Technetium-99m Pyrophosphate Uptake in Experimental Viral Perimyocarditis

Sequential Study of Myocardial Uptake and Pathologic Correlates

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SUMMARY The myocardial uptake of technetium-99m pyrophosphate (99mTc-PYP) in perimyocarditis induced by coxsackievirus B3 in BALB/c mice was studied. 99mTc-PYP uptake ratio, measured by the ratio of counts/min per gram for the heart to counts/min per gram for the skull, began to increase 5 days after virus inoculation when myocardial necrosis was evident. On the seventh day after the inoculation, 99mTc-PYP ratio reached a maximum. Histologically, fine, dystrophic calcification appeared in the necrotic fibers. 99mTc-PYP uptake was in proportion to the extent of the myocardial lesions. Thereafter, cellular infiltrations increased with time and were most severe on the fourteenth day, but 99mTc-PYP ratio had begun to fade. On the twenty-eighth day, perimyocardial fibrosis increased and calcification was still present, but 99mTc-PYP ratio had decreased. Myocardial uptake with severe pathologic lesions was visualized on a whole-body image on the seventh day after inoculation with the virus. The findings may provide a basis upon which 99mTc-PYP imaging may be applied to viral perimyocarditis in humans.

ALTHOUGH VIRUSES can seldom be demonstrated in the myocardium and clinical evidence suggesting viral infection can be obtained in only a few instances, myocarditis is probably more often caused by viral infection than has previously been suspected.1 Coxsackie B viruses are considered to be the most common cause of viral myocarditis in man.1-4 Coxsackie B myopericarditis is well accepted and numerous case reports have been documented.5-7

The diagnosis of viral myocarditis is often difficult because the clinical presentation shows wide variations, ranging from total absence of clinical manifestations to sudden, unexpected death. It appears unlikely that different viruses cause specific or characteristic cardiac manifestations, although patients may have other specific systemic manifestations associated with certain viruses.1

In our previous study,8 we showed that coxsackieviruses B3 and B4 produced significant myocarditis in mice. More recently, we found a high incidence of severe perimyocarditis in the right ventricle in weanling BALB/c mice inoculated with coxsackievirus B3 (Nancy strain).9

Using this experimental model, we studied myocardial uptake of technetium-99m stannous pyrophosphate (99mTc-PYP) and scintigraphic imaging, and found that 99mTc-PYP myocardial scintigraphy may be useful for diagnosing viral perimyocarditis.

Methods

Induction of Experimental Viral Perimyocarditis

Coxsackievirus B3 (Nancy strain) was used. The virus stock was prepared in cultures of HeLa cells in serum-free 199 medium. Control fluids from HeLa cell culture were also prepared. Both virus and control fluids were stored at −70°C until used.

Inbred BALB/c mice were obtained from Charles River, Japan. This strain has been maintained continuously by brother-sister matings. At 3–4 weeks of age, mice were inoculated intraperitoneally with 0.1 ml of virus suspension containing 10⁴.⁵ TCD₅₀ (50% tissue culture infective doses) per 0.1 ml. The mice were sacrificed 3, 5, 7, 14 and 28 days after the inoculation.

Gross inspection of the heart was made for any alteration in myocardial appearance. The degree of gross pathologic involvement in each sample was graded from 0, 1+, 2+, 3+ and 4+, depending on whether the lesion was absent or present in 25, 50, 75 or 100%, respectively, of the surface of the right ventricle. After inspection, hearts were processed for histologic and virologic studies.

Three- to 4-week-old mice serving as the controls were inoculated intraperitoneally with 0.1 ml of virus-free HeLa cell culture fluid and sacrificed 7 days after the inoculation. Hearts were processed and examined in the same manner as those from mice given the virus-containing fluid.

Distribution Studies of 99mTc-PYP

Twelve mice treated with virus-free control fluid and 115 mice inoculated with coxsackievirus B3, weighing 6.5–24.5 g, were sacrificed and organs were excised 1 hour after the injection of 10 μCi of 99mTc-PYP through a vein in the tail. Tissue uptake of the tracer in the skull, heart, lung, liver, kidney, spleen

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and blood was counted in a well-type gamma scintillation counter as counts/min per gram of tissue. The concentration of the tracer was calculated by the ratio of counts/min per gram for the tissues to counts/min per gram for the skull.

