Comparison of Different Radioactive Renal Agents in Cisplatin-Induced Tubular Toxicity in Rats

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The efficacy of five different radiodiagnostic agents for detecting renal tubular dysfunction induced with cisplatin in rats was compared to controls. Diethylenetriaminepentaacetic acid (DTPA) labeled with $^{99m}$Tc or $^{111}$In was administered simultaneously with each of the other four agents ($^{99m}$Tc]glucoheptonate, $^{99m}$Tc]dimercaptosuccinic acid, [111]I]hippuran and [111]In]lysozyme) as a standard to normalize for differences in functional impairment from animal to animal from the same dose of cisplatin. The 2-hr plasma clearance and computer-generated 2- to 3-min uptake in the two kidneys with $^{99m}$Tc]dimercaptosuccinic acid were significantly inferior to similar measurements with the other agents in differentiating abnormal from normal function. The 2-hr uptake of $^{99m}$Tc]glucoheptonate and [111]In]lysozyme proved of no value in this differentiation. The late renal retention of $^{99m}$Tc]dimercaptosuccinic acid well separated the cisplatin from control rats, but the greatest difference was observed by the 2-hr uptakes of [111]I]hippuran and DTPA.


In clinical nuclear medicine, one must decide which of several renal radiodiagnostic agents will best differentiate abnormal from normal renal function in different diseases of the kidneys. Is one agent as good as another, or is one agent optimal for all kidney lesions? In attempting to answer these questions, we have compared several diagnostic agents previously in models simulating different spontaneous human renal diseases induced in rats compared with controls. In puromycin-induced glomerular damage simulating minimal change glomerulonephritis or the nephrotic syndrome in humans, no conventional agents (technetium-99m $^{99m}$Tc) GHA, $^{99m}$TcDMS, or iodine-131 ($^{131}$I) hippuran proved better than diethylenetriaminepentaacetic acid (DTPA) chelates (1). However, an unconventional agent, indium-111 ($^{111}$In] amdex (cationic aminated dextran) differentiated the glomerular dysfunction from controls better than DTPA (2). In rats rendered hypertensive by induced unilateral renal artery stenosis, no agent was identified, with or without captopril, that could better demonstrate the differences in function between the stenotic and contralateral kidney than DTPA (3). For this lesion, and the glomerular damage model, [111]I]hippuran appeared inferior to DTPA. For differentiating acute cyclosporine nephrotoxicity (probably primarily a result of vasoconstriction), no agent again appeared to be superior to DTPA (4). In this paper, we have compared five different renal agents in drug-induced renal tubular damage in rats. We selected cisplatin nephrotoxicity as the model because the histologic and functional abnormalities have been studied exhaustively in rats, and this entity remains a major problem in cancer chemotherapy.

MATERIALS AND METHODS

Except for the drug administration, the methods used were similar to those reported previously in rats with cyclosporine nephrotoxicity (4). Male Sprague-Dawley rats were given 7.5 mg/M$^2$ Cisplatin for injection (Platinol, Bristol Laboratories, Syracuse, NY) intravenously daily for 5 days. This dose is equivalent to 1.10 mg/kg in a rat weighing 230 g. Radionuclide studies were performed 10–11 days after the last drug dose in groups of six rats per day. Twelve technically satisfactory

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radionuclide studies were obtained for each renal agent in cisplatin rats. Actually, an average of 18 cisplatin rats was required because of technical failures and/or severe loss of renal function. Every animal in the study was used for only one experiment.

The following renal agents were used: $[^{113} \text{In}] \text{DTPA}$ (Amersham Corp., Arlington Heights, IL), and dimercaptosuccinic acid (DMS) (Medi-Physics, Richmond, CA), and kits of glucoheptonate (GHA) and DTPA prepared in-house for $^{99m} \text{Tc}$ labeling. Hippuran was labeled with $^{131} \text{I}$ by exchange iodination as reported previously (1) and purified by recrystallization. Human milk lysozyme was coupled with DTPA cyclic dihydroxide for labeling with $^{111} \text{In}$ as described previously (2), as a model low molecular weight protein accumulating in the renal cortex. The rats were anesthetized with sodium pentobarbital 5 mg/100 g body weight intraperitoneally, weighed, and the renal agents injected intravenously through a tail vein, as follows:

