Effect of Methylprednisolone upon Technetium-99m Pyrophosphate Assessment of Myocardial Necrosis in the Canine Countershock Model

Ricky M. Schneider, M.D., John P. Hayslett, M.D., S. Evans Downing, M.D., Harvey J. Berger, M.D., Richard K. Donabedian, M.D., and Barry L. Zaret, M.D.

SUMMARY Repeat DC countershock reproducibly results in myocardial necrosis in dogs. In this model, myocardial technetium-99m pyrophosphate (PYP) uptake correlates linearly with tissue creatine kinase depletion ($r = -0.83$). The effect of pretreatment with methylprednisolone (MP) was studied with PYP in 25 dogs. In myocardium damaged by countershock, 12 MP dogs had higher tissue radioactivity samples:normal (S:N) ratios than control ($P < 0.05$), suggesting increased tissue injury. However, by several other measures of tissue damage, the two groups did not differ. MP-elevated PYP S:N ratios were explained by reduced PYP activity in normal myocardium of MP dogs. Further experiments in 21 dogs revealed that renal PYP clearance, which correlated with glomerular filtration rate (GFR) as measured by creatinine clearance, was increased in MP dogs, resulting in accelerated urinary excretion of PYP ($46.9 \pm 3.6$ vs $35.8 \pm 2.4$ percent injected dose in one hour, $P < 0.01$), and reduced blood PYP.

Thus, MP does not modify countershock-induced myocardial injury. However, by increasing GFR, MP increased PYP excretion, resulting in lowered blood and normal zone myocardial PYP, thereby spuriously affecting myocardial PYP tissue uptake data.

TECHNETIUM-99m STANNOUS PYROPHOSPHATE (PYP) imaging is a newly developed approach to the assessment of myocardial injury. In acute myocardial infarction (MI) both myocardial imaging and tissue uptake studies demonstrate zones of necrosis as regions of increased radionuclide concentration. This technique also identifies zones of abnormal PYP uptake following repeated DC countershock in dogs, an experimental model in which substantial myocardial necrosis is regularly produced.

PYP distribution within a myocardial region is dependent upon at least two factors: tissue injury and a blood flow-related delivery of the radiopharmaceutical to the injured zone. Because of this blood flow dependence, PYP tissue levels are not of value in determining the degree of necrosis following MI. However, in the canine countershock model, myocardial blood flow in regions of tissue necrosis is normal 24 hours following injury. Thus, myocardial PYP tissue levels in this model, as opposed to MI, should directly reflect the extent of regional necrosis.

Controversy still exists concerning whether corticosteroids modify the extent of ischemic necrosis following MI. Conflicting data have been presented in both clinical and experimental infarction. Countershock-induced myocardial necrosis represents a model where steroid pretreatment might modify tissue damage. In this model, PYP studies should provide relevant information.

This report describes experiments which utilize quantitative PYP techniques in the canine countershock model to assess the effects of corticosteroid pretreatment on the degree of myocardial injury. In the course of the study, our initial observations necessitated further evaluation of the effects of corticosteroids on PYP clearance and distribution.

From the Sections of Cardiology and Nephrology, Department of Internal Medicine, and the Departments of Diagnostic Radiology, Pathology and Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut.

Supported in part by NHLI Contract N01HV52988, and by American Heart Association Grant 75-723. Dr. Zaret is an Established Investigator of the American Heart Association.

Address for reprints: Barry L. Zaret, M.D., Yale University School of Medicine, Section of Cardiology, 87 LMP, 333 Cedar Street, New Haven, Connecticut 06510.

Received June 13, 1977; revision accepted July 25, 1977.

Methods

Effects of Methylprednisolone on Countershock-Induced Myocardial Injury

Initial countershock experiments were performed in twenty-five mongrel dogs of either sex ranging in weight from 12.3 to 19.5 kg. Animals were anesthetized with intravenous sodium pentobarbital (30 mg/kg), the trachea intubated, and respiration maintained with a Harvard respirator. Twelve dogs weighing $16.8 \pm 0.6$ (SEM) kg were given methylprednisolone sodium succinate (MP), 30 mg/kg, intravenously. A weight-matched group of 13 dogs ($17.5 \pm 0.3$ kg) received an equivalent volume of drug vehicle. Ninety minutes following either drug or vehicle, each animal received two 400 watt-second DC electroshocks. The two countershocks were separated by one minute, and were administered via a B-D Electrodyne Model ELD-5B DC pulse external defibrillator. Animals lay on their sides with the right chest closest to the table. The two paddle electrodes (4 cm diameter) were applied with Redux electrode paste at the point of maximal cardiac impulse on the shaved left chest, and directly opposite this point on the shaved right chest. Animals were premedicated with i.v. lidocaine. Countershock-induced ventricular ectopy, if sustained, was treated with additional 50 mg doses of lidocaine.

