Tc-99m DMSA Renal Uptake: Influence of Biochemical and Physiologic Factors

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Thirty-eight female Sprague-Dawley rats were studied to determine the effects of (a) tubular blockade and (b) commonly encountered changes in hydration and acid-base balance, on the urinary excretion and renal localization of Tc-99m dimercaptosuccinic acid (DMSA). Ten additional rats were studied to quantitate the in vivo protein binding of Tc-99m DMSA, and a final group of 12 animals was used to quantitate DMSA distribution in animals with diminished functional renal mass.

Both osmotic diuresis and dehydration by water deprivation for 24 hr resulted in a plasma clearance of DMSA slower than in control animals. Acid-base imbalances significantly affected the renal accumulation of DMSA, and acidosis was associated with markedly increased background due to increased liver accumulation. The protein-bound portion of Tc-99m DMSA in the plasma was high, reaching 89% within the first 5 min, and rising very slightly (n.s.) with time. The unbound portion of DMSA had a plasma clearance slightly higher than the GFR. Ablation of large amounts of renal tissue, resulting in significant decreases in GFR, did not significantly affect the renal localization of DMSA in the intact portions of the kidneys. These data demonstrate that commonly encountered changes in acid-base balance and hydration will significantly alter the biologic distribution of Tc-99m DMSA. These factors should be controlled when carrying out clinical studies.

J Nucl Med 22: 1054–1058, 1981

Available data are inadequate concerning the physiologic mechanisms of localization of the major technetium-labeled renal imaging agents, although they are achieving increasing clinical use. A few reports of studies of these compounds describe their organ distribution in laboratory animals (*1–3*) and their potential for clinical use in humans (*4,5*). Arnold and co-workers reported high renal cortical concentrations of DMSA in the dog and in human studies (*6*), which appeared similar to those of the mercury chlormerodrin compounds (*7*). These data have been quoted to support the suggestion that Tc-99m DMSA may be the best available technetium-labeled agent for determining relative functional renal mass.

It has been shown that organ distribution of DMSA can be altered markedly by the method of preparation, and especially by the original pH (*8–10,22*). The greatest renal localization of Tc-99m DMSA is achieved after reduction by SnCl at pH 2. Since patients referred for renal studies often have reduced renal function and abnormalities of acid-base balance, it is important to evaluate the possible effects of these pathologic conditions on the excretion and renal localization of Tc-99m DMSA. Our study was designed to determine the effects of acidosis, alkalosis, osmotic diuresis, and dehydration. These variables are frequently encountered in patients with renal disease. The organ distribution of DMSA in animals with diminished functional renal mass was also investigated to determine whether the distribution of DMSA is affected by changes in renal function. Renal tubular blocking agents and protein binding were studied to gain further insight into the mode of excretion and renal accumulation of the compound.

Received Dec. 29, 1980; revision accepted July 18, 1981.

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METHODS

Thirty-eight female Sprague-Dawley rats weighing 200–300 g each were studied by the single-injection

clearance technique (*12*) after anesthetization with ether. Silastic catheters (o.d. 0.37 in., i.d. 0.20 in.) were placed in the left carotid artery, left jugular vein, and urinary bladder. The rats were then placed in Plexiglas restraining cages, allowed to awaken, and divided randomly into six groups for study. Group 1 consisted of six control animals. Animals in Group 2 (n = 5) were given food *ad libitum* but were not allowed access to water for 24 hr before the study. Each rat in Group 3 (n = 7) was given osmotic diuresis with a loading dose of 250 mg of mannitol in a volume of 1 ml, followed by an infusion of solution containing 750 mg of mannitol (3 ml) added to 7 ml of heparinized saline. The volume administered ranged from 4.5 to 5 ml (337–375 mg). A fourth group of animals (n = 8) was given 100 mg of probenecid per 100 g of body weight, by gavage, to achieve proximal tubular blockage (*13*) one hour before the study. The urine was alkalinized in Group 5 (n = 6) by giving 800 mg of sodium bicarbonate in 2 ml of distilled water by gavage, 70–80 min before the start of the study. Acidification was achieved in Group 6 (n = 6) with 0.1 mg of NH_4Cl in 1.0 ml of distilled water, given by gavage 90 min before injection of the DMSA. The rats were allowed water, but all food was removed from the cage 24 hr before the study.

