Clinical Evaluation of Tc-99m N,N′-Bis(Mercaptoacetyl)-2,3-Diaminopropanoate as a Replacement for I-131 Hippurate: Concise Communication

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A clinical comparison of Tc-99m N,N′-bis(mercaptoacetyl)-2,3-diaminopropanoate (Component A) (Tc-99m CO_2-DADS-A) and I-131 hippurate was conducted in a series of five normal volunteers and 18 patients. Each subject was studied in one session with Tc-99m CO_2-DADS-A and I-131-hippurate; digital and analog images were recorded for 30 min and after voiding. In the normal volunteers, digital images with Tc-99m CO_2-DADS-A gave a kidney-to-background ratio at 3 min that was greater relative to I-131 hippurate, a leading-edge parenchymal transit time that was similar to I-131 hippurate, and a percent injected dose in the urine at 30 min that was slightly less than I-131 hippurate ($p < 0.05$). In patients (serum creatinine 1.0 to 14.3 mg/dl), deceasing renal function impaired excretion of Tc-99m CO_2-DADS-A more than that of I-131 hippurate ($p < 0.01$). In analog images, Tc-99m CO_2-DADS-A always gave superior spatial resolution. No evidence of hepatobiliary excretion was detected with either radiopharmaceutical. We conclude that Tc-99m CO_2-DADS-A and similar compounds should be pursued as possible replacements for I-131 hippurate.

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The commonly used renal radiopharmaceuticals, Tc-99m DTPA and I-131 hippurate, both have significant disadvantages. Tc-99m DTPA is cleared by glomerular filtration, so its renal extraction efficiency is limited to 20–25% (*1*). I-131 hippurate is cleared mainly by tubular cell secretion with a relatively high renal extraction efficiency of about 65%–80% (*1*), but its iodine-131 label limits the spatial resolution in the resulting images.

An ideal renal radiopharmaceutical for routine use would possess both a technetium-99m label and a high extraction efficiency. Good extraction can only be achieved with clearance by tubular secretion. The discovery that Tc-99m N,N′-bis(mercaptoacetyl)-ethylenediamine (Tc-99m DADS) is excreted by the tubular cells provided a significant lead in the development of an improved renal radiopharmaceutical (*2–4*), but animal and clinical evaluations of Tc-99m DADS indicate that its biological properties are somewhat inferior to those of I-131 hippurate (*5,6*). Consequently, a series of analogs of Tc-99m DADS has been synthesized in an attempt to find a better agent (*7*). One of these, Tc-99m N,N′-bis(mercaptoacetyl)-2,3-diaminopropanoate, Component A (TC-99m CO_2-DADS-A), has demonstrated superior biological properties in normal animals (*8*). In this report we present the results of a clinical comparison between Tc-99m CO_2-DADS-A and I-131 hippurate in normal volunteers and patients.

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METHODS AND MATERIALS

Radiopharmaceutical preparation. Tc-99m CO_2-DADS-A was prepared essentially as described earlier (*8*). The following modifications were made in order to obtain higher specific activity in the desired form and to

utilize a chromatography solvent system that provides an injectable product. Approximately 100 mCi of pertechnetate (Tc-99m) in generator eluate was made basic by the addition of 5*N* NaOH (four parts eluate to one part NaOH). The radioactivity was extracted twice with methyl ethyl ketone of analytical reagent grade and the extracts were combined and evaporated to dryness in a nitrogen stream with warming. The hydrolyzed ligand was prepared from 0.5 mg of ethyl N,N′-bis(benzoylmercaptoacetyl)-2,3-diaminopropanoate; it was dissolved in 0.3 ml ethanol, treated with 30 μl of ethyl N,N′ - bis(benzoylmercaptoacetyl) - 2,3 - diaminopropanoate that was dissolved in 0.3 ml ethanol, treated with 30 μl of 5 *N* NaOH, diluted with 0.3 ml H_2O, and finally heated for 15 min at 90°C. The hydrolyzed ligand solution (ca. 0.25 ml) was added to the pertechnetate residue. Freshly dissolved 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, injected onto an octadecylsilyl high-performance liquid chromatography (HPLC) column (250 × 4.6 mm), and eluted with 4% ethanol in 0.01 *M* sodium phosphate (pH 6). The column effluent was collected in samples as Component A (first) and Component B (second); the two components were sterilized by passage through a 0.22 μm filter. The yield of Component A was 40 to 50 mCi in 45 to 60 min. The pH of Component A, as injected for imaging, was 6.0.

