Renal Handling of Technetium-99m DMSA: Evidence for Glomerular Filtration and Peritubular Uptake

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The finding of an enhanced excretion of [^{99m}Tc]dimercaptosuccinic acid (DMSA) in patients with tubular reabsorption disorders prompted us to investigate the role of filtration in the renal handling of [^{99m}Tc]DMSA. Our studies in human serum indicated that binding to serum proteins was ~90%. Chromatography of human urine and studies in rats showed that the complex was excreted unaltered into the urine. Renal extraction of [^{99m}Tc]DMSA in a human volunteer was 5.8%. Continuous infusion of [^{99m}Tc]DMSA in 13 individuals with normal renal function gave the following results (mean ± s.d.): plasma clearance of [^{99m}Tc]DMSA 34 ± 4 ml/min, urinary clearance of [^{99m}Tc]DMSA 12 ± 3 ml/min. The calculated filtered load of [^{99m}Tc]DMSA closely resembled the urinary clearance, whereas the plasma clearance was about three times faster. This indicates that peritubular uptake accounts for ~65% and filtration for ~35% of the renal handling of [^{99m}Tc]DMSA.

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Technetium-99m dimercaptosuccinic acid ([^{99m}Tc]DMSA) was introduced in 1974 (*1–4*) and is still the agent of choice for static renal scintigraphy. Despite its frequent use, little is known about the mechanism of uptake in the kidney. It has been shown that the tracer is concentrated in the proximal tubular cells of the kidney (*5*).

Technetium-99m DMSA could enter the proximal tubular cell either by glomerular filtration and subsequent reabsorption or by direct uptake from the peritubular capillaries. Because [^{99m}Tc]DMSA is largely bound to serum proteins (*1,4,6,7*), it is generally assumed that glomerular filtration is insignificant and that the uptake takes place at the peritubular side of the cell (*8*).

We found a low renal uptake and a high concentration of [^{99m}Tc]DMSA in the urine of patients with the Fanconi syndrome and other forms of tubular reabsorption dysfunction that are accompanied by tubular proteinuria (*9,10*). This prompted us to investigate whether or not at least part of this small anionic tracer is filtered by the glomerulus. We therefore studied the kinetics of [^{99m}Tc]DMSA by measuring plasma protein binding, by investigating whether it was the intact complex that appeared in the urine, and by measuring renal extraction rate, plasma clearance, and urinary clearance of [^{99m}Tc]DMSA.

MATERIALS AND METHODS

A commercial kit for labeling DMSA with $^{99m}TcO_4^-$ was used (Mallinckrodt Diagnostica, Petten, Holland). The kit, containing 1.2 mg dimercaptosuccinic acid, 6 mg $SnCl_2$, and 30 mg inositol was labeled with 60 MBq of $^{99m}TcO_4^-$ for continuous infusion studies, with 500 MBq for protein binding and renal scintigraphy studies in humans and with 1.6 GBq for the rat experiments. An oxygen-free solution of NaCl 0.9% was used for dilution to 5 ml; labeling was always carried out at a temperature of 20°C. All studies were carried out within 4 hr after preparation of the radioactive complex. Radiochemical purity was checked regularly by thin layer chromatography in n-butanol saturated with 0.3 N HCl and was always higher than 95%.

Protein Binding

Protein binding of [^{99m}Tc]DMSA was determined using two different methods: ultrafiltration and trichloroacetic acid

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precipitation. For the ultrafiltration study, serum was taken from three normal persons, two children with proximal tubular dysfunction and two renal transplant recipients. Serum (5 ml) was incubated in vitro with 0.1 MBq [^{99m}Tc]DMSA, resulting in a concentration of radioactivity slightly higher than immediately after injection of [^{99m}Tc]DMSA in a renal scintigraphy study. One milliliter of this mixture was placed in a reservoir for ultrafiltration (Amicon Micropartition System MPS-1) and centrifuged at 2,000 *g* for 20 min. Radioactivity was measured in a well-type gamma counter in 100 μl of ultrafiltrate and in 100 μl taken from the original serum-[^{99m}Tc]DMSA mixture to determine the free (C_f) and total (C_t) concentration of [^{99m}Tc]DMSA. The degree of protein binding (P) was calculated according to the formula $P = (C_t - C_f)/C_t$.

