anterior descending artery. Thus, further experience with CCU patients with cardiac chest pain and normal conventional studies for myocardial necrosis seems needed before specific conclusions are drawn.

References


Mummification of the Infarcted Myocardium by High Dose Corticosteroids

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SUMMARY There is evidence that glucocorticoids reduce infarct size but their use in myocardial infarction remains controversial because of their potential adverse effects on healing of the infarct. To investigate the healing process, rats received either four parenteral doses of 50 mg/kg of methylprednisolone (MP) or saline 5 min, 3, 6 and 24 hr after coronary occlusion and their hearts were examined by light and electron microscopy 48 hr and seven days after occlusion. At 48 hr, in five untreated rats, only 12 ± 7% of injured myocytes showed the persistence of striations and a relatively intact sarcolemma despite loss of nuclei and hence appeared "mummified" whereas in six MP-treated rats 72 ± 8% of myocytes exhibited this appearance (P < 0.001). In treated rats there were fewer phagocytes than in controls. At seven days, in seven MP rats, mummified cells were still more prominent than in five untreated rats and there were fewer phagocytes and less collagen. In conclusion, high dose MP delays the inflammatory process and retards the disintegration of necrotic myocytes, resulting in impaired healing.

THE USE OF GLUCOCORTICOIDs to decrease myocardial infarct size still remains controversial. A number of experimental studies have shown that steroids decrease myocardial infarct size,1,4 while another has failed to show this.4 Recently, in a rat model of myocardial infarction produced by coronary occlusion it was shown that glucocorticoids decreased infarct size when the hearts were examined after both 48 hr and 21 days of coronary occlusion, but high doses of methylprednisolone (MP) resulted in significantly thinner scars after 21 days of occlusion.8 Accordingly, this study was done to determine whether there are light and electron microscopic changes induced by high-dose MP treatment which can explain the poor scar formation.

Methods

Albino Sprague-Dawley male rats weighing approximately 250–300 g were lightly anesthetized with ether. Their left main coronary artery was occluded, as described in detail previously.8 Briefly, thoracotomies were performed in the fifth or sixth left intercostal space and the heart was extruded from the thoracic cavity. The left main coronary artery was ligated approximately 2 mm from its origin by a 4-0 silk suture on anatraumatic needle. Follow-
ing occlusion, the heart was placed back into the thoracic cavity and the rats were allowed to recover. Rats received either MP (50 mg/kg intravenously 5 min after occlusion and intramuscularly at 3 hr, 6 hr and 24 hr after occlusion) or saline. They were sacrificed either 48 hr (five untreated and six MP-treated rats) or 7 days after the occlusion (seven untreated and five MP-treated rats). Just prior to excision of the heart, carbon black (1 mg/kg) was injected intravenously to determine whether a successful occlusion had occurred and to distinguish perfused tissue (black areas) from tissue to which coronary flow was reduced (pink areas).

Transmural sections for electron microscopy were obtained from both the midventricular septum, which is well perfused in this model, and from three sites on the non-perfused anterior ventricular wall. The epicardium and endocardium were trimmed from the muscle. Tissue from each site was cut into six or seven 1–2 mm cubes under cold 1% osmic acid and allowed to fix for one hour in the osmic acid. Two randomly chosen cubes from each of the four sites were then dehydrated in graded alcohol, passed through propylene oxide and a 1:1 mixture of propylene oxide plus Epon 812 overnight prior to embedding in Epon 812. Sections approximately 1 micron thick (i.e., thick sections) were stained with toluidine blue for light microscopy. Thin sections were mounted on plain copper grids and stained with aqueous uranyl acetate and lead citrate and examined on a Philips 201 electron microscope. The groups were compared qualitatively using the thin sections and semiquantitatively using thick sections.

From each thick section an estimate of the percentage of injured myocardial cells in the nonperfused zone, showing either necrotic breakdown of cells (with loss of nuclei and striations and sarcolemmal membrane) or “mummification” of cells (with preservation of striations and sarcolemmal membrane but degeneration of nuclei) was made. An overall mean percentage of injured cells showing either necrotic breakdown or mummification was made by averaging the percentages of cell types from each thick section. The degree of round cell, macrophage and fibroblast infiltration was graded 0-4 (0 = absent; 1 = occasionally present; 2 = more numerous; 3 = very frequent; 4 = present in large sheets). Collagen infiltration was graded 0-4 (0 = no collagen deposition; 1 = occasional thin bundles (< 5 µ thick) of collagen; 2 = occasional thin plus occasional thick bundles (> 5 µ thick); 3 = numerous thin plus occasional thick bundles of collagen; 4 = numerous thick bundles or wide sheets of collagen. A mean grade (weighted average 0-4 or 0-3) was calculated for each rat and compared between groups.