- 3.7 MBq (100 μCi) $^{99m} \text{Tc}$GHA plus 1.11 MBq (30 μCi) $^{113} \text{In} \text{DTPA}$
- 7.4 MBq (200 μCi) $^{99m} \text{Tc}$DMS plus 1.1 MBq $^{111} \text{In} \text{DTPA}$
- 3.7 MBq $^{131} \text{I}$hippuran plus 1.11 MBq $^{99m} \text{Tc}$DTPA
- 3.7 MBq $^{111} \text{In}$lysozyme plus 1.11 MBq $^{99m} \text{Tc}$DTPA
- 3.7 MBq $^{99m} \text{Tc}$DTPA plus 2.22 MBq (60 μCi) $^{111} \text{In}$ DTPA

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

The early renal uptake from 30 to 90 sec after injection was quantitated by a modified Gates gamma camera-computer technique (5) previously described in detail (1). As an exception, the uptake of $^{99m} \text{Tc}$ DMS was measured by 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. At 2 hr, the abdomen was opened and the last sample of blood drawn from the inferior vena cava. The animals were killed and 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 diluted 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 from the serial plasma samples obtained up to 2 hr; as an exception, only the samples up to 1 hr were used for the hippuran clearances. The cisplatin rats were compared with equal groups of control rats reported previously (4) that received only the anesthetic and radioactive agents. Using both cisplatin and control series, linear regressions were performed between the clearances and both the computer estimated early renal uptake and the 2-hr uptake for each agent. The data were examined by random block analysis of variance and differences between pairs of renal agents (cisplatin/control ratios) tested by Duncan's multiple-range procedure (6).

RESULTS

The mean body weight of the cisplatin rats (224 g, ± 31 s.d.) was not significantly different from that of the

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*(Cis) rats and controls; mean of 12 animals in each group, s.d. in parentheses.
control rats (238 g, ± 18 s.d.), although animals have lost weight with other cisplatin dosage regimens. The mean weight of the two kidneys of the cisplatin rats (1.99 g) was greater than in controls (1.72 g) because of edema. Likewise, the percent body weight of the two kidneys in the cisplatin rats (0.87%) was greater than in controls (0.73%). The mean DTPA clearance in the entire group of 60 cisplatin rats was 0.319 ml/min/100 g (s.d. ± 0.203) compared with a clearance of 0.863 ml/min/100 g (s.d. ± 0.170) in the 60 control rats (p < 0.001 by unpaired t-test).

The biodistribution data for the five groups of rats are compared in Table 1. Each group contains cisplatin and control rats and a different agent in combination with a DTPA chelate of 99mTc or 111In. The control rat data reported previously (4) are included in the table to facilitate the comparison with the cisplatin rats. The criterion for identifying a superior agent to detect the drug-induced tubular dysfunction was a cisplatin/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. Because of obvious differences in the degree of renal damage for the same dose of cisplatin from animal to animal and in different groups, the ratios for the different agents were normalized with the corresponding DTPA clearances in the same animal. To complete the analysis of variance for the normalized ratios, a group of animals studied with [111In]DTPA and normalized with [99mTc]DTPA was required.

The ratios of clearances were derived as follows. Pairs of cisplatin and control rats first were assigned by the random block method (6)—

\[
\frac{\text{agent clearance cis rat A}}{\text{DTPA clearance cis rat A}} \times \frac{\text{grand mean DTPA clearance cis rats (0.319)}}{\text{agent clearance control rat B}} \times \frac{\text{DTPA clearance control rat B}}{\text{grand mean DTPA clearance control rats (0.863)}}.
\]

The computer-generated early renal uptakes and 2-hr uptakes were normalized similarly with the above clearance values. The normalized mean ratios are listed in Table 2. It will be noted that these ratios are markedly different from those which could be obtained from the mean values listed in Table 1.

By random block anovar, there was a significant difference between groups of renal clearances (F = 11, p < 0.005). By Duncan’s multiple range test, DMS was significantly worse (highest ratio) than all other agents. Although hippuran had the best (lowest) ratio, this was not significantly different from that of DTPA or lysozyme.

The anovar of the computer-generated uptake of the two kidneys between groups showed a significant difference (F = 4, p < 0.005). By Duncan’s criteria, the only significant relationship was that DMS was significantly poorer than the other agents.

The anovar of the 2-hr uptake of the two kidneys measured by direct organ assay again showed a significant difference between groups (F = 11, p < 0.005). The 2-hr uptakes of GHA and lysozyme in cisplatin and control rats were so similar that these values were of no use in their differentiation. The 2-hr renal retention of DMS was significantly less in cisplatin rats than in controls. The late renal retention of both hippuran and DTPA was marked, compared with low values in controls, so that cisplatin/control ratios were high. For the analysis of variance, the reciprocals of these ratios were compared with the cisplatin/control ratios of the other agents. Using this procedure, these inverted ratios were better (lower) than that of DMS; although hippuran showed the greatest difference between cisplatin and controls it was not significantly better than that of DTPA. In another analysis of variance including cisplatin/control ratios of clearances, early computer uptakes and 2-hr renal uptakes, the best differentiation between the treated and control rats was observed with the 2-hr uptake of hippuran and DTPA.

