Tubular Reabsorption of Technetium-99m-DMSA

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Intrarenal handling of ^{99m}Tc-DMSA is still controversial, particularly in the existence of tubular reabsorption from the tubular fluid. Experiments were performed with micropuncture technique on the rat kidney in an attempt to elucidate this question. **Methods:** The concentration profile of ^{99m}Tc-DMSA along the nephron was measured in fluid from Bowman's space of surface glomeruli and from the proximal and distal tubules collected by micropuncture. Superficial loops of proximal tubules were micropunctured and microperfused with ^{99m}Tc-DMSA or [^{99m}Tc] pertechnetate for 10 or 20 min at physiological flow rates; the recovery of activity was measured in the final urine. **Results:** Bowman's space urine contained only 14% of the ^{99m}Tc activity of arterial plasma, indicating low filtration of ^{99m}Tc-DMSA, likely due to high plasma protein binding. Tubular fluid-to-plasma activity ratios of 0.31 in the proximal tubules and 1.31 in the distal tubules suggest that ^{99m}Tc-DMSA is neither secreted nor reabsorbed along the nephron. Ninety-eight percent of the ^{99m}Tc-DMSA activity was recovered in the final ipsilateral urine, while only 0.5% was found in the urine of the contralateral kidney. **Conclusion:** A low fraction of ^{99m}Tc-DMSA enters the tubule by glomerular filtration and is not reabsorbed from the tubular fluid. Thus, only peritubular extraction by the tubular cell is responsible for renal uptake of ^{99m}Tc-DMSA.

Key Words: technetium-99m-DMSA; technetium-99m-pertechnetate; glomerular filtration; tubular reabsorption; micropuncture technique

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Different hypotheses exist about the handling of ^{99m}Tc-DMSA in the kidney. In particular, the mechanism responsible for ^{99m}Tc-DMSA uptake in the proximal tubular cells to ensure high quality renal imaging for several hours after injection is not clear. The conflicting results of studies on the renal handling of ^{99m}Tc-DMSA have been reviewed by Taylor (*1*), and the potential role of tubular reabsorption of ^{99m}Tc-DMSA from the tubular fluid has been especially debated. Some investigators (*2–4*) postulate tubular reabsorption as the predominant route for ^{99m}Tc-DMSA uptake by the kidney, but others (*5–10*) suggest peritubular uptake and no tubular reabsorption of ^{99m}Tc-DMSA in the kidneys. Most hypotheses, however, arise from indirect results. For instance, a study that used a nonfiltering kidney and that revealed no effect on the ^{99m}Tc-DMSA uptake, thus indicating no tubular reabsorption (*6*), has been questioned because of limitations in the nonfiltering kidney model (*1*). We therefore took another approach: to measure directly the amount of ultrafiltration of ^{99m}Tc-DMSA.

Earlier investigations on ^{99m}Tc-DMSA filtration were based on in vitro determination of ^{99m}Tc-DMSA protein binding and the assumption that the nonprotein-bound portion of ^{99m}Tc-DMSA appears in the ultrafiltrate. Not only is this approach indirect, but the amount of bound protein varies considerably in different studies, as does the amount of estimated, nonprotein-bound, filtered ^{99m}Tc-DMSA.

We sought to determine how much ^{99m}Tc-DMSA is filtered and reabsorbed in the kidney using the more direct approach of micropuncture and microperfusion techniques. Control experiments were performed using ^{99m}Tc-DTPA, which is known to be handled like inulin, i.e., without tubular transport, or [^{99m}Tc]pertechnetate, which serves as an indicator of proximal tubular reabsorption (*11*).

METHODS

Two different series of experiments were performed. One consisted of micropuncture collection experiments from surface glomeruli and from proximal and distal tubules during constant intravenous infusion of ^{99m}Tc-DMSA. The other involved microperfusion of proximal tubules and recovery measurements in the final urine. Both series of animal experiments were approved by the local ethical committees for animal research.

