Radionuclide Imaging of Experimental Myocarditis

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SUMMARY The ability of \(^{67}\)Ga citrate and \(^{99m}\)Tc pyrophosphate cardiac imaging to detect myocarditis was assessed in an experimental rabbit model. Twenty-three rabbits were imaged approximately 72 hours after infusion of i.v. norepinephrine. Diffuse cardiac uptake was found in 13 of 15 rabbits scanned with \(^{67}\)Ga. Tissue distribution studies documented significant myocardial uptake of \(^{67}\)Ga in those with positive scans. Precordial imaging and tissue distribution studies revealed no cardiac uptake in the eight rabbits scanned with \(^{99m}\)Tc. Histologic examination of all 23 hearts revealed qualitatively similar, typical lesions of myocarditis. This study suggests that cardiac imaging with \(^{67}\)Ga may be useful in the detection of myocarditis.

MYOCARDITIS, an inflammatory condition involving the heart, can lead to sudden unexpected death or severe congestive heart failure.\(^1\)\(^2\) Yet, its accurate detection is frequently difficult.\(^3\)\(^4\) The clinical manifestations are often nonspecific,\(^1\)\(^3\) and conventional diagnostic tests such as the ECG, serum cardiac enzymes and chest x-ray are insensitive as well as nonspecific.\(^5\)\(^6\)

Recently, a brief clinical report\(^8\) and two experimental studies\(^6\)\(^7\) have suggested that radionuclide cardiac imaging can detect the presence of myocarditis. In this report, we present the results of radionuclide cardiac imaging with \(^{99m}\)Tc pyrophosphate and \(^{67}\)Ga citrate in an experimental model of myocarditis.

Methods and Materials

Myocarditis was created in 23 adult New Zealand white rabbits using a modification of the technique of Downing and Lee.\(^8\) Each rabbit was anesthetized with i.v. pentobarbital (30 mg/kg) and a 25-gauge butterfly needle was secured in a marginal ear vein. Norepinephrine was infused at a rate of 3 \(\mu\)g/kg/min for 100 minutes using a Harvard constant infusion pump.

Radionuclide Imaging with \(^{67}\)Ga Citrate

Myocardial imaging was performed in 15 rabbits 70–74 hours after the norepinephrine infusion. Five normal control rabbits were also imaged. All rabbits received 1.3 mCi of \(^{67}\)Ga citrate through a marginal ear vein 24 hours before cardiac imaging.

After i.v. pentobarbital anesthesia, the rabbits were immobilized in the supine position. A single 10° left anterior oblique image of the heart was obtained using a scintillation detection camera and a pinhole collimator with a 5.9-mm aperture. The pulse-height analyzer settings were centered on the 184 keV and 296 keV emissions of gallium, and a double-isotope summation technique was used. One hundred thousand counts were obtained in each image. The images were recorded on 70-mm film. All scintigrams were interpreted independently by two observers. Scintigrams were considered positive when distinct uptake was present in the cardiac region.

Analysis of Tissue Radionuclide Uptake

Upon completion of in vivo imaging, each animal was sacrificed with a lethal dose of i.v. pentobarbital. The heart was excised intact, washed free of blood and clot, and weighed. The excised hearts from 10 of the rabbits were imaged again. Twenty-five thousand counts were obtained in each image. In each rabbit, the radioactivity content of heart and thigh muscle was individually assayed in a Capintec dose calibrator. Background counts were recorded and net counts were expressed in \(\mu\)Ci/g of tissue.

Radionuclide Imaging with \(^{99m}\)Tc Pyrophosphate

Cardiac scintigrams were performed on eight additional rabbits 70–74 hours after norepinephrine infusion. All rabbits received 5 mCi of \(^{99m}\)Tc pyrophosphate via a marginal ear vein, and 2–3 hours later, a single 10° left anterior oblique image of the cardiac region was obtained. Precordial images were recorded as described above, except that the pulse-height analyzer settings were centered on 140 keV.