For several of the agents in the current study, the hepatic uptake was higher in the cisplatin rats than in the control rats. Although the 2-hr urine data also were incomplete, the excretion of DTPA and DMS appeared decreased with cisplatin toxicity whereas that of lysozyme was somewhat increased.

Linear correlations between renal clearances, the computer estimated early uptake and the 2-hr retention in both kidneys are listed in Table 3 and representative graphs plotting renal clearances versus renal uptakes are shown in Figures 1 and 2.

**DISCUSSION**

Cisplatin localizes in the nucleus, microsomes, and cytoplasm of renal tubular cells, presumably damaging them directly. Histologically in rats, widespread pale
zones of degeneration and necrosis are seen in the outer stripe of the medulla (S3 segment of the proximal tubule) extending into the cortex. The changes reach a peak 5 days after drug administration (7). As in mercury nephrotoxicity, there is an associated fall in protein-bound sulphydryl groups along the brush border of the proximal tubule. In cisplatin rats the GFR falls and no inulin tubular leakage occurs, yet the glomeruli appear normal histologically. Polyuria occurs despite the fall in GFR because of the decreased concentration of sodium and urea in the papillae as a result of abnormal function of the proximal tubules or collecting ducts (8). Cisplatin does not inhibit the transport of PAH (organic anion transport), but does inhibit cation transport (9).

In the present project, therefore, it is highly unlikely that the distribution of [\textsuperscript{111}In]DTPA was influenced by cisplatin inhibition. Very large therapeutic doses of 2, 3-dimercaptosuccinic acid (DMS) administered to rats lowers the platinum renal concentrations because it is a heavy metal chelator, but nevertheless does not prevent renal toxicity (10). In this project, it is unlikely that the cisplatin per se influenced the distribution of the \textsuperscript{99mTc}DMS.

The dose of cisplatin administered in the present study of 7.5 mg/M\textsuperscript{2} is much smaller than in one clinical protocol in the drug manufacturer’s package insert...
however, well differentiates this tubular dysfunction from normal, and this measurement correlates closely with GFR, as shown previously (1) in glomerular lesions in rats \( r = 0.92 \). This close association between the late tubular concentration of this agent and the GFR (Fig. 1D) could be because of a tubuloglomerular feedback mechanism. The parameters which display the greatest difference between cisplatin and control rats were the 2-hr retention of \([^{111}\text{In}]\)hippuran (Fig. 2B) and, second, DTPA (Fig. 2D). The difference between

(FIGURE 1)
Correlation of clearances and computer-generated early uptakes in two kidneys. A: GHA, B: lysozyme, C: DMS, D: correlation of clearance of \([^{111}\text{In}]\)DTPA with the 2-hr uptake of DMS in two kidneys. Solid triangles-cisplatin rats; open circles-controls.
these two parameters was not statistically significant (p > 0.05). The 2-hr renal retention of $^{99m}$Tc|GHA and $^{111}$In lysozyme were of no value in detecting this tubular dysfunction; previously, the delayed renal uptake of GHA was found of no value also for the detection of glomerular lesions.

In an earlier report (11), the histologic findings in the kidneys with the same dose regimen of cisplatin were described. The 3-hr renal uptake cisplatin/control ratio of $^{131}$I|hippuran in that report was 0.05 for the same dose regimen of cisplatin, similar to the current 2-hr value (Table 2). Dehydration was found to be an important factor in increasing the late renal retention of hippuran with little or no change in its clearance. Abnormal parenchymal retention of $^{99m}$Tc|methylene diphosphonate also was demonstrated after cisplatin treatment with associated increases in parenchymal calcium concentration.

Factors other than direct tubular cytotoxicity probably contribute to the renal dysfunction from cisplatin administration. Continuous infusion measurements on the same day before and after cisplatin infusions in
patients showed a mean drop of 15% in ERPF and little if any change in GFR, suggesting an increase in renal vascular resistance (12). Renal vasoconstriction has been observed also in rats early after administration of cisplatin (13). In the present experiments, the interval of 10–11 days between the last dose of cisplatin and the radionuclide experiments may have minimized or eliminated possible vasoconstrictive effects of the drug.

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