Results

I. Nonischemic Myocardium

Myocardial tissue from the nonischemic midventricular septum (which always was perfused with carbon black) showed normal myocardial cells in both untreated and MP-treated groups. No differences in myocyte structure or collagen deposition between the two groups was noted.

Figure 1. a) Necrotic breakdown of myocardial cells from the center of an infarct after 48 hours of coronary occlusion in an untreated cell. The dark dots represent mitochondria. Cell architecture is disrupted and individual myofibers and striations cannot be distinguished. Light microscopy; toluidine-blue stained × 1380. b) Mummified myocardial cells from the center of an infarct after 48 hours of coronary occlusion in a rat which received high dose methylprednisolone. Note the preservation of striations with wide I bands (arrow). Clear areas adjacent to the intercalated discs are present (double arrows); degenerated nucleus (d). Light microscopy; toluidine-blue stained × 1380.
Figure 2.  
a) Electron micrograph of a 48-hour-old infarct in an untreated rat. The myocardial cell (lower right side of picture) is disrupted with loss of sarcomere and sarcolemmal integrity. Mitochondria (arrows) are condensed and contain large amorphous densities. Degenerated myofilaments (my) are present. A macrophage (m) appears to be actively phagocytizing the myocardial cell and contains damaged mitochondria (arrow heads) which are presumably from a dead myocardial cell. It also contains large lipid droplets. × 11,200.  
b) Myocardial cell from an infarct after 48 hours of coronary occlusion in a methylprednisolone-treated rat. Despite preservation of sarcomeres and myofilaments, the nucleus is degenerated as manifest by loss of nuclear membrane integrity (arrows) and degenerated chromatin. Mitochondria (m) contain amorphous dense bodies. Wide I bands are present. × 14,000.  
c) Mummified myocardial cell from an infarct after 48 hours of occlusion in a methylprednisolone-treated rat. Mitochondria (m) are condensed and contain amorphous matrix densities. Intermyofibrillar edema (e) is present. Myofibrils, Z bands (Z) and portions of the sarcolemmal membrane can still be recognized. A large clear space is adjacent to the intercalated disc giving the appearance of a fracture (f). × 14,000.
II. Ischemic Myocardium

Forty-eight Hour Infarcts

At 48 hours after coronary occlusion in untreated rats, examination by light microscopy of 1 micron sections revealed that most necrotic muscle cells showed loss of striations, sarcolemmal disruption, and they were being actively phagocytized (fig. 1a). Electron microscopy confirmed these findings. In addition, the individual membranes of mitochondria often appeared dense, contained large amorphous densities and mitochondrial cristae were compressed. The sarcolemmal membrane, the cell nucleus, Z bands, and individual myofibrils were not recognizable. A second type of injured myocardial cell was present in smaller numbers. These cells had persistent striations but absent nuclei. They were present only in the center of the infarct and were never seen at the infarct edge. Microscopic foci of hemorrhage and occasional thrombotic vessels were present. Large macrophages were numerous and contained debris from the necrotic muscle cells, including damaged mitochondria and large lipid droplets (fig. 2a). Besides the large macrophages, a profuse pleomorphic population of mononuclear cells was present which included cells with large nuclei and small amounts of cytoplasm (mostly lymphocytes and some endothelial cells) and cells with large nuclei and large amounts of cytoplasm (macrophages, endothelial cells and undifferentiated cells). Neutrophils and fibroblasts were infrequent.

In MP-treated rats, few myocardial cells showed loss of striations and disruption when examined by light microscopy. Instead, the second type of injured myocardial cell which was seen only in the center of the infarct in untreated rats was more prominent. These cells were present in large sheets at both the center and the edge of the infarct. They showed striations, still had intact portions of their sarcolemmal membranes and clearly defined sarcomeres but appeared injured as manifest by wide I bands, large clear spaces adjacent to the intercalated discs and degenerated or absent nuclei (fig. 1b).

By electron microscopy, these cells contained Z bands and myofibrils but their nuclei were absent or disrupted. Their mitochondria were dense with degenerated and compressed cristae and they contained amorphous dense bodies (fig. 2b, c). Wide I bands were present as well as intermyofibrillar edema. The sarcolemmal membrane often could be identified. In addition, these cells often had wide apparently empty spaces adjacent to their intercalated discs. Since these cells exhibited ultrastructural evidence of irreversible injury including degenerated nuclei, but were architecturally well preserved after two days of coronary occlusion, they may be referred to as mummified. Besides changes in myocardial cells, infiltration of macrophages and other mononuclear cells was significantly less prominent in MP-treated rats.