Analysis of Tissue Radionuclide Uptake

Upon completion of in vivo imaging, tissue radionuclide uptake was determined as described above, except that the excised hearts were not imaged again and autoradiographs were attempted in four rabbits according to the technique of Buja and colleagues.\(^8\) Five transverse sections of the heart 3–4 mm thick were obtained consecutively from the apex to the base of the heart. These specimens were prepared using Carnoy's solution and absolute alcohol. Sections 6-\(\mu\) thick were
cut and approximately 24 hours later dipped in Kodak NTB-2 photographic emulsion. The emulsion was wiped from the bottom of the slide. Slides were placed in light-tight boxes. They were exposed for varying periods of time on an individual basis, depending on the findings from the previously developed sections.

Pathology
Myocardial sections stained with hematoxylin and eosin were obtained from all rabbits for analysis by light microscopy.

Results
Radionuclide Imaging with $^{67}$Ga Citrate
Distinct diffuse cardiac uptake of tracer was visualized by in vivo precordial imaging in 13 of 15 rabbits 72 hours after norepinephrine infusion (figs. 1 and 2). No definite cardiac uptake was found in the five normal control rabbits (fig. 3).

Analysis of Tissue Radionuclide Uptake
The rabbit hearts with positive precordial images had net radioactivity values of 0.36–2.0 $\mu$Ci/g (mean 0.97 $\mu$Ci/g). The two rabbit hearts with negative precordial images had net radioactivity values of 0.04 and 0.03 $\mu$Ci/g (fig. 4). Net radioactivity values for thigh muscle ranged from 0.06–0.09 $\mu$Ci/g (mean 0.05 $\mu$Ci/g). The ratio of heart radioactivity to thigh muscle radioactivity ranged from 0.8–3.6 (mean 2.0) in the 13 rabbits with positive precordial images. The ratios of heart radioactivity to thigh muscle radioactivity were 0.7 and 0.25 (mean 0.5) for the two rabbits with negative precordial scans. Distinct cardiac radioisotopic uptake was visualized in all 10 excised rabbit hearts (figs. 4 and 5).

Radionuclide Imaging with $^{99m}$Tc Pyrophosphate
No cardiac pyrophosphate uptake was visualized by in vivo precordial imaging in the eight rabbits imaged 72 hours after norepinephrine infusion (fig. 4).

Analysis of Tissue Radionuclide Uptake
Heart radioactivity ranged from 0.2–0.5 $\mu$Ci/g (mean 0.3 $\mu$Ci/g) (fig. 4). Thigh muscle radioactivity ranged from 0.05–1.4 $\mu$Ci/g (mean 0.55 $\mu$Ci/g). The ratio of heart radioactivity to thigh muscle radioactivity ranged from 0.4–2.6 (mean 1.0). The autoradiographs revealed no evidence of tracer uptake.

Pathology
All 23 rabbit hearts had qualitatively similar histologic changes (figs. 6 and 7). There was diffuse edema of the myocardium, focal degeneration and necrosis of the myocardial fibers and foci of inflam-
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Positive images from both excised heart (10/10) and precordium (67-Ga citrate)
- Precordial images only (67-Ga citrate)
- Precordial images with 99m-Technetium Pyrophosphate (0/8)

Figure 4. Myocardial radioactivity content correlated with radionuclide imaging.

There is uniform distribution of tracer in the heart.

Inflammatory cells that were predominantly histiocytes with occasional plasma cells and polymorphonuclear neutrophils. These lesions were more numerous and extensive in the left ventricle, particularly the inner two-thirds of the wall. They were often located around small myocardial blood vessels.

Figure 5. Image of the excised heart shown in figure 1. There is uniform distribution of tracer in the heart.

Figure 6. Edema, focal myocardial necrosis and white blood cell infiltration around small blood vessels. Hematoxylin and eosin; magnification × 28.
Discussion

Myocarditis, an inflammatory process involving the heart, has been reported after bacterial, viral, rickettsial, mycotic and parasitic disease and in association with collagen vascular disease, acute rheumatic fever and in an idiopathic form.1, 10, 11

The reliable diagnosis of myocarditis is frequently difficult because its clinical presentation is often entirely nonspecific.1, 8 Traditional diagnostic tests are of little value. In some instances, abnormalities can be noted in the ECG, chest x-ray, and with serum cardiac enzyme determinations.1, 3, 4, 11 However, these abnormalities are nonspecific and infrequent. A definitive diagnosis of myocardial inflammation can be obtained by transvenous right ventricular endomyocardial biopsy.12 However, this invasive technique involves some risk and discomfort to the patient, requires considerable operator skill, and is not readily available at many medical centers.13

