Myocardial Hydroxyproline Reduced by Early Administration of Methylprednisolone or Ibuprofen to Rabbits with Radiation-induced Heart Disease

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SUMMARY The ability of methylprednisolone (MP) and ibuprofen (IB) to reduce the severity of the late state of radiation-induced heart disease was assessed in 57 New Zealand white rabbits. Before and shortly after cardiac irradiation, 15 rabbits received i.v. MP, 30 mg/kg twice daily for 3 days, and 15 others received IB, 12.5 mg/kg twice daily for 2 days. No drug was administered to 14 irradiated rabbits, and neither irradiation nor drugs were administered to 13 rabbits that served as controls. All 15 rabbits treated with MP and 13 of the 15 treated with IB lived for 100 days. Only seven of the untreated, irradiated rabbits lived that long. Longevity of each treated group of rabbits was better (p < 0.01 and 0.05) than that of the untreated, irradiated rabbits. Surviving rabbits were killed 100 days after irradiation. Pericarditis (p < 0.05) and pericardial effusion (p < 0.01) were less frequent in the treated, irradiated groups than in the untreated, irradiated rabbits. At least some rabbits in each irradiated group had microscopic evidence of myocardial fibrosis. The fibrosis was quantitated by determination of myocardial hydroxyproline concentrations (MHP). MHP concentration in the untreated, irradiated rabbits was greater than in those treated with MP (p < 0.05) or IB (p < 0.01) and in the untreated, unirradiated rabbits (p < 0.01). Early administration of MP or IB retarded the development of myocardial fibrosis, pericarditis and pericardial effusion, and improved survival in this experimental model of radiation-induced heart disease.

CLINICALLY SIGNIFICANT cardiac disease develops in a small percentage of patients who have received incidental cardiac irradiation during radiotherapy for chest malignancies. The pathology of radiation-induced heart disease has been well characterized in an experimental rabbit model. These rabbits develop late cardiac lesions identical to those in man months after radiation. Myocardial and pericardial fibrosis dominate the late stage of the disease. The fibrosis appears to result from microcirculatory insufficiency and subsequent ischemia. The early administration of either methylprednisolone or ibuprofen has preserved ischemic myocardium after acute coronary occlusions in animal models. In a brief, preliminary study we found that early administration of corticosteroids reduced left ventricular myocardial fibrosis in experimental, radiation-induced heart disease. This report describes our subsequent experience with methylprednisolone and ibuprofen.

Methods

Preparation of the Animal Model

Fifty-seven young, male New Zealand white rabbits that weighed 1.8–2.4 kg were used in this investigation. Using a modification of the method of Stewart et al., we irradiated the hearts of 44 rabbits. We administered 2000 rad (20 Gy) at 300 rad (3 Gy) per minute from a single anterior port with an 18-MeV electron beam. The dose was calculated 2 cm beneath the skin, approximately in the center of the heart. Using a simulator, we had localized the heart before irradiation. We had immobilized but not anesthetized each rabbit in a supine position.

The irradiated rabbits were divided into three groups. Fifteen rabbits were given i.v. methylprednisolone, 30 mg/kg, 2 hours before irradiation and then twice daily at 9 a.m. and 4 p.m. for 3 consecutive days. A second group of 15 rabbits were given i.v. ibuprofen, 12.5 mg/kg, 2 hours before irradiation and then twice daily at 9 a.m. and 4 p.m. for 2 consecutive days. Fourteen rabbits received no drugs. The remaining 13 rabbits received neither drugs nor irradiation and served as controls. The rabbits were killed with a lethal dose of pentobarbital 100 days after irradiation.

Pathology and Hydroxyproline Analysis

The hearts from every rabbit, including those that died spontaneously, were excised intact and examined for excessive pericardial fluid (> 1 ml). A transverse section cut from each heart 1 cm proximal to the cardiac apex and 2 mm thick was placed in formalin. Each transverse section included both right and left ventricles with epicardium and endocardium. These were stained with hematoxylin and eosin and Masson's trichrome stains. A pathologist unaware of the experimental design of the study determined the presence or absence of myocardial fibrosis and pericarditis. The extent of myocardial fibrosis was graded by the method of Fishbein et al.: 0 = absent, 1+ = mild, 2+ = moderate and 3+ = severe.