The precipitation was carried out in serum of 12 normal humans without evidence of renal disease. Serum was incubated with [^{99m}Tc]DMSA and the serum proteins were precipitated by adding 1 ml trichloroacetic acid (TCA) 20% to 1 ml of serum. The precipitate (R) and supernatant (S) were separated and counted, and protein binding was calculated as $P = R/(R + S)$.

Urine Chromatography

During the first 2 hr after injection of the tracer, urine was collected from two patients with normal renal function who underwent a renal scintigraphy study with [^{99m}Tc]DMSA (dosage 75 MBq). Using thin layer chromatography (TLC) in n-butanol saturated with 0.3 N hydrochloric acid, three solutions were studied: (a) the original [^{99m}Tc]DMSA solution, (b) patient urine, after i.v. [^{99m}Tc]DMSA injection, containing ~1 MBq of radioactivity in 20 ml urine, and (c) the same urine after addition of 1 MBq of the original [^{99m}Tc]DMSA. The TLC paper was cut into pieces of 0.5 cm which were counted in a well-type gamma counter.

Rat Experiments

A female Wistar rat (200 g) received 20 MBq of [^{99m}Tc]DMSA intravenously. After 1 hr, a pinhole gamma camera image was made. Urine was then collected by catheterization. A portion of the urine (0.3 ml), containing 0.2 MBq of radioactivity, was injected intravenously in a second rat and the distribution in this rat was examined 1 hr later using a gamma camera. The experiment was repeated in two other rats, in the first of which dysfunction of the proximal tubules was induced by injecting Na-maleate (2 mmol/kg body weight) intraperitoneally 2 hr prior to the study (*11*).

Extraction

The extraction (E) of [^{99m}Tc]DMSA in the kidney was measured in a human volunteer by taking arterial (A) and renal venous (V) serum samples during continuous infusion of iodine-131 (^{131}I) hippurate and [^{99m}Tc]DMSA by the method described below. Iodine-131 and ^{99m}Tc were counted in a well-type gamma counter and a correction was made for ^{131}I counts in the ^{99m}Tc window. Extraction of [^{131}I]hippurate and [^{99m}Tc]DMSA was calculated as $E = (A - V)/A$.

Renal Clearance

In 13 individuals with normal renal function, the renal clearance of [^{99m}Tc]DMSA was measured by adding [^{99m}Tc]DMSA to the infusion fluid during the continuous infusion of [^{131}I]hippurate and [^{125}I]iothalamate for the measurement of effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) (*12*). Iodine-131 hippurate containing <1% free iodide was used. In short, a sustaining solution was prepared by dissolving 2.0 MBq of [^{131}I]hippurate, 1.5 MBq of [^{125}I] iothalamate, and 3.0 MBq of [^{99m}Tc]DMSA in 100 ml of a 0.9% NaCl solution; 20 ml of this solution, to which 0.3 MBq of [^{125}I]iothalamate and 1.5 MBq of [^{99m}Tc]DMSA were added, was used as a priming solution. Injection of the priming solution was followed by continuous infusion of the sustaining solution for 5.5 hr at a rate of 12.0 ml/hr. After an equilibration time of 1.5 hr, plasma concentrations of the three radiopharmaceuticals were stable. In the next 4 hr, plasma samples were taken every hour and urine was collected during two periods of 2 hr. Thereafter, ^{131}I and ^{99m}Tc were counted in blood and urine samples and a correction was made for ^{131}I counts in the technetium window. After decay of ^{99m}Tc, ^{131}I, and ^{125}I were counted and a correction was made for ^{131}I counts in the ^{125}I window. ERPF and GFR were calculated (*12*). Since after equilibration the rate of infusion equals the rate of elimination of [^{99m}Tc]DMSA, the plasma clearance (C_p) of [^{99m}Tc]DMSA could be calculated:

$C_p = IV/P$,

where I = concentration of [^{99m}Tc]DMSA in the infusion fluid (cpm/ml)

V = infusion rate (ml/min)

P = plasma concentration of [^{99m}Tc]DMSA (cpm/ml).