In the untreated group 88 ± 7% of damaged myocardial cells showed necrotic breakdown without striations whereas only 28 ± 8% (P < 0.001) in the glucocorticoid-treated group showed necrotic breakdown. In contrast, the remainder, i.e., 72 ± 8% of damaged myocardial cells, were mummified in MP-treated rats compared to only 12 ± 7% in the untreated group (P < 0.001). Further semiquantitative results are shown in table I and confirm that round cell and macrophage infiltration was greater in the untreated animals.

Seven Day Infarcts

In the untreated rats, there were few intact myocardial cells present in the ischemic anterior ventricular wall seven days after coronary occlusion. Much of the wall was replaced by granulation and connective tissue and hence fibroblasts and collagen were the predominant findings by both light and electron microscopy (figs. 3a, 4a). The myocardial cells which were present usually showed necrotic breakdown (figs. 3a, 4b) or were normal, but a few mummified cells persisted. There were several other prominent morphologic features at seven days including mononuclear round cell infiltration and vascular proliferation (fig. 4c). Large macrophages were present (fig. 4d) but less numerous than at 48 hours.

In MP-treated animals, examination of 1µ thick sections by light microscopy revealed that large sheets of mummified cells still were present (figs. 3b, 5). These cells still contained striations but nuclei had dropped out leaving large lacunae in the cells. However, there were more myocardial cells showing necrotic breakdown and being phagocytized and there was more round cell infiltration at one week than had been present at 48 hours in MP-treated rats. Spaces presumably representing interstitial edema were more evident in MP-treated rats compared to an interstitium with more abundant collagen in untreated rats. By electron microscopy, the seven day mummified cells differed from the 48 hr mummified cells in that at 48 hours both Z bands and myofilaments were present, whereas at seven days Z bands were still present but individual myofilaments could not be distinguished and appeared as granular debris (fig. 5).

<table>
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<tr>
<th>Table 1. Cellular Infiltration in Infarcts (Mean Scores 0-4)</th>
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<tr>
<td><strong>Mononuclear cells</strong></td>
</tr>
<tr>
<td>Large nucleus, small cytoplasm</td>
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<tr>
<td><strong>48 Hours</strong></td>
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<tr>
<td>Untreated</td>
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The number of dead cells in the infarct remaining in untreated rats at one week made up less than approximately 10% of the infarcted tissue. Those that were present demonstrated necrotic breakdown. Mummified cells were still present in large sheets in the MP-group, making up 52 ± 15% of the damaged cells while 48 ± 15% showed necrotic breakdown. The degree of interstitial collagen infiltration was smaller in the MP-treated (0.9 ± 0.3) compared to untreated rats (2.3 ± 0.3, P < 0.01) (table 1). Although the number of fibroblasts appeared smaller in MP rats, the difference was not statistically significant (table 1).

At seven days, mononuclear cells with small amounts of cytoplasm were more frequent in the MP group; whereas those with large amounts of cytoplasm were more frequent in the untreated group (table 1).

Discussion

The results of studies concerning the effect of glucocorticoids on myocardial infarct size in experimental animals have been controversial in that some have shown a beneficial effect while others have not. Similarly, some clinical studies have shown reduced mortality and infarct size in patients with acute myocardial infarction treated with glucocorticoids while others have not confirmed a beneficial effect of glucocorticoids. Roberts et al. found that multiple dose MP actually increased infarct size and the incidence of ventricular arrhythmia.

One theory concerning the deleterious effects of steroids in acute myocardial infarction is the phenomenon of delayed or poor healing. In the rat model of myocardial infarction, this phenomenon appears to be dose dependent. Rats subjected to a standardized coronary occlusion which received either intravenous hydrocortisone (50 mg/kg) or one intravenous injection (50 mg/kg) of MP showed smaller infarcts at 48 hours and three weeks than did untreated controls and these steroid treated groups did not show thinning of myocardial scars, while rats which received four doses of MP (50 mg/kg) also showed smaller infarcts at 48 hours and three weeks but significantly thinner scars.

In the rat model of infarction myocardial necrosis peaks between 24-48 hours after coronary occlusion. By routine light microscopy, most necrotic cells exhibit increased eosinophilia, loss of striations and increased granularity of their cytoplasm and nuclear pyknosis or karyolysis. Infiltration of neutrophils is notably sparse in rats compared to human infarcts and peaks at 48 hours. Infiltration of lymphocytes and mononuclear phagocytes becomes prominent by 48-72 hours, peaks at seven days and then gradually decreases until at 21 days only occasional mononuclear cells are present. At the same time that myocardial cells are undergoing necrosis and being phagocytized, fibroblasts are being mobilized and collagen is deposited. This begins as early as three days postinfarction. Between the fourth and twenty-first day there is continued prominence of fibroblasts with increased deposition of collagen until the entire necrotic zone is replaced. The process is similar in humans, although it is slower, so that healing usually is complete in rats by three weeks compared to 4-8 weeks in man; this difference may be related to the rat’s higher metabolic rate and smaller body size.
ADVERSE EFFECTS OF GLUCOCORTICOIDS/Kloner et al.