**99mTc-PYP Imaging**

For myocardial imaging, 10 nontreated mice and 10 mice with severe cardiac lesions on the seventh day after virus inoculation were given 100 μCi of 99mTc-PYP 1 hour before sacrifice. Whole body images were obtained in the posteroanterior view, using a Searle Radiographics Pho/Gamma HP scintillation camera fitted with a 4.7-mm pinhole collimator. Thirty thousand counts were accumulated for whole-body images and 1000 counts for excised heart images at the energy peak of 140 keV using a 25% window. After whole-body images were obtained, the heart was excised and imaging of the excised heart and the residual body was performed.

All scintigrams were interpreted independently by two observers who were unaware of the experimental conditions.

**Pathologic and Virologic Studies**

For attempts at virus isolation, the heart was ground with sea sand in 2.0 ml of minimum essential medium (MEM). The suspension was centrifuged and 0.1 ml of each of the supernatant was inoculated into tube culture of HeLa cells containing 1.0 ml of MEM supplemented with 2% fetal calf serum. Tubes were observed daily for the appearance of a characteristic cytopathic effect. Tissues were fixed in 10% formalin solution, sectioned transversely at the midportion of the ventricle, embedded in paraffin and stained with hematoxylin-eosin and von Kossa.

**Statistical Analysis**

Statistical analysis was performed by analysis of variance. Results were expressed as the mean ± sd.

**Results**

**Pathologic and Virologic Findings**

Yellowish-white patches were seen on the surface of the right ventricle of the heart on the seventh to the twenty-eighth day after inoculation with the virus. Before the third day, no histologic abnormality was noted. On the fifth day, myocardial fibers appeared fragmented and swollen and were intensely eosinophilic. Cross striations were not evident. Inflammatory cells were sparse or had not yet appeared (figs. 1A and 1B). By the seventh day, fine dystrophic calcification was seen in the necrotic fibers stained with von Kossa (figs. 1C and 1D). These fine granules of calcification were enlarged and became somewhat homogeneous on the fourteenth day. At this stage, cellular infiltration was evident and such consisted of mainly mononuclear cells (figs. 1E and 1F). After the twenty-eighth day, perimyocardial fibrosis increased and cellular infiltrations decreased but calcification was still present (figs. 1G and 1H). These changes were limited exclusively to the pericardial side of the right ventricle. In three severely affected mice, there were also small focal lesions in the ventricular septum and/or the left ventricle.

Coxsackievirus B3 was isolated from the hearts of 10 of 10 mice on the fifth day, six of 10 on the seventh and three of 10 on the fourteenth day after the inoculation with the virus. Attempts to yield a virus in mice treated with virus-free HeLa cell fluid was unsuccessful in all 12 mice.

**Tissue Distribution Studies**

Table 1 represents tracer distribution studies performed on the seven control mice and seven infected mice with positive myocardial lesions. The 99mTc-PYP was found essentially in the bone and in the myocardium of positive lesions. There was no statistically significant difference between the mean activity ratios of the tissues other than the heart in control and inoculated mice.

The uptake of 99mTc-PYP of hearts inoculated with coxsackievirus B3 did not increase before the third day (0.036 ± 0.013, mean ± sd) (fig. 2). The 99mTc-PYP ratio of hearts with positive lesions appeared to increase 5 days after virus inoculation (0.333 ± 0.194), but there was no statistically significant difference. The uptake ratio reached a maximum on the seventh day (0.752 ± 0.586, p < 0.001 compared with controls) and decreased significantly on the fourteenth day (0.527 ± 0.576, p < 0.05), but remained positive compared with controls (p < 0.001). On the twenty-eighth day, there were no significant differences between the hearts with positive lesions (0.159 ± 0.165) and those of controls.

On the seventh day after the inoculation, 99mTc-PYP uptake ratios in grade 0, 1+, 2+, 3+ and 4+ (each group consisted of six mice) were 0.039 ± 0.012, 0.223 ± 0.057, 0.498 ± 0.088, 1.030 ± 0.220 and 1.840 ± 0.674, respectively (fig. 3). Grade 1+ appeared high compared with controls (0.036 ± 0.011), but the difference was not significant. Grade 2+ was higher than controls (p < 0.01), grade 3+ higher than 2+ (p < 0.005) and grade 4+ higher than 3+ (p < 0.001). Increases in pathologic grade tended to be associated with increases in 99mTc-PYP ratio.

