Quality Control of Tc-99m DTPA for Measurement of Glomerular Filtration: Concise Communication

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When technetium-99m DTPA is used to measure glomerular filtration rate (GFR), the accuracy depends on the supplier of the radiopharmaceutical. The error in GFR is due to protein binding, as we have shown by direct measurement. In 19 patients, GFR measured with Tc-DTPA and corrected for protein binding agreed with that measured simultaneously using Yb-169 DTPA (correlation coefficient 0.991). Without correction, Tc-DTPA gave falsely low values in patients having good renal function, in whom unbound activity cleared rapidly while bound activity remained in the circulation. When Tc-DTPA is used to measure GFR, the in vivo protein binding should be measured and used to correct the data.

J Nucl Med 24: 722–727, 1983

Numerous investigators have proposed Tc-99m DTPA as an agent to measure renal glomerular filtration rate (GFR), either by blood clearance (*1–10*) or by external detection (*4,11–17*). However, it has been shown that the accuracy depends, at least in Europe, on the supplier of the radiopharmaceutical (*6*). To assess use of this agent for clinical GFR measurements, we therefore conducted simple screening experiments to compare the products available in the United States. Chromatography and biodistribution studies in mice showed differences that were small but statistically significant. At that time, it seemed doubtful whether these differences were of clinical significance.

For initial studies in patients, we nevertheless took the precaution of checking results simultaneously against another tracer, Yb-169 DTPA, that is also cleared by glomerular filtration (*18–20*). We were surprised to discover a substantial discrepancy, which was tentatively attributed to partial binding of the Tc-99m DTPA by plasma proteins. Protein binding had been recognized at the time of the first clinical studies with this agent, but its extent was considered too small to prejudice a GFR measurement (*1*). Since binding of the commercially available agents could differ from that of the preparations originally described, we began to measure in vivo protein binding routinely as part of the GFR measurement. This revealed that protein binding was often extensive, and that it increased with time after injection. The protein binding measured for each patient was used to correct the GFR calculation for that patient. Such correction was found to eliminate most of the discrepancy between Tc-99m DTPA and Yb-169 DTPA measurements.

Received Dec. 21, 1982; revision accepted Mar. 21, 1983.

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MATERIALS AND METHODS

Preparations studied. Kits from three commercial sources of Tc-99m (Sn)DTPA (diethylenetetraminepenta-acetic acid) were studied. These were designated A, B, and C. Their compositions when reconstituted for chromatographic and biodistribution studies were:

A. Lyophilized kit containing 20.6 mg $CaNa_3DTPA$, 0.11–0.21 mg $SnCl_2$, adjusted to pH 4.0 with HCl. Reconstituted volume 1 ml.

B. Lyophilized kit containing 10 mg $CaNa_3DTPA$, 0.50 mg $SnCl_2$, adjusted to pH 4 with HCl. Reconstituted volume 3 ml.
C. Lyophilized kit containing 5 mg "Na salt of DTPA," 0.25 mg $SnCl_2$. Reconstituted volume 2 ml.

When reconstituted for human use, 20 mCi of pertechnetate was added in 5–7 ml physiologic saline, and 5 mCi of this was administered to the patient.

Calculation of glomerular filtration rate. The GFR was calculated from the formula:

$$GFR = \text{dose} \Big/ \int_0^\infty a_{plasma} dt$$

(This equation is derived by writing the differential mass balance equation for the kidney, and then integrating, with the assumption that the entire dose is excreted by glomerular filtration.) The integral in the denominator was obtained by least-squares fit of the plasma time-activity curve to a sum of exponentials. Eight points were measured on the time-activity curve, at intervals from 10 to 240 min after injection, by drawing blood through a heparin lock. The same method was used both for Tc-99m DTPA and Yb-169 DTPA, but to correct the Tc-99m DTPA data for binding by plasma proteins, unbound plasma activity was substituted for total plasma activity in the above equation to obtain the "corrected GFR."

The same method can be derived by assuming a linear compartmental model. However, as shown by the above derivation, the validity of the method does not depend on assuming a compartmental model, only that (a) the plasma disappearance curve is well fitted by a sum of exponentials, (b) that there is no extrarenal site of excretion or binding, and (c) that the agent is truly excreted by glomerular filtration alone. In practice, the curve from 10 to 240 min was well fitted by a sum of two exponentials except for patients with very low renal function, in which a single exponential better described the curve. (This meant using a single exponential fit whenever the Gauss-Newton iterative two-exponential fit failed to converge.)

Measurement of in vivo protein binding. Two methods were used to measure protein binding. The first was based on ultrafiltration, using a centrifugal micropartition system* with membrane filters† at room temperature. These were used exactly according to the manufacturer's instructions.

