Evaluation of Cyclosporine Nephrotoxicity in Rats with Various Renal Radioactive Agents

John G. McAfee, F. Deaver Thomas, Gopal Subramanian, Marsha Roskopf, and Bradford Hellwig

Division of Nuclear Medicine and Radiological Sciences, Department of Radiology, SUNY Health Science Center at Syracuse, Syracuse, New York

The efficacy of different radiodiagnostic agents for demonstrating the decline in renal function from cyclosporine (CyA) nephrotoxicity was assessed in rats receiving a standard dose of the drug for 2 wk, compared with control rats. The agents included [^{99m}Tc]DTPA, [^{131}I]hippuran, [^{111}In]lysozyme, [^{99m}Tc]glucoheptonate (GHA), [^{99m}Tc]dimercaptosuccinate (DMS) and [^{111}In] aminated dextran (amdex). A small dose of [^{99m}Tc]-or [^{111}In]DTPA was administered simultaneously to normalize the results for variations in drug response from one animal to another. There were statistically significant differences in the detectability of the renal functional impairment by plasma clearance, early and 2-hr renal uptake among the different agents. However, none was clearly superior to DTPA. This conclusion is consistent with previous studies which showed a parallel decline in glomerular filtration rate (GFR) and effective renal plasma flow in acute CyA toxicity probably due primarily to vasoconstriction.

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Cyclosporine (Cyclosporin A) is a lipophilic cyclic undecapeptide (mol wt 1,202 D) immunosuppressive drug erratically absorbed from the GI tract, highly bound to plasma proteins, lipoproteins and red cells, metabolized extensively by the liver but with a renal clearance in humans of only 1 ml/min (*1*). In combination with prednisone, it is the most effective immunosuppressive used in renal, cardiac, liver and marrow transplant patients. It blocks the production of interleukin-2 (IL-2, a T-cell lymphokine) by lymphocytes, prevents the generation of cytotoxic cells and indirectly inhibits monocyte function, gamma-interferon, IL-1 and macrophage chemotactic factor. It is neither cytotoxic nor myelosuppressive. However, it is nephrotoxic. Even in heart transplant recipients without pre-existing renal disease on low doses of 7.4 mg/kg/day for 1 year or more, inulin clearance is 45% lower and PAH clearance 33% lower than in patients on azathioprine (*2*). The PAH renal extraction efficiency drops to ~73% and the transport of neutral dextran-40 is restricted, suggesting an intrinsic loss of filtration capacity. In one series (*3*), 19% of cadaveric renal transplants were lost; ~40% of these losses were a result of cyclosporine (*3*) and 40% of those induced by cyclosporine were lost within the first 2 wk.

Three clinical renal syndromes are ascribed to CyA (*4*). Episodes of acute renal insufficiency, often beginning 1 mo post-transplant are characterized by elevation of the serum creatinine and reduced GFR and ERPF. These episodes are difficult to distinguish from rejection, but promptly reversed by reduction of the daily dose of the drug. CyA is not primarily a tubular toxin, as originally thought, and the vacuolar changes and eosinophilic inclusions in the tubules appear to be markers of CyA therapy rather than toxicity (*1*). The acute episodes are probably due to renal vasoconstriction, perhaps related to renal prostaglandin and thromboxane production (*5*), but not primarily mediated by the renin-angiotensin system. The second syndrome, subacute severe renal failure extending beyond one week is less common, but recovery of renal function on withdrawal on CyA is incomplete. Histologically, there is diffuse interstitial fibrosis and an arteriopathy of interlobar and arcuate arteries of the renal cortex with intimal hyperplasia and hyalinosis, fibrin and platelet deposition in both renal transplants (*3*) and in patients without organ transplants (*6*). In the third syndrome of chronic nephropathy, there is a sustained increase in serum creatinine after 1 year, more common in cadaveric renal transplants, with prominent interstitial fibro-

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For reprints contact: John G. McAfee, MD, Div. of Nuclear Medicine and Radiological Sciences, Dept. of Radiology, SUNY Health Science Center at Syracuse, 750 East Adams St., Syracuse, NY.

sis, atrophy of cortical tubules with thickening of the basement membrane, focal sclerosis of glomeruli and focal hyalinosis of small arteries. These abnormalities have been found also in cardiac transplant patients on high doses of the drug (*2*).

