarct (Fig. 4 *A*). However methylprednisolone-treated animals showed a marked suppression of inflammatory cells in the infarct zone. At 3 d inflammatory cell infiltration was present in 62.44±2.4% of the infarct area among saline-treated but only 14.28±3.7% of the infarct area among methylprednisolone-treated infarcts ($P < 0.001$). Among methylprednisolone-treated infarcts, hypereosinophilic myocytes were intact and some myocytes still retained pyknotic nuclei (Fig. 4 *B*). These changes are consistent with delayed necrosis and "mummification" previously reported by Bulkley and Roberts (2) and Kloner et al. (4). Furthermore, no rupture of necrotic

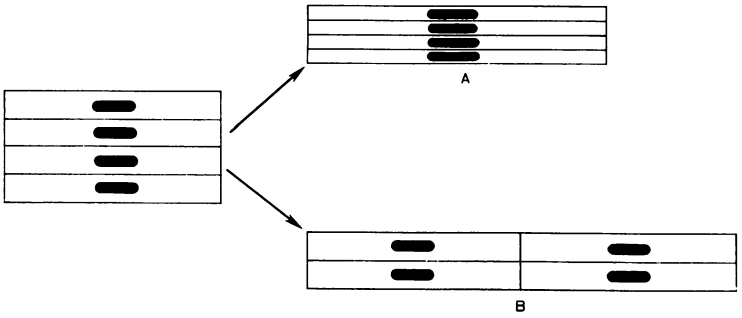

Figure 2. Possible mechanisms of infarct wall thinning. (*A*) Necrotic myocytes may stretch, thereby reducing their cell diameters with no change in the number of cells across the myocardial wall. (*B*) Necrotic myocytes could slip past one another so that the number of cells across the wall is reduced without change in cell diameter.

Table I. Morphologic Findings

	n	Expanded	Infarct size	Wall thick[¶]	Cavity volume
			% left ventricular volume	*mm*	*mm³*
3-d					
SHAM-SAL*	7	—	—	2.6±0.2	52.5±2.9
SHAM-MP[‡]	8	—	—	2.6±0.1	49.8±3.9
MI-SAL[§]	15	10	35.0±2.4	1.35±0.08**	89.8±5.3**
MI-MP[‖]	15	11	35.2±2.6	0.99±0.12‡‡	102.7±8.4**
P (ANOVA)	—	—	NS	<0.001	<0.001
7-d					
SHAM-SAL	7	—	—	2.5±0.1	49.7±2.6
SHAM-MP	8	—	—	2.7±0.1	51.3±2.8
MI-SAL	13	9	25.2±2.1	1.07±0.09**	91.9±6.4**
MI-MP	15	11	29.0±2.3	0.68±0.06‡‡	106.2±7.2‡‡
P (ANOVA)	—	—	NS	<0.001	<0.001

Values are means±SE. * Sham operated, saline treated. ‡ Sham operated, methylprednisolone treated. § Infarcted with coronary ligation, saline treated. ‖ Infarcted with coronary ligation, methylprednisolone treated. ¶ Infarct region wall thickness or the equivalent region in shams. ** Significantly different than shams. ‡‡ Significantly different than shams and saline infarcts.

myocytes was seen within the infarct zone of either methylprednisolone- or saline-treated groups at 3 d. Fibroblasts with little or no collagen deposition were seen only at the margins of the infarct in both saline- and methylprednisolone-treated infarcts at 3 d. Masson's trichrome showed no significant collagen staining in either saline- or methylprednisolone-treated infarcts at 3 d. At 7 d, in saline-treated infarcts most of the infarct zone was replaced by granulation and connective tissue with only rare necrotic myocytes present. However, in methylprednisolone infarcts large numbers of "mummified" myocytes and myocytes undergoing necrotic degeneration were still present. Although inflammatory cells and fibroblasts were seen throughout the infarct region, the collagen content was significantly reduced in 7-d methylprednisolone-treated infarcts compared with saline-treated infarcts. No significant difference in interstitial edema as assessed histologically was noted between steroid- and saline-treated infarcts at either 3 or 7 d. However at 24 h methylprednisolone suppressed the edema in the infarct zone. Among saline-treated infarcts, the infarct region increased in water content from 71.5±0.6% (SE) of the total weight (in the noninfarcted septum) to 76.2±0.4% water content ($P < 0.01$). Methylprednisolone treatment completely suppressed this water weight gain of the infarct region (70.6±0.6% water in the septum vs. 71.1±0.8% water in the infarct region).