The other method was gel filtration, using mini-columns‡ prepacked with Sephadex G-25 M. These columns were eluted with 0.15 *M* NaCl, 0.002 *M* NaH_2PO_4, with 0.002% chlorhexidine added as bacteriostat, and pH adjusted to 7.4 with NaOH. The samples were counted both for Tc-99m and, after adequate decay, for Yb-169 ($T_{1/2}$ = 32 days). Since the protein-bound fraction amounted to only a few percent of the total activity, it was critical to avoid even slight contamination of the void-volume fraction by later fractions. To cut as sharply as possible after the void volume, the minimum visible amount of blue dextran was added to the sample before application, and elution was terminated as soon as the blue band had left the column. (If one elutes the fixed volume specified by the manufacturer, more is included in the eluate than just the void-volume peak.)

To calculate the GFR, the membrane-filtration data were used, since these more closely simulate glomerular ultrafiltration. In principle, ultrafiltration measures both irreversibly and reversibly bound activity, whereas gel filtration measures only the component that is irreversibly bound and cannot dissociate while on the column. In practice, the two methods gave very similar results, and it appears that either can be used to correct the GFR.

Chromatography. The method used for ion-exchange paper chromatography was derived from a method of column ion-exchange chromatography described previously (*21*). Anion-exchange paper§ was equilibrated by means of continuous overnight development with aqueous 0.15 *M* NaCl, 0.01 *M* NaH_2PO_4, adjusted to pH 7.0 with NaOH. One-microliter samples (containing about 1 μCi Tc-99m) were applied to the wet paper without stopping eluent flow, and development was continued in the descending direction for 2 hr. In that time, Tc-99m DTPA migrated 9 cm and pertechnetate 4 cm.

The amount of activity in each chromatographic fraction was determined by cutting out the corresponding segment of the paper strip and counting it in a scintillation well counter. The results were expressed as fractions of total activity on the strip. Since migration distances were sensitive to minor changes in operating conditions, the locations of the Tc-99m DTPA spot and of a control pertechnetate spot were determined on each strip by autoradiography. One segment was cut to include the Tc-99m DTPA spot, one to include the pertechnetate spot, and one to include the point of sample application. The remainder of the strip was also counted, but contained little activity; it was included in total activity to calculate percentages.

Distribution of activity in mice. Male outbred albino mice, weighing 25–35 gm, were injected by tail vein with 0.1 ml of the sample. Three hours later the animals were killed and tissue specimens obtained for determination of activity in a scintillation counter. Specimens of whole blood, liver, and spleen were obtained. The stomach was counted intact, including contents. The tail was also counted in order to exclude animals in which there was significant extravasation of the dose; the criterion for rejection was a tail count differing by three or more standard deviations from the mean tail count.

TABLE 1. CHROMATOGRAPHY OF THREE DIFFERENT Tc-DTPA PREPARATIONS: PERCENTAGE OF TOTAL ACTIVITY IN INDIVIDUAL FRACTIONS (MEAN ± STANDARD ERRORS OF N REPLICATES). REPLICATES ARE DIFFERENT VIALS FROM SINGLE LOT

	Ion-exchange method (N = 5)		
Preparation	Tc-DTPA*	Origin* ("Hydrolyzed-reduced")	TcO_4^-
A	97.3 ± 0.6	0.5 ± 0.1	1.5 ± 0.5
B	93.0 ± 1.2	2.3 ± 0.6	3.7 ± 0.5
C	93.9 ± 0.5	3.2 ± 0.4	2.4 ± 0.4
TcO_4^-	0.17 ± 0.05	0.11 ± 0.05	99.71 ± 0.06

* The differences among A, B, and C are statistically significant (one-way analysis of variance, $p < 0.05$).

RESULTS

Autoradiograms of the chromatographic strips showed Tc-99m DTPA to migrate as a discrete spot, neither bound at the point of application nor moving with the solvent front, and well separated from pertechnetate. The results of cutting the strips into segments and measuring activity in a well counter are presented in Table 1. Ninety percent or more of the applied activity was found in the Tc-99m DTPA fraction. These data indicated that material from supplier A was of higher purity than that from either B or C; analysis of variance showed this difference to be significant at the 95% confidence level.

The tissue distributions of all three preparations in mice are shown in Table 2. The blood clearance of preparation A was more rapid than those of the other two. Analysis of variance again confirmed that the difference was significant at the 95% confidence level. The preparation found to be of the highest purity by ion-exchange chromatography was thus found to have the most rapid blood clearance in mice.

Initial clinical studies were performed with kits from supplier B. Results from the first eight patients are shown in Fig. 1. Falsely low values for GFR were obtained with Tc-99m DTPA, especially when the GFR was high. These findings were contrary to what we had expected after the animal studies, but agreed with previous clinical findings by Carlsen and others (*6*).