Because the pathophysiology of CyA nephrotoxicity in renal transplant patients is often confounded by coexisting rejection and other complications, numerous studies have been carried out in experimental animals. The rat has been the favorite model for acute and subacute nephrotoxicity (*7*). However, there is no satisfactory model for CyA chronic nephropathy (*4*). Only meager information is available on the effect of CyA on the clearance and biodistribution of renal radiodiagnostic agents. The goal of this paper is to explore the similarities and differences in behavior of various radioactive agents in response to CyA administration.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 170–190 g received 20 mg/kg cyclosporine Sandimmune (cyclosporine) i.v. Sandoz, Inc., East Hanover, NJ, dissolved in polyoxyethylate castor oil and ethanol; diluted four times with 0.9% sodium chloride intraperitoneally daily, five times per week (Monday through Friday) for 2 wk. The histologic changes in the kidneys from a similar dosage regimen have been described previously (*8*). Four days after the last dose of CyA, radionuclide studies were performed in groups of six rats per day, to a total of 24 rats for each renal agent, including 12 controls and 12 receiving CyA. The control animals did not receive any drugs other than anesthesia and radioactive agents; every animal in the study was used for only one experiment.

The following renal agents were used: commercial kits of indium-111 (^{111}In) DTPA (Amersham Corp., Arlington Heights, IL) and dimercaptosuccinic acid (Medi-Physics, Inc., Richmond, CA) (DMS), and kits of glucoheptonate (GHA) and DTPA prepared in-house for technetium-99m (^{99m}Tc) labeling. Hippuran was labeled with iodine-131 (^{131}I) by exchange iodination and purified by high performance liquid chromatography as reported previously (*9*). Amdex (dextran, mol wt 5,000, rendered cationic by amination) and human milk lysozyme (mol wt 14,000) were coupled with DTPA cyclic dianhydride for labeling with as described previously (*10*). Labeled amdex was developed to demonstrate the loss of anionic charge on the glomerular basement membrane, and labeled lysozyme as a model low molecular weight protein accumulating in the renal cortex.

After the rats were anesthetized with sodium pentobarbital 5 mg/100 g intraperitoneally, the animals were weighed and the renal agents were injected intravenously through a tail vein in six groups of rats as follows:

100 μCi [^{99m}Tc]DTPA plus 60 μCi [^{111}In]DTPA;
50 μCi [^{131}I]hippuran plus 100 μCi [^{99m}Tc]DTPA;
100 μCi [^{111}In]lysozyme plus 100 μCi [^{99m}Tc]DTPA;
200 μCi [^{99m}Tc]GHA plus 30 μCi [^{111}In]DTPA;
30 μCi [^{99m}Tc]DMS plus 30 μCi [^{111}In]DTPA;
100 μCi [^{111}In] Amdex plus 100 μCi [^{99m}Tc]DTPA.

In each group, DTPA labeled with either ^{99m}Tc or ^{111}In was used as a "standard" agent to normalize the results.

The early renal uptake from 30 to 90 sec after injection was quantitated by a modified Gates (*11*) gamma camera-computer technique previously described in detail (*9*). As an exception, the uptake of [^{99m}Tc]DMS was measured between 2 and 3 min because its earlier uptake was so low. Heparinized blood samples of 0.2 ml were drawn from the warmed tail vein at 5, 10, 20, 30, 40, 60, 80, and 100 min to measure plasma clearances (*9,10*). At 120 min, the abdomen was opened and the last sample of blood drawn from the inferior vena cava. The animals were killed by excising the heart. The kidney depths from the posterior surface were measured by insertion of needles for attenuation corrections of the early in vivo renal uptakes.

The kidneys, liver, and bladder (containing urine) were dissected, weighed and counted in a well scintillation detector in comparison with a dilute standard of the administered activity. The concentration data were expressed as the percent dose per organ. The plasma clearances were calculated as ml/min/100 g body weight. Linear regressions were performed between the clearances and both the computer estimated early renal uptake and the 2-hr uptake of each agent. The data were examined by random block analysis of variance and differences between pairs of renal agents (CyA/control ratios) tested by Tukey's method (*12*).

