Effect of Hydration and Dehydration on Technetium-99m CO_2 DADS Renal Studies in Normal Volunteers

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Ten normal volunteers were studied in the hydrated and dehydrated states with the new renal radiopharmaceutical technetium-99m *N,N'*-bis(mercapto acetyl)-2,3-diaminopropanoate ([^{99m}Tc]CO_2 DADS). The data were used to determine the effect of hydration and dehydration and to determine the normal range in each state. Visual evaluation of the images indicated that the first appearance of tracer in the collecting system was approximately the same in either state, that the concentration of tracer in the collecting system was always higher in the dehydrated state ($p < 0.01$), and that the ureters always appeared more segmented in the dehydrated state ($p < 0.01$). Quantitative analysis of the images indicated that the kidney to background ratio 1–2 min after injection was somewhat greater in the dehydrated state (13.5 ± 4.0) than in the hydrated state (9.8 ± 2.2) ($p < 0.05$), that the size of the bladder was always greater in the hydrated state ($p < 0.05$), and there was no difference in the amount of tracer in the bladder at 30 min after injection. The results define the normal hydrated and dehydrated [^{99m}Tc]CO_2 DADS renal study and identify several differences between the two states which can be explained primarily by differences in urine flow rates.

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The new renal radiopharmaceutical technetium-99m *N,N'*-bis(mercaptoacetyl)-2,3-diaminopropanoate [^{99m}Tc]CO_2 DADS), which is labeled with ^{99m}Tc and possesses biological properties similar to iodine-131 (^{131}I)hippuran, appears to have all of the advantages, but none of the disadvantages, of the two standard renal imaging radiopharmaceuticals, [^{99m}Tc]diethylenetriaminepentaacetic acid (DTPA) and [^{131}I]hippuran (1–7). Technetium-99m DTPA has the advantages of a ^{99m}Tc label, but since it is cleared by glomerular filtration, the disadvantage of an extraction efficiency of only 20–25% (*8*). Iodine-131 hippuran has the advantage of a high extraction efficiency of 65–80%, but the disadvantage of limited spatial resolution because of its ^{131}I label (6).

One disadvantage of [^{99m}Tc]CO_2 DADS is that labeling of the ligand with ^{99m}Tc results in chelate ring epimers with only one of the isomers having the desired biological properties (*6,9*). This fact requires separation of the epimers by high performance liquid chromatography (HPLC) after labeling. However, this requirement should not represent a significant preparation or economic problem to a centralized nuclear pharmacy, but it will discourage in house preparation in most hospitals. In this study we determined the normal appearance of [^{99m}Tc]CO_2 DADS renal studies in both the hydrated and dehydrated states.

MATERIALS AND METHODS

Radiopharmaceutical preparation

Technetium-99m CO_2 DADS was prepared essentially as previously described (*7*). The hydrolyzed ligand was prepared from 0.5 mg of ethyl *N,N'*-bis(benzoylmercaptoacetyl)-2,3-diamino propanoate which was dissolved in 0.3 ml ethanol, treated with 30 μl of 5 N NaOH, diluted with 0.3 ml H_2O, and heated for 15 min at 95°C. To the hydrolyzed ligand solution was

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added about 100 mCi of [^{99m}Tc]pertechnetate. Sodium dithionite (1 mg in 30 μl H_2O) was added and the mixture was heated at 95°C for 15 min in order to optimize the product ratio. The mixture was neutralized with 20 μl of 6 N HCl and injected onto a 5 μ, 250 mm × 4.6 mm, octadecylsilyl HPLC column* and eluted with 0.01*M* sodium phosphate, pH 6 (96%) and ethanol (4%). The column effluent was collected in samples as component A (first) and component B (second). Component A, the one with the desired biological properties, was sterilized by passage through a 0.22 μm filter into a sterile vial. The yield of component A was 40 to 50 mCi in an overall preparation time of 45 to 60 min; the pH of component A as injected was 6.0.

Volunteer selection

Each volunteer had a normal serum creatinine, a normal urinalysis, no history of renal disease, and no disease at the time of the study. All volunteers gave informed consent and no volunteer was used more than once.

Imaging protocol

Each volunteer was studied twice, once hydrated and once dehydrated, on different days. The volunteer was prepared for the hydrated study by reporting to the nuclear medicine section at ~7:30 a.m. and then drinking 500 ml of water at 0, 10, and 20 min of a 30-min period for a total water intake of 1,500 ml. At the end of the 30-min period the radiopharmaceutical was injected and imaging was begun.

