Quality Control of Technetium-99m DTPA: Correlation of Analytic Tests with In Vivo Protein Binding in Man

Charles D. Russell, Katherine Rowell, and Johnny W. Scott

Division of Nuclear Medicine; University of Alabama Hospitals; and Nuclear Medicine Service, Birmingham V.A. Medical Center, Birmingham, Alabama

When $[^{99m}Tc]$DTPA is administered, a small fraction of the activity (presumably an impurity) is bound to plasma proteins. This causes an error in the calculation of glomerular filtration rate from plasma clearance. This paper presents two methods of laboratory quality control for measuring the fraction that binds to plasma proteins. One method involves in vitro binding to human serum albumin followed by gel filtration. The other method involves descending paper chromatography on wet pre-equilibrated anion exchange paper. In a series of 80 patients, correlation was demonstrated between laboratory characteristics and actual clinical performance of the $[^{99m}Tc]$DTPA preparation. Both laboratory methods appear suitable for routine quality control.


When technetium-99m diethylenetriaminepentaacetic acid ($[^{99m}Tc]$DTPA) is administered intravenously, a small fraction of the activity is bound to plasma proteins (1,2). The protein-bound fraction is thought to represent an impurity and not true $[^{99m}Tc]$DTPA. Since it remains in circulation while unbound activity is excreted, it can lead to significant errors in the calculation of glomerular filtration rate (GFR) from plasma clearance (2,3). Accurate GFR measurement is possible in the face of significant protein binding, if the binding is measured for each individual patient and the appropriate correction made; however, this is cumbersome. It would be better to have laboratory tests capable of predicting the extent of protein binding, in order to select lots of $[^{99m}Tc]$DTPA for which protein binding was within acceptable limits. Until now, no laboratory tests have been available that have the proven ability to predict protein binding in man. In this paper, we compare the results of two analytic quality control methods with protein binding in 80 patients. Both of the analytic methods were found to correlate with in vivo protein binding, and thus appear suitable for screening $[^{99m}Tc]$DTPA kits for GFR measurement.

MATERIALS AND METHODS

Plasma samples were obtained from 80 patients who were undergoing $[^{99m}Tc]$DTPA renal function studies for routine clinical indications. The GFR was estimated from the activity in a single plasma sample obtained 3 hr after i.v. administration (4). The sample was collected in a standard ethylenediaminetetraacetic acid-containing vacuum tube and the protein-bound fraction was measured using two methods, ultrafiltration and gel filtration, as described elsewhere (2). In brief, for ultrafiltration, the plasma sample was passed through a membrane filter that retained plasma proteins, and the activity in the protein-free ultra-filtrate was compared with that in the original plasma. For gel filtration, the sample was applied to a conventional dextran gel size-exclusion column, and the activity in the high-molecular-weight fraction that eluted at the void volume was compared with the activity in the original plasma.

To compare different formulations of $[^{99m}Tc]$DTPA, four different commercial formulations were employed. For three of these, kits from at least two different lot numbers were evaluated. Each lot was subjected to two different in vitro quality control tests, one using ion-exchange paper chromatography (2) and the other using gel filtration after mixing with human serum albumin. The method for in vitro measurement of binding to human serum albumin was as follows. One microliter of the dose was added to 1 ml of a solution containing 1% human serum albumin, 0.15M NaCl, 0.002M NaH$_2$PO$_4$, 0.002% chlorhexidine as bacteriostat, with pH adjusted to 7.4 with NaOH. This was allowed to stand for 15 min at room temperature and then the bound fraction was
FIGURE 1
Correlation between in vivo and in vitro binding measurements, mean ± s.e.e. for four different formulations
determined by gel filtration, using the method described for plasma samples (2).

The commercial [99mTc]DTPA kits were formulated as follows.
A. 20.6 mg CaNa$_3$DTPA, 0.21 mg SnCl$_2$, reconstituted to 1–8 ml.
B. 5 mg "sodium salt of DTPA" 0.25 mg SnCl$_2$, reconstituted to 2–8 ml.
C. 10 mg CaNa$_3$DTPA, 0.50 mg SnCl$_2$, reconstituted to no more than 5 ml.
D. 3.0 mg CaNa$_3$DTPA, 0.15 mg SnCl$_2$, reconstituted to 1.3 ml as unit dose.
All measurements were made in duplicate and averaged, using at least nine vials from each lot.