The remainder of the right and left ventricles and interventricular septum were analyzed for hydroxyproline content by the Blumenkrantz method.
Statistical Analysis
The numbers of rabbits that survived and the pathologic results in the untreated, irradiated group and in the treated, irradiated groups were compared by chi-square analysis. The hydroxyproline concentrations in untreated, unirradiated rabbits and in each of the three groups of irradiated rabbits were compared by analysis of variance followed by the Student-Newman-Keuls multiple range test. Hydroxyproline values in the 0, 1+, and 2+ myocardial fibrosis categories were also compared with analysis of variance followed by the Student-Newman-Keuls multiple range test. A p value less than 0.05 was considered statistically significant.

Results

Longevity
All rabbits treated with methylprednisolone and 13 of the 15 rabbits treated with ibuprofen lived until they were killed 100 days after irradiation. More rabbits that received methylprednisolone (p < 0.01) or ibuprofen (p < 0.05) lived 100 days after irradiation than the untreated, irradiated rabbits (table 1). One rabbit that received ibuprofen died 89 days and another 93 days after irradiation. Untreated rabbits died 83, 87, 88, 91, 92, 94 and 99 days after irradiation.

Pathologic Examination

Pericarditis
One rabbit treated with methylprednisolone, one treated with ibuprofen and eight untreated, irradiated rabbits had adhesive pericarditis on gross inspection. All of these rabbits and three methylprednisolone and two untreated, irradiated rabbits had microscopic evidence of pericarditis (table 1). The pericarditis was characterized by focal or diffuse fibrinous deposits with proliferation of mesothelial cells and mononuclear cells (fig. 1). No pericarditis was evident in the untreated, unirradiated group.

Pericardial Effusion
Nine rabbits treated with methylprednisolone had excessive clear pericardial fluid (3–12 ml, mean 8 ml) (table 1). Nine ibuprofen rabbits had large accumulations of clear pericardial fluid (3–17 ml, mean 8 ml).

All 14 untreated, irradiated rabbits had excessive hemorrhagic pericardial fluid (2–8 ml, mean 4 ml). No excessive pericardial fluid was evident in the untreated, unirradiated group.

Myocardial Fibrosis
Figure 2 shows representative areas of fibrosis in irradiated rabbits and normal myocardium from an unirradiated rabbit. A pathologic grade of 0 was assigned to one methylprednisolone and three ibuprofen rabbits. A grade of 2+ was given to two untreated, irradiated rabbits and three ibuprofen rabbits. A grade of 1+ was given to the remaining rabbits in the irradiated groups. No rabbit had 3+ fibrosis. The fibrosis was patchy and the right ventricle was more extensively scarred than the left. No histologic abnormalities were evident in the untreated, unirradiated group.

The mean hydroxyproline concentrations in the 2+ group (83.8 ± 6.1 mg/g) (± SEM) were significantly greater than those in the 1+ (47.7 ± 6.4 mg/g, p < 0.05) and 0 (32.2 ± 8.9 mg/g, p < 0.01) groups.

Hydroxyproline Content
Mean hydroxyproline concentrations (per g of dry tissue) ranged from 48.7 ± 6.2 mg/g in the rabbits treated with methylprednisolone to 72.0 ± 5.3 mg/g in the rabbits irradiated but not treated with drugs (fig. 1, table 1). The hydroxyproline concentration was 49.1 ± 7.1 mg/g in the rabbits treated with ibuprofen, and 34.4 ± 2.2 mg/g in the untreated, unirradiated rabbits (fig. 3, table 1).

The hydroxyproline concentrations in the two groups that were treated and irradiated were less (p < 0.01 or 0.05) than that in the untreated but irradiated rabbits, and did not differ from the concentration in the untreated and unirradiated rabbits.

