Renal Handling of Technetium-99m DMSA in Rats with Proximal Tubular Dysfunction

Abraham P. Provoost and Matthijs Van Aken

Department of Pediatric Surgery, Laboratory for Surgery, Erasmus University, Rotterdam, The Netherlands

The renal handling of technetium-99m dimercaptosuccinic acid ([^{99m}Tc]DMSA) was studied in rats before and after treatment with Na-maleate (2 mmol/kg i.v.). In the control period, when measured 2 hr after the intravenous injection of [^{99m}Tc]DMSA, 39.9% of the injected dose was in the kidneys and 14.6% was in the bladder. After Na-maleate treatment, only 6.4% of the injected dose of [^{99m}Tc]DMSA was retained in the kidneys while 37.9% was found in the bladder. Subsequent studies revealed that Na-maleate produced a fall in the glomerular filtration rate, the effective renal plasma flow, and a generalized proximal tubular dysfunction. The latter was characterized by polyuria and an increased excretion of glucose, protein, albumin, calcium, and inorganic phosphate. It was concluded that proximal tubular dysfunction markedly alters the renal handling of [^{99m}Tc]DMSA. Whether this augmented urinary excretion is due to an inhibition of reabsorption or an enhanced cellular efflux of [^{99m}Tc]DMSA remains to be answered.

J Nucl Med 26:1063–1067, 1985

In 1974, technetium-99m 2,3 dimercaptosuccinic acid ([^{99m}Tc]DMSA) was introduced as a substitute for radioactive mercury containing compounds for static imaging of the kidney (*1*). Technetium-99m DMSA has been used not only to visualize the kidneys to study renal morphology (*2–5*), but also to assess individual kidney function (*4,5*). Technetium-99m DMSA is generally used to determine differential renal function (i.e., the percentile contribution of each kidney to the total renal function). Experimentally (*6,7*), as well as clinically (*8–10*), good correlations have been obtained between the differential renal [^{99m}Tc]DMSA uptake and indices of renal function such as glomerular filtration rate (GFR), creatinine clearance, renal blood flow, and effective renal plasma flow (ERPF).

Despite its clinical use in assessing relative renal function, the mechanism(s) by which [^{99m}Tc]DMSA is taken up by the kidneys is still not completely known. Two major probabilities are either tubular reabsorption of the filtered [^{99m}Tc]DMSA from the luminal side or uptake from the peritubular capillaries. Irrespective of the mechanism of renal uptake, specific forms of proximal tubular dysfunction may lead to alterations in the renal handling of [^{99m}Tc]DMSA. Such alterations have recently been described in nine pediatric patients with proximal tubular dysfunction, four of them with idiopathic Fanconi syndrome. Despite a relatively well maintained GFR, there was a low renal uptake and an intense bladder visualization (*11*). In animals, treatment with sodium-maleate (Na-maleate) produces a renal syndrome, consisting of glucosuria, aminoaciduria, phosphaturia, and an increased excretion of bicarbonate, closely resembling the clinical picture of Fanconi syndrome (*12,13*). In the present study, we investigated the effects of Na-maleate on the renal handling of [^{99m}Tc]DMSA in rats.

MATERIALS AND METHODS

The experiments were carried out using adult male rats of a Wistar strain (WAG/Rij) with a body weight (BW) of about 270–300 g. The animals had tap water and normal rat chow available ad libitum.

Received Jan. 7, 1985; revision accepted June 4, 1985.

For reprints contact: Abraham P. Provoost, PhD, Dept. of Pediatric Surgery, Laboratory for Surgery, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

Experimental setup

Three experiments were carried out to establish the effects of Na-maleate. The first experiment concerned the renal handling of [^{99m}Tc]DMSA. In the second experiment, we studied the GFR and the ERPF, while the third experiment focused on the proximal tubular function (i.e., the reabsorbtion of glucose, protein, albumin, calcium and phosphate). In all experiments, Na-maleate was injected i.v. in a dose of 2 mmol/kg BW (400 mg/kg), and measurements always included a control period before Na-maleate administration.