The volunteer was prepared for the dehydrated study by taking nothing by mouth from the time he went to bed until the imaging study was over; the dehydrated study was started at ~8:00 a.m.

For each study the volunteer was injected with ~7.5 mCi of [^{99m}Tc]CO_2 DADS (the A component). The exact amount was calculated by measuring the radioactivity in the syringe before and after injection.

Imaging was performed by placing the volunteer supine over a 15-in. field-of-view gamma camera; a low-energy, high resolution collimator was used. The patient was positioned so that the kidneys would be in the upper portion of the field of view. Following a bolus injection, analog images were acquired every 3 sec for 30 sec and then at 1, 5, 10, 15, 20, and 25 min. The 1-min image was acquired for 750 thousand counts and subsequent images were acquired for the same time as the 1-min image.

In addition, digital 128 × 128 images were acquired every 3 sec for 30 sec and then every minute for 29 min beginning at 1 min. Finally, a 1-min analog and digital image were acquired of the bladder in the posterior projection, usually after moving the volunteer up relative to the gamma camera.

Data analysis

The analog images were used to compare the concentration of tracer in the collecting systems and the degree of ureteral segmentation in the hydrated and dehydrated states.

The digital images were used to measure the kidney to background ratios (an index of renal clearance), the time of first appearance of tracer in the collecting systems (the leading edge parenchymal transit time), and the amount of radioactivity in the bladder (an index of excretion) (*5,7*).

The kidney to background count ratio was calculated from the 1.0- to 2.0-min digital data. A background region of interest (ROI) was placed under each kidney and the kidney to background ratios were calculated individually and as an average for each volunteer after normalizing the background region of interest to 100 pixels. The time of first appearance of tracer in the collecting systems was visually estimated from serial 1-min digital images; the times were estimated individually for each kidney and as an average for each volunteer.

The amount of tracer in the bladder at 30 min after injection in the hydrated and dehydrated states was calculated by placing regions of interest over the bladder in the 30-min digital images. It was assumed that attenuation in the two studies in the same volunteer would be similar and cancel. In addition, the area occupied by the bladder ROI was measured as an index of bladder size.

All visual evaluations were made by two observers (WCK and SAH) independently without knowledge of whether the study was a hydration or dehydration study. When the observers disagreed, the results were averaged.

RESULTS

Volunteer data

The age range of the ten volunteers was 22 to 57 yr with an average of 34.8 yr. Their serum creatinine range was 0.9 to 1.2 mg/dl (normal: 0.7–1.4 mg/dl) and all had normal urinalyses. Nine underwent the hydration study first and one underwent the dehydration study first. The average time between hydrated and dehydrated studies was 1.8 days and the maximum time was 7 days.

Analog images

The two observers agreed in 32 of 37 (86%) determinations. In those qualitative determinations in which they disagreed, the disagreement was never between opposite categories, but between a significant compared with no significant difference. Data from all ten volunteers was not available for analysis for all parameters for technical reasons.

