INVESTIGATIVE NUCLEAR MEDICINE

Technetium-99m Pyrophosphate in Diagnosis of Acute Cardiac Rejection in the Rat with Effect of Cyclosporine: Concise Communication

Christopher G. A. McGregor, Rudolph Hatz, Salim Aziz, Margaret B. Billingham, and I. Ross McDougall

Stanford University Medical Center, Stanford, California

Tc-99m pyrophosphate (PPi) uptake has been measured in a rat model of heterotopic heart transplant at 5 days after transplantation. Comparison of tracer uptake, as a ratio between the heterotopic transplanted heart and the recipient's native heart, was made in four groups of animals. Group 1, with transplants between animals of isogeneic strain showed a lower ratio, significantly different from the ratio in Group 2 in which transplants were between nonisogeneic animals. The ratio of uptake after transplantation between nonisogeneic animals treated with 10 mg/kg-day of cyclosporine (Group 3), was not significantly different from Group 1. In contrast, the ratio of uptake between nonisogeneic animals treated with subtherapeutic doses of cyclosporine (Group 4), was not significantly different from Group 2. In Groups 1, 2, and 3 there was a correlation between the histology of the transplanted hearts, graded 1 to 5 according to the severity of rejection, compared with the uptake of Tc-99m PPi. This agent can therefore be used to diagnose cardiac rejection in a rat model, and the results correlate well with the severity of rejection as assessed histologically.

J Nucl Med 25: 870–873, 1984

The uptake of technetium-99m pyrophosphate (Tc-99m PPi) has been shown to be a sensitive and specific indicator of ischemic myocardial injury in animals and man (*1*,*2*). In a heterotopic transplant model between rats of isogeneic strain, Tc-99m PPi uptake in the transplanted heart correlated with other parameters of myocardial injury such as creative kinase activity (*3*) and long-term histological changes (*4*). In these experiments the myocardial injury was ischemic, in the form of storage of the hearts before transplantation. Tc-99m PPi uptake in myocardial injury caused by acute cardiac rejection has not been described in animal experiments, and one clinical report was inconclusive (*5*).

We therefore used this readily available radionuclide to investigate uptake during acute cardiac rejection in the experimental model of heterotopic cardiac transplantation between nonisogeneic rats, to study its potential clinical value in the noninvasive diagnosis of cardiac rejection after heart transplantation. To date endomyocardial biopsy remains the only dependable method of diagnosis (*6*).

Cyclosporine has been shown to be a powerful immunosuppressive agent in the rat after transplantation of the heart, kidney, pancreas, skin, liver, and small bowel (*7–12*), and is now in widespread clinical use after transplantation of many organs (*12–14*). Graft survival time, as judged by the presence of the heartbeat, has been the index of the depth of immunosuppression by cyclosporine (*7*) and other drugs after rat heart transplantation (*15–17*).

We further evaluated Tc-99m PPi uptake as a measure of myocardial injury secondary to cardiac rejection, as modified by cyclosporine in therapeutic and subtherapeutic doses, after heart transplantation between nonisogeneic rats.

Received Feb. 7, 1984; revision accepted Apr. 13, 1984.

For reprints contact: Dr. Christopher G. A. McGregor, Dept. of Cardiovascular Surgery, Stanford University Medical Center, Stanford, CA 94305.

TABLE 1. TRANSPLANT-TO-RECIPIENT HEART UPTAKE RATIOS FOR Tc-99m PYROPHOSPHATE IN RATS

Groups	Tc-99m PPi uptake (mean ± s.d.)*	p values
Group 1 (N = 6)	1.85 ± 0.9	Group 1 compared with Group 2 $p < 0.01$
		Group 1 compared with Group 3 p NS
Group 2 (N = 11)	3.23 ± 0.96	Group 2 compared with Group 3 $p < 0.01$
		Group 2 compared with Group 4 p NS
Group 3 (N = 6)†	2.17 ± 0.5	
Group 4 (N = 4)	2.81 (2.09 − 3.66)‡	

* Uptake per gram of tissue per 100 μCi injected activity: transplanted heart over recipient's heart.
† One result excluded because transplanted heart showed large acute myocardial infarction.
‡ Range.
NS = not significant.