Multiposition precordial electrocardiographic recordings were made before and five and 60 minutes after countershock at 15 predetermined sites on the chest wall corresponding to five sites, from sternum to mid-axillary line, in the left fourth, fifth and sixth intercostal spaces. ST-segment amplitude was measured in all recordings 0.08 sec after the onset of the QRS complex, with the PR segment taken as baseline. The sum of all ST amplitudes exceeding 2 mm ($\Sigma ST$) and the number of lead sites with such ST elevation ($N ST$) were determined for each study.

Twenty-four hours following countershock, each dog received 10 mCi PYP intravenously. One hour later, the animals were sacrificed. Animals were then studied with a variety of techniques, which included tissue radioactivity measurements, quantitative imaging, tissue enzyme assay and histopathology.
In all dogs, the hearts were removed, trimmed of epicardial fat, and cut into approximately 25 1–2 g transmural samples. These samples included all grossly visible abnormal tissue, adjacent border zones, and five normal uninvolved samples from the posterior left ventricle. Samples were divided into epicardial and endocardial halves, weighed and counted for PYP activity ($^{99m}$Tc) in a Picker Spectrocaler IIIA well-type scintillation counter with a 3" $\times$ 3" sodium iodide crystal at a window of 100–140 keV. Sample radioactivity was calculated as counts per minute (cpm) per gram of tissue, corrected for decay of $^{99m}$Tc, and normalized for injected dose. PYP activity in abnormal samples was also expressed as an activity ratio between the specific myocardial sample and the mean of five clearly normal samples obtained remote from the site of injury — the sample: normal (S:N) ratio. The mean of the five most abnormal S:N ratios was obtained for each animal.

In five hearts from control and four from MP treated dogs, 40–80 mg full-thickness portions of each tissue sample were assayed for creatine kinase (CK) activity according to previously described techniques.4 CK data in these nine animals were expressed both as activity ratios and as absolute activities (mIU/mg). The relationship between PYP uptake and CK depletion was analyzed further in fifty transmural samples from grossly abnormal zones as well as apparently normal border zones in three additional untreated countershock animals. CK and PYP activities each were expressed as S:N activity ratios.

A total of 26 transmural biopsies were taken for histopathologic study from eight control and eight MP dogs. Tissue was fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, Masson trichrome and hematoxylin basic fuchsin picric acid (HBFP) stains. Sections were examined for evidence of necrosis, edema and cellular reaction, and for depth of myocardial injury. The sections were graded semiquantitatively in a blind manner by one of the authors according to the following scheme: 0, no definite necrosis; 1+, scattered, mild, but definite necrosis; 2+, subepicardial necrosis (limited to outer $\frac{1}{2}$ of myocardium) with moderate to severe tissue reaction; 3+, transmural necrosis (greater than $\frac{1}{2}$ full-thickness) with moderate to severe tissue reaction.

Before dissection, seven excised hearts from each group were imaged quantitatively using a computerized multiscrystal scintillation camera (Baird-Atomic System 77). The great vessels and atria were removed. The ventricles were opened and imaged en face with the anterior epicardial surface 5 cm from the parallel hole collimator. Computerized data processing of each image permitted expression of abnormal zone PYP uptake as radioactivity per unit area (counts/cm$^2$).

Effects of MP on Plasma PYP Concentration and Metabolic Clearance

The uptake of PYP in myocardial tissue following MP could be influenced by altered levels of unbound radionuclide in plasma water or by changes in radionuclide clearance. Therefore, studies were performed to evaluate the effect of MP on the degree of plasma protein binding and on the metabolic clearance of PYP, as reflected by the rates of bone uptake and renal clearance of PYP. In these studies 10 animals (mean weight 19.4 ± 0.9 kg) received MP (30 mg/kg i.v.) and 11 animals (mean weight 19.2 ± 0.8 kg) received its vehicle 24 hours prior to PYP injection, as in previous studies.