Experiment 1

The renal handling of [^{99m}Tc]DMSA (18.5 MBq in 0.3 ml, i.v.) was studied in seven rats. Static images, with 5-min counting time, were obtained at 1, 2, 4, 6, and 24 hr after injection with the aid of a gamma camera.* The data were stored and analyzed on a dedicated computer system.† Four regions of interest were distinguished from the stored data involving the left or right kidney, the bladder, or a background area. After correction for background activity, the radioactivity in kidneys and bladder was calculated as a percentage of the injected amount.

The [^{99m}Tc]DMSA scans were performed 1 wk before Na-maleate treatment, on the day of treatment, and 1 wk after treatment. On the day of treatment, the [^{99m}Tc]DMSA was injected 2 hr after the Na-maleate.

Experiment 2

The GFR and ERPF were determined in seven rats 1 wk before, on the day of Na-maleate treatment as well as 1 and 2 wk after treatment. On the day of maleate treatment, the GFR and ERPF were obtained 2-3 hr after the maleate administration. The GFR and ERPF were measured as the plasma clearances of Chromium-51 EDTA and iodine-125 iodohippurate, by a method allowing for repeated use in the same animal (*14*).

Experiment 3

Urine collected from 6 rats was placed in metabolic cases, to determine the osmolality and the excretion of glucose, total protein, albumin, calcium, and phosphate. After two control periods of 24 hr, Na-maleate was injected i.v. Subsequently, urine was collected 0-2, 2-24, and 24-48 hr after injection. One week after injection of Na-maleate urine was again collected for two 24-hr periods.

Analytical procedures

Glucose was measured with a glucostick on a reflectance colorimeter (Ames). Total protein was determined spectrophotometrically by the Biuret method. Albumin was measured spectrophotometrically after reaction with bromocresolgreen. Calcium was measured spectrophotometrically as o-cresolphtalein complex. Inorganic phosphate was measured spectrophotometrically after reaction with molybdate. Urine osmolality was determined by freezing-point depression.

Statistics

Statistical differences between the mean values obtained after treatment with the values obtained in the same animals before treatment, were determined using a paired Student's t-test. Statistical significance was considered to be significant when a value $p < 0.05$ was found.

RESULTS

Renal handling of [^{99m}Tc]DMSA

In the control period, the major part of the [^{99m}Tc]DMSA injected was retained by the kidneys. At 2 hr after injection, 39.9% of the injected dose was in the kidneys and 14.6% was in the bladder. This indicated that 73% of the amount cleared by the kidneys remained in the renal parenchyma, while 27% was excreted into the bladder (Table 1). After treatment with Na-maleate, 37.9% of the injected dose of [^{99m}Tc]DMSA was found in the bladder, whereas the kidneys retained only 6.4% of the injected dose. Consequently, after

TABLE 1
The Renal Handling of ^{99m}Tc DMSA Before and After Treatment with Sodium Maleate*

Item	BW† (gram)	1 hr % Kidney‡	1 hr % Bladder‡	2 hr % Kidney	2 hr % Bladder	4 hr % Kidney	24 hr % Kidney
Pre	273 ± 10	36.8 ± 2.1	11.8 ± 1.7	39.9 ± 2.4	14.6 ± 1.9	44.6 ± 3.3	48.7 ± 2.6
Day 0	260 ± 11	7.5 ± 2.5	34.0 ± 11.1	6.4 ± 1.8	37.9 ± 8.9	8.1 ± 4.1	14.7 ± 2.1
Day 7	267 ± 11	34.5 ± 4.3	17.3 ± 1.9	38.6 ± 4.8	20.0 ± 5.5	39.5 ± 3.3	47.1 ± 3.6

* All data are Mean ± s.d. for seven rats.
† BW is body weight.
‡ Kidney and bladder data represent the percentage of the injected dose.