Table II. Quantitative Histologic Findings

	% Infarct area with inflammatory cells (±SE)	Collagen content grade			
		0	1+	2+	3+
3-d					
MI-SAL	62.44±2.4	6 (100%)	—	—	—
n	15				
MI-MP	14.28±3.7	6 (100%)	—	—	—
n	15				
P	<0.001	NS			
7-d					
MI-SAL	78.2±4.4	—	—	2 (33%)	4 (67%)
n	13				
MI-MP	56.3±6.2	1 (17%)	5 (83%)	—	—
n	15				
P	<0.001		<0.001 (Chi-square)		

Effect of methylprednisolone on severity of early expansion (3 d). At 3 d, there was no significant difference in wall thickness and cavity volume between sham groups (Table I). Fig. 5 show the differences in the data distributions for infarct wall thickness and cavity volume between the sham animals and both infarct groups. All animals with infarcts had significantly thinner infarct walls and larger left ventricular cavity volumes than sham-operated animals ($P < 0.001$). Furthermore, methylprednisolone-treated animals had significantly thinner infarcts than saline-treated animals with infarcts ($P < 0.001$). Although infarcts in both treated groups underwent thinning, only the more severely expanded infarcts underwent cavity dilatation. The severely expanded infarcts from the methylprednisolone-treated group had markedly larger left ventricular cavities than those from the saline-treated group. Therefore, methylprednisolone increased the severity of infarct expansion with no effect on infarct size or prevalence of expansion.

Effect of methylprednisolone on severity of expansion during the early healing phase (7 d). At 7 d, wall thickness and cavity volume were not significantly different between sham groups but were significantly different between sham and infarct groups ($P < 0.001$). For all infarcts, infarct wall thinning was greater at 7 d than at 3 d ($P < 0.001$). That is, infarct thinning progressed with time regardless of treatment. The 7-d methylprednisolone-treated infarcts had significantly thinner walls (0.68±0.06 mm vs. 1.07±0.09 mm; $P < 0.001$) and larger cavity volumes (106.2±7.2 mm^3 vs. 91.9±6.4 mm^3; $P < 0.01$) than saline-treated infarcts.

Because both saline- and methylprednisolone-treated infarcts showed progressive thinning and dilatation over time, the differences seen between the two groups at 7 d could be explained by two alternative hypotheses. First, methylprednisolone could promote early thinning and dilatation by 3 d without any further effects afterward. In this situation methylprednisolone infarcts would have thinner walls and larger cavities at 7 d because they