Micropuncture Collection Experiments

Five adult male Wistar rats weighing 250–440 g were used. They had free access to normal rat chow and tap water. After intraperitoneal anesthesia with 100–120 mg/kg thiobarbital, the trachea was cannulated and polyethylene catheters were placed in the femoral artery for continuous blood pressure monitoring and arterial blood sampling. The femoral or jugular vein was also cannulated for infusion of ^{99m}Tc-DMSA dissolved in saline to give a total infusion rate of 0.5 ml/hr/100 g body weight. Technetium-99m-DMSA (30 MBq/ml) was prepared from a kit approximately 1–3 hr before onset of infusion, and tubular collection was started 1 hr after onset of continuous infusion. The radiochemical purity of the ^{99m}Tc-DMSA kit was greater than 95%, according to the manufacturer. In our own quality control measurements, we obtained a purity of 99%. In one instance, the purity of DMSA was 98.0–99.2% directly after preparation (n = 30) and remained unchanged up to 6 hr after storage at room temperature without any signs of significant decomposition (Jansson B, *personal communication*). Thus, a relatively pure and stable radiopharmaceutical

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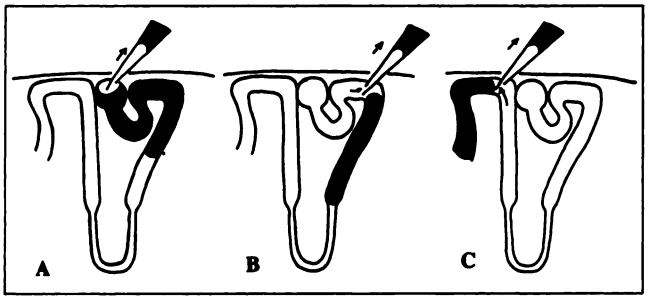

FIGURE 1. Schematic drawing of the micropuncture technique for collecting tubular fluid from the Bowman space of the glomerulus (A), the proximal tubule (B) and the distal tubule (C).

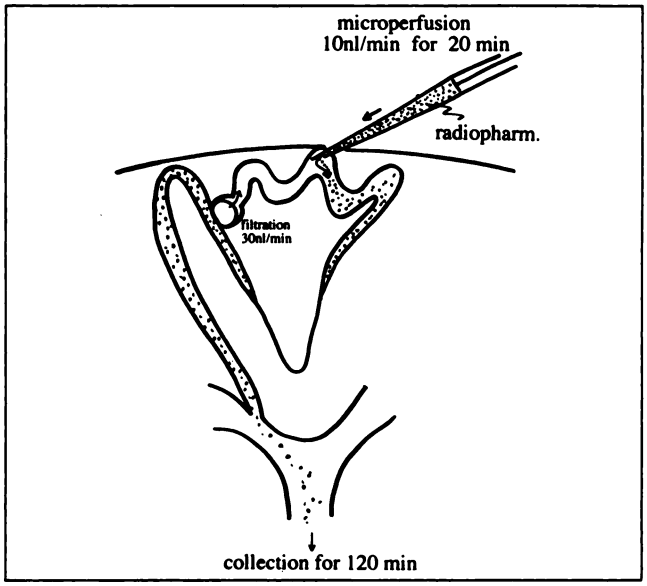

FIGURE 2. Schematic drawing of the micropuncture microperfusion experiment. A surface proximal tubule was microperfused with the aid of a microcapillary filled with ^{99m}Tc-DMSA and connected to a high-precision microperfusion pump. During microperfusion and up to 120 min thereafter, the final urine sample was collected for recovery measurements.

was used in this study and the length of the constant infusion did not affect glomerular and tubular kinetics.

A polyethylene catheter was placed in the ureter for urine sampling. The left kidney was prepared by peeling off the connective tissue, placing it in a lucite cup, affixing it with cotton and covering it with mineral oil. The kidney surface was illuminated by a fiberoptic light source.

Micropuncture was performed as described earlier (*12*) and is shown schematically in Figure 1. Surface glomeruli, visible at the surface as capillary balls, were punctured with sharpened glass pipets (5–7 μm outer diameter) filled with Sudan-stained rhicinus oil. The oil was injected into the Bowman space and the emerging proximal tubule and ultrafiltrate were collected by applying slight suction. Proximal tubules were selected randomly and tubular fluid was aspirated by collection pipets as previously described. Glomeruli of the same nephrons were identified by injection of oil upstream from the puncture site into the glomerule and reaspiration prior to proximal tubular collection. Thus, in some cases, both glomerular and tubular collection were performed in the same individual nephron. Distal tubules were identified by a single-shot injection of lissamin-green colored Ringer solution into a randomly selected proximal tubule with a separate pipet (1–3 μm diameter). The dye appeared at the distal puncture site after passage through Henle's loop. All samples were collected in front of the injected oil block (Fig. 1).