All of the rats except the mannitol group received a constant infusion of heparinized saline. The infusion was delivered at a rate of 2 ml/hr by Sage pump.

The Tc-99m DMSA was prepared from a commercial kit that contained 0.547 mg dimercaptosuccinate and 0.29 mg SnCl. Before use, purity was verified by paper chromatography (*14*). The pertechnetate (Tc-99m) was eluted from a commercial generator. Twenty to forty minutes after labeling, 0.1 ml of Tc-99m DMSA (50–60 μCi) and 0.1 ml of I-125 iothalamate (10 μCi) were injected through the jugular catheter. Arterial blood and urine samples were collected 5-min intervals up to 20 min, then at 10-min intervals during the following 80 min.

The animals were killed immediately after the clearance studies and the distribution of the dose in whole blood, plasma, urine, liver, and kidneys was determined by counting multiple samples in a well scintillation counter. Plasma clearance and urinary accumulation of I-125 iothalamate and Tc-99m DMSA were plotted on semilogarithmic paper for the clearance calculation, as described earlier (*15*). Each kidney was counted separately and the percent dose localized in the kidneys was used for comparison between the controls and the five other groups. All results are expressed as means ± one s.e.

Protein-binding study. Five additional normal control animals and five rats with 50% of one kidney infarcted by segmental arterial ligation 3 wk earlier were studied as described above. Blood samples were collected at 5, 10, 20, 40, 60, and 80 min. The blood was centrifuged immediately at each collection period and 100 μl of plasma was used for the determination of the protein-bound fraction of the injected Tc-99m DMSA. The protein-bound portion was precipitated with 1 ml of a 10% solution of trichloracetic acid, and two washings were carried out. The supernatants (unbound Tc-99m DMSA) and the precipitate were counted, and plasma clearances were calculated separately for the bound, unbound, and combined portions.

Infarcted kidneys. A third group of twelve female Sprague-Dawley rats, 200–300 g, was studied 1–3 wk after infarction of 50% of each kidney. Two to three weeks before the study, each rat had ~50% of the blood supply to each kidney obstructed by a 6-zero silk ligature around a branch vessel. The infarcted portions of the kidneys were demarcated by a distinct color change, and the percent infarction was estimated visually. Protein-binding fraction, plasma clearance, and renal and liver localization studies were done using the methods described above. The kidneys were removed at the end of the clearance study and divided into infarcted and non-infarcted portions. Multiple samples were counted and weighed, and the percent of the dose per gram of tissue calculated.

RESULTS

Plasma clearances of the iothalamate (GFR) were not significantly different, compared with control, for any of the test groups (Fig. 1). There appeared to be a tendency for decreased clearance in the dehydrated group, but this was not statistically significant. The amount of DMSA in the urine was significantly reduced ($p < 0.05$) in the dehydrated group (Table 1). There was a statistically significant reduction of the Tc-99m DMSA concentrated by the kidneys during acid-base imbalance (alkaline urine $p < 0.05$, acid urine $p < 0.1$) (Table 2). The animals treated with ammonium chloride showed marked decrease in renal accumulation, and a concom-

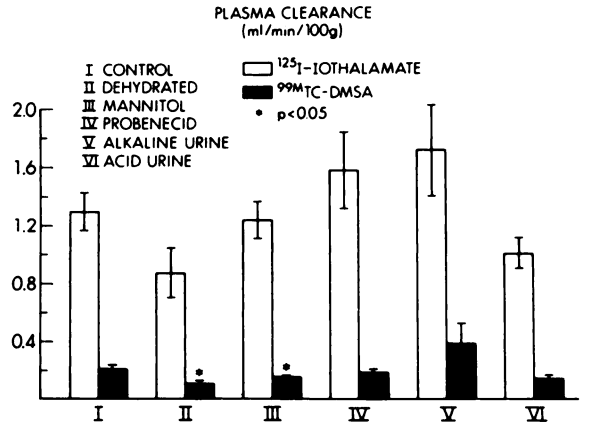

FIG. 1. Groups 2 through 6 versus Group 1. The GFR was not significantly changed for any of the test groups. However, a significantly slower plasma clearance of DMSA was demonstrated in the dehydrated and mannitol groups.