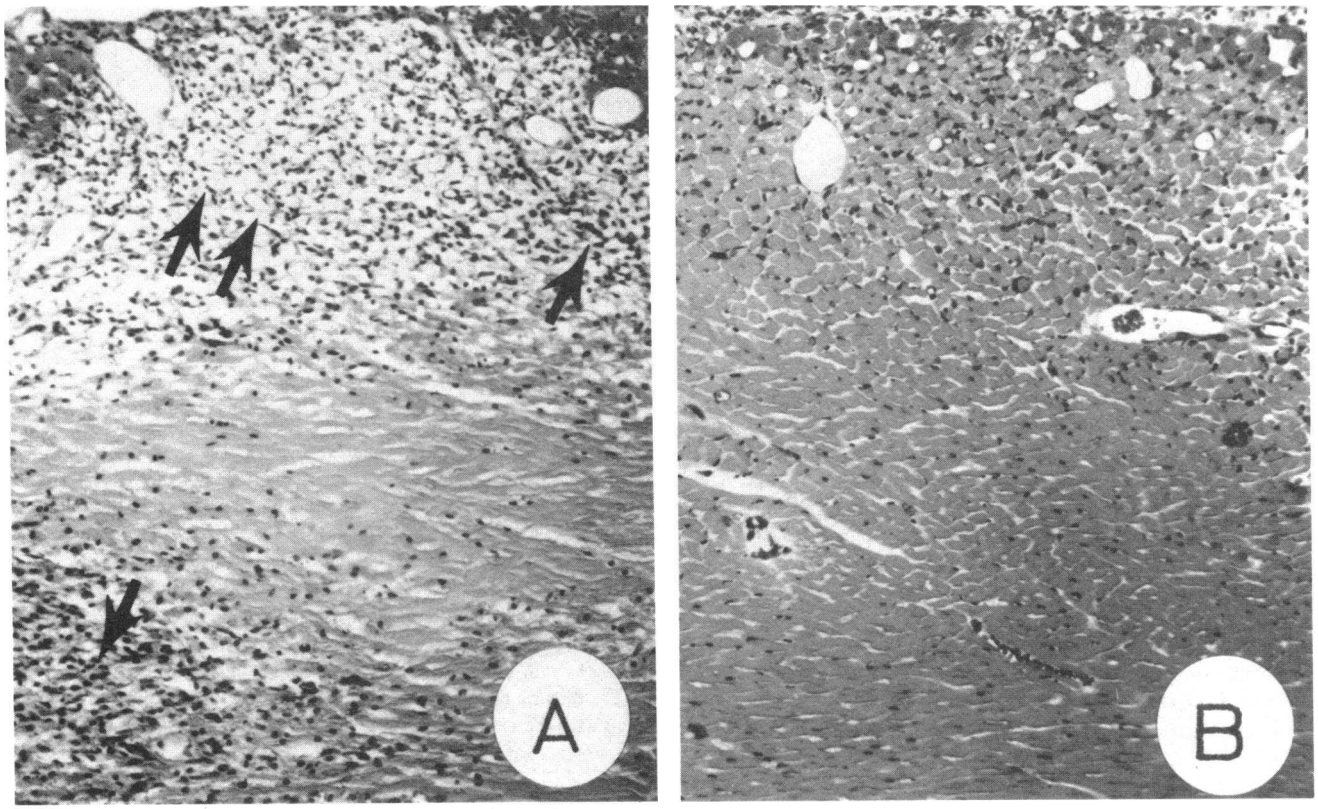

Figure 4. Histopathology of 3-d infarcts. (Hematoxylin-eosin × 100 original magnification). (*A*) Saline control infarct shows dense polymorphonuclear infiltrate (*single arrow*) with patches of myocyte dropout (*double arrows*). (*B*) Methylprednisolone-treated infarct shows suppression of polymorphonuclear cells with preservation of hypereosinophilic necrotic myocytes.

have undergone greater infarct expansion by 3 d (Figs. 6 *A* and 7 *A*). The differences between methylprednisolone infarcts at 3 d vs. 7 d would be due to the effect of time alone. Alternatively, methylprednisolone might exert an additional effect over time beyond 3 d (Figs. 6 *B* and 7 *B*). In this case, the differences between the groups at 7 d would be caused not only by the early methylprednisolone effects at 3 d and the time-related effects seen in both groups, but also by an interaction between treatment and time (beyond 3 d). An interaction between two variables is defined statistically as one where the effect of one variable (treatment) depends on the level of the other variable (time). Therefore, the interaction between treatment and time was tested using two-way analysis of variance. This analysis showed no significant interaction between treatment and time beyond 3 d (interaction term for infarct wall thickness, F ratio = 1.231; $P = 0.27$; interaction term for cavity volume, F ratio = 0.994; $P = 0.32$). The differences in wall thickness and cavity volume between methylprednisolone- and saline-treated infarcts at 7 d could be explained entirely by the differences between the groups already present at 3 d (Figs. 6 *C* and 7 *C*). Thus, methylprednisolone treatment did not cause incremental infarct wall thinning or cavity dilatation between 3 and 7 d.