Collection times varied from 2 to 5 min and samples (10 to 100 nl) were collected and transferred to precalibrated constant bore capillaries (Microcaps, 0.5 μl, Drummond) to measure the volume. After transferring the samples into counting vials filled with 0.5 ml water, their activity was measured in a well-type counter. Plasma samples and final urine samples were also analyzed for their radioactivity as above. Activity was measured the same day and corrected for decay.

The plasma protein binding of ^{99m}Tc-DMSA was 90%, as measured in three rats using the centrifugation-filtration method in special vials (Amicon, Centrifree, Beverly, MA) at 3000 g.

Micropuncture Perfusion Experiments

In nine nephrons of four male Wistar rats, microperfusion experiments using ^{99m}Tc-DMSA and [^{99m}Tc]pertechnetate were performed. The animals, weighing 165–185 g and prepared as above, received an intravenous infusion of Ringer solution at 1.5 ml/hr/100 g body weight. The ureters of both kidneys were cannulated and the final urine was collected. Urine flow rates of 1 to 9 μl/min were obtained from the perfused left or nonperfused contralateral right kidney.

Microperfusion, applied as described earlier (*13,14*) is depicted schematically in Figure 2. Technetium-99m-DMSA, dissolved in physiological saline at 80 MBq/ml, was injected into randomly selected proximal tubules at a rate of 5 nl/min for 20 min (n = 2) or 10 nl/min for 10 min with the aid of a high precision and thermoinsulated microperfusion pump. Both before and after successful microperfusion, aliquots of the perfusate were collected at the same perfusion rates and identical time intervals and transferred to counting vials filled with 0.5 ml water. Means of activity of these aliquots served to determine the infused amount. Leakage of the perfusate observable through microscope was minimal (seen as a droplet of less than 0.5 nl under the oil surface). During microperfusion and at least 120 min thereafter, urine was collected from both kidneys (Fig. 3). Urine samples were weighed and transferred into counting vials and water was added to obtain a counting volume of 0.5 ml. The percentage of recovery in the final urine of the perfused kidney and the percentage of leakage to the final urine of the contralateral kidney were calculated from the calibrated infused amount. Recovery was defined as the total amount of activity in the final urine divided by the total infused amount, given as a percentage. Leakage was defined as the total amount of activity in the urine of the contralateral kidney during the same time period divided by the infused amount, given as a percentage. To assure the reliability of the method, control experiments were performed in two nephrons of one rat using ^{99m}Tc-DTPA. Leakage of ^{99m}Tc-DTPA was 1.3% and the recovery 98.7%. Thus, negligible leakage and almost complete recovery were found using this technique with ^{99m}Tc-DTPA. Similar results were reported for inulin by Hellberg and Källskog (*14*).

The radioactivity in the urine and infusion aliquots was measured directly after collection in a well-type counter and correction for decay.

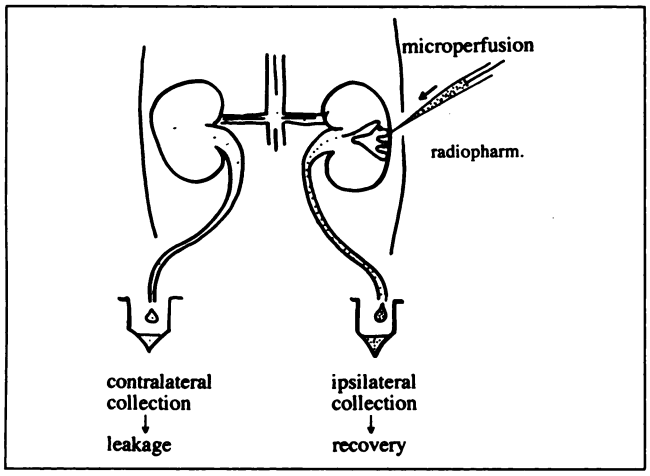

FIGURE 3. Schematic drawing of the recovery and leakage measurement of ^{99m}Tc-DMSA in the rat during microperfusion of a proximal tubule, as shown in Figure 2. The final urine sample from both kidneys was collected separately for measurements of ^{99m}Tc-DMSA recovery (perfused kidney) and leakage (contralateral kidney).