Iodine-131 hippurate was obtained commercially. The percentage of free iodine-131 was not determined, and the agent was not further purified.

Subject selection. All subjects gave informed consent and none was studied under this protocol more than once. The normal volunteers each had a serum creatinine done on the day of the study. The patients were selected from those with either native or transplant kidneys who were referred for radionuclide renal studies. Serum creatinine levels were obtained from all patients.

Imaging protocol. No attempt was made to regulate the state of hydration of the normal volunteers or patients. Following an intravenous injection of 15 mCi of Tc-99m CO_2-DADS-A, both analog and digital images were acquired with the patient supine. The gamma camera had a 15-in. (diam) detector. Anterior projection was used for transplanted kidneys and posterior for native kidneys. A high-resolution, low-energy collimator was used, with a 20% symmetrical window. Rapid serial 3-sec analog images were acquired for 30 sec following injection. Delayed analog images were acquired at 1, 5, 10, 15, 20, and 30 min. The 1-min image was acquired for 750 Kcounts, and subsequent images used the same counting time. In addition, serial digital images were acquired during the first 30 min at 2 frames per second for 30 sec, 4 frames/min for 4 min, and 1 frame/min for 26 min. At 30 min a urine specimen was collected; then five sequential 1-min postvoid analog and digital images were acquired. At approximately 35 min, a single anterior analog image of the upper abdomen, including the hepatobiliary system, was recorded.

Following the Tc-99m CO_2-DADS-A study, the patient was injected intravenously with 500 μCi of I-131 hippurate, and a second set of anterior analog and digital images were acquired with a similar camera but a high-energy collimator. Again the patient was supine, and a 20% symmetrical window was used, bracketing the 364-keV gamma peak. Ten analog images of 3 min each were acquired over 30 min. Serial digital images were acquired at one for the first 30 sec, then 1 frame/30 sec frame/min for 30 min. At 30 min a urine specimen was voided and five postvoid analog and digital images were obtained as before.

Data analysis. The analog images were used to compare anatomic spatial resolution and to assess the degree of hepatobiliary excretion of Tc-99m CO_2-DADS-A. The digital images were used to quantitate the relative kidney-to-background ratio, parenchymal transit time, and urinary excretion of the Tc-99m CO_2-DADS-A and I-131 hippurate. The regions of interest were placed visually by one of us (VMS) and their position was recorded on film. In most cases identical regions of interest were used for both the Tc-99m CO_2-DADS-A study and the I-131 hippurate study.

The kidney-to-background ratio was calculated from the 2.5- to 3.5-min digital data. When the subject had two kidneys, two background regions of interest were used and the results for the two kidneys averaged. Parenchymal transit time was visually estimated from the leading-edge transit time (appearance time of radioactivity in calyces, pelvis, ureter, or bladder) in serial 1-min digital images of Tc-99m CO_2-DADS-A and I-131 hippurate. For this evaluation each pair of studies was examined independently by two observers without knowledge of the patient's identity; if the observers disagreed, their results were averaged. In subjects with two kidneys, the leading-edge transit times were averaged when there was no reason to suspect asymmetrical function (e.g., in normal volunteers); the subject was excluded when function was presumed asymmetrical (e.g., in unilateral renal artery stenosis). Urinary excretion was evaluated by quantitating the percent of injected dose excreted in the urine by 30 min after injection. This was done by comparing the radioactivity in the voided specimen with a standard and correcting for retained urine in the bladder using a bladder time-activity curve (*5*). The five postvoid images allowed backward extrapolation of residual bladder radioactivity to 30 min (*5*).

The serum creatinine values were used to determine whether decreased renal function had a greater effect on the uptake, transit, and excretion of Tc-99m CO_2-DADS-A or I-131 hippurate.