Myocardial cell slippage vs. stretch. We next examined the possible cellular mechanisms by which methylprednisolone treatment causes early infarct wall thinning. Because no rupture of necrotic myocytes was seen in either group at 3 d, there are two other possible mechanisms by which steroids could promote early infarct expansion. Methylprednisolone might promote the stretch of necrotic myocytes in the infarct region. As the myocytes stretch, their fiber diameters decrease without change in number of cells across the wall resulting in infarct thinning and dilatation. Alternatively, methylprednisolone might increase the slippage of necrotic myocytes, whereby myocytes would slip past one another and rearrange so that fewer myocytes compose the thickness of the infarct wall, thereby resulting in more severe thinning and dilatation. To study this, counts of the number of myocytes across the infarct region were performed in a total of 16 hearts: 4 shams, 6 saline-treated, and 6 methylprednisolone-treated infarcts. The number of myocytes per unit width of wall thickness (myocyte density) was used as a measure of change in individual myocyte dimension. There was no significant difference in the regional myocyte density across the wall in hearts of the sham group or either infarct group. Myocyte density averaged 5.1±0.1 cells per 100 μ in sham hearts, and 5.9±0.04 cells per 100 μ within the infarct region of the saline-treated infarct group, a difference of 16%. We have previously shown that this increase in myocyte density among hearts with infarcts can be explained by a reduction in myocyte cell diameter (myocyte stretch) (11). However, this degree of change in myocyte density does not explain the degree of thinning seen between sham and infarct hearts. Mean infarct wall thickness was 2.7±0.2 mm in the sham group and 1.2±0.1 mm in the saline-treated infarct group a difference of 56%. The 16% increase in myocyte density would account for a ~ 13% reduction in wall thickness. Moreover, no significant difference in myocyte density between methylprednisolone (6.0±0.04) and saline (5.9±0.04) -treated 3-day infarcts was seen. Thus, the difference in infarct wall thinning between methylprednisolone- and saline-treated infarcts by day 3 must occur by a mechanism other than cell stretch. In Fig. 8, mean transmural myocyte number is plotted against in-

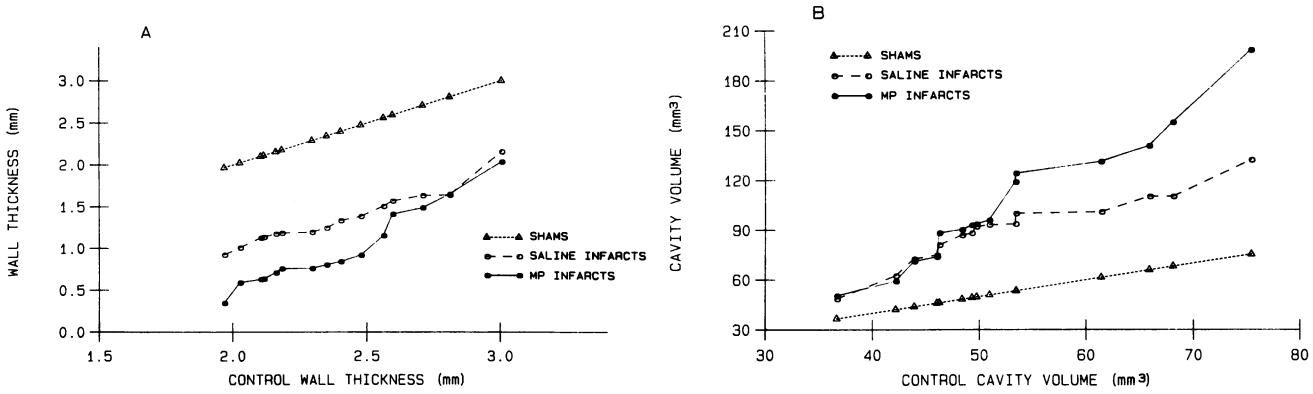

Figure 5. Empirical quantile–quantile plots of data for infarct region wall thickness and cavity volume in 3-d hearts. The plots are constructed by ordering the data from each group from lowest to highest value. The ranked values called quantiles of the sham group are plotted along the x axis. The quantiles of all groups are plotted on the y axis. Because the sham quantiles are plotted on both axes, they form the line $y = x$. If the distribution of values for each infarct group was identical to the sham group, all points would fall exactly on the line $y = x$. Departures from this line give information about how the distribution of each infarct group differs from the sham group and from one another (8). (*A*) Both infarct groups had significantly thinner walls than the sham group. The lowest value in each infarct group was lower than the lowest sham value. The next lowest values in the infarct groups were lower than the next lowest in the sham group. A similar comparison between each successive value of each group through to the highest value can be made and in each case the infarct values are less than shams. Also it can be seen that the methylprednisolone group is skewed toward having thinner walls than the saline-treated group. The thinnest infarcts in the methylprednisolone group are much thinner than the thinnest infarct in the saline-treated group. (*B*) Both infarct groups had significantly larger cavities than the sham group. As with wall thickness, a similar skewing toward extreme values is seen with cavity volumes in the methylprednisolone group compared with the saline group.