Furthermore, the urinary clearance (C_u) of [^{99m}Tc]DMSA was calculated using the formula $C_u = UV/P$,

where U = concentration of [^{99m}Tc]DMSA in urine (cpm/ml);

V = urine volume (ml/min);

P = plasma concentration of [^{99m}Tc]DMSA (cpm/ml).

RESULTS

Protein Binding

Since protein binding influences both the distribution volume as well as the amount of [^{99m}Tc]DMSA available for glomerular filtration, the binding of [^{99m}Tc]DMSA to serum proteins was measured.

Using ultrafiltration, in three normal persons values of 89%, 94%, and 94% were found, respectively. In serum of two patients with proximal tubular dysfunction, showing enhanced excretion of [^{99m}Tc]DMSA into the urine, a similar binding of [^{99m}Tc]DMSA to serum proteins was found: 88% and 93%. In two renal transplant patients, protein binding of [^{99m}Tc]DMSA was 96% and 97%.

Protein binding of [^{99m}Tc]DMSA, determined with trichloroacetic acid precipitation, averaged 90% (n = 12, range 85–94%).

Urine Chromatography

Before measuring and calculating plasma and urinary clearance of [^{99m}Tc]DMSA based upon radioactivity measurement, we investigated whether the intact molecule appeared in the urine. Using thin layer chromatography, we examined the original [^{99m}Tc]DMSA solution, patient urine 2 hr after i.v. injection of [^{99m}Tc]DMSA and the same urine after addition of [^{99m}Tc]DMSA. Each time, one peak was obtained with an R_f

value (relative front) between 0.30 and 0.40. This suggests that all three peaks represent the same compound. The impurities most likely to occur, hydrolyzed-reduced technetium and free pertechnetate, have R_f values of 0.0–0.15 and 1.0, respectively. None of these impurities were detected.

Rat Experiments

To test whether [^{99m}Tc]DMSA after excretion into the urine still had the same biologic characteristics, we administered i.v. the urine of a rat, after i.v. [^{99m}Tc]DMSA injection, into another rat. The experiment was repeated in two other rats after induction of proximal tubulopathy with an injection of Na-maleate in one rat.

The results are shown in Figure 1. In the first rat, as expected, a normal distribution of [^{99m}Tc]DMSA at 1 hr after injection was seen: 39% of the injected dose was localized in the kidneys and 5% had been excreted into the urine. One hour after injection of this urine into the second rat, 21% of the radioactivity injected was retained in the renal cortex and urinary excretion was 9%. The third rat showed a pattern of dysfunction of the proximal tubules induced by Na-maleate. In this situation, a large amount (21%) of the tracer had been excreted in the urine. When this urine was injected intravenously in the fourth rat, the radioactivity was distributed in a similar way as in the first normal rat.