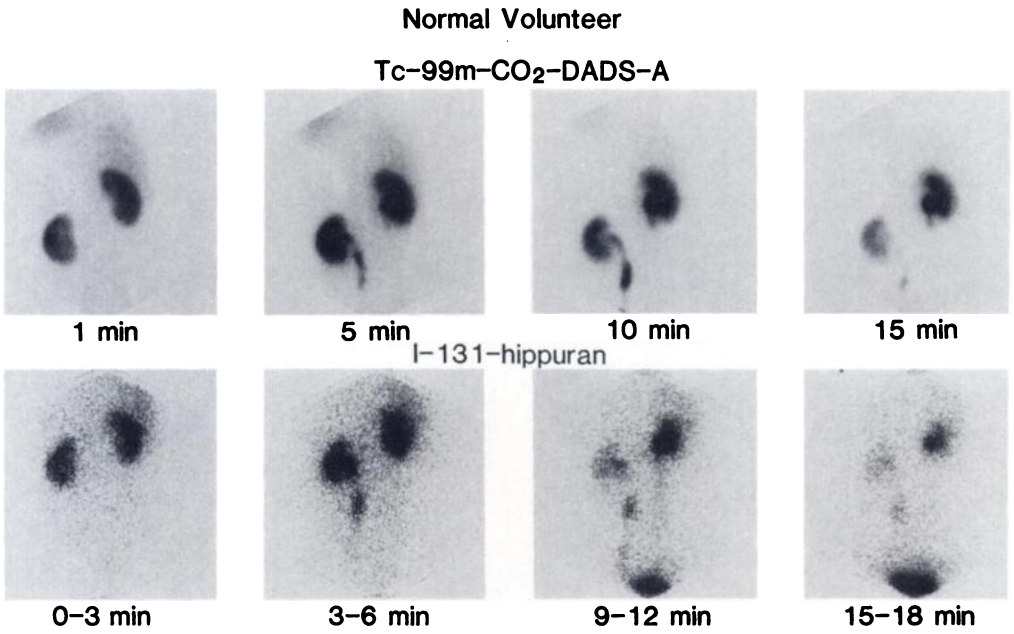

FIG. 1. Sequential Tc-99m CO_2-DADS-A and I-131 hippurate studies in normal volunteer show higher kidney-to-background ratio with Tc-99m CO_2-DADS-A, similar leading-edge transit times, and probably slower parenchymal washout of Tc-99m CO_2-DADS-A. Bladder is outside field of view in Tc-99m CO_2-DADS-A study. Spatial resolution in Tc-99m CO_2-DADS-A images is clearly superior to that of I-131 hippurate images.

Tc-99m CO_2-DADS (component B). Two additional normal volunteers were studied with both HPLC components of Tc-99m CO_2-DADS (Components A and B) on successive days. These studies were analyzed in the same way as the comparison studies between Tc-99m CO_2-DADS-A and I-131 hippurate.