MATERIALS AND METHODS

Heterotopic transplantation used the technique of Ono and Lindsey (*18*), in which the transplanted heart is placed in the peritoneal cavity and the recipient's own heart is in situ. Twenty-eight consecutive operations were done using inbred LBN and ACI rats. Four groups of animals were studied.

Group 1: Transplants between untreated isogeneic rats (LBN → LBN), in six animals.

Group 2: Transplants between untreated nonisogeneic rats (ACI → LBN), in 11 animals.

Group 3: Transplants between nonisogeneic rats (ACI → LBN), treated with a therapeutic dose of cyclosporine (10 mg/kg/day), in seven animals.

Group 4: Transplants between nonisogeneic rats (ACI → LBN), treated with a subtherapeutic dose of cyclosporine (1 mg/kg-day), in four animals.

Cyclosporine was given by orogastric injection once a day at a standard time. Transplants in Groups 1 and 2 were carried out as controls of (a) the operative procedure, as ischemic injury could cause increased Tc-99m PPi uptake, and (b) the uptake of Tc-99m PPi after transplantation between untreated nonisogeneic rats respectively.

Radionuclide studies were carried out on all animals on the fifth postoperative day when the hearts were beating, but cardiac rejection can be expected. In a previous study from this institution, survival of transplanted hearts between these strains lasted 6 ± 0.7 days (*19*). One hundred microcuries of Tc-99m PPi complexed to 0.24 mg of stannous pyrophosphate was given in 0.3 ml of normal saline by direct injection into the femoral vein after cutdown in anesthetized animals. The activity given was determined by counting the syringe before and after injection. Two hours after injection, the animals were killed. The transplanted heart, the recipient's own heart, and a blood sample were removed, weighed, and counted in an automated well counter using the settings recommended for Tc-99m. All transplanted hearts were beating at the time of death. Tracer uptake per gram of tissue per 100 μCi injected activity was calculated, and a ratio was obtained for uptake in the transplanted heart relative to the recipient's own heart acting as a control.

Microscopic cross sections of each heart were assessed "blind" by a pathologist and graded 1 to 5 according to the severity of rejection:

Grade 1: Normal endocardium and myocardium.

Grade 2: Sparse pyroninophilic mononuclear cells, mainly around small vessels and the endocardium. Interstitial edema may be present.

Grade 3: Definite perivascular and/or interstitial focal infiltrate of pyroninophilic mononuclear cells, but without myocyte necrosis.

Grade 4: Prominent interstitial inflammatory infiltrate of mononuclear cells, with focal myocardial necrosis where the infiltrate is greatest.

Grade 5: Extensive myocardial necrosis with a significant inflammatory infiltrate of neutrophils as well as mononuclear cells. Vascular necrosis with interstitial hemorrhage now ensues.

Histological evaluation was available in six rats in Group 1, seven in Group 2, and seven in Group 3. Student's t-test was used to compare Tc-99m PPi uptake between groups and Spearman's rank correlation was used to compare Tc-99m PPi uptake with histological grading in 20 hearts.

RESULTS

The Tc-99m PPi uptake ratios (transplant/inborn) for each of the groups are shown in Table 1. There was

TABLE 2. COMPARISON OF Tc-99m PYROPHOSPHATE UPTAKE IN TRANSPLANTED HEARTS IN ISOGENEIC RATS (GROUP 1), NONISOGENEIC RATS (GROUP 2) AND NONISOGENEIC RATS TREATED WITH CYCLOSPORINE (GROUP 3)