Discussion
Marked pericardial and myocardial fibrosis may follow incidental cardiac irradiation received during radiation therapy for chest malignancies. Stewart and Fajardo reported a 5.8% incidence of carditis among 411 such patients. Applefeld and associates reported a 38% incidence of pericardial disease in 81 patients treated for Hodgkin’s disease between 1968

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rabbits</th>
<th>100-day survival</th>
<th>Pericarditis</th>
<th>Pericardial effusion</th>
<th>Hydroxyproline concentration (mg/g dry tissue) (mean ± SEM)</th>
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<tr>
<td>Irradiation +</td>
<td>15</td>
<td>15*</td>
<td>4*</td>
<td>9†</td>
<td>48.7 ± 6.2*</td>
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<tr>
<td>Irradiation +</td>
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<td>13*</td>
<td>3*</td>
<td>9†</td>
<td>49.1 ± 7.1†</td>
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<tr>
<td>ibuprofen</td>
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<tr>
<td>Irradiation</td>
<td>14</td>
<td>7</td>
<td>10</td>
<td>14</td>
<td>72.0 ± 5.3</td>
</tr>
<tr>
<td>None</td>
<td>13</td>
<td>13†</td>
<td>0†</td>
<td>0†</td>
<td>34.4 ± 2.2†</td>
</tr>
</tbody>
</table>

*p < 0.05 vs irradiation group.
†p < 0.01 vs irradiation group.
‡p < 0.005 vs irradiation group.
and 1972. In recent years the development of the linear accelerator and modification of thoracic mantle field techniques for the administration of therapeutic radiation have decreased the incidental radiation dose received by the heart. These innovations may result in a decreased incidence of radiation-induced heart disease in subsequent years. However, this trend would be partially offset by the increase in the number of patients alive and at risk of developing radiation-induced heart disease, because the prognosis of patients with thoracic neoplasms has improved after treatment with radiation and chemotherapy.

In related studies, Fajardo and Stewart reported on the acute and late stages of experimental radiation-induced heart disease in rabbits exposed to a single dose of 2000 rad or fractionated doses totaling up to 10,000 rad. The disease is characterized by progressive capillary endothelial damage with microthrombi and capillary plugging, resulting in microcirculatory insufficiency. The late lesions did not begin to appear until 70 days after irradiation and consisted primarily of progressive pericardial fibrosis, pericardial effusion, and diffuse myocardial fibrosis. These sequelae were identical to those in man. Further, they established that a single dose of 2000 rad was equivalent in terms of pathologic changes to a dose of 5400 rad in 12 fractions, and that these changes occurred in 94% of the rabbits.

In animal models, the early administration of either systemic corticosteroid or ibuprofen has preserved myocardium during ischemia resulting from acute coronary occlusions. In the study described here, early administration of these drugs retarded the severity of the late myocardial fibrosis. This can be deduced from the difference between hydroxyproline concentrations in the rabbits treated with methylprednisolone or ibuprofen and then irradiated and those only irradiated. The significance of these findings is even more striking when one considers that many of the irradiated, untreated rabbits did not live long enough for maximal development of myocardial fibrosis. The

and differences between groups in longevity and prevalences of pericarditis and pericardial effusion also support the concept that these drugs protect the myocardium.

Although rabbits with widespread myocardial

Figure 1. Fibrin deposits and a mesothelial/histiocytic infiltrate are seen in this thickened portion of pericardium. Hematoxylin and eosin stain; magnification × 400.

Figure 2. (A) Section of normal heart. (B) Heart section showing 1+ fibrosis. (C) Heart section showing 2+ fibrosis. Magnification × 170.
fibrosis on microscopic examination had greater amounts of myocardial hydroxyproline than rabbits with less extensive or no histologic evidence of fibrosis, myocardial fibrosis was evident in all irradiated groups and did not separate the treated, irradiated groups from the untreated, irradiated rabbits. We previously noted that determination of myocardial collagen content by hydroxyproline analysis was superior to light microscopy in the analysis of myocardial fibrosis in rabbits.\textsuperscript{12}

The mechanism of the protective effect of methylprednisolone and ibuprofen is not known, but may be due to inhibition of the migration of inflammatory cells and prevention of the subsequent release of lysosomal enzymes.\textsuperscript{17-19} Both ibuprofen\textsuperscript{17, 18} and corticosteroids\textsuperscript{20} protect lysosomes. The acidotic conditions in hypoxic cells disrupt these organelles, which then release acid hydrolases that may contribute to early irreversibility of cellular damage.

In conclusion, early administration of methylprednisolone or ibuprofen improves survival, decreases the incidence of pericarditis and pericardial effusion and reduces myocardial fibrosis in the experimental rabbit model of radiation-induced heart disease.

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