To determine the amount of PYP bound to plasma protein and to estimate levels of free radionuclide for clearance calculations, plasma was prepared from whole blood in five pairs of control and MP treated dogs. Plasma protein was precipitated using a modification of the method of Somogyi.10 Direct determination of cpm of $^{99m}$Tc in plasma and the protein fraction in paired samples provided an estimate of the amounts of free and bound radionuclide.

The urinary excretion rate of PYP and fractional excretion rate of the radionuclide were determined in two groups of animals. In six pairs of control and MP animals, the clearance of PYP was calculated during the intravenous infusion of 5 percent dextrose in water (D5W), at a rate of 300 ml per hour. In the second group of five pairs of control and experimental animals, fractional excretion, C$_{PYP}$/C$_{Cr}$, was determined during infusion of D5W at 450 ml per hour to facilitate accurate urine collections. Such water-loading would not be expected to alter C$_{Cr}$ or C$_{PYP}$ measurements. In this study exogenous creatinine (Cr) was administered initially in a bolus of 20 mg/kg in 20 ml of D5W, and then by constant infusion at 6 mg per minute to provide constant plasma levels. Clearance studies were begun 30 min before injection of PYP and were continued for 60 min thereafter. Urine collections were made at 10 min intervals and arterial blood samples were obtained for Cr and PYP levels before administration of the nuclide and at the end of each urine collection period. Plasma and urine Cr concentrations were measured on a Technicon autoanalyzer by a modification of the method of Folin and Wu.37

The renal clearance of PYP (C$_{PYP}$) and creatinine (C$_{Cr}$) was estimated from the expression

$$C = \sum \left( \frac{U_i \cdot V_i}{P_i} \right) / 60 \text{ minutes},$$

where $U_i$ and $P_i$ represent creatinine concentration (mg per ml) or PYP activity (cpm/ml) in 10 minute samples of urine and plasma, while $V_i$ represents urine volume during each timed collection. The average value for 60 minutes after PYP injection was determined. In the dog, C$_{Cr}$ is a precise indicator of glomerular filtration rate (GFR).

In an additional six pairs of control and experimental animals, 2–3 g of cortical bone was excised (three samples per biopsy site) from the right and left fourth ribs, the posterior iliac crests and the femoral heads. All bone samples were weighed and counted for $^{99m}$Tc activity as described previously.

All statistical analyses were performed by the Student’s $t$-test unless otherwise specified.

Results

Relationship between $^{99m}$Tc-PYP Uptake and CK Depletion in Countershock Injury

The validity of utilizing myocardial PYP uptake as an index of myocardial necrosis in the countershock model was examined by comparison of radionuclide levels with tissue CK depletion (fig. 1). There was an inverse linear correlation between S:N ratios of tissue PYP and tissue CK ($r = -0.83$). Thus, in the nonischemic countershock model,
as opposed to the situation of acute MI, tissue PYP uptake correlates with CK depletion. This observation supports the use of tissue PYP levels in assessing myocardial damage in this model.

Effects of MP on Countershock Injury: PYP Assessment (Table 1)

The MP group had a significantly greater mean PYP S:N ratio than control, 41.4 ± 6.5 vs 27.0 ± 2.2 (P < 0.05) (fig. 2). This difference initially suggested that the MP group sustained greater necrosis than control. However, absolute PYP radioactivity in abnormal zones (cpm/g/mCi) did not differ significantly in the two groups; in fact, the control group’s mean was higher. Absolute PYP activity in the normal zone was, on the other hand, significantly less in the MP group (P < 0.025). Thus, the increased abnormality of the PYP S:N ratio in MP dogs would appear to be a function of reduced normal zone PYP activity rather than increased abnormal zone PYP activity. The latter condition would be expected if the change in the PYP activity ratio truly reflected increased tissue necrosis.

An estimate of abnormal zone mass was made by summing the weights of all epicardial and endocardial tissue samples with abnormal PYP accumulation, defined arbitrarily by a S:N ratio greater than 3:1. There was no significant difference between the MP dogs (10.3 ± 1.1 g) and controls (10.4 ± 1.2 g) (fig. 3). Likewise, abnormal zone PYP count densities derived from en face computerized imaging of the excised hearts did not significantly differ in the two groups (fig. 3).

Effects of MP on Countershock Injury: CK Assessment and ST-Segment Mapping

There were no differences between the two groups in mean CK activity ratios, which were 0.21 ± 0.06 in the control group and 0.24 ± 0.05 in the MP group (fig. 4). Likewise, absolute tissue CK in both abnormal and normal zones were similar in the two groups. These results are similar to those recently reported by Vogel et al. in a model of ischemic myocardial necrosis.