Group	Tc-99m PPi uptake (mean ± s.d.)	Histological grade of rejection mean (range)
Group 1 (N = 6)	1.85 ± 0.9	1.75 (1.0–2.0)
Group 2 (N = 7)	3.2 ± 0.7	4.14 (4.0–5.0)
Group 3 (N = 7)*	2.17 ± 0.51	1.83 (1.0–3.0)

* One heart showed acute myocardial infarction and was excluded from analysis.

a statistical difference between Group 1 and Group 2 transplants ($p < 0.01$) and between Group 2 and Group 3 ($p < 0.01$). One of the rats treated with cyclosporine (Group 3) was found to have a large anterior and septal myocardial infarct, and that animal was excluded from statistical analysis. Table 2 shows the correlation between uptake of Tc-99m PPi in the transplanted heart and histological grading of transplant rejection in Groups 1, 2, and 3.

Group 4 animals treated with suboptimal doses of cyclosporine showed Tc-99m PPi uptake results that could not be distinguished from those in untreated nonisogeneic rats.

Using Spearman's rank correlation for nonparametric measurements, the degree of Tc-99m PPi uptake in the transplanted heart and the grade of histological rejection showed a marked agreement ($r = 0.643$, $p < 0.01$).

DISCUSSION

This study confirms that the uptake of Tc-99m PPi is significantly increased in myocardial injury due to cardiac rejection. This uptake also seems a good measurement of the effectiveness of immunosuppression in this model, in that uptake was significantly increased in underimmunosuppressed and nonimmunosuppressed, nonisogeneic grafts compared with grafts in animals treated with therapeutic doses of cyclosporine and in grafts between isogeneic rats. This model allows assessment of rejection, and therefore the efficacy of immunosuppression, at an early period in the transplanted heart while it remains viable and beating, rather than when it is dead and showing histological changes of autolysis and organization. Cyclosporine is clearly an effective immunosuppressive agent in the dose of 10 mg/kg-day used in this study, but had little demonstrable effect at the lower dose of 1 mg/kg-day. The timing of assessment of rejection at 5 days in this study is arbitrary and may not be at the peak of the rejection process. Provided ischemic damage to the heart can be excluded, the potential clinical application of Tc-99m PPi for the noninvasive diagnosis of rejection after cardiac transplantation, for which endomyocardial biopsy remains the only dependable method (*6*), merits further investigation.

REFERENCES

1. WILLERSON JT, PARKEY RW, BONTE FJ, et al: Pathophysiologic consideration and clinicopathological correlates of technetium-99m stannous pyrophosphate myocardial scintigraphy. *Semin Nucl Med 10*:54–69, 1980
2. LYONS KP, OLSON HG, ARONOW WS: Pyrophosphate myocardial imaging. *Semin Nucl Med* 10:168–177, 1980
3. MCGREGOR CGA, HANNAN J, SMITH AF, et al: A study of cold cardioplegic myocardial protection in rats: an experimental model using the uptake of technetium 99m pyrophosphate and enzyme activity as parameters of injury. *Cardiovasc Res* 17:70–74, 1983
4. MCGREGOR CGA, MCCALLUM HM, HANNAN J, et al: The long term effects of cold cardioplegic myocardial protection in the rat. *J Thorac Cardiovasc Surg:* in press
5. MCKILLOP JH, MCDOUGALL IR, GORIS ML, et al: Failure to diagnose cardiac transplant rejection with Tc-99m-PYP images. *Clin Nucl Med 6*:375–377, 1981
6. CAVES PK, BILLINGHAM ME, STINSON EB, et al: Serial transvenous biopsy of the transplanted human heart: Improved management of acute rejection episodes. *Lancet i*:821–826, 1974
7. KOSTAKIS AJ, WHITE DJG, CALNE RY: Prolongation of rat heart allograft survival by Cyclosporin A. IRCS Med Sci 5:280, 1977 (abst)
8. HOMAN WP, FABRE JW, WILLIAMS KA, et al: Studies on the immunosuppressive properties of Cyclosporin A in rats receiving renal allografts. *Transplantation* 29:361–366, 1980
9. RYNASIEWICZ JJ, SUTHERLAND DER, KAWAHARA K, et al. Cyclosporin A prolongation of segmental pancreatic and islet allograft function in rats. *Trans Proc 12*:270–274, 1980
10. WHITE DJG, PLUMB AM, PAWALEC G, et al. Cyclosporin A: An immunosuppressive agent preferentially active against proliferating T cells. *Transplantation* 27:55–58, 1979
11. ZIMMERMAN FA, WHITE DJG, GRANZOW R, et al. Immunosuppressive therapy in liver and small bowel grafts in the rat. *Z Kinderchir* 30:177–180, 1980
12. CALNE RY, WHITE DJG, TNIRU S, et al. Cyclosporin-A in clinical organ grafting. *Transplant Proc* 13:349–358, 1981
14. OYER PE, STINSON EB, JAMIESON SW, et al: One year experience with cyclosporin A in clinical heart transplantation. *Heart Transplant* 1: 285–290, 1982
15. MILLER CM, MARTINELLI GP, RACELIS D, et al. Prolongation of rat cardiac allografts by pretransplant administration of blood transfusion and Cyclosporin A. *Transplantation* 33:335–337, 1982
16. SALAMAN JR: Influence of steroid dosage on the survival of cardiac allografts in the rat. *Transplantation* 35(5):510–511, 1983.