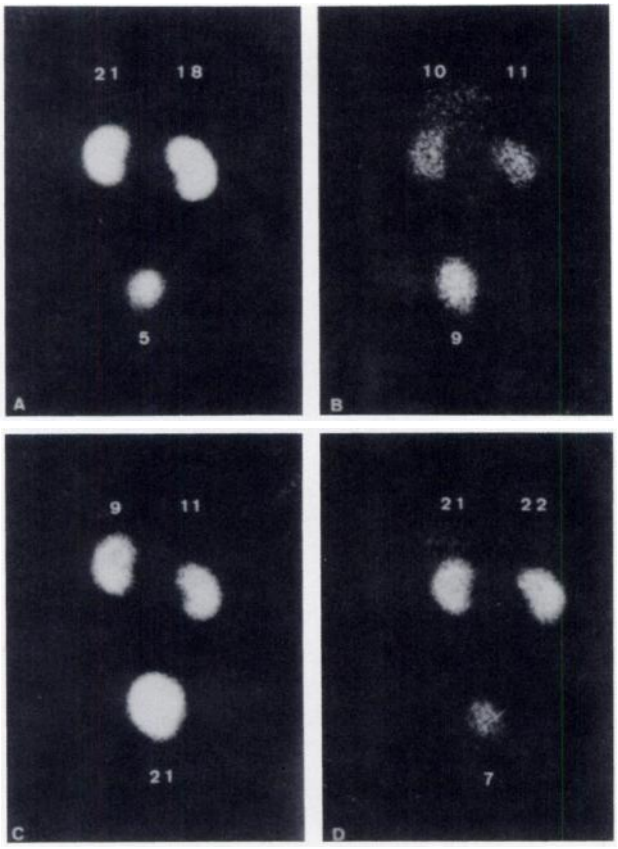

FIGURE 1
Renal uptake and urinary excretion of [^{99m}Tc]DMSA in four rats (anterior views). Figures represent absolute amounts of [^{99m}Tc]DMSA as percentage of the injected dose. Rat A received 20 MBq of [^{99m}Tc]DMSA. The radioactive urine of rat A was injected intravenously in rat B. In rat C, proximal tubular dysfunction was induced by an i.p. injection of Na-maleate prior to the injection of 20 MBq [^{99m}Tc]DMSA. The radioactive urine of rat C was administered to rat D.

Renal Extraction

In order to establish the renal contribution to the clearance of [^{99m}Tc]DMSA, we measured the renal extraction capacity in one normal individual. After catheterization of the right renal vein, renal extraction of [^{99m}Tc]DMSA was shown to be 5.8 ± 0.2% in three paired arterial and renal venous blood samples. The extraction of [^{131}I]hippurate, measured in the same blood samples, was 75 ± 3%.

[^{99m}Tc]DMSA Clearance

Eventually, the actual plasma clearance and urinary clearance of [^{99m}Tc]DMSA were measured, together with effective renal plasma flow (ERPF) and glomerular filtration rate (GFR), in order to correlate them with the data on protein binding and renal extraction of [^{99m}Tc]DMSA. Because [^{99m}Tc]DMSA is largely retained in the renal cortex, the plasma clearance exceeds the urinary clearance.

Continuous infusion of [^{99m}Tc]DMSA, together with [^{131}I]hippurate and [^{125}I]iothalamate in 13 individuals with normal renal function gave the following results (mean ± s.d.; the individual values are shown in Table 1): ERPF: 558 ± 85 ml/min, GFR: 123 ± 15 ml/min, plasma clearance of [^{99m}Tc]DMSA: 34 ± 4 ml/min, urinary clearance of [^{99m}Tc]DMSA: 12 ± 3 ml/min.

DISCUSSION

Of many radiopharmaceuticals used in nuclear medicine imaging today, the exact mechanisms of uptake and excretion remain obscure. This is particularly true for the renal imaging agent [^{99m}Tc]DMSA, that is accumulated in the proximal tubular cells (*5*). However, the exact mechanism of uptake are unclear. As [^{99m}Tc]DMSA is mostly bound by serum proteins, it was reasoned that the free fraction of [^{99m}Tc]DMSA was very small and that uptake thus would take place at the peritubular side of the cell (*8*). Recently, the findings of an enhanced urinary clearance of the tracer in children with the Fanconi syndrome, a dysfunction of proximal tubular reabsorption (*9,10*), and of a decreased renal [^{99m}Tc]DMSA uptake in patients with renal artery stenosis after captopril treatment (*13*), raised interest in the possibility of glomerular filtration of the tracer. We therefore studied the renal handling of [^{99m}Tc]DMSA by measuring protein binding, metabolism, renal extraction, plasma clearance and urinary clearance of the tracer.

Binding to plasma proteins, determining the fraction

TABLE 1
Renal Function and [^{99m}Tc]DMSA Clearance Values in 13 Individuals with Normal Renal Function*

Patient no.	ERPF	GFR	DMSA (plasma)	DMSA (urine)
1	551	142	35.4	12.4
2	537	110	29.2	9.9
3	688	105	30.0	11.1
4	736	149	31.3	11.9
5	588	120	27.9	8.1
6	418	113	32.7	11.0
7	557	126	35.6	8.5
8	588	127	41.0	15.4
9	560	140	37.6	6.5
10	560	132	35.9	14.4
11	507	124	40.0	17.1
12	451	99	36.7	13.5
13	514	108	30.2	9.9
Mean	558	123	34.1	11.5
s.d.	85	15	4.2	3.0

* All values are in ml/min and corrected for body surface area.