RESULTS

As shown in Table 1, which gives the results of the individual micropuncture collection experiments, the amount of ^{99m}Tc-DMSA in the ultrafiltrate of the normal rat was very low, with a ultrafiltrate-to-plasma ratio of 0.14 ± 0.02. The ^{99m}Tc-DMSA concentration increased along the nephron, as seen from a tubular fluid-to-plasma concentration ratio of 0.31 ± 0.04 at the proximal tubular site and 1.31 ± 0.34 at the distal tubular site, reaching a ratio of 38 ± 7.1 in the final urine. The duration of the constant infusion of ^{99m}Tc-DMSA did not affect glomerular and tubular kinetics.

Results of the tubular microperfusion experiments (Table 2) show that almost all (97.9%) of the injected ^{99m}Tc-DMSA was recovered in the final urine after passage through the entire nephron. Only 0.5% of the injected activity was found in the final urine of the contralateral kidney. These values agree with those reported for inulin (*14*) as well as our measurements with ^{99m}Tc-DTPA.

When [^{99m}Tc]pertechnetate was used in the perfusion fluid, 57% was recovered in the final urine and 4.5% was found in the urine of the contralateral kidney, indicating significant outward movement of activity from the tubular lumen mediated by reabsorption or passive diffusion. This finding is consistent with earlier observations on pertechnetate (*11*).

DISCUSSION

We identified two important events in this study: First, low fractions ^{99m}Tc-DMSA enter the tubular lumen by glomerular filtration; second, ^{99m}Tc-DMSA in the tubular lumen is not actively or passively reabsorbed by the tubular epithelium.

Both results were obtained by direct in vivo measure-

TABLE 1
Micropuncture Collection Data

Rat	Nephron	Glomer. UF/P	Proximal TF/P	Distal TF/P	Final urine UF/P
1	1	0.20	0.50	2.45	29
	2	0.17	0.13		
	3	0.14	0.20		
	4	0.11	0.14		
	5		0.33		
	6		0.16		
2	1	0.10	0.25	0.78	16
	2	0.21	0.17	1.05	
	3		0.36	0.60	
	4		0.26		
	5		0.51		
3	1	0.11	0.28		41
4	1		0.28		56
5	1	0.11	0.30	1.70	49
	2		0.28		
	3		0.20		
	4		0.85		
Mean ± s.e.m		0.14 ± 0.02	0.31 ± 0.04	1.31 ± 0.34	38.2 ± 7.1
Number	17	8	17	5	5

UF/P = ratio between activity of the ultrafiltrate in Bowman space to that of arterial plasma; TF/P = ratio between activity of tubular fluid to that of arterial plasma; U/P = ratio of activity of the final urine to that of arterial plasma.

TABLE 2
Microperfusion Data

Rat	Nephron	^{99m}Tc-DMSA		[^{99m}Tc]pertechnetate	
		Recov	Leak	Recov	Leak
1	1	98.7	0.3		
2	1	100.6	0.4		
	2	98.8	0.4		
	3	96.2	0.3		
3	1	108.0	0.6		
	2	84.2	1.1		
	3	99.4	0.5		
	4			61.3	5.1
4	1			52.7	4.0
Mean ± s.e.m		97.9 ± 2.6	0.5 ± 0.1	57	4.5
Number	9	7	7	2	2

Recov = percent of microperfused amount collected in the final urine of the ipsilateral kidney; leak = percent of microperfused amount collected in the final urine of the contralateral kidney.