17. HARDY MA, OLUWOLE S, FAWWAZ R, et al: Selective lymphoid irradiation. Prolongation of cardiac xenografts and allografts in presensitized rats. *Transplantation 33*(3): 237-242, 1982
18. ONO K, LINDSEY ES: Improved technique of heart transplantation in rats. *J Thorac Cardiovasc Surg* 57:225-229, 1969
19. JAMIESON SW, BURTON NA, REITZ BA, et al: Survival of heart allografts in rats treated with azathioprine and sodium salicylate. *Lancet* 1:130-133, 1979

Southeastern Chapter
Society of Nuclear Medicine
25th Annual Meeting

November 1-3, 1984 **Hyatt Regency** **Lexington, Kentucky**

The Southeastern Chapter of the Society of Nuclear Medicine will hold its 25th Anniversary Celebration November 1-3, 1984 at the Hyatt Regency in Lexington, Kentucky.

A Continuing Education Program entitled "Nuclear Medicine 1984" will be held, including outstanding faculty. The program is approved for 14 hr AMA Category 1 credit and 1.4 CEU's as is designated by ACPE.

The scientific program will feature recent work from centers throughout the Southeastern Chapter.

There will be two technologists workshops:

Ethics and Legalities of Nuclear Medicine Procedures
Cardiac Nuclear Medicine

For registration information and forms contact:

Dr. Vince Sodd
5987 Turpin Hills Drive
Cincinnati, Ohio 45244

Information Processing in Medical Imaging
9th International Conference

June 10-14, 1985 **Washington, D.C.**

Announcement and Call for Papers

The Scientific Review Committee welcomes abstracts on all aspects of computer and mathematical analysis and processing of in vivo digital images (nuclear medicine, CT, ultrasound, NMR, etc.). The conference objective is to share in advances that have been made in these fields; constructively criticize the presented work; develop directions for future efforts. The format of the program is structured to foster this objective with approximately 45 minutes for papers plus ample discussion time for each presentation. Full papers will be published in book form.

The total conference attendance is limited to fewer than 100 participants—either authors or co-authors of submitted papers or those researchers who wish to take an active part in the ensuing discussions. All attendees must register in advance. Abstracts must be less than 2 pages long, including supporting data, typed on 8½" by 11" paper, single spaced, in camera ready form. Limited financial support may be available in certain circumstances.

Send abstracts to, or request further information and registration forms from:

Stephen L. Bacharach, Ph.D.
National Institutes of Health
Building 10, Room 1C401
Bethesda, MD 20205 USA

Abstract deadline: December 10, 1984.