Precordial ST-segment mapping revealed no significant difference between the two groups in either SST or NST (fig. 5).

Effects of MP on Countershock Injury: Histopathologic Assessment

There were consistently two visually distinct epicardial areas of right ventricular and left ventricular damage, corresponding to entrance and exit wounds. All biopsies studied showed qualitatively similar transmural disruption of muscle fibers, interstitial edema, and mixed cellular infiltration, with mononuclear leukocytes predominating over polymorphonuclear leukocytes. There was no clear difference in mean histopathologic grade between the two groups (MP 2.4 ± 0.2; control 2.6 ± 0.2).

Effect of MP on Plasma PYP Concentration and Metabolic Clearance

An analysis of blood PYP concentrations and the metabolic clearance rate of the radionuclide was performed to evaluate the cause of reduced myocardial PYP levels in MP treated animals as compared to control. When similar amounts of PYP were administered to both control and MP treated animals in a protocol identical to that used in the countershock study, whole blood concentration of PYP 60
minutes after PYP injection was significantly reduced from the control level of 3.94 ± 0.62 to 2.27 ± 0.39 × 10^4 cpm/ml/mCi (P < 0.025) in the MP group. There was no difference in the amount of PYP bound to plasma protein, which averaged 83.4 ± 1.2% in controls and 77.7 ± 3.8% in experimental animals.

Reduction in circulating PYP after MP could have resulted from changes in its metabolic clearance, either by increased urinary elimination or by increased uptake by bone, the principal PYP reservoir. Since an increase in urinary excretion of PYP could result from an increase in filtered load or from an alteration in the renal tubular handling of PYP, fractional clearance studies were performed. The fractional clearance of PYP, estimated from C_{PYP}/C_{Cr}, was not different between groups, and approached one (1.19 ± 0.08 in control and 1.12 ± 0.09 in experimental dogs). Moreover, there was a linear relationship between C_{PYP} and C_{Cr}, with r = 0.78. This suggests that in the dog, PYP like Cr is neither secreted nor reabsorbed in the renal tubule. In fact, PYP could be used as a tracer for measurement of GFR.

Renal PYP clearance measurements showed that MP markedly increased the urinary elimination of the nuclide during the 60 minute interval after administration (fig. 6). C_{PYP} averaged 111.1 ± 13.4 ml/min in control dogs and 163.4 ± 25.7 ml/min in MP dogs (P < 0.05). Estimated as the amount of injected dose excreted within one hour, steroid-treated animals excreted 46.9 ± 3.6% of the initial dose, compared to 35.8 ± 2.5% in controls (P < 0.01). These data indicate that in the MP group, an increase in filtered load of PYP, through an elevated GFR, significantly accelerated urinary PYP elimination.

In contrast, there was no evidence that MP influenced PYP uptake in samples of bone tissue, which averaged 0.018 ± 0.004% injected dose per g in controls and 0.017 ± 0.003% injected dose per g in experimental dogs.

**Discussion**

In the countershock model, corticosteroid pretreatment did not modify the degree of myocardial necrosis. This finding was supported by data obtained with a variety of
techniques. Steroid pretreatment did result in altered regional myocardial PYP radioactivity ratios associated with reduced normal zone or background PYP levels, rather than increased abnormal zone uptake. Further study revealed the phenomenon to be due to decreased circulating PYP resulting from a steroid-induced acceleration of urinary PYP clearance involving an increase in GFR.

The experiments described in this report demonstrate how commonly employed pharmacologic agents may modify radionuclide myocardial uptake, thereby erroneously altering interpretation of the degree of tissue damage as assessed by radioactive tracer techniques. Clearly, the intrinsic effects of an intervention upon radionuclide kinetics must be established prior to using that radionuclide to evaluate the intervention's efficacy.

Binding of PYP to plasma proteins was not affected by steroid treatment. A reduction in plasma protein-binding in the MP group could have accounted for more rapid urinary excretion secondary to increased filtered load. Since PYP is a bone avid radionuclide, increased bone PYP uptake in the MP group could have accounted for reduced circulating PYP and reduced normal myocardial PYP. However, again, there was no difference in PYP bone accumulation in steroid-treated and control dogs.