TABLE 2
The Glomerular Filtration Rate and Effective Renal Plasma Flow Before and After Treatment with Sodium-Maleate*

Item	BW† (gram)	GFR‡ (ml/min/100 g BW)	ERPF** (ml/min/100 g BW)	GFR/ERPF
Pre	276 ± 9	0.69 ± 0.06	1.60 ± 0.08	0.43 ± 0.03
Day 0	277 ± 8	0.42 ± 0.10	0.86 ± 0.16	0.48 ± 0.04
Day 7	283 ± 9	0.53 ± 0.02	1.49 ± 0.05	0.36 ± 0.02
Day 14	290 ± 9	0.63 ± 0.03	1.56 ± 0.07	0.41 ± 0.02

* All data are Mean ± s.d. of seven rats.
† BW is body weight.
‡ GFR is glomerular filtration rate.
** ERPF is effective renal plasma flow.

maleate treatment, the renal parenchyma retained only 14% of the amount cleared by the kidneys.

The effects of Na-maleate were reversible. Repeat scans after seven days showed an almost normal handling of the injected [^{99m}Tc]DMSA.

Glomerular filtration rate and effective renal plasma flow

As shown in Table 2, treatment with Na-maleate caused a reversible fall in both the GFR and the ERPF. Immediately after Na-maleate treatment, the GFR dropped to 61% and the ERPF to 54% of the pretreatment value. One wk after treatment, the GFR was still slightly reduced, but after 2 wk both the GFR and ERPF were back to control values.

Proximal tubular function

As shown in Table 3, injection of Na-maleate caused an increase in the urinary excretion of water, glucose, total protein, albumin, calcium, and inorganic phosphate. These changes lasted for at least 48 hr. The most severe changes were observed during the first day, especially the first 2 hr after injection. At Day 7, an almost normal pattern of urinary excretion was observed.

DISCUSSION

The present experimental findings clearly show that a generalized proximal tubular dysfunction markedly affects the renal handling of [^{99m}Tc]DMSA. A low renal uptake and a high activity in the bladder suggest that the [^{99m}Tc]DMSA, normally retained intracellularly, is either not taken up or released at an enhanced rate. The renal handling of [^{99m}Tc]DMSA in rats with a Na-maleate-induced experimental Fanconi syndrome, closely resembles the changes reported in children with idiopathic Fanconi syndrome and other forms of proximal tubular dysfunction (*11*).

Unfortunately, the use of Na-maleate to alter proximal tubular function does not allow conclusions to be drawn on the specific mechanism by which [^{99m}Tc]DMSA is taken up in the tubular cells. The increased urinary excretion of the various solutes may be explained in different ways, which alter the interpretation of the maleate-induced changes in renal [^{99m}Tc]DMSA handling. Initially, the inhibition of proximal tubular reabsorption was thought to be the mechanism of action of Na-maleate. However, an alternative explanation has been suggested by Bergeron et al. (*15*). They postulated that an increased membrane permeability may cause the transport defects observed in experimental Fanconi syndrome. Unfortunately, it is by no means easy to make a distinction between these two mechanisms. An increased permeability of the tubular cell membrane, from lumen into the cells, for inulin and lissamine green has been found (*16*). These substances normally remain within the tubular fluid and should not appear intracellularly. For substances, that may be present in the tubular fluid after filtration as well as in the cell after reabsorption, a distinction between inhibition of reabsorption and increased cellular efflux is almost impossible. Both mechanisms may explain the enhanced urinary excretion of glucose, amino acids, bicarbonate, sodium, phos-

TABLE 3
The Urinary Excretion of Glucose, Protein, Albumin, Calcium, and Phosphate Before and After Treatment with Maleate*