farct region wall thickness for control hearts and for methylprednisolone- and saline-treated infarct hearts at 3 d. For all hearts, wall thickness in the infarct region correlated well with transmural myocyte number ($r = 0.989$; $P < 0.001$). This correlation also held when infarcts alone were analyzed ($r = 0.982$; $P < 0.001$). Among the hearts with infarcts, the number of cells across the wall at the site of the infarct was 74.2±6.1 cells in the saline-treated hearts, and 56.3±2.1 in the steroid-treated hearts. From this difference in the number of cells across wall, the expected infarct wall thickness in the methylprednisolone-treated animals would be 0.90 mm. This estimate is extremely close to the actual mean value of 0.88 mm. Therefore, the decrease in the thickness of the infarct wall observed in 3-d methylprednisolone-treated animals compared with saline-treated animals can be completely accounted for by increased myocardial cell slippage.

With regard to myocyte branching we felt our counting method adequately recognized branching myocytes both in cross-sectional and longitudinal planes. However even if we overestimated total cell counts through failure to recognize branched myocytes, the differences in cell counts between the groups would not be affected because the branching pattern of myocytes is presumably the same in the control and treatment groups. It would mean however that the determined cell number per 100 μ wall thickness and the number of myocytes across the wall are overestimates in absolute terms.

Discussion

To study possible early mechanisms of steroid-induced scar thinning and dilatation, we used the rat model of acute myocardial infarction. The rat model has been useful in the study of infarct expansion (11) and has been used to study steroid-induced structural changes in the infarct region (1). Methylprednisolone was administered in doses similar to those used by other investigators (1, 3, 4). Animals were sacrificed to assess the severity of expansion both before (3 d) and after (7 d) early collagen deposition begins. Histochemical studies to confirm these assumptions concerning collagen deposition were performed. We noted no significant collagen staining with Masson's trichrome in the infarct zone of either methylprednisolone- or saline-treated animals at 3 d, suggesting that no significant new collagen deposition had occurred by this time. These findings are in accord with those of Fishbein et al. (13) who also found scant collagen staining by Masson's trichrome in the 3-d-old rat infarct. Therefore effects of steroids on infarct structure by 3 d is likely, independent of effects on collagen deposition.

At 7 d, our histochemical results also confirmed findings by Kloner et al. (4) that methylprednisolone-treated animals undergoing infarction have markedly reduced collagen staining in the infarct zone compared with control animals with infarcts. Thus the 7-d time period is appropriate to study the influence of delayed collagen deposition on early aneurysmal dilatation.

The results of this study show that high-dose methylprednisolone administered within the first 24 h after infarction did not change the prevalence of infarcts that underwent expansion. By 3 d, however, methylprednisolone markedly aggravated the severity of early infarct expansion without any effect on infarct size. That is, in this study, methylprednisolone did not cause expansion in transmural infarcts that would otherwise not expand. But among infarcts that did expand, expansion was more severe in methylprednisolone-treated hearts. Although methylprednisolone delayed collagen deposition and healing in 7-d infarcts, it caused no further infarct thinning or cavity dilatation beyond the initial 3 d. Therefore, enhanced early infarct expansion before the beginning of collagen deposition appears to be the major component of steroid-induced aneurysmal dilatation. This implies that a delay in collagen deposition leading to a prolonged phase during which necrotic myocardium might be more susceptible to thinning and dilatation is not the explanation of steroid-induced aneurysmal dilatation. Our results also suggest