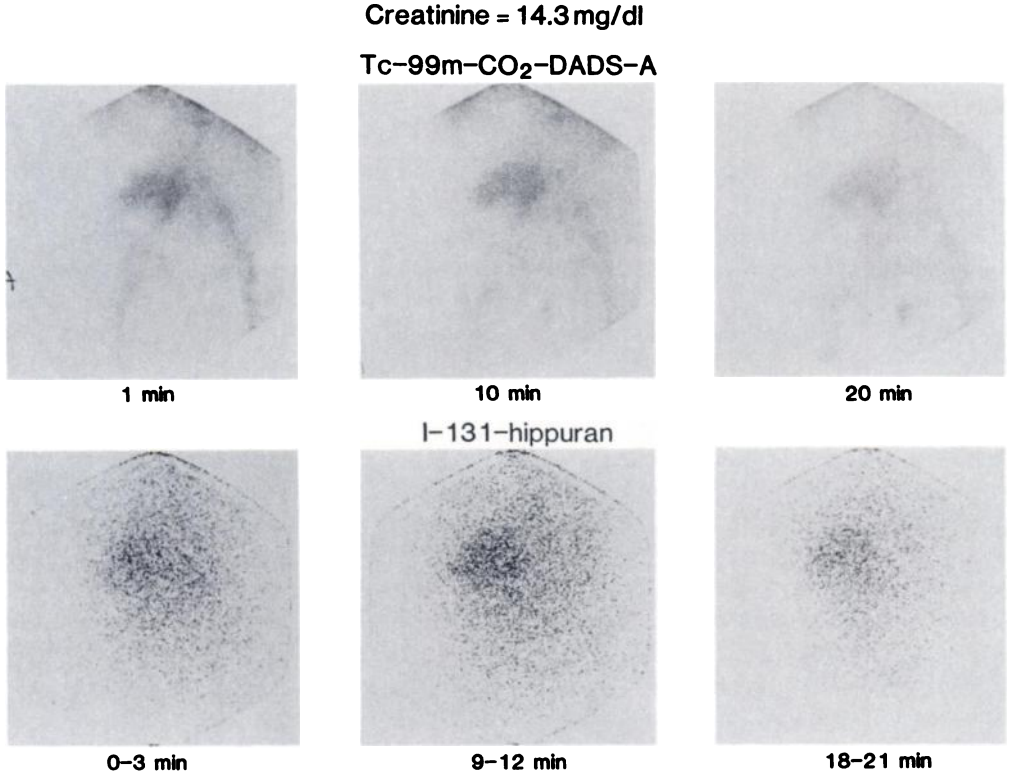

FIG. 2. Sequential Tc-99m CO_2-DADS-A and I-131 hippurate studies in patient with renal transplant in right iliac fossa, chronic rejection, and serum creatinine of 14.3 mg/dl. Note similar kidney-to-background ratios, no evidence of renal excretion through 20 min, and similar degrees of parenchymal washout. General spatial resolution and delineation of large vessels is superior with Tc-99m CO_2-DADS-A.

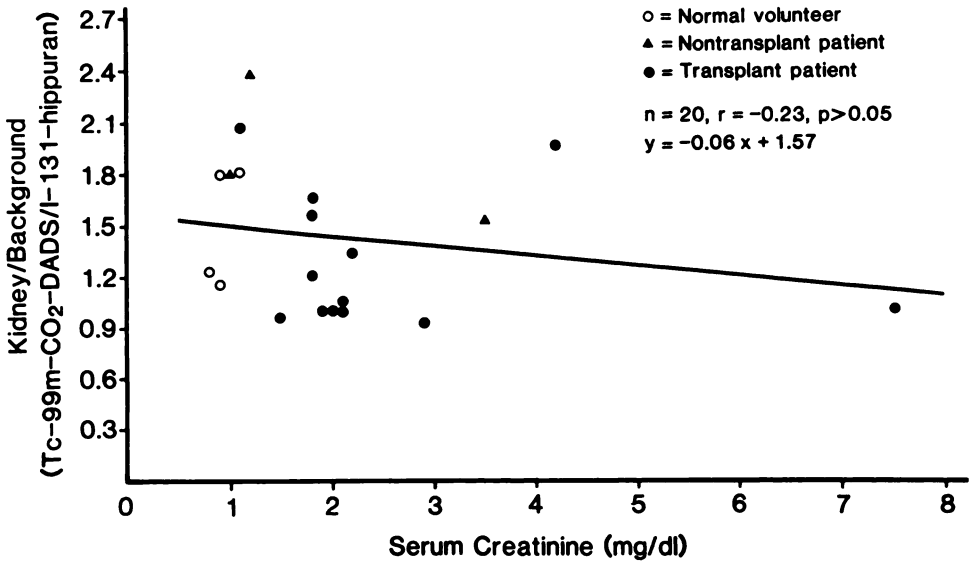

FIG. 3. Quotient for two kidney-to-background ratios (Tc-99m CO_2-DADS-A ÷ I-131 hippurate) is plotted against serum creatinine. In almost every subject kidney-to-background ratio is higher with Tc-99m CO_2-DADS-A. With decreasing renal function (increasing serum creatinine), renal uptake of Tc-99m CO_2-DADS-A may be affected slightly more than that of I-131 hippurate. However, both uptake-to-background ratios show wide variability at all levels of kidney function.

RESULTS

Subject data. In the five normal volunteers the serum creatinine levels ranged from 0.8 to 1.1 mg/dl (mean = 0.9) and were drawn on the day of the radionuclide studies.

Of the 18 patients 15 had renal transplants and three had native kidneys. The diagnoses in the transplant patients were: rejection in six, normal in four, infarcts with good function in two, acute tubular necrosis in two, and cyclosporin-A toxicity in one. The renal diagnoses in the three patients without transplanted kidneys were: normal in one, arteriovenous malformation in one, and renal artery stenosis in one. The serum creatinine levels ranged from 1.0 mg/dl to 14.3 mg/dl (mean = 3.7); the serum creatinine specimen was drawn on the day of imaging in 17 patients and the day before in one.