Item	BW (gram)	V (ml/24 hr)	U glu.V (μmol/24 hr)	U prot.V (mg/24 hr)	U alb.V (mg/24 hr)	U Ca. V (mmol. 24 hr)	U Pi. V (mmol/24 hr)
Control	285 ± 8	8.4 ± 1.2	<10	31.0 ± 4.9	7.2 ± 1.3	7.1 ± 4.7	0.46 ± 0.09
Day 0	287 ± 7	27.5 ± 5.2	565 ± 309	114.4 ± 17.8	45.3 ± 9.9	37.5 ± 5.9	0.92 ± 0.15
Day 1	272 ± 7	16.1 ± 2.9	592 ± 174	90.3 ± 13.1	31.6 ± 7.6	39.2 ± 17.6	0.65 ± 0.10
Day 7	279 ± 9	8.4 ± 1.6	<10	27.8 ± 4.4	6.6 ± 0.9	12.2 ± 2.6	0.45 ± 0.15

* All data are mean ± s.d. for six rats.
BW = body weight.
V = 24-hr urine excretion.
U glu.V = 24-hr urinary glucose excretion.
U prot.V = 24-hr urinary protein excretion.
U alb.V = 24-hr urinary albumin excretion.
U Ca.V = 24-hr urinary calcium excretion.
U Pi.V = 24-hr urinary inorganic phosphate excretion.

phate, and protein observed after treatment with Na-maleate (*12,13,17-19*). Consequently, any of the proximal tubular reabsorptive mechanisms may be involved in the renal uptake of [^{99m}Tc]DMSA after filtration of the free (nonprotein bound) fraction of this compound (*20*). On the other hand, leakage of [^{99m}Tc]DMSA that has entered the tubular cell by uptake from peri-tubular capillaries and subsequent fixation is equally feasible. Regarding this renal uptake, tubular secretion is not of great importance since probenicid, an inhibitor of this transport, had no effect on the renal handling of [^{99m}Tc]DMSA (*20*).

A third possible action of maleate in the kidney may well enhance cellular leakage. In the cells, maleate reacts with the sulfhydryl groups of glutathione, cysteine, and proteins (*21,22*), probably after being converted to maleyl-CoA (*21*). This action reduces the number of -SH binding sites. Following the injection of [^{99m}Tc]DMSA, a Tc(DMSA)2 complex is thought to circulate and to bind to the proximal tubular cell membrane. After cellular uptake, this complex is assumed to split, one of the DMSA molecules leaks into the urine, and the Tc-DMSA moiety binds to proteins containing sulfhydryl groups (*23*). Intracellular localization studies have shown that [^{99m}Tc]DMSA is bound mainly to soluble cystoplasmic proteins (*24*). This binding is important to retain the compound intracellularly. A depletion of these binding sites would lead to a diffusion of Tc-DMSA out of the cells into the urinary space and finally into the bladder. Following administration of mercury salts, which are known to bind to sulfhydryl groups in the proximal tubular cells, an enhanced excretion of [^{99m}Tc]DMSA has been found (*23,25*).

In the present experiment, the effect of a change in the GFR on the renal uptake is considered to be small. Although there was a 40% reduction in the GFR at the time of the first [^{99m}Tc]DMSA scan after Na-maleate injection, the total amount of [^{99m}Tc]DMSA handled by the kidneys (i.e., kidney plus bladder activity) was about 81% of that during the control scan. In rats, in which the clearance of Iodine-125 iothalamate was reduced to 52% by 50% infarction of both kidneys, the amount of [^{99m}Tc]DMSA handled by the kidneys was still 74% of that of control rats (*20*). Although mannitol-induced diuresis did decrease the plasma clearance of [^{99m}Tc]DMSA, there was but little effect on the ratio of urinary to kidney ^{99m}Tc activity, which was similar to that in controls. This may indicate that the diuresis observed after Na-maleate as such was not altering the renal handling of [^{99m}Tc]DMSA. Alterations in the renal handling of [^{99m}Tc]DMSA may be anticipated when the compound is not optimally prepared (*23,26*) or when the acid-base balance is being disturbed (*20*). Neither condition occurred in the present experiments.