Prior acute infectious myocarditis or chronic recurrent myocarditis is a possible cause of unexplained congestive cardiomyopathy.1, 5, 14, 18 The recognition of myocardial inflammation in these cases has permitted the institution of immunosuppressive therapy, which occasionally resulted in short-term resolution of the signs and symptoms of the congestive heart failure.5, 12 A sensitive noninvasive technique for detecting myocardial inflammation could prove to be a useful tool for detecting these patients and monitoring their response to various therapeutic interventions. A reliable noninvasive test would also be useful in detecting the presence of myocarditis in the infant or child where symptoms of heart failure due to acute myocarditis can be very subtle and mimic extracardiac disease,16 or when cardiac fungal infection is suspected, as myocarditis is frequently present but difficult to recognize in this setting.17

Recently, a brief clinical report suggested that cardiac imaging with ⁶⁷Ga citrate may be useful in detecting myocarditis.6 Gallium decays by electron capture, has a half-life of 78 hours, and produces four gamma rays with energies suitable for scanning. There is no β emission, and the radiation dose to the patient is well within the acceptable range.18 ⁶⁷Ga citrate myocardial imaging has been useful in detecting inflammatory lesions of the heart, including myocardial abscess, bacterial endocarditis, and the inflammatory response accompanying acute myocardial infarction.19-21 Also, cardiac localization of gallium has recently been noted in three patients with unexplained congestive cardiomyopathy presumably due to myocardial inflammation.8 Incorporation of ⁶⁷Ga into inflammatory lesions appears to be mediated chiefly by the binding of the nuclide within inflammatory white blood cells.22

Technetium-99m pyrophosphate cardiac scintigraphy is useful for detecting irreversible myocardial cellular damage from a variety of causes.23 This technique might be useful in detecting the cellular damage accompanying myocarditis, as myocardial

Figure 7. Myocardial necrosis and infiltration by histiocytes. Hematoxylin and eosin; magnification × 430.
uptake of $^{99mTc}$ stannous pyrophosphate has been noted in experimental viral myocarditis.\textsuperscript{6, 7}

We evaluated the ability of these radionuclides to detect myocarditis induced by norepinephrine in rabbits. This model was chosen because of the characteristic reproducible lesions produced by the norepinephrine exposure.\textsuperscript{8, 24-26} Histologically, myocarditis consists of myocardial cellular damage accompanied by an inflammatory leukocytic infiltration of predominantly mononuclear cells, the identical histologic features of norepinephrine-induced myocarditis.\textsuperscript{1, 3, 6, 24-26}

In this investigation, $^{99mTc}$ pyrophosphate cardiac scintigraphy was not helpful in detecting myocarditis. Although myocardial cellular necrosis was present in our model, the magnitude of damage did not appear to be sufficient to allow pyrophosphate uptake. However, $^{67}$Ga citrate myocardial imaging proved to be a sensitive technique for detecting myocarditis in our experimental model. This finding is not surprising, as there were widespread areas of white cell infiltration, the cellular substrate essential for $^{67}$Ga uptake.

The differences between $^{67}$Ga citrate and $^{99mTc}$ pyrophosphate may be less in certain clinical circumstances than we observed in this experimental study. The amount of $^{99mTc}$ that can be given in man is several times greater than the amount of $^{67}$Ga that can be safely administered. Consequently, if the two agents were administered at clinically relevant dose ratios, the difference observed might be minimized in some instances. More important, however, is the possibility that the myocarditis might result in a relatively greater amount of myocardial cellular necrosis than accompanying inflammatory response, in which case $^{99mTc}$ pyrophosphate scans might be superior to those of $^{67}$Ga citrate.

Our results suggest that further investigation of $^{67}$Ga citrate myocardial imaging for the detection of myocarditis is warranted.

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References

13. Ferrans VJ, Roberts WC: Myocardial biopsy: a useful diagnostic procedure or only a research tool? Am J Cardiol 41: 965, 1978
Radionuclide imaging of experimental myocarditis.
W C Reeves, G L Jackson, F W Flickinger, H G Kwee, E J Schwiter, J Werner, L Whitesell, M A Biddle, G Copenhaver, B S Shaikh and R Zelis

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