of [^{99m}Tc]DMSA that is available for filtration, was shown by us by two different methods to be ~90%. The concentrations of [^{99m}Tc]DMSA we used were, however, approximately four times higher than those used in a renal scintigraphy study and 200 times higher than in the continuous infusion studies, so that our results might underestimate the protein binding in vivo. Furthermore, our results might be influenced by the concentrations of unlabeled DMSA. This is not likely, since the concentrations used are low (~10^{-6} mol/l or less) but this could be a subject of further study. Our results agree quite well with those obtained by Yee et al. (*6*), but differ from those of others (*4,7,14,15*) who found binding to serum proteins to be between 66 and 75%. We do not have an explanation for these discrepancies, other than differences in the techniques used.

To test whether the intact [^{99m}Tc]DMSA complex appeared in the urine, we investigated its behavior on thin layer chromatography and in an in vivo experiment in rats. Both studies indicated that biologic behavior of the tracer had not changed. This was even the case after induction of proximal tubulopathy with maleic acid: the increased urinary excretion of [^{99m}Tc]DMSA observed in this condition is not due to metabolic alterations of the compound.

It is not possible to measure the filtered load of [^{99m}Tc]DMSA in humans directly. We therefore measured other parameters of [^{99m}Tc]DMSA kinetics in humans: renal extraction efficiency, plasma clearance and urinary clearance. Renal extraction efficiency in one normal individual was found to be 5.8%. In 13 normal individuals, plasma clearance and urinary clearance were 34 ± 4 ml/min and 12 ± 3 ml/min, respectively. As 34 ml/min in these individuals represented 6% of their ERPF, there was good agreement between the plasma clearance and the renal extraction efficiency, indicating that there was little elimination of [^{99m}Tc]DMSA from the blood by other organs than the kidney.

We did not measure the protein binding of [^{99m}Tc]DMSA in vivo in the individuals that underwent the clearance studies. It is probable, however, that in these studies the protein binding was the same as in the in vitro studies. The binding of [^{99m}Tc]DMSA to plasma proteins, ~90% in our study, determines the fraction available for filtration, so the filtration of [^{99m}Tc]DMSA in persons with a normal GFR of 125 ml/min can be calculated to be $0.10 \times 125 = 12.5$ ml/min. This almost equals the urinary clearance of [^{99m}Tc]DMSA that was 12 ml/min, while the plasma clearance of [^{99m}Tc]DMSA is approximately three times faster. This indicates that peritubular uptake accounts for ~65%, and filtration for ~35% in the renal handling of [^{99m}Tc]DMSA. Theoretically, it is possible that [^{99m}Tc]DMSA that has been filtered is excreted completely without reabsorption. It is, however, also possible that the filtered amount of [^{99m}Tc]DMSA is partially or even completely reabsorbed, compensated for by secretion into the lumen of an equal amount of [^{99m}Tc]DMSA. Other techniques will be necessary to settle this question.

On the other hand, if the serum protein binding of [^{99m}Tc]DMSA is 70%, as has been found by some investigators (*4,7,14,15*) the filtration of [^{99m}Tc]DMSA would be $0.30 \times 125 = 38$ ml/min. In that case the renal handling of [^{99m}Tc]DMSA could be explained by filtration followed by reabsorption of two-thirds of the filtered amount. There are, however, strong indications that peritubular uptake plays a role in the renal handling of [^{99m}Tc]DMSA. After cessation of glomerular filtration in an isolated perfused rat kidney by ligation of the ureter, it was found that [^{99m}Tc]DMSA uptake did not change (*16*). Taylor (*17*) and Provoost (*11*) conducted experiments by ligation of the ureter and by preloading rats with unlabeled DMSA, and both concluded that there was at least some peritubular uptake of [^{99m}Tc]DMSA. In conclusion, both mechanisms, glomerular filtration and peritubular uptake play a role in the renal handling of [^{99m}Tc]DMSA. Our measurements of protein binding and clearance suggest that glomerular filtration accounts for about 35% and peritubular uptake for ~65% of the plasma clearance of [^{99m}Tc]DMSA. At this moment it is not clear whether the filtered [^{99m}Tc]DMSA is reabsorbed and in that way contributes to the uptake in the renal cortex.

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