Recent preliminary results from rat studies gave us some more information on the different role of filtration and fixation in the renal handling of [^{99m}Tc]DMSA (*25*). The data obtained in rats after loading with nonradioactive DMSA can be interpreted as indicating a major role for peritubular fixation in the renal accumulation and of glomerular filtration in the urinary excretion of [^{99m}Tc]DMSA. According to these findings, the effect of maleate, would then be enhanced leakage from the tubules with a depletion of sulfhydryl binding sites a likely explanation for this leakage.

Measurement of the renal clearance of nonprotein bound [^{99m}Tc]DMSA could provide information about the importance of mechanisms other than glomerular filtration in the renal handling of this compound. That is, if peritubular uptake is of importance, the total renal clearance (kidneys plus urine) should be greater than the GFR. Clearance studies in rats have been performed (*20*) and show that the clearance of free [^{99m}Tc]DMSA is slightly higher than the GFR. Unfortunately, it is not clear whether these clearance figures refer to either the urinary or the total renal clearance. In humans, it was found that during continuous i.v. infusion of [^{99m}Tc]DMSA in the urinary clearance, not corrected for plasma-protein binding, was about 8-10% of the GFR (*27*). When these data are corrected for protein binding, ~90% in rats (*20*), urinary clearance would almost equal the GFR. At the same time, the amount of [^{99m}Tc]DMSA accumulated in the kidneys is about four times the amount present in the urine (*5,10*). Consequently, the total renal clearance of [^{99m}Tc]DMSA would be about five times the urinary clearance, a figure comparable to the renal uptake plasma flow. Thus in humans, a peritubular renal uptake appears important in the renal clearance of [^{99m}Tc]DMSA.

In conclusion, the present experimental data together with the similar clinical observations (*11*), indicate that knowledge of the proximal tubular function is essential for the correct interpretation of the static renal images obtained with [^{99m}Tc]DMSA.

FOOTNOTES

* Siemens Medical Systems, Iselin, NJ. (formerly: Nuclear Chicago, Chicago, IL)

† Gamma 11, Digital Equipment Corp., Maynard, MA.

ACKNOWLEDGMENT

We are very grateful to the Department of Nuclear Medicine for supplying a gamma camera for experimental purposes. We also thank them for preparing the technetium-99m DMSA. We thank Mrs. A. Ribbink-Goslinga for her stylistic help.

This work was presented at the 31st Annual Meeting of The Society of Nuclear Medicine, Los Angeles, California, June 5-9, 1984 and at the 18th Annual Meeting of The European Society for Pediatric Nephrology, Dubrovnik, Yugoslavia, September 17-20, 1984.