RESULTS

The body weight of the 72 CyA rats (258g ± 21 s.d.) compared with the control rats (238g ± 18 s.d.) was significantly greater ($p < 0.001$ by unpaired t-test). Likewise, the weight of the two kidneys of the CyA rats (1.90g ± 0.22 s.d.) was greater than in control rats (1.72g ± 0.17 s.d.) ($p < 0.001$). However, the percent body weight of the two kidneys was the same for both groups (0.73%). This indicated that the dosage regimen of CyA did not cause detectable renal atrophy during the study.

Despite considerable variability in renal functional response from animal to animal, CyA produced a definite fall in DTPA clearance (GFR). Comparing the 72 CyA rats to the 72 controls, the mean clearance in the treated animals was significantly lower (0.67 ± 0.15 s.d. cf 0.87 ± 0.17 ml/min/100g, $p < 0.001$ by unpaired t-test).

The biodistribution data for the six groups of rats are compared in Table 1. Each group contains CyA and control rats, and a different agent in combination with a diethylenetriaminepentaacetic acid (DTPA) chelate of ^{99m}Tc or ^{111}In. The criterion for identifying a superior agent to detect cyclosporine nephrotoxicity was a CyA/control ratio of clearances or early computer uptake statistically significantly lower than that of DTPA. For the 2-hr renal uptake, a superior agent could be significantly lower or higher than that of DTPA, since late retention of an agent could also be indicative of nephrotoxicity. Because of obvious differences in the degree

TABLE 1
Comparison of Renal Agents in Cyclosporine (CyA) Rats and Controls

	Clearance ml/min/100 g	Early computer uptake % dose, two kidneys	2 kidney	2-hr uptake by radioassay liver	% dose urine
CyA [^{99m}Tc]DTPA	0.645 (0.0823)	9.72 (1.46)	1.34 (0.496)	0.313 (0.0742)	75.5 (6.88)
Con	0.899 (0.105)	11.0 (1.19)	0.817 (0.123)	0.243 (0.0250)	82.0 (4.98)
CyA [^{111}In]DTPA	0.641 (0.0813)	8.17 (1.14)	1.29 (0.601)	0.287 (0.0863)	75.9 (7.15)
Con	0.854 (0.0679)	11.1 (1.70)	0.672 (0.129)	0.249 (0.0233)	88.9 (4.66)
CyA Hipp	1.40 (0.406)	13.00 (2.18)	0.533 (0.543)	0.183 (0.0711)	81.8 (8.82)
Con	2.06 (0.402)	21.3 (2.45)	0.271 (0.201)	— —	— —
CyA [^{99m}Tc]DTPA	0.591 (0.162)	— —	3.03 (1.55)	0.459 (0.167)	71.8 (9.55)
Con	0.782 (0.184)	— —	1.86 (0.749)	— —	— —
CyA In-Lys	0.443 (0.0843)	11.9 (1.28)	50.3 (7.69)	2.68 (0.765)	35.0 (6.55)
Con	0.754 (0.0844)	14.5 (2.06)	50.5 (3.62)	1.71 (1.04)	28.6 (4.66)
CyA [^{99m}Tc]DTPA	0.718 (0.212)	— —	1.61 (0.920)	0.324 (0.0674)	89.7 (9.92)
Con	0.745 (0.116)	— —	1.70 (0.881)	0.273 (0.117)	75.9 (14.3)
CyA [^{99m}Tc]GHA	0.422 (0.112)	10.1 (1.13)	12.9 (2.61)	0.740 (0.148)	64.4 (8.83)
Con	0.653 (0.150)	14.3 (1.82)	13.5 (1.07)	— —	— —
CyA [^{111}In]DTPA	0.747 (0.207)	— —	1.39 (0.793)	0.301 (0.110)	84.6 (12.2)
Con	0.900 (0.281)	— —	1.38 (0.559)	— —	— —
CyA [^{99m}Tc]DMS	0.129 (0.0273)	18.2 (3.97)	50.4 (3.34)	3.30 (0.925)	9.05 (2.29)
Con	0.201 (0.0319)	18.9 (1.80)	56.9 (3.25)	2.41 (0.263)	17.9 (5.81)
CyA [^{111}In]DTPA	0.659 (0.118)	— —	1.11 (0.489)	0.269 (0.0296)	84.9 (6.83)
Con	0.987 (0.0951)	— —	0.878 (0.156)	0.306 (0.126)	78.2 (10.2)
CyA In-Amdex	0.879 (0.132)	10.3 (1.51)	1.45 (0.348)	0.703 (0.279)	81.4 (8.88)
Con	0.947 (0.112)	15.2 (1.20)	0.980 (0.311)	1.27 (0.261)	63.5 (13.0)
CyA [^{99m}Tc]DTPA	0.691 (0.0800)	— —	1.40 (0.268)	0.329 (0.047)	79.5 (5.26)
Con	0.944 (0.185)	— —	1.52 (0.598)	0.375 (0.0873)	66.2 (12.7)