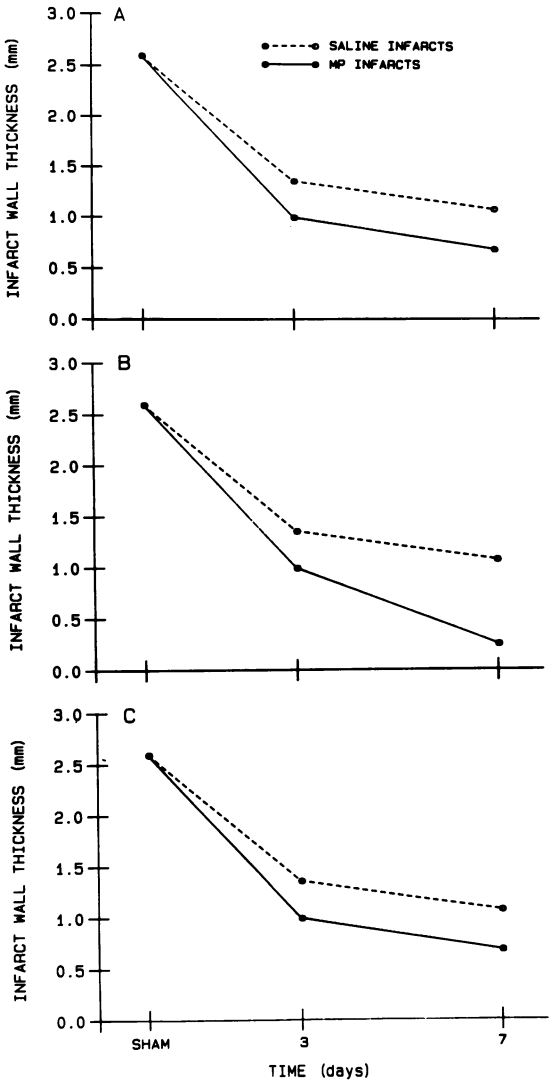

Figure 6. Hypothetical (*A* and *B*) and actual (*C*) plots showing change in wall thickness over time according to treatment group. In the first hypothesis (*A*) methylprednisolone promotes thinning only within the first 3 d. At 7 d, the differences between control and treatment groups are the same as the differences seen at 3 d. After 3 d further thinning is due to the effect of time alone (no interactive effect). In the second hypothesis (*B*) methylprednisolone causes incremental thinning over time. The differences between control and treatment groups at 7 d are greater than the differences seen at 3 d due to an interaction between methylprednisolone treatment and time. After 3 d, methylprednisolone continues to promote additional thinning over time. (*C*) Actual mean infarct wall thickness plotted over time for both treatment groups. This plot is similar to (*A*). Two-way analysis of variance showed that differences between saline- and methylprednisolone-treated infarcts at 7 d after infarction could be explained entirely by the differences already seen at 3 d. Thus methylprednisolone treatment caused no further infarct thinning beyond 3 d and all further thinning thereafter is caused by time-related effects alone. Overall SD, 0.41 mm.

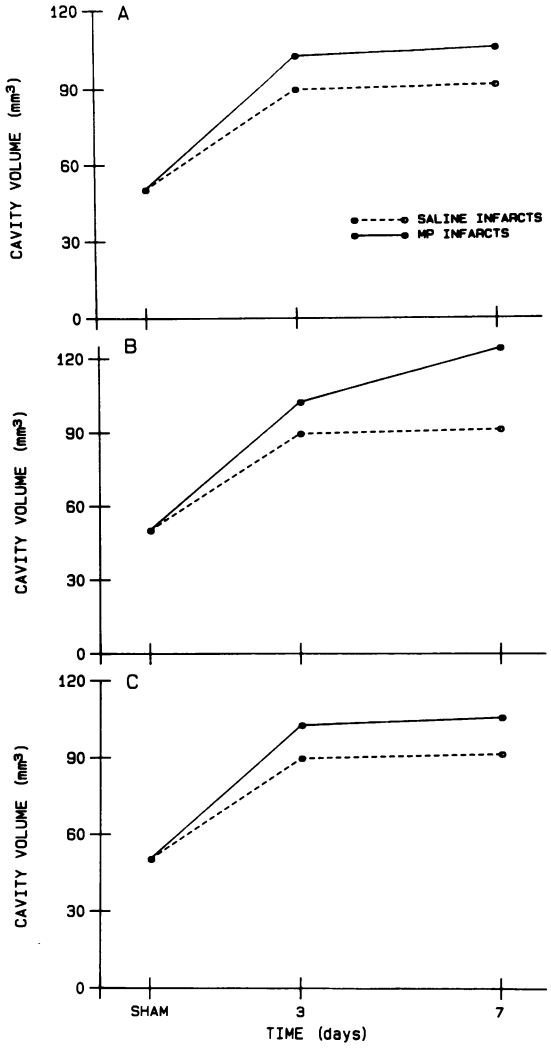

Figure 7. Hypothetical (*A* and *B*) and actual (*C*) plots showing change in cavity volume over time according to treatment group. The first hypothesis (*A*) shows no interactive effect of time and treatment beyond 3 d. The second hypothesis (*B*) shows an interactive effect of treatment and time to 7 d. (*C*) Actual mean cavity volume plotted over time for both treatment groups. This plot is similar to (*A*), indicating that there is no effect of methylprednisolone treatment on cavity dilatation beyond 3 d. Overall SD, 31.9 mm³.