TABLE 1
Comparison of In Vitro with In Vivo Measurements for Four Different [99mTc]DTPA Formulations*

<table>
<thead>
<tr>
<th>Formulation</th>
<th>In vitro</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HSA (% bound)</td>
<td>Ion exchange (% at origin)</td>
</tr>
<tr>
<td>A</td>
<td>0.79 ± 0.19</td>
<td>0.65 ± 0.08</td>
</tr>
<tr>
<td>B</td>
<td>1.09 ± 0.17</td>
<td>1.01 ± 0.19</td>
</tr>
<tr>
<td>C</td>
<td>3.3 ± 0.3</td>
<td>4.4 ± 0.5</td>
</tr>
<tr>
<td>D</td>
<td>2.8 ± 0.4</td>
<td>3.5 ± 0.4</td>
</tr>
</tbody>
</table>

* Mean ± s.e.e. for N samples, where N = 26 formulation A, 19 for B, 21 for C, and 9 for D.

RESULTS

The two different analytic tests agreed with each other and with the in vivo protein binding. This is presented graphically in Fig. 1 for one of the analytic tests. The numerical results for both analytic tests are presented in Table 1.

Kits with two separate lot numbers were tested for formulations A through C, but no statistically significant lot-to-lot variation was found. Technetium generators from three different manufacturers were used, also without significant differences. However, preliminary data showed that the time interval between compounding and use was important (Fig. 2). This variable was not studied in detail. Instead, its effect was limited by administering the dose and beginning analytic measurements at the fixed time of 30–60 min. All measurements in Table 1 and Fig. 1 were made in this manner.

DISCUSSION

Because the fraction of [99mTc]DTPA that remains immobile on anion exchange paper agrees quantitatively with the fraction that binds to human serum albumin in vitro (Table 1), we shall assume for the present that these two tests measure the same impurity
or combination of impurities. The apparent volume of
distribution of this impurity is 11.7 l, calculated from
the 3-hr plasma samples (dividing administered dose of
impurity by the bound plasma activity per unit vol-
ume). This volume is substantially larger than plasma
volume, so that the complex with plasma proteins must
either penetrate the extravascular space or else slowly
dissociate. Attributing protein binding to an impurity
rather than to $^{99m}$TcDTPA itself (2,5,6) explains why
binding depends so strongly on such details of prep-
eration as the source of the kits and the time interval
after compounding.

What level of purity is required for use in GFR
measurement? Suppose that GFR is to be estimated
from a single 3-hr plasma sample. Using the observed
3-hr distribution volume of 11.7 l for the bound fraction
together with an equation that relates GFR to plasma
activity (4) leads to the following error estimates: An
impurity level of 1% of the dose will cause an error of
1.2 ml/min in the GFR when the GFR is 5 ml/min, an
error of 1.9 ml/min when the GFR is 40 ml/min, and
an error of 6.7 ml/min when the GFR is 150 ml/min.
These errors, when compared with other errors inherent
in the procedure (4), are negligible when GFR is low
and acceptable when GFR is high. A 1% impurity level
is therefore acceptable when GFR is to be measured
from a single 3-hr plasma sample. Inspection of Table
1 shows that this standard can be met in practice.

We have demonstrated the validity of two methods
of quality control. We suspect that some readers will
prefer to draw inferences from our data instead of
applying these methods in their own environment.
Ideally, quality control measurements should be re-
peated for each separate lot of radiopharmaceutical and
under the actual conditions of use.

CONCLUSION

Two analytic methods have been presented that pre-
dict protein binding of $^{99m}$TcDTPA. These can be
used to screen preparations intended for GFR meas-
urements. If GFR is to be estimated from a single 3-hr
plasma sample, the impurity that binds to plasma
proteins should not exceed 1% of the dose.

ACKNOWLEDGMENTS

The authors thank Ms. Dorothea Ballard for help with this
manuscript. This work was supported by the Birmingham
Veterans Administration Medical Center, Birmingham, Al.

REFERENCES

of $^{99m}$Tc-DTPA for the measurement of glomerular
control of Tc-99m-DTPA for measurement of glo-
merular filtration: Concise communication. J Nucl
of four commercial Tc-99m (Sn) DTPA preparations
used for the measurement of glomerular filtration rate:
1980
4. Russell CD, Bischoff PG, Kontzen FN, et al: Measure-
ment of GFR: Single injection plasma clearance
method without urine collection. J Nucl Med
26:1243–1247, 1985
5. Kempi V, Persson RBR: Tc-99m-DTPA(Sn)—dry-kit
preparation. Quality control and clearance studies.
distribution and excretion of DTPA labeled with Tc-