REFERENCES

1. Lin TH, Khentigan A, Winchell HS; A ^{99m}Tc-chelate substitute for organoradiomercurial renal agents. *J Nucl Med* 15:34-35,1974
2. Enlander D, Weber PM, Dos Remedios LV: Renal cortical imaging in 35 patients: Superior quality with ^{99m}Tc-DMSA. *J Nucl Med* 15:743-749, 1974
3. Handmaker H, Young BW, Lowenstein JM: Clinical experience with ^{99m}Tc-DMSA (dimercaptosuccinic acid): A new renal-imaging agent. *J Nucl Med* 16:28-32, 1975
4. Daly MJ, Henry RE: Defining renal anatomy and function with ^{99m}Tc-dimercaptosuccinic acid: Clinical and renographic correlation. *J Urol* 126:5-9, 1981
5. Taylor A, Jr: Quantitation of renal function with static imaging agents. *Semin Nucl Med* 12:330-344 1982
6. Daly MJ, Jones W, Rudd TG, et al: Differential renal function using technetium-99m dimercaptosuccinic acid (DMSA): In vitro correlation. *J Nucl Med* 20:63-66, 1979
7. Powers TA, Stone WJ, Grove RB, et al: Radionuclide measurement of differential glomerular filtration rate. *Invest Radiol* 16:59-64, 1981
8. Kawamura J, Hosokawa S, Yoshida O, et al: Validity of ^{99m}Tc dimercaptosuccinic acid renal uptake for an assessment of individual kidney function. *J Urol* 119:305-309, 1978
9. Taylor A, Jr: Delayed scanning with DMSA: A simple index of relative renal plasma flow. *Radiology* 136:449-453, 1980
10. Abbou CC, Moretti JL, Chopin D, et al: Le scintigraphie au dimercaptosuccinate de technetium: ^{99m}Tc-DMSA. *Nouv Presse Med* 10:1475-1478, 1981
11. Van Luijk WHJ, Ensing GJ, Piers DA: Low renal uptake of ^{99m}Tc-DMSA in patients with proximal tubular dysfunction. *Eur J Nucl Med* 8:404-405, 1983
12. Berliner RW, Kennedy TJ, Hilton JG: Effect of maleic acid on renal function. *Proc Soc Exp Biol* Med 75:791-794, 1950
13. Harrison HE, Harrison HC: Experimental production of renal glycosuria, phosphaturia, and aminoaciduria by injection of maleic acid. *Science* 120:606-608, 1954
14. Provoost AP, De Keijzer MH, Wolff ED, et al: Development of renal function in the rat. The measurement of GFR and ERPF and correlation to body and kidney weight. *Renal Physiol* 6:1-9, 1983
15. Bergeron M, Dubord L, Hausser C: Membrane permeability as a cause of transport defects in experimental Fanconi syndrome. *J Clin Invest* 57:1181-1189, 1976
16. Maesaka JK, McCaffery M: Evidence for renal tubular leakage in maleic acid-induced Fanconi syndrome, *Amer J Physiol* 239:F507-F513, 1980
17. Wen SF: Micropuncture studies of glucose transport in the dog: Mechanism of renal glycosuria. *Amer J Physiol* 231:1024-1032, 1976
18. Guenther R, Silbernagl S, Deetjen P: Maleic acid induced aminoacidurla: Studied by free flow micropuncture and continuous microperfusion. *Pfluegers Arch* 382:109-114, 1979
19. Brewer ED, Senekjian O, Ince A, et al: Maleic acid-induced reabsorptive dysfunction in proximal and distal nephron. *Amer J Physiol* 254:F339-F344, 1983
20. Yee CA, Lee HB, Blaufox MD: Tc-99m DMSA renal uptake: Influence of biochemical and physiological factors. *J Nucl Med* 22:1054-1058, 1981
21. Szczepanska M, Angielski S: Prevention of maleate-induced tubular dysfunction by acetoacetate. *Amer J Physiol* 239:F50-F56, 1980
22. Morgan EJ, Friedmann E: Maleic acid as inhibitor of enzyme reactions induced by SH-compounds. *Biochem J* 32:862-870, 1983
23. Moretti JL, Rapin JR, Saccavini JC, et al: 2,3 Dimercapto succinic acid chelates: Their structure and biological behaviour. In *Nuclear Medicine and Biology II,* Raynound C, ed, Paris, Pergamon Press, 1982, 1651-1654
24. Vanlic-Razumenic N, Petrovic J: Biochemical studies of the renal radiopharmaceutical compound dimercaptosuccinate. Subcellular localisation of ^{99m}Tc-DMSA complex in the rat in vivo. *Eur J Nucl Med* 6:169-172, 1981
25. Provoost AP, Van Aken M: Investigations into the renal handling of Tc-99m DMSA in rats. Fourth International Symposium of Nephrology at Montecatini, p 20, 1985 (abstr)
26. Ikeda I, Inoue O, Kurata K: Preparation of various technetium-99m dimercaptosuccinate complexes and their evaluation as radiotracers. *J Nucl Med* 18:1222-1229, 1977
27. Van Luijk WHJ, Ensing GJ, Meijer S, et al: Is the relative ^{99m}Tc-DMSA clearance a useful marker of proximal tubular dysfunction? *Eur J Nucl Med* 9:439-442, 1984