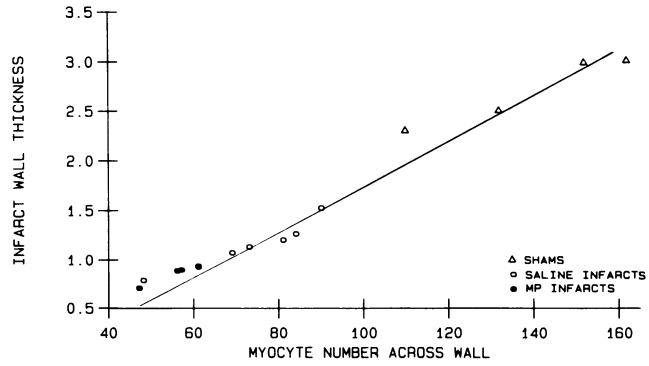

Figure 8. Correlation of infarct region wall thickness vs. number of myocytes across the infarcted wall in hearts sacrificed at 3 d. For sham and infarct hearts regardless of treatment, wall thickness exhibits a high degree of correlation with number of myocytes across the wall ($r = 0.99$; $P < 0.001$). This correlation also held when only the infarct hearts were analyzed ($r = 0.98$; $P < 0.001$).

that scar stretch is not the primary mechanism by which the administration of steroids early after transmural myocardial infarction causes accentuated aneurysm formation. The findings focus on the first 3 d after transmural myocardial infarction as the critical time during which steroids increase infarct expansion. At this time necrotic myocytes, inflammatory cells, and edema

dominate the infarct. Steroid action on the myocardium at this early time likely relates to its effect on these structural elements within the infarct region.

We therefore examined possible cellular mechanisms by which steroids worsen early infarct expansion. Our results show that the entire additional decrease in infarct thickness seen with steroid therapy could be explained by a decrease in transmural myocyte number without evidence of myocyte stretch or myocyte rupture. This implies that infarct thinning occurs by myocyte slippage. These results agree with the findings of other investigators. By counting the number of myocytes across the wall and cross-sectional area of myocytes, Linzbach et al. (9) showed that ventricular dilatation due to chronic volume overload could not be explained by myocyte stretch but rather by a sliding displacement or slippage of heart layers leading to a decrease in the number of muscle layers in the ventricular wall. Spotnitz et al. (10) also reported a strong correlation between changes in wall thickness and changes in transmural cell number but not cell density when studying rat hearts postmortem, passively distended with varying left ventricular volume. These findings suggested that most of the wall thinning is not mediated by individual myocyte stretch but rather by a change in the geometric arrangement of groups of myocytes perhaps by a sliding between myocyte bundles (slippage). In addition, we have previously shown that the structural changes of infarct expansion are for the most part mediated by the slippage of necrotic myocytes (decrease in the number of cell across the wall) both within and outside the infarct zone (15).

How do steroids lead to decreased numbers of myocardial fibers across the wall? There are two possibilities. First, steroids might alter the volume load and augment wall stress on the expanded ventricle, resulting in layers of cells slipping apart with aggravated expansion. Although we did not measure hemodynamics, several studies have shown no significant hemodynamic change when high-dose methylprednisolone is administered acutely after infarction (20–23). Thus, it is unlikely that steroids alter load significantly enough to independently worsen expansion.

A second possibility is that steroids primarily alter the material properties of the infarcted myocardium leading to worsening cell slippage and further expansion. Caulfield and Borg (24) have suggested that the viscoelastic properties of myocardium are determined by a weblike network of loose collagen around bundles of myocytes. These loose collagen connections between myocyte bundles could permit the slippage and rearrangement of these bundles as seen in acute cardiac dilatation.

Immediately after coronary occlusion, the ischemic segment becomes noncontractile and systolic bulging is seen (25). Forrester et al. (7) hypothesized that systolic bulging might be caused by passive stretch and ultimate anatomic disruption of noncontractile myocardial fibers and could account for the dilatation of the ischemic segment. However, our analysis showed no significant myocyte stretch and no evidence of myocardial fiber rupture. We postulate instead that the degree to which ischemic myocyte bundles slip determines the extent to which the ischemic zone thins and dilates. Early systolic bulging of ischemic myocardium might then represent the enhanced slippage of myocyte bundles to the extent allowed by the loose collagen connections between these bundles.