Analog images. The Tc-99m CO_2-DADS-A images provided greater spatial resolution in all subjects; the larger vascular structures were always seen (Fig. 1, 2). Upper abdominal images were available in all five normal volunteers and in 16 of 18 patients; no evidence of hepatobiliary excretion was seen in any of these subjects.

Digital images. Results were available for each parameter in somewhat fewer than all 23 subjects because of technical problems, e.g., computer malfunction, or inability to obtain adequate urine collections due to urinary diversion through an ileal loop. In the visual evaluation of the leading-edge parenchymal transit time, each kidney in patients with two kidneys was counted separately. The two observers disagreed in 5 out of 40 (12.5%) determinations; the disagreements were never by more than 1 min.

In four normal volunteers the kidney-to-background ratio for Tc-99m CO_2-DADS-A was (150 ± 37)% (mean ± s.d.) of that for I-131 hippurate; the range was 116% to 179%. The kidney-to-background ratio was determined in a total of 20 subjects; it was higher for Tc-99m CO_2-DADS-A in 16, higher for I-131 hippurate in three, and equal in one ($p < 0.05$). For all subjects, the quotient for the two kidney-to-background ratios (Tc-99m CO_2-DADS-A ÷ I-131 hippurate) failed to correlate well with the serum creatinine level: $r = -0.23$, $p > 0.05$ (Fig. 3). The linear regression equation was $y = -0.06x + 1.57$.

In three normal volunteers the leading-edge transit time of Tc-99m CO_2-DADS-A was (108 ± 18)% (mean ± s.d.) re I-131 hippurate; the range was 95% to 129%. The leading-edge parenchymal transit time was determined in a total of 17 subjects; it was longer for Tc-99m CO_2-DADS-A in ten, longer for I-131 hippurate in one and equal in six ($p < 0.05$). For all subjects the quotient for the two leading-edge transit times (Tc-99m CO_2-DADS-A ÷ I-131 hippurate) correlated poorly with serum creatinine level: $r = -0.03$, $p > 0.05$ (Fig. 4). The linear regression equation was $y = -0.01x + 1.24$.

In five normal volunteers the percentage of injected dose excreted in the urine at 30 min following injection for Tc-99m CO_2-DADS-A was (81 ± 4)% re I-131 hippurate ($p < 0.05$); the range was 77% to 85%. The percent of injected dose excreted into the urine in 3 hr was determined in a total of 17 subjects; it was lower for Tc-99m CO_2-DADS-A in all 17 ($p < 0.05$). For all subjects the quotient for the two percentages of dose excreted in the urine (Tc-99m CO_2-DADS-A ÷ I-131 hippurate) correlated inversely with serum creatinine

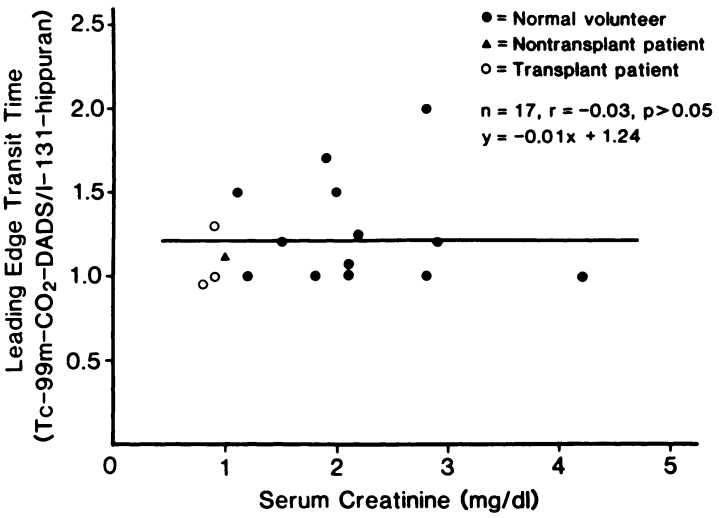

FIG. 4. Quotient for two values of leading-edge transit time (Tc-99m CO_2-DADS-A ÷ I-131 hippurate) is plotted against serum creatinine. This time in patients is almost always longer with Tc-99m CO_2-DADS-A. Impairment of renal function affects two transit times about equally.

level: r = −0.83, p <0.01 (Fig. 5). The linear regression equation was y = −0.07x + 0.80. The absolute percent of injected dose excreted in the urine in 30 min was (58 ± 4)% for Tc-99m CO_2-DADS-A and (73 ± 2)% for I-131 hippurate (p <0.01).