To ascertain whether protein binding could account for this error, the binding was measured directly in subsequent clinical studies (Fig. 2). The binding was substantial, and increased with time after administration. Similar results were obtained with either membrane filtration or gel filtration. After finding extensive protein binding with preparation B in four patients, and with preparation C in one patient, preparation A was tried and found to have considerably less protein binding. On the basis of these few clinical measurements (which should not be construed as any sort of valid clinical comparison between suppliers), preparation A was chosen for subsequent GFR measurements in patients. All data were corrected for protein binding, even though the correction was small for this agent. The pooled results are shown in Fig. 3, which involves kits from all three suppliers. After correction, the Tc-99m DTPA clearance correlated closely (correlation coefficient 0.991) with that of Yb-169 DTPA. The difference between the regression line and the line of identity was small, though statistically significant (Student's t-test: $p < 0.05$). The four measurements made with preparation B, and one made with preparation C, also agreed with Yb-169

TABLE 2. TISSUE DISTRIBUTIONS OF THREE DIFFERENT Tc-DTPA PREPARATIONS IN MICE AT 3 HR AFTER INJECTION (1% DOSE/1% BODY WEIGHT, MEAN ± STANDARD ERROR OF N REPLICATES). REPLICATES ARE DIFFERENT ANIMALS AND INCLUDE AT LEAST THREE DIFFERENT VIALS, ALL FROM A SINGLE LOT

Preparation	N	Blood*	Liver	Spleen	Stomach†
A	6	0.0047 ± 0.0014	0.029 ± 0.002	0.011 ± 0.002	0.040 ± 0.021
B	6	0.0117 ± 0.0007	0.044 ± 0.005	0.028 ± 0.014	0.030 ± 0.006
C	8	0.0098 ± 0.0007	0.032 ± 0.002	0.0098 ± 0.0007	0.023 ± 0.006

* Differences between A, B, and C are statistically significant (one-way analysis of variance, $p < 0.05$).
† % Dose/organ.

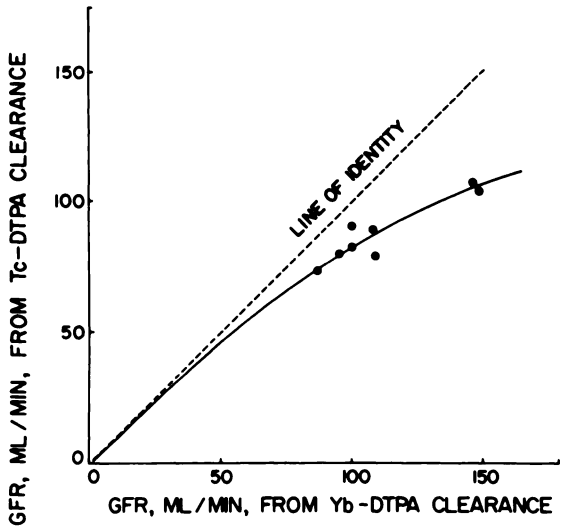

FIG. 1. Glomerular filtration rate in man calculated from Tc-DTPA plasma clearance compared with that calculated from ytterbium-DTPA clearance (using eight samples from 10 to 240 min after administration). Results from Tc-DTPA are falsely low, especially when GFR is high. Similar findings have been reported by Carlsen et al. (*6*). Tc-DTPA kits used for this figure were all from supplier B.

DTPA after correction, although the protein binding was greater and the correction was consequently larger.

DISCUSSION

Hauser and others, in introducing Tc-99m DTPA, reported that 5 to 10% of the dose was bound to plasma proteins at 1 hr after administration to patients (*22*). Similar results were reported by Klopper and others who investigated its use for GFR measurements (*1*). They concluded that Tc-99m DTPA gave results accurate enough for clinical use, despite slight protein binding (leading to 8% underestimation in their hands when compared with iothalamate). Hosain found the kits to deteriorate on storage, with appearance of a slow extra

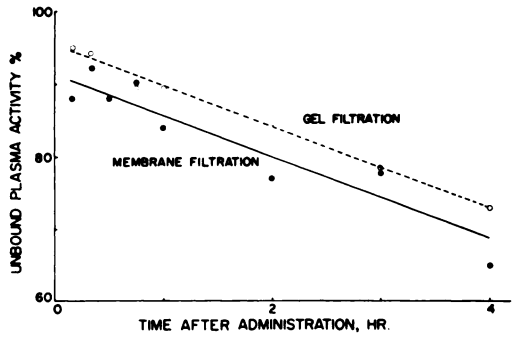

FIG. 2. Free (unbound) Tc-DTPA in human plasma compared with time after administration, as measured by two different methods in representative patient. Proportion of free Tc-DTPA decreases with time, presumably because of renal excretion. Kit from supplier C. Some Tc-DTPA preparations give much less protein binding than this (*1*).