Systolic bulging of the infarct zone partially but not completely resolves progressively after the first few hours of ischemia and infarction (26). Some hearts, however, particularly those having sustained transmural infarction, remain with a fixed increase in diastolic infarct segment length. This persistent early segmental dilatation of the infarct zone represents infarct expansion. On the cellular level this might represent irreversible myocyte bundle slippage due to overstretch or rupture of the loose collagen connections between these myocyte bundles. In hearts that show partial resolution of systolic bulging, in the early hours after infarction, several investigators have reported a concomitant increase in the local diastolic stiffness of the infarct zone (26, 27). This stiffness continues to increase over the next few days before collagen deposition begins. Thus, this early increase in stiffness that anatomically might represent the resistance to myocyte bundle slippage may be important in limiting the extent of infarct expansion.

Changes in the material properties of the infarcted myocardium could account for the early increase in infarct wall stiffness. Reimer and Jennings (28) have reported that in the early hours after coronary occlusion the infarct region gains weight by the accumulation of edema. Over the next few days the infarct region gains further weight by the addition of cellular elements. The development of edema and subsequent inflammatory cell infiltrate might be the anatomic elements that explain the early increase in stiffness measured in the infarct zone. On the cellular level these components of the interstitial space could buttress the loose collagen network and limit the extent of necrotic myocyte bundle slippage and infarct expansion. Steroids have been shown to suppress tissue edema in the early hours after inflammation (29, 30). These effects are dose dependent and last for only several hours after they are administered (30). We have shown that high-dose methylprednisolone completely suppressed the edema in 24-h infarcts. In addition, inflammatory cell infiltration was markedly suppressed at 3 d by methylprednisolone. One could surmise therefore that reduced early edema and decreased inflammatory cell infiltration associated with high-dose steroids could promote myocyte bundle slippage and thus worsen infarct expansion. These results support findings by Hammerman et al. (31) who showed that indomethacin, a nonsteroidal antiinflammatory agent, promoted early infarct expansion in the dog. These authors postulated that suppression of early edema by indomethacin promotes expansion. However, because they studied 7-d infarcts they were unable to detect the differences in edema between treatment and control groups that we demonstrated at 24 h.

A possible limitation of our results and assumptions concerning the mechanisms of enhanced infarct expansion by steroids relates to our method of quantifying collagen in the infarct zone. We recognize that Masson's trichrome stain may not be sensitive enough to detect small amounts of collagen laid down by the rare fibroblasts seen at the margins of 3-d-old infarcts. Thus, it remains to be determined by using more sensitive methods such as hydroxyproline assays whether small amounts of collagen are laid down at the margins or the infarct and whether early steroid treatment affects this deposition. Nevertheless, because at 3 d no fibroblasts are seen throughout most of the infarct zone, including the thinnest central region, we believe that an effect by steroids on early collagen deposition, even if demonstrable, would not significantly alter the severity of infarct expansion at this time.

Likewise in 7-d infarcts Masson trichrome stain was again used to assess infarct region collagen content. However, the differences in collagen staining between steroid- and saline-treated animals with 7-d-old infarcts are so dramatic that it is unlikely

that these results would be substantially changed by utilizing more precise quantitative methods of assessing collagen content in the infarct zone.

In conclusion, this study shows that high-dose steroids lead to infarct thinning and cavity dilatation within 3 d of infarction and that there is no further effect on infarct expansion after this time. We also showed that steroids promote expansion by enhancing the slippage of necrotic myocytes. Furthermore, steroids were shown to reduce edema content and the inflammatory cell infiltration within the infarct zone during the time when expansion occurs. The steroid enhanced slippage of necrotic myocytes focuses attention on the potential importance of the connections between myocyte bundles and also the extracellular matrix in determining the severity of expansion. Edema and inflammatory cell infiltration may be the elements of this extracellular matrix that limit myocyte bundle slippage and hence infarct expansion independent of steroid treatment. Further studies are needed to test these hypotheses and further clarify the mechanisms that determine the severity of expansion.

Acknowledgments

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