Figure 6 shows time-activity curves from a renal region of interest in a typical normal volunteer. A kidney was selected that showed little pooling in the calyces, and the region of interest was drawn to exclude excreted radioactivity so that the curves would reflect the time courses of the radiopharmaceuticals in the renal parenchyma. The Tc-99m CO_2-DADS-A and I-131 hippurate curves were correlated for background and normalized for the dose injected. The Tc-99m CO_2-DADS-A curve shows a higher peak, although the times of peak radioactivity are approximately the same. However, Tc-99m CO_2-DADS-A showed a slower postpeak decrease (washout) of parenchymal radioactivity: the I-131 hippurate curve is ~70% of Tc-99m CO_2-DADS-A at their peaks, but only ~28% at 30 min.

Tc-99m CO_2-DADS (component B). The creatinine levels in these two normal volunteers were 0.8 and 0.9 mg/dl. No evidence of hepatobiliary excretion was seen in either volunteer with either radiotracer component. The kidney-to-background ratio for Tc-99m CO_2-DADS-A was 160% of that for Tc-99m-CO_2-DADS-B, and the percent of injected dose excreted in the urine for Tc-99m CO_2-DADS-A was 290% of that for Tc-99m CO_2-DADS-B. The absolute percent of injected dose excreted in the urine in 30 min for Tc-99m CO_2-DADS-B was 18.5%. The percent difference between the two components re leading-edge transit time could not be determined because radioactivity did not pool in the

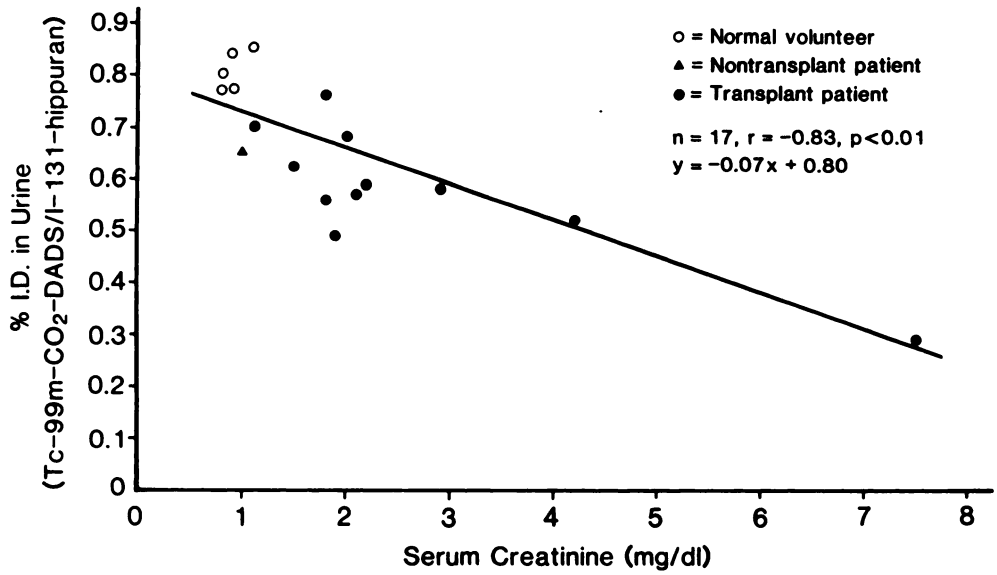

FIG. 5. Percent of injected Tc-99m CO_2-DADS-A excreted in 30 min, over same for I-131 hippurate, is plotted against serum creatinine. With normal kidneys, 30-min fraction for Tc-99m CO_2-DADS-A is about 80% of that for I-131 hippurate, and renal impairment affects the former to greater extent (p <0.01).