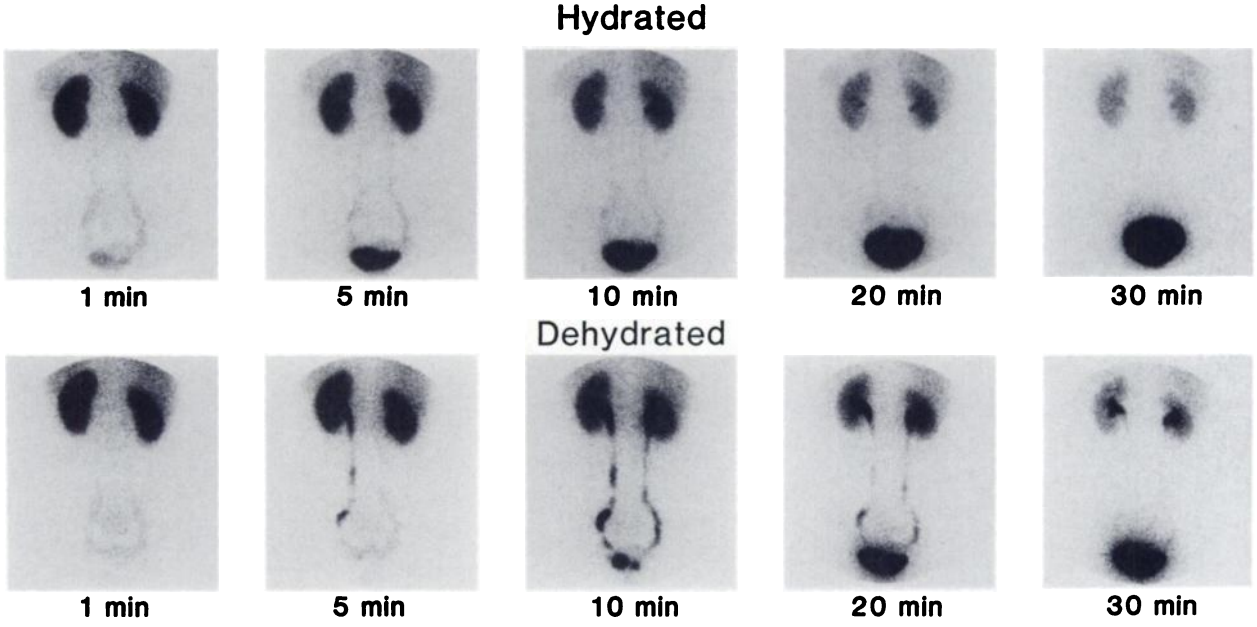

FIGURE 1
[^{99m}Tc]CO_2 DADS renal studies are shown from normal volunteer during hydration and dehydration. Study during hydration shows lower concentration of tracer in collecting system, lack of segmentation of ureters, and larger bladder in contrast to dehydrated study

TABLE 1
Visual and Qualitative Comparison of Hydrated and Dehydrated [^{99m}Tc]CO_2 DADS Studies*

Parameter	Hydrated> dehydrated	Hydrated< dehydrated	Hydrated= dehydrated	Total
First appearance of tracer in collecting system	4,3	1,5	4,1	9,9
Tracer concentration in collecting system	0,0	9,9†	0,0	9,9
Segmentation in ureters	0,0	10,8†	0,2	10,10
Kidney/background ratio	1	7	1	9
Bladder size	9†	0	0	9

* Number of volunteers, one for each of two observers for first three parameters; single number for kidney/background parameter represents qualitative evaluation of numerical ratio.
† Statistically significant ($p < 0.05$).

The concentration of tracer in the collecting systems was higher in the dehydrated state in all ten volunteers. The degree of segmentation of tracer in the ureters was greater in the dehydrated state in almost all volunteers (Fig. 1 and Table 1). In fact, segmentation was rarely seen in the hydrated state.

Digital images

The kidney to background ratio was not significantly different between right and left kidneys in either the hydrated or dehydrated states. However, the kidney to background ratio in the hydrated state was significantly less than in the dehydrated state for right kidneys, left kidneys and the average of right and left kidneys (Tables 2 and 3). The kidney to background ratio in the hydrated state was less in both kidneys in seven volunteers, greater in one volunteer, and opposite in the two kidneys in one volunteer (Table 1).

The leading edge parenchymal transit time was not significantly different between right and left kidneys or between the hydrated and dehydrated states (Tables 1–3). The observers judged the leading edge transit time the same in both kidneys in 16 of 18 determinations in

TABLE 2
Normal Range for Hydrated and Dehydrated [^{99m}Tc]CO_2 DADS Studies*

Parameter	Hydrated	Dehydrated
Kidney/background ratio		
Right	10.3 ± 2.5	13.6 ± 3.6†
Left	9.3 ± 1.8	13.2 ± 4.5†
Average (R and L)	9.8 ± 2.2	13.5 ± 4.0†
Right/left	1.09 ± 0.09	1.07 ± 0.13
Transit time (min)		
Right	3.33 ± 0.90	2.78 ± 0.97
Left	3.22 ± 0.97	3.17 ± 1.12
Average (R and L)	3.28 ± 0.93	2.97 ± 1.02
Right/left	1.05 ± 0.09	0.89 ± 0.15
Bladder radioactivity (cts/mCi)	11,852 ± 1,699	11,942 ± 2,950
Bladder area	6.27 ± 2.19	3.36 ± 1.23

* All values given as mean ± s.d.
† Statistically significant ($p < 0.05$).

TABLE 3
Normal Range Hydrated Compared with Dehydrated [^{99m}Tc]CO_2 DADS Studies*

Parameter	Hydrated/dehydrated ratio
Kidney/background	
Right	0.77 ± 0.27†
Left	0.78 ± 0.22†
Average (R and L)	0.78 ± 0.24†
Transit time	
Right	1.28 ± 0.50
Left	1.07 ± 0.36
Average (R and L)	1.16 ± 0.39
Bladder radioactivity	1.02 ± 0.19
Bladder area	2.19 ± 1.58

* All values are given as mean ± s.d.
† Statistically significant ($p < 0.05$).

the hydrated state and in 11 of 18 determinations in the dehydrated state; differences between the two observers were never greater than 1 min, the minimum increment.