**99mTc-PYP Imaging**

Tracer uptake in the chest in mice with severe perimyocarditis was high. After the removal of the heart, repeated imaging was performed and a definite defect was evident in the chest. The image of the excised heart was compatible with imaging defect in the chest (fig. 4). In control mice, whole-body images were unchanged after the removal of the heart and the image of the excised heart was not visualized.

**Discussion**

This study demonstrated that measurement of 99mTc-PYP uptake of the heart is an extremely sen-
TABLE 1. Technetium-99m Pyrophosphate Uptake Ratio in Tissues of Control and Inoculated Mice

<table>
<thead>
<tr>
<th></th>
<th>Skull</th>
<th>Heart</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Blood</th>
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</thead>
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<tr>
<td>Controls (n = 7)</td>
<td>1.000</td>
<td>0.036</td>
<td>0.076</td>
<td>0.051</td>
<td>0.028</td>
<td>0.343</td>
<td>0.083</td>
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<tr>
<td>± S.D.</td>
<td>±0.011</td>
<td>±0.056</td>
<td>±0.008</td>
<td>±0.005</td>
<td>±0.061</td>
<td>±0.019</td>
<td></td>
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<tr>
<td>Seven days after inoculation (n = 7)</td>
<td>1.000</td>
<td>1.128</td>
<td>0.102</td>
<td>0.081</td>
<td>0.049</td>
<td>0.399</td>
<td>0.081</td>
</tr>
<tr>
<td>± S.D.</td>
<td>±0.561</td>
<td>±0.031</td>
<td>±0.062</td>
<td>±0.032</td>
<td>±0.113</td>
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<td>p</td>
<td>&lt; 0.005</td>
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Figure 1. Histologic findings of the heart of mice inoculated with coxsackievirus B3. On the fifth day after the inoculation (A and B), necrosis of myocardial cell is evident but cellular infiltrations are minimal. Von Kossa positive calcification is not noted. On the seventh day (C and D), there is fine calcification in the necrotic fibers. Fine granules of calcification become enlarged and somewhat homogeneous on the fourteenth day. Cellular infiltrations are marked in this stage (E and F). On the twenty-eighth day, perimyocardial fibrosis increases, cellular infiltration decreases but calcification is still present (G and H). All of these changes are exclusively limited to the right ventricle. Hematoxylin and eosin stain were used for A, C, E and G and von Kossa stain for B, D, F and H; A and B magnification × 160, C-H magnification × 80.

Figure 2. Sequential study of technetium-99m pyrophosphate (99mTc-PYP) uptake ratio of the heart of mice inoculated with coxsackievirus B3. (+) = positive, (-) = negative pathologic findings. The uptake ratio was highest on the seventh day and decreased significantly on the fourteenth day, but remained high compared with controls.

Sensitive technique for detecting acute viral perimyocarditis in the experimental animal. The 99mTc-PYP uptake ratio of the heart began to increase 5 days after virus inoculation when myocardial necrosis was evident but cellular infiltration was sparse or had not yet appeared. On the seventh day after the inoculation, 99mTc-PYP ratio of the heart reached a maximum and histologically, fine dystrophic calcification was seen in the necrotic fibers that were stained with von Kossa. At this stage, increases in pathologic grade tended to be associated with increases in 99mTc-PYP ratio. Thereafter, cellular infiltrations increased over time and were most severe on the fourteenth day, but 99mTc-PYP ratio had begun to fade. After the twenty-eighth day, perimyocardial fibrosis increased and cellular infiltrations gradually decreased, but calcification was still present. The 99mTc-PYP uptake ratio had decreased at this stage. Why the pathologic lesions were exclusively limited to the right ventricle is not known, but genetic factors are probably involved, as these lesions were not limited to the right ventricle in other strains of mice (e.g., A/J strain) inoculated with coxsackievirus B3 (unpublished data).