TABLE 1. I-125 IOTHALAMATE

Group (n)	Urine	Both kidneys (% of injected dose)	Liver
1 Control (6)	82.18 ± 3.1	0.69 ± 0.2	2.8 ± 0.9
2 Dehydrated (5)	42.56 ± 15.9*	3.19 ± 1.3	3.8 ± 1.7
3 Mannitol (7)	78.23 ± 6.7	1.58 ± 0.6	1.8 ± 0.3
4 Probenecid (8)	76.40 ± 3.0	0.79 ± 0.4	1.5 ± 0.4
5 Alkaline urine (6)	71.39 ± 9.7	0.48 ± 0.1	1.1 ± 0.3
6 Acid urine (6)	50.17 ± 9.2	1.33 ± 0.4	2.5 ± 0.3

Mean ± s.e.
* $p < 0.05$.

itant significant increase in liver accumulation. The last column of Table 2 tabulates the marked reduction in the ratio of right kidney to liver under acidosis. There appears to be a direct relationship between urinary pH and the amount of DMSA in the liver.

The mean urinary pH at 80 min was 9.18 ± 0.13 ($\bar{x}$ ± s.e.) after alkalinization with a mean plasma pH of 7.87 ± 0.03. The mean urinary pH was 5.9 ± 0.4 after acidification with a mean plasma pH of 7.69 ± 0.14.

In all six groups the left kidneys were compared with the right kidneys as to percentage of Tc-99m DMSA dose/gram of tissue (Fig. 2). There was excellent correlation between the concentrations of activity in the two kidneys. There did not appear to be any disturbance of the symmetry of uptake with the changes in urinary flow rates, or during the acid-base imbalances.

Eighty-nine percent of the Tc-99m DMSA was found in the precipitated plasma protein within 5 min of injection in the five control animals (Fig. 3). This protein-bound portion rose slightly but not significantly during the course of the study. The protein-bound fraction of the plasma DMSA was not significantly different in the animals with one kidney 50% infarcted, nor in those with 50% infarction of both kidneys.

Plasma clearances of the protein-bound fraction of Tc-99m DMSA, and of the combined bound and unbound fractions, were significantly lower ($p < 0.025$) than for the I-125 iothalamate (GFR). Linear regression analysis of the supernatants (free Tc-99m DMSA) indicates a slightly faster plasma clearance than would be expected for an agent excreted solely by GFR (Fig. 4). The rats with 50% infarction of both kidneys had significant reductions in GFR and urinary excretion of the iothalamate. The plasma clearance and urinary excretion of the DMSA also was reduced in these animals, but the excretion was not reduced to statistically significant levels. There was a significant increase in the amount of iothalamate retained in the infarcted kidneys, and a decrease in the amount of DMSA present when the animals were killed. A significant increase in liver accumulation of DMSA was noted in the infarcted animals (Table 3).

When the kidney parts were evaluated separately—i.e., control versus the infarcted portions of the kidneys—there was no significant difference between the control and noninfarcted tissue. Between infarcted and noninfarcted tissue, however, the ratio for DMSA uptake per gram was 0.088.