FIGURE 4. a) Myocardial infarction in an untreated rat following seven days of coronary occlusion. Evidence of healing is present as manifest by the large fibroblast (f) in the center of the picture and the abundant collagen (c) surrounding it. A portion of a cell in the upper left corner contains a large amount of rough endoplasmic reticulum, suggesting that protein synthesis is active. ×8,400. b) A degenerated myocardial cell in an untreated rat following one week of occlusion. No evidence of myofibrils or striations is present. Mitochondria are condensed and contain large amorphous dense bodies. ×15,000. c) Vascular proliferation in a one-week-old infarct from an untreated rat. New capillaries are being formed in the interstitium and new endothelial cells can be recognized by their large nuclei. ×6,000. d) Large macrophages from a one-week-old untreated infarct. Note that they contain large lipid droplets (1). ×5,000.
The present study examined the morphologic aspects of the delayed healing phenomenon in this rat model. The most striking finding was the presence of large sheets of dead myocytes with nuclear degeneration but with preservation of striations and membranes. These apparently well-preserved dead cells, or mummified cells, were numerous at both 48 hours and seven days in high dose MP-treated rats and significantly less common in untreated animals. At 48 hours of ischemia, both Z bands (striations) and myofilaments were present in these cells. At seven days, these cells still showed striations due to Z bands but individual myofilaments were absent.

Bulkley and Roberts described a patient who received large doses of corticosteroids for Dressler's syndrome following a myocardial infarction; after 63 days of survival the patient still had necrotic myocardial cells present in the infarct. It has been noted that humans receiving large doses of steroids during acute myocardial infarction may be more prone to develop aneurysms or ventricular rupture.

Hence, while steroids may preserve ischemic myocardial cells and decrease infarct size as shown in some studies, multiple doses of steroids also preserve dead myocardial cells and hence prevent the normal breakdown process and healing of the infarct. Whether the prevention of myocardial cell breakdown is due to a direct effect of steroids on the myocardial cell (such as by stabilizing lysosomal and other membranes) or is due to the effects of steroids on decreasing inflammatory cell infiltration and phagocytosis cannot be distinguished from this study.

While it is difficult to quantitate collagen deposition without biochemical measurements of tensile strength, we did attempt to determine by morphologic examination whether there were differences between treated and untreated rats. At seven days after coronary occlusion collagen deposition is not as prominent as at 21 days when scar formation in the rat heart is complete; yet even at this early stage of healing, collagenization appeared suppressed by MP.

In untreated rats, 48 hours after occlusion, a heterogeneous
population of mononuclear cells consisting of lymphocytes, macrophages (with and without cytoplasmic debris), endothelial cells, and undifferentiated cells was observed in the area of infarction. At this time, in MP treated rats, all of these cell types were less frequent. By seven days there was no longer a difference in the number of macrophages containing debris. However, cells with large nuclei and large amounts of cytoplasm (endothelial cells, macrophages without debris, and undifferentiated cells) were still less frequent. Cells with large nuclei and scant cytoplasm, most of which were small lymphocytes, were more frequent. The reasons for this delayed increase in lymphocytes is unknown but may represent recovery from an earlier retarding effect on the influx of these cells into the injured area by MP.

Other anti-inflammatory drugs, including cobra venom factor and ibuprofen have been demonstrated to decrease myocardial infarct size in the same rat model. Review of the histologic features of infarcts at 48 hours and 21 days by routine light microscopy in rats treated with these drugs failed to reveal enhancement of mummification or thinning of scars (unpublished observations). Roughly 10–20% of myocardial cells in infarcts from untreated, cobra venom factor-treated and ibuprofen-treated rats showed mummification in contrast to high dose MP which results in more prominent mummification. Thus, the structural changes produced by MP which result in thinner scars appear to be unique for high doses of glucocorticoids and not due to a simple anti-inflammatory effect per se. Similarly non-anti-inflammatory agents such as hyaluronidase and propranolol which reduce infarct size also have been shown not to delay healing and/or to impair scar formation.

In conclusion, when high multiple-doses of MP were administered to rats with acute coronary occlusion, delayed healing of the infarct occurred. This delayed healing appeared largely due to the presence of mummified (dead, but architecturally well preserved) and phagocytosed myocardial cells in the infarct. The high dose steroids also resulted in an early reduction of inflammatory cell reaction and later in the reduction of collagen deposition. These early morphologic alterations induced by high dose steroids most likely contributed to the presence of thinner scars three weeks following coronary occlusion.

Acknowledgment

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