Previous studies in experimental animals and man have indicated that 99mTc-PYP is an extremely sensitive technique for detecting acute myocardial infarction.\(^{11-13}\) Abnormal 99mTc-PYP uptake was also observed in instances of left ventricular aneurysm,\(^{14}\) experimental and human myocardial contusion,\(^{15}\) cardiomyocyte,\(^{16, 17}\) calcific valvular heart disease\(^{18}\) and experimental streptococcal endocarditis.\(^{19}\)

Although the mechanism by which the infarct imaging agent accumulate in the damaged myocardium is unknown, it is clear that these agents concentrate selectively in an acutely necrotic myocardium, irrespective of the cause of the cardiac necrosis.\(^{20}\) Buja and co-workers\(^{21}\) considered uptake of 99mTc-PYP and
related phosphate in infarcted myocardium and other tissues to be a multifactorial phenomenon in which concentration of the agents results from complexing with various soluble and insoluble forms of tissue calcium stores, including amorphous calcium phosphate, crystalline hydroxyapatite and calcium complexed with organic macromolecules, possibly supplemented by calcium-independent complexing with tissue constituents. On the other hand, Dewanjee and Kahn proposed that the binding of \( ^{99m} \text{Tc-PYP} \) in the soluble muscle proteins and enzymes in different cardiac abnormalities probably plays a major role and calcium phosphate has only a minor role.

The \( ^{99m} \text{Tc-PYP} \) uptake in viral perimyocardial lesions may be related to the presence of both calcium and denaturated protein macromolecule in the necrotic debris. The necrotic and cellular process resulting from viral perimyocarditis may be a model for further study of the mechanism of \( ^{99m} \text{Tc-PYP} \) cardiac uptake.

Whole-body scans do not convincingly demonstrate the myocardial uptake of \( ^{99m} \text{Tc-PYP} \), even when a pinhole collimator is used. However, imaging of the excised heart and observation of the imaging defect in the chest on the residual-body image clearly demonstrated uptake of \( ^{99m} \text{Tc-PYP} \) by the cardiac tissue. In the excised heart, right and left ventricular myocardium could not be discriminated because of the small size of the heart.

In the experimental work of myocardial infarction, \( ^{99m} \text{Tc-PYP} \) images were positive when the extent of myocardial infarction was greater than 1 g. The weight of the heart at the time of our scintigraphic study was 61.5 ± 11.9 mg. Mice may be too small to obtain a completely adequate scintigram. Studies are under way to obtain a better scintigram using a larger animal.

Olson and co-workers reported 11 patients with acute pericarditis who had a positive \( ^{99m} \text{Tc-PYP} \) scintigram, a localized pattern in six patients and diffuse pattern in five. A positive \( ^{99m} \text{Tc-PYP} \) scintigram during acute pericarditis is more likely if ST-segment elevation is present on the ECG. A positive scintigram has also been reported in a case of cholesterol pericarditis and in a case of pericarditis after acute myocardial infarction.

Other workers have described normal \( ^{99m} \text{Tc-PYP} \) images in patients with acute pericarditis. However, it cannot be ruled out that the disease was inactive or in a healing phase at the time of study. Alternatively, as

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**Figure 3.** Relationship of pathologic grade to technetium-99m pyrophosphate (\( ^{99m} \text{Tc-PYP} \)) uptake ratio on the seventh day after virus inoculation. Pathologic grade correlated closely with \( ^{99m} \text{Tc-PYP} \) uptake ratio. Each group included six mice.

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**Figure 4.** Technetium-99m pyrophosphate (\( ^{99m} \text{Tc-PYP} \)) images (posteroanterior view) of a mouse with severe perimyocarditis. Note higher uptake in the chest on a whole body image (A) and after the removal of the heart, definite defect was evident in the chest (B, arrow). The excised-heart image (C) was compatible with imaging defect in the chest. Accumulation of the tracer was evident in the skeleton, kidneys and tail. The tracer was injected into a tail vein.
shown in an experimental study, the absence of subepicardial necrosis may have resulted in a negative imaging.

The marked sensitivity of \textsuperscript{99m}Tc-PYP uptake in viral perimyocarditis, at least in the experimental model, offers great promise as a reliable, noninvasive method. The present findings provide a basis upon which radionuclide imaging may be applied to perimyocarditis in humans.

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