TABLE 2. Tc-99m DMSA

Group (n)	Urine	Kidneys % of injected dose	Liver	R.K./liver (% dose/g)
1 Control (6)	15.22 ± 2.0	54.92 ± 3.4	9.8 ± 2.1	35.3 ± 17.5
2 Dehydrated (5)	10.31 ± 2.9	59.48 ± 5.6	12.1 ± 2.5	24.5 ± 15.4
3 Mannitol (7)	14.64 ± 1.4	49.83 ± 1.1	8.0 ± 1.9	29.1 ± 17.5
4 Probenecid (8)	14.32 ± 0.9	50.36 ± 2.2	4.6 ± 0.3	55.9 ± 16.9*
5 Alkaline urine (6)	20.89 ± 3.4	43.62 ± 1.6*	5.4 ± 0.2	33.9 ± 4.5
6 Acid urine (6)	15.55 ± 3.5	20.95 ± 1.0†	18.1 ± 1.1†	4.8 ± 1.0†

Mean ± s.e.
* $p < 0.05$.
† $p < 0.01$.

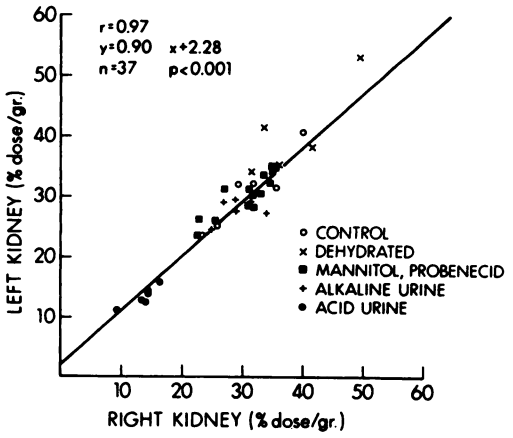

FIG. 2. Left kidney versus right kidney (percent of DMSA dose/gram of tissue). Symmetry of uptake of the Tc-99m DMSA was not affected by any of the systemic disturbances.

DISCUSSION

The decreased renal concentration of DMSA in rats producing either acid or alkaline urine suggests that acid-base imbalance will significantly alter DMSA kinetics. Acidosis may markedly increase the background activity during renal scanning, owing to concomitant increased liver accumulation. This may well impair the clinician's ability to compare sequential renal studies in a patient with changing biochemical and physiologic status.

Several other problems may also occur. Changing kidney/liver ratios may cause major problems when the relative renal uptake between the two kidneys is calculated. False overestimates of right renal activity may be expected to occur when there is an increased hepatic contribution to the count rate. Abnormalities of acidification of the urine in asymmetrical renal disease may theoretically affect the renal uptake disproportionately. In this situation, again, the ratio of renal uptake may be misleading.

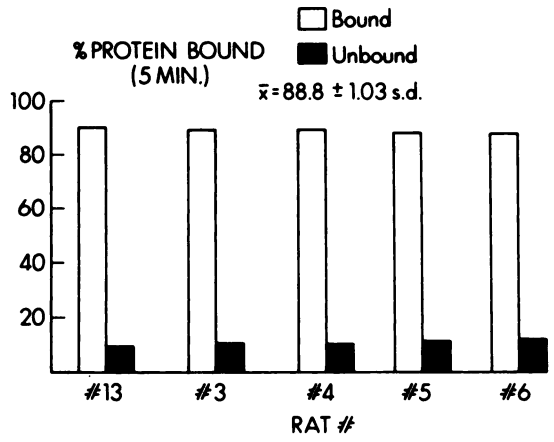

FIG. 3. Protein binding in five control animals. Bound fraction was 0.89 at 5 min, and did not change significantly thereafter.

Mannitol diuresis in normal animals causes an increased renal medullary blood flow (*16*). This may decrease the renal extraction fraction of the DMSA through its effect on transit time, thus accounting for the slower plasma clearance in this group as compared with the control group. Our finding of a marked decrease in plasma clearance of DMSA under dehydration noted here is probably caused by a reduction in size of the intravascular compartment and decreased renal perfusion. In addition, there appears to be a weak inverse correlation between the renal and hepatic accumulation and the urinary flow rate. Maintaining adequate hydration, then, becomes an important factor in decreasing background levels of Tc-99m DMSA as well as in decreasing the absorbed radiation dose to the kidneys and bladder.