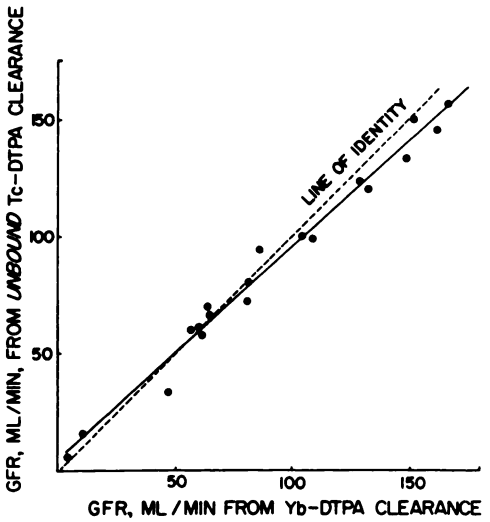

FIG. 3. GFR calculated from free (unbound) Tc-DTPA plasma clearance compared with that from ytterbium-DTPA plasma clearance. Results are closely correlated ($r = 0.991$) but not quite identical. Kits from several different suppliers were used.

component in the plasma time-activity curve (*23*). Kempi and Persson also identified a slow (17-hr) component in the plasma clearance curve, corresponding to 2% of the administered dose, which they attributed to plasma-bound activity (*24*). They suggested that protein binding was correlated with the amount of "hydrolyzed reduced" technetium found by gel-filtration chromatography. Recently, Carlsen and others reported that the accuracy of GFR measurements depended on which manufacturer supplied the kits (*6*). They did not identify the source of error, but mentioned variable protein binding as one possibility. Moore and Rhodes, using chromatographic quality-control procedures, raised the question of whether the quality of commercial kits has deteriorated in recent years (*25*).

Our findings show that protein binding can indeed cause substantial error when Tc-99m DTPA is used to measure GFR. The error can be nearly eliminated by measuring protein binding separately for each patient and making appropriate corrections; this is cumbersome but not necessarily impractical. The fraction of total plasma activity bound to protein increases with time after injection, apparently because the unbound activity is preferentially excreted by the kidneys. Since the unbound activity is excreted more rapidly when GFR is high, the residual bound activity increases more rapidly and the error is greater at high GFR. This effect of greater error at high GFR can be seen in Fig. 1, and also in similar data published by Carlsen and others (*6*). That the bound activity dissociates only slowly from the plasma proteins is shown by quantitative agreement between membrane-filtration and gel-filtration measurements (Fig. 2). The former measures total bound activity whereas the latter measures only the portion of the bound activity that dissociates from plasma protein

too slowly to separate during passage through the column.

Yb-169 DTPA has been less studied than some other GFR agents, though reports indicate that, like other metal chelates, it is excreted solely by glomerular filtration (*18–20*). For purposes of this paper, it can be regarded simply as a stable benchmark against which the lot-to-lot variation in Tc-99m DTPA can be readily measured. Cr-51 EDTA would have been preferable for this purpose, though unfortunately not available for intravenous use in the United States. Iodine-125 iothalamate falls in a different chemical class, so that observed differences would be harder to interpret; they would be apt to reflect genuine differences between agents rather than impurities in the Tc-99m DTPA. No protein binding by Yb-169 DTPA was detected in this study, although for Yb-169 DTPA the activity remaining in the plasma after the first hour, in patients with normal renal function, was too low for accurate measurement of protein binding.

A quality-control method that correlates with human in vivo protein binding would be very useful. Several conventional methods were tried by Carlsen, but to no avail (*6*). The 3-hr blood clearance in the mouse (Table 2) is clearly not a good model for three-hour blood clearance in man. The 24-hr clearance in mice recommended by the USP is even less likely to be useful as an index of short-term clearance in man (*26*). Clearance in mice at shorter time intervals may be useful, but this has not yet been investigated. Dual-tracer measurements of plasma clearance in dogs, as performed by Hosain, are most promising (*23*). The chromatographic methods of Table 1 are capable of distinguishing between different commercial kits, but whether they can be relied upon to predict clinical performance has not yet been determined. We have laboratory evidence that the properties of Tc-DTPA preparations change with time after preparation, and probably also with the source of the generator and the amount of activity added. However, we have not yet confirmed the laboratory data by measurement of protein binding in humans. Until detailed studies verify the validity of some test for the quality control of Tc-99m DTPA, it is safer for clinical purposes to measure the protein binding in each patient and then to correct for it.

FOOTNOTES

* Amicon MPS-1.
† Amicon YMB.
‡ Pharmacia PD-10.
§ Whatman DE 81.

ACKNOWLEDGMENTS

We are indebted to Ms. Dorothea Ballard for assistance with the manuscript and to the commercial suppliers for the donation of kits.

This work was supported by the Veterans Administration Medical Center, Birmingham, Alabama, and by the National Cancer Institute, DHEW, Grant #1-R01-CA27252.

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