Several studies have demonstrated a GFR increase after adrenocorticotropic hormone (ACTH) or corticosteroid administration in man. In dogs, ACTH, cortisone and dexamethasone have been found substantially to increase GFR. Methylprednisolone, which has little or no mineralocorticoid effect, has been shown to increase inulin clearance in the dog and in the rat. The mechanism by which glucocorticoids increase GFR is not entirely clear, but several studies have demonstrated an associated increase in renal plasma flow.

These experiments were undertaken with the belief that countershock-induced myocardial necrosis would provide a situation where pharmacologic treatment prior to injury might be logically employed. However, in this model MP did not modify tissue damage. Several potential explanations exist: a) corticosteroids may not truly reduce myocardial injury; b) the electrical energy employed in these experiments may have been too great to permit salvage of myocardium; c) countershock necrosis may differ from ischemic necrosis in the potential for tissue salvage. The issue of corticosteroid-related reduction in the extent of injury following myocardial infarction remains controversial. The most frequently offered explanation for corticosteroid modification of cellular injury involves stabilization of bi membranes, including lysosomal membranes.

Countershock injury differs pathologically from ischemic injury. Certain non-ischemic cellular injuries, such as countershock, result from direct attack on the cell membrane, and cause rapid, uniform cell death and lysis. Two histopathologic hallmarks distinguish countershock injury from ischemic coagulation necrosis: a) an immediate cytoplasmic disorganization; and b) the absence of major polymorphonuclear leukocyte infiltration. These changes, occurring in the presence of an intact microcirculation, appear to fit into the pattern of myofibrillar degeneration. The possibility that countershock necrosis and ischemic infarction originate as different forms of injury could, in part, explain different responses to corticosteroid intervention.

Effects of corticosteroids on myocardial metabolism and hemodynamics have been studied intensively. Corticosteroids appear to increase coronary blood flow, an effect of potential relevance for salvage of ischemic myocardium. If myocardial blood flow was increased by MP administration in our experiments, then myocardial delivery of PYP increased as well. This conceivably resulted in increased myocardial radionuclide uptake. However, abnormal zone PYP uptake was not different in the two groups, and normal zone PYP activity was clearly less in MP dogs than control. This indicates that augmented myocardial blood flow, if present, potentially opposed and minimized the observed effect on myocardial PYP distribution.

Acknowledgment

The authors are grateful for the technical assistance of Mario Addabbo, Carol Orr and John Grelia and the secretarial assistance of Coleta Sawyer. Methylprednisolone was kindly supplied by the Upjohn Company.

References

Discrete Membranous Subaortic Stenosis

Report of 31 Patients, Review of the Literature, and Delineation of Management

NEVIN M. KATZ, M.D., MORTIMER J. BUCKLEY, M.D., AND RICHARD R. LIBERTHSON, M.D.

SUMMARY The presentation, management, and follow-up of 31 patients with discrete membranous subaortic stenosis (DMSS) is presented. DMSS comprised 16% of 185 patients with congenital left ventricular (LV) obstruction. Only one patient was older than 40 years. The rarity of DMSS in older patients in both our population and in the literature is noted, and possible explanations are discussed. One-quarter of these patients had dyspnea, chest pain, or syncope combined with electrocardiographic left ventricular hypertrophy (LVH) and strain, and these all had peak LV outflow gradients (PSG) greater than 85 mm Hg. One-quarter had neither symptoms nor electrocardiographic abnormalities and all had PSG < 90 mm Hg. Bacterial endocarditis was found in 13%, and in 13% an immediate family member also had congenital LV obstruction. Following surgical resection (25 patients), 18 were asymptomatic, two had residual fibromuscular obstruction, and four developed new fibromuscular obstruction after one to six years (leading to one to late sudden death). Thus, even after resection, these patients require continued re-evaluation for residual or new LV obstruction.

WHILE DISCRETE MEMBRANOUS SUBAORTIC STENOSIS (DMSS) has been well described,1-9 and is reported to comprise between 8 and 30% of patients with congenital obstruction of left ventricular (LV) outflow,1-9 a number of aspects concerning this entity warrant further clarification, emphasis, and delineation, including particularly its natural history, and its peri- and late postoperative course and management.

In this study, we report our experience with 31 patients with DMSS, present their history and clinical course, their diagnostic evaluation and management, their peri- and late postoperative complications, and review the reported literature.

Methods

Between 1949 and 1977, 31 patients with DMSS were managed at the Massachusetts General Hospital. In all pa-
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Circulation. 1977;56:1029-1034
doi: 10.1161/01.CIR.56.6.1029

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