The slightly faster than GFR plasma clearance of the unbound (free portion) of the Tc-99m DMSA may suggest a small secretory component in its renal excretion, or more likely an extrarenal compartment. Since the unbound portion constitutes only 10% of the plasma Tc-99m DMSA activity, there is little effect on the overall clearance rate. This also is supported by the results of the probenecid-treated group, in which no change in renal or urinary DMSA was shown during tubular blockade. Probenecid probably does not block the renal enzyme system that concentrates DMSA. Since liver concentration during probenecid blockade is reduced, there may be some hepatic inhibition by this agent.

The marked decrease in the amount of functional tissue in the group with bilaterally infarcted kidneys did not affect the uptake of the DMSA in the intact portions of the kidneys in spite of the significantly decreased GFR. The significantly higher amount of iothalamate in the infarcted kidneys, compared with the controls, may be a reflection of the markedly decreased plasma clearance, and possibly may be due to counts from the blood contained within the kidneys, or to intratubular obstruction with urinary stasis.

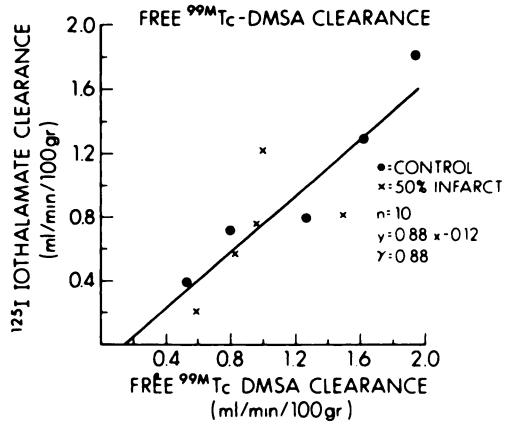

FIG. 4. Clearance of unbound Tc-99m DMSA versus GFR. Free Tc-99m DMSA clearance was slightly faster than GFR, which is compatible with a small secretory component in its renal excretion, or more likely an extrarenal site of localization or excretion.

TABLE 3. 50% INFARCTION OF BOTH KIDNEYS

	Plasma clearance	Urine	Both kidneys	Liver	Normal tissue		Infarcted tissue	
	ml/min/100 gr		% of injected dose		L.K. %/g	R.K.	L.K. %/g	R.K.
CONTROL I-125	1.30 ± 0.13	82.18 ± 3.13	0.69 ± 0.22	2.85 ± 0.91	0.40 ± 0.13	0.41 ± 0.14		
INFARCT I-125	0.67 ± 0.08†	49.70 ± 4.89†	3.07 ± 0.67*	4.87 ± 0.92	1.82 ± 0.41*	2.00 ± 0.44*	0.82 ± 0.27	0.80 ± 0.20
CONTROL DMSA	0.21 ± 0.02	14.22 ± 1.99	55.09 ± 3.38	9.76 ± 2.07	30.83 ± 2.39	30.81 ± 2.53		
INFARCT DMSA	0.15 ± 0.02*	11.61 ± 1.31	39.76 ± 2.86*	19.48 ± 2.76†	28.26 ± 2.74	27.24 ± 2.59	1.32 ± 0.33†	1.13 ± 0.34†

Mean ± s.e.
* $p < 0.05$.
† $p < 0.01$.
Control (n = 6).
Infarct (n = 12).

It is apparent from these studies that plasma clearance of DMSA does not strictly follow the GFR. There is a weak inverse correlation of the renal and hepatic accumulation with urinary flow rates, and there appears to be no significant tubular secretion or reabsorption of DMSA. Acid-base imbalances cause decreased renal localization, especially during acidosis, and there is a significant rise in liver accumulation with acidosis, contributing to an increased right renal background. Protein binding of a large fraction of the DMSA occurs very shortly after intravenous injection and does not change significantly with time.

In view of the increasing popularity of DMSA for the evaluation of renal function, it is imperative to establish more clearly the pathologic states that may affect its renal uptake. The findings reported here suggest several situations in which renal uptake of DMSA may be dissociated from functional renal mass. These considerations are particularly important in the evaluation of serial changes in renal function and in diseases with unilateral renal involvement.

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