The amount of tracer in the bladder at hydration and dehydration was not significantly different (Tables 2 and 3). However, the bladder size was always greater in the hydrated state although no attempt was made to have the volunteers void prior to beginning each study (Table 1).

DISCUSSION

Despite the fact that routine radionuclide renal studies are usually done without patient preparation, the results of this study demonstrate significant differences between hydrated and dehydrated [^{99m}Tc]CO_2 DADS studies in normal volunteers. In the hydrated state in comparison to the dehydrated state the concentration of tracer in the collecting systems is consistently lower, the volume of urine in the ureters is consistently higher (lack of segmentation), and the bladder size is consistently larger. These findings presumably all reflect the increased production of urine by the nephrons in the hydrated state.

In addition, the kidney to background ratio at 1 to 2 min was significantly higher in the dehydrated state although not to a large extent. The explanation for this finding is not entirely clear. It is most likely secondary to a small amount of excreted tracer by 2 min, with a greater amount of tracer remaining in the calyces and pelvis and, thus, in the renal region of interest in the dehydrated state (Fig. 1). Background radioactivity should be mainly from blood because [^{99m}Tc]CO_2 DADS is highly protein bound (*6*) and there is no reason to expect the percent of blood volume within the background ROIs to change from hydration to dehydration.

There was no significant difference in leading edge transit times between hydration and dehydration; this suggests that as urine volume in the tubules increases or decreases with hydration and dehydration, respectively, the volume of the tubules changes in parallel so that the transit time remains constant (*10*). The amount of tracer reaching the bladder in 30 min was similar in the hydrated and dehydrated states.

The findings together suggest that parameters such as renal blood flow, tubular clearance, and renal excretion of tracer were unaffected over the range of hydration and dehydration tested. The expected change in urine production and volume can account for the higher concentration of tracer in the collecting system, segmentation of ureters, and smaller bladder size seen in the dehydrated state.

In addition to determining qualitative differences between hydrated and dehydrated renal studies, the data allow determination of quantitative normal ranges for [^{99m}Tc]CO_2 DADS renal studies for kidney to background ratios (an index of renal clearance), time of first appearance of tracer in the collecting systems (an index of parenchymal transit time), and amount of tracer in the bladder (an index of amount of excretion). These data are necessary to maximize sensitivity and specificity for detection of minimal unilateral and bilateral renal disease. These measurements were selected so that blood or urine sample collection would not be necessary since sample collection is unlikely to be performed in the clinical setting.

Previous studies of normal findings in radionuclide renal studies in the hydrated and dehydrated states have focused on the renogram (*11–14*). It has been demonstrated that renograms in the dehydrated state are more sensitive, but less specific, than in the hydrated state (*11–14*). Some authors have recommended studying patients in both the hydrated and dehydrated states routinely (*15*), but in general there has been no consensus on the optimal state of hydration for radionuclide renal studies. Renogram time-activity curves suffer from the fact that the regions of interest defined by probes or even camera-computer systems do not adequately separate radioactivity in the collecting systems from that in the surrounding renal parenchyma.

The differences in the visual appearance of hydrated and dehydrated studies raises the possibility of reevaluating a stress renal test. Since renal disease often leads to the inability to produce either concentrated or dilute urine, both hydration and dehydration constitute a stress to renal function. Previous investigations in this area have dealt with time-activity curves, [^{131}I]hippuran images with their low count rates, or [^{99m}Tc]DTPA images which reflect only glomerular filtration—a process in which the kidney is passive. Studies would be needed to determine whether hydration, dehydration, or both constitute the optimum stress

study. In addition, this study raises the question of whether the routine renal radionuclide study should be performed with a specified degree of hydration or dehydration.

The lack of significant right to left renal asymmetry in any of the parameters which were evaluated raises the possibility that the [^{99m}Tc]CO_2 DADS renal study in the hydrated and/or dehydrated state might be useful as a screening test for renovascular hypertension. The availability of a ^{99m}Tc-labeled tubular agent would make investigation of this possibility significantly different from previous attempts.

In summary, we have defined the normal range and appearance of several parameters in [^{99m}Tc]CO_2 DADS renal studies and demonstrated that the state of hydration has a significant effect on several of these parameters. The data presented here should be helpful in maximizing the usefulness of [^{99m}Tc]CO_2 DADS renal studies if [^{99m}Tc]CO_2 DADS or a similar agent attains widespread use.

FOOTNOTE

* Bechman-Altex Ultrasphere, 5μ, Berkeley, CA.

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