*Mean of 12 animals in each group s.d. in parentheses

of renal damage for the same dose of CyA (mg/kg) in different groups, the ratios for the different agents were normalized with the corresponding DTPA values in the same animal. To complete the analysis of variance for the normalized ratios, therefore, a group of animals comparing [^{99m}Tc]DTPA with [^{111}In]DTPA was required.

The ratios of clearances shown in Table 2 were derived as follows: (CyA rat agent/DTPA ratio) ÷ (control rat agent/DTPA). There was a significant difference between groups in the random block anovar of renal clearances ($p < 0.005$). Lysozyme and hippuran had the lowest CyA/control ratios, and GHA the highest ratio. Nonetheless, none of the agents had a ratio significantly better than DTPA by Tukey's comparison of pairs.

The anovar of the computer-generated uptake of the two kidneys between groups showed a significant difference ($p < 0.005$). Hippuran had the lowest ratio, and DMS the highest. By Tukey's test, DMS was significantly poorer than other agents, but no agent was significantly better than DTPA.

The anovar of the 2-hr renal uptakes measured by direct organ assay showed a significant difference between groups ($p < 0.001$). This difference was due to the high values of hippuran, lysozyme and amdex. However, this late renal retention appeared to be a poor criterion for detecting changes in renal function with CyA, because some of the standard deviations (Table 1) were relatively large, and differences between CyA and control values relatively small; indeed, the mean values for lysozyme were identical. As in previous studies in rats (*9,10*) with and without glomerular disease, the cumulative 2-hr urine radioactivity also was a poor

TABLE 2
Mean CyA/Control Ratios Normalized by DTPA Values

	DTPA*	Hipp	Lys	GHA	DMS	Amdex
Clearance	0.931	0.694	0.659	1.38	1.02	1.26
Early computer renal uptake	0.928	0.677	0.715	0.691	1.13	0.746
2-hr renal uptake by radioassay	0.902	1.76	1.74	1.06	1.07	1.59

*Technetium-99m DTPA/indium-DTPA ratios

discriminator. For many of the agents in the current study, the hepatic uptake was higher in the CyA than in the control rats.

In the previous studies in rats (*9,10*), a good linear correlation was observed generally between the renal clearances and either the computer estimated early renal uptake or the late uptake ($r \approx 0.85$). However, in the current study with cyclosporine and control rats, these linear correlations were relatively poor ($r \leqq 0.74$). Representative graphs comparing renal clearances with renal uptakes are shown in Figure 1.

DISCUSSION

The mechanism of cyclosporine nephrotoxicity remains controversial (*13*). Renal vasoconstriction is the favored mechanism for acute toxicity (*4*). Acute infusions of 20 mg/kg result in a prompt 44% decrease in renal blood flow measured with radiolabeled microspheres, increased renal vascular resistance, peripheral renin activity and prostacyclin (*14*). An acute fall in GFR is prevented by renal denervation and a chronic fall by the alpha-adrenergic blocker, prazosin (*15*). Less acute toxicity is accompanied by changes in fluid volume. In rats given 10 mg/kg/day intramuscularly for 7 days (*16*), plasma volume with Evan's blue is reduced 33% because of hypoalbuminemia, whereas the inulin extracellular volume is generally increased 29%, but variable. Acute volume expansion with saline at least partially corrects the low GFR and renal blood flow, suggesting that the hypovolemia may contribute to the renal functional defect (*17*). The increase in body and kidney weight from CyA observed in the present study probably can be attributed to alterations in fluid balance, with a relative increase in interstitial rather than intravascular fluid volume.