ments of micropuncture techniques in rat kidneys. The amount of ^{99m}Tc-DMSA filtered from plasma within the glomeruli was assessed by analyzing fluid from Bowman's space directly. The 0.14 concentration ratio between plasma and ultrafiltrate in the glomerulum corresponded to the approximately 10% of protein-free fraction of DMSA, as measured in vitro using the ultrafiltration centrifugation. Thus, the plasma protein binding of DMSA might be the cause of its retention during glomerular filtration. The amount of filtered DMSA will therefore be low, only 14% of the glomerular filtration rate or 4.2% of the renal plasma flow (assuming a filtration fraction of 30%; i.e., $0.30 \times 0.14 = 0.042$). For humans, decreased fractions would be expected since the filtration fraction is lower (approximately 20%) and the amount of unbound DMSA in the plasma is the same as in the rat (approximately 10%). Interestingly, in a study by de Lange et al. (*8*), indirect estimation of the filtered amount, based on a filtration rate of 123 ml/min and the assumption that only unbound fraction is available for filtration, resulted in a filtration fraction of 10%. This equals the final excretion of DMSA in the urine. Thus, their findings in humans support the hypothesis of absent tubular reabsorption or secretion of DMSA during passage through the nephron.

It is therefore reasonable to assume that further increase of ^{99m}Tc-DMSA activity in tubular fluid along the nephron is caused solely by water extraction similar to that for inulin (*15,16*). In two nephrons shown in Table 1 (rat 1, nephron 2; rat 2, nephron 2), the concentration ratio in the early proximal tubule was lower than that in the glomerule. Since this is in the scatter of estimate for nanoliter-samples of low radioactivity, it may not represent a true decrease of DMSA activity in the proximal tubule.

The issue of tubular reabsorption was further addressed by direct measurements using microperfusion re-collection experiments. This technique is highly sensitive for measuring reabsorption or leakage of intratubularly applied substances (*13,14*). Technetium-99m-pertechnetate showed considerable reabsorption along the nephron, as postulated earlier (*11*) and documented by our direct measurements. Technetium-99m-DMSA and ^{99m}Tc-DTPA, however, showed no tubular reabsorption in this study and thus were handled like inulin when present in the tubular lumen (*14*). Our results support the hypothesis that ^{99m}Tc-DMSA is not reabsorbed from the tubular fluid (*5–10*). This is, however, at variance with the hypothesis of others (*2*), who argue for tubular reabsorption as the main pathway of renal uptake of DMSA. One explanation for the controversy over tubular reabsorption of ^{99m}Tc-DMSA may be that the amount of protein-bound ^{99m}Tc-DMSA in the study of Peters et al. (*2*) was considerably smaller than other reported values (*9*) or those stated in this study, which resulted in different estimates of filtered DMSA.

For obvious reasons, such direct measurements cannot be made in human kidneys, and species differences must also be considered when explaining the different results. Nevertheless, the values for plasma protein binding of ^{99m}Tc-DMSA in human blood (*8,9*) correspond with our results in rats. Thus, the same conditions of low glomerular filtration and absence of tubular reabsorption are probably also present in the human kidney. Although the present study cannot specifically address the issue of any secretion of DMSA along the nephron, our data do not support the postulation that there is secretion. If this were true, however, a higher concentration profile of DMSA along the nephron would be expected.

CONCLUSION

Present knowledge of intrarenal handling of ^{99m}Tc-DMSA indicates that:

1. Technetium-99m DMSA is highly bound to plasma proteins in the circulating blood after injection and penetrates the glomerular filter at very low rates.
2. The amount of ^{99m}Tc-DMSA present in the tubular system is completely excreted and not reabsorbed from the tubular fluid.
3. Peritubular extraction must account for the uptake of ^{99m}Tc-DMSA in the proximal tubular cells of the renal cortex. Technetium-99m-DMSA is then bound to the cell plasma proteins (*17–19*), presumably with a high binding constant, and not further removed, i.e., it accumulates with time in the kidney.

Peritubular uptake occurs by an active process that depends on aerobic metabolism (*6*) and may also be influenced by acidbase disturbances (*5*) and tubular dysfunction, as seen in Fanconi syndrome (*3,4*). Thus, the renal uptake of ^{99m}Tc-DMSA, as shown on gamma camera studies after intravenous injection, is based on the visualization of cell uptake from peritubular capillaries into the renal cortex.

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