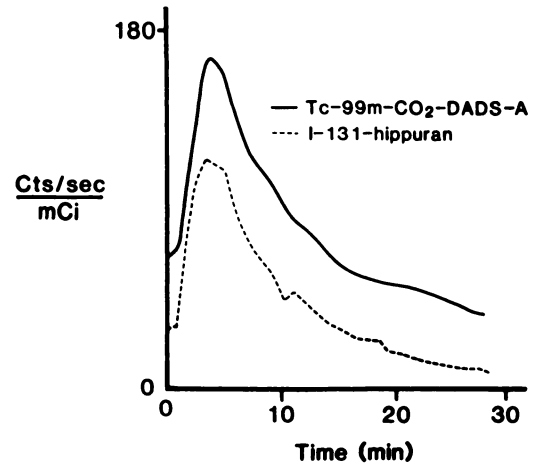

FIG. 6. Time-activity curves from renal region of interest in normal volunteer who was studied with both Tc-99m CO_2-DADS-A and I-131 hippurate. Peak uptake per mCi of Tc-99m CO_2-DADS-A exceeds that for I-131 hippurate, and parenchymal washout (counts at 30 min over peak counts) is slower for Tc-99m CO_2-DADS-A.

upper collecting systems and the bladder was not included in the field of view in either subject.

DISCUSSION

In normal volunteers Tc-99m CO_2-DADS-A, relative to I-131 hippurate, demonstrates a 50% higher kidney-to-background ratio at 3 min, an 8% longer leading-edge transit time, and a 19% lower percent of injected dose excreted in 30 min. These results are superior to those obtained for Tc-99m DADS in a similar previous study (*6*). In that study, in patients with normal renal function, Tc-99m DADS, relative to I-131 hippurate, showed a 10% lower kidney-to-background ratio, a 23% longer leading-edge transit time, and a 61% lower percent of injected dose excreted in 30 min (*6*).

Tc-99m CO_2-DADS-A, like the previously reported Tc-99m DADS (*6*), gives images with high spatial resolution, clearly demonstrating the kidneys and larger vascular structures. These findings result from the Tc-99m label and the fact that Tc-99m CO_2-DADS-A is 93–94% protein bound compared with 56–64% for I-131 hippurate (*8*). However, Tc-99m CO_2-DADS-A, unlike Tc-99m DADS, shows no evidence of hepatobiliary excretion.

In patients with decreased renal function, there was a greater effect on the percent of injected dose excreted in the urine for Tc-99m CO_2-DADS-A than for I-131 hippurate. These findings are consistent with the previously reported results in animals, in which probenicid (an inhibitor of tubular secretion) caused a greater reduction in renal excretion of Tc-99m CO_2 DADS-A than of I-131 hippurate (*8*). However, there was no significantly greater effect of decreased renal function on the kidney-to-background ratio or the leading-edge transit time. These findings are different from the results with Tc-99m DADS, in which decreasing renal function affected all three parameters to a greater extent than with I-131 hippurate (*6*).

The higher kidney-to-background ratio of Tc-99m CO_2-DADS-A relative to I-131 hippurate is of interest since Tc-99m CO_2-DADS-A was not superior to I-131 hippurate in the other two biological parameters, namely leading-edge transit time and percent dose excreted in the urine. Since both radiopharmaceuticals were cleared almost solely by the kidneys, the higher kidney-to-background ratio for Tc-99m CO_2-DADS-A may be secondary to a higher extraction efficiency, a smaller volume of distribution, or both. Since the volume of distribution of Tc-99m CO_2-DADS-A is significantly smaller than that of I-131 hippurate because of the greater degree of protein binding of Tc-99m CO_2-DADS-A (*8*), the extraction efficiency of Tc-99m CO_2-DADS-A may not be greater than the approximately 65–80% that has been measured for I-131 hippurate (*1*).

Preparation of Tc-99m CO_2-DADS-A requires fractionation with a high-performance liquid chromatograph, since labeling of the CO_2-DADS ligand with Tc-99m results in two epimeric compounds (*9*), only one of which possesses the desired biological properties (*8*). The additional expense and expertise needed for HPLC will probably limit the clinical use of Tc-99m CO_2-DADS-A to larger hospitals or those served by centralized nuclear pharmacies.

We tentatively conclude that Tc-99m CO_2-DADS-A is superior to I-131 hippurate for renal imaging because of its technetium-99m label and its higher kidney-to-background ratio. However, because its biological properties are in some ways inferior to those of I-131 hippurate, and because fractionation by HPLC following labeling is required, the search should continue for more desirable Tc-99m-labeled replacements for I-131 hippurate.

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