Relatively few clinical radionuclide studies of the kidneys during the administration of CyA have been reported. In 14 imaging studies without quantitation in nine patients after liver transplantation (*18*), nine showed a decrease in the parenchymal uptake of [^{99m}Tc]DTPA greater than the decreased perfusion, and four had an equal decrease in perfusion and uptake. The decrease in [^{131}I]hippuran parenchymal uptake was similar to that of [^{99m}Tc]DTPA. In contrast, camera-computer studies in patients within 36 hr of renal transplantation showed an absent first transit peak of ^{99m}Tc in 83% of patients on CyA, compared to only 13% on azathioprine (*19*). In another report (*20*) of 32 patients with cadaveric renal transplants on cyclosporine, there was invariably a depressed uptake of [^{131}I]hippuran attributed to drug-induced vasoconstriction, because many of the biopsies were negative for rejection. Renal images with [^{111}In]oxine-labeled platelets were almost always positive in severe CyA nephrotoxicity, corresponding to platelet and fibrin deposition in cortical arteries seen histologically (*6*).

In the current series of experiments, the possible influence of anesthesia on the results is not known. The dose of CyA in mg/kg administered to the rats was in the range used previously in humans. With a similar protocol of dosage intraperitoneally, histologic changes have been observed invariably in rats by both light and electron microscopy (*8*).

All of the radioactive agents we examined showed decreased renal function in CyA rats compared with controls. The reduction in renal function with the same dose of CyA adjusted to body weight, however, appeared more variable than in other rat models of human

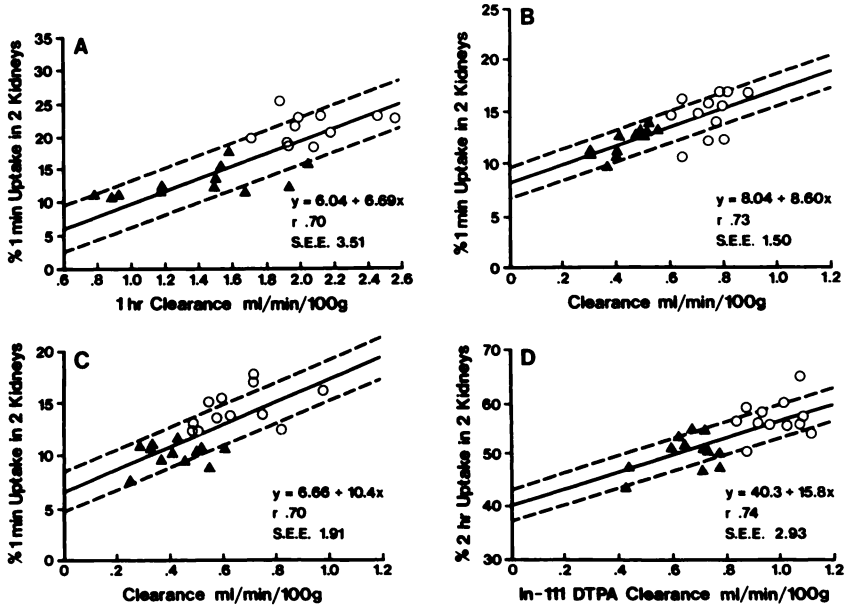

FIGURE 1
Linear correlations between plasma clearances and uptake in two kidneys; open circles-control rats; solid triangles cyclosporine-treated rats. (A) [^{131}I]hippuran (B) [^{111}In]lysozyme (C) [^{99m}Tc]GHA (D) Two-hour plasma clearance of [^{111}In]DTPA versus 2-hr renal uptake of [^{99m}Tc]DMS.

pathology. On comparing the CyA and control mean values for the various agents (Table 1), it would appear that some agents distinguish the treated from untreated groups better than others. Nonetheless, on normalizing the data using DTPA as a "standard agent" in each animal, most of the differences disappeared. We did not identify a renal agent which was clearly better than labeled DTPA for the detection of CyA nephrotoxicity, as assessed by either renal clearance or computer-estimated early renal uptake. The similar degree of CyA-induced reduction in renal function with the various agents is compatible with the present theory incriminating vasoconstriction rather than direct tubular toxicity.

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REFERENCES

1. Bennett WM, Norman DJ. Action and toxicity of cyclosporine. *Ann Rev Med* 1986; 37:215–224.
2. Myers BD, Ross J, Newton L, et al. Cyclosporine-associated chronic nephropathy. *N Engl J Med* 1984; 311:699–705.
3. Sommer BG, Innes JT, Whitehurst RM, et al. Cyclosporine-associated renal arteriopathy resulting in loss of allograft function. *Am J Surg* 1985; 149:756–764.
4. Myers BD. Cyclosporine nephrotoxicity. *Kidney Intern* 1986; 30:964–974.
5. Coffman TM, Carr DR, Yarger WE, et al. Evidence that renal prostaglandin and thromboxane production is stimulated in chronic cyclosporine nephrotoxicity. *Transplantation* 1987; 43:282–285.
6. Nahman NS, Cosio FG, Kolkin S, et al. Cyclosporine nephrotoxicity without major organ transplantation. *Ann Int Med* 1987; 106:400–402.
7. Whiting PH, Thomson AW, Blair JT, et al. Experimental Cyclosporin A nephrotoxicity. *Br J Exp Pathol* 1982; 63:88–94.
8. Siminton SC, Rynasiewicz J, Sibley RK. Light microscopic and electron microscopic features of experimental Cyclosporin A nephrotoxicity. *Lab Invest* 1983; 48:78A–79A.
9. McAfee JG, Thomas FD, Subramanian G, et al. Detection of diffuse glomerular lesions in rats. I. Comparison of conventional radioactive agents. *J Nucl Med* 1986; 27:502–512.
10. McAfee JG, Thomas FD, Subramanian G, et al. Detection of diffuse glomerular lesions in rats. II. Comparison of In-111 cationic small macromolecules with technetium-99m DTPA. *J Nucl Med* 1986; 27:513–520.
11. Gates GF. Glomerular filtration rate: estimation from fractional renal accumulation of ^{99m}Tc DTPA (stannous). *Am J Roentgenol* 1982; 138:565–570.
12. Steel RGD, Torrie JH. Principles and procedures of statistics: a biomedical approach. New York: McGraw-Hill, 1980: 137–194.
13. Thiel G. Experimental Cyclosporin A nephrotoxicity: a summary of the International Workshop (Basle, April 24–26, 1985). *Clin Nephrol* 1986; 25(suppl 1):S205–S210.
14. Paller MS, Murray BM, Ferris TF. Decreased renal blood flow after cyclosporine infusion [Abstract]. *Kidney Intern* 1984; 27:346.
15. Murray BM, Paller MS. Beneficial effects of renal denervation and prazosin on GFR and renal blood flow after cyclosporine in rats. *Clin Nephrol* 1986; 25(suppl 1):S37–S39.
16. Kaskel FJ, Devarajan P, Moore LC. Disturbances in plasma and extracellular volumes in chronic cyclosporine nephrotoxicity (CCN) [Abstract]. *Fed Proc* 1987; 4:1327.
17. Devarajan P, Kaskal FJ, Moore LC, et al. Reversal of hemodynamic deficit in chronic cyclosporine nephrotoxicity (CCN) by volume expansion [Abstract]. *Kidney Intern* 1986; 31:366.
18. Klintmalm GBG, Klingensmith WC, Iwatsuki S, et al. ^{99m}Tc-DTPA and ^{131}I-hippuran findings in liver transplant recipients treated with cyclosporin A. *Radiology* 1982; 142:199–202.
19. Thomsen HS, Munck O. Use of ^{99}Tc^{m} radionuclides to show nephrotoxicity of Cyclosporin A in transplanted kidneys. *Acta Radiologica* 1987; 28:59–61.
20. Thomsen HS, Nielsen SL, Larsen S, et al. Renography and biopsy-verified acute rejection in renal allotransplanted patients receiving Cyclosporin A. *Eur J Nucl Med* 1987; 12:473–476.