jnm/ RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

The Effect of Carrier Technetium in the Preparation of ^{99m}Tc-Human Serum Albumin

William C. Porter, Howard J. Dworkin, and Robert F. Gutkowski

William Beaumont Hospital, Royal Oak, Michigan

Accumulation of daughter ^{99}Tc in ^{99}Mo–^{99m}Tc generator systems may adversely affect the preparation of certain ^{99m}Tc radiopharmaceuticals. In a study of the effect of "carrier" technetium on the radiochemical purity of ^{99m}Tc-human serum albumin, varying amounts of ^{99}Tc were added to a commercially available electrolytic kit. The maximum amount of ^{99}Tc that could be added to such a kit and still maintain radiochemical purity above 90% was about 3×10^{15} atoms. When kits containing 10^{16} ^{99}Tc atoms were electrolyzed for the conventional 42 sec, the radiochemical purity was only 50.8%. However, when the electrolysis time was varied, it was found that electrolysis for 80 sec yielded a product with greater than 90% radiochemical purity. Four solutions to the problem created by the ^{99}Tc carrier effect are described.

J Nucl Med 17: 704–706, 1976

Human serum albumin labeled with ^{99m}Tc (^{99m}Tc-HSA) is a valuable radiopharmaceutical widely employed for static and dynamic imaging of vascular spaces. A convenient method for preparing this agent electrolytically is marketed commercially in kit form (*1*). The electrolytic method of preparation routinely yields binding efficiencies* (BE) in excess of 95%, as determined by thin-layer chromatography. Occasionally, we have encountered low BEs of 40–60% for which no obvious cause was evident. A review of our quality control records for the last 12 months revealed that all of the unacceptable preparations (BE < 90%) had been prepared with sodium pertechnetate obtained from the initial elution of a ^{99}Mo–^{99m}Tc generator. Some contaminant present in this first eluate was thought to be responsible for the unusually low labeling efficiencies.

Molybdenum-99 decay results in the formation of either ^{99}Tc or ^{99m}Tc atoms, and decay of the metastable ^{99m}Tc isomer also results in the formation of ^{99}Tc. The initial eluate of a ^{99}Mo–^{99m}Tc generator (usually obtained on a Monday) contains a much greater number of ^{99}Tc than ^{99m}Tc atoms. We hypothesized that these high concentrations of ^{99}Tc atoms were producing a carrier effect. The following study was undertaken to test this hypothesis.

MATERIALS AND METHODS

1. Thirty ^{99m}Tc-human serum albumin kits (New England Nuclear Corp., North Billerica, Mass.) were prepared following the manufacturer's instructions. In order to minimize the ^{99}Tc carrier effect, the eluate was obtained within 6 hr after a previous elution. Fifteen millicuries of ^{99m}Tc-sodium pertechnetate obtained from a 500-mCi Ultra-TechneKow FM generator, containing a maximum of 2×10^{13} technetium atoms, was used for each ^{99m}Tc-HSA kit. One-tenth milliliter of 25% human serum albumin (Armour Lot M67211) was added to each kit. In order to examine the effect of increasing amounts of

* For ascending chromatography of ^{99m}Tc-HSA on Gelman instant thin-layer chromatographic media type SG, 85% methanol solvent, the binding efficiency is defined as

$$BE = \frac{^{99m}\text{Tc counts at origin}}{\text{Total } ^{99m}\text{Tc counts on strip}} \times 100\%.$$

Received Oct. 28, 1975; revision accepted Feb. 4, 1976.

For reprints contact: W. C. Porter, Nuclear Pharmacy, Dept. of Nuclear Medicine, William Beaumont Hospital, Royal Oak, MI 48072.

^{99}Tc carrier, dilutions of ^{99}Tc in 0.9% NaCl were prepared from a stock solution of $NH_4{}^{99}TcO_4$ (New England Nuclear Lot 1741), which contained 3.53 $\times$ 10^{20} ^{99}Tc atoms (1 mCi). Carrier technetium was added to each kit in amounts ranging from 10^{13} to 10^{18} atoms. Prior to electrolysis, the volume of each kit was adjusted to 6.0 ml with 0.9% NaCl for injection. Each kit was electrolyzed for 42 sec at 100 mA and then allowed to incubate for 30 min before buffering.

Radiochemical purity was determined for each ^{99m}Tc-HSA preparation soon after buffering, and the results were confirmed by protein precipitation of the final product with 25% trichloroacetic acid.

2. In an effort to prepare ^{99m}Tc-HSA with binding efficiencies greater than 90% when 10^{16} ^{99}Tc atoms were present, we examined the effect of increasing the electrolysis times. Twenty commercial kits were prepared as follows. Fifteen millicuries of ^{99m}Tc-sodium pertechnetate and 0.1 ml of 25% HSA were added to each kit as in Experiment 1. After the addition of 10^{16} ^{99}Tc atoms, the volume of each kit was adjusted to 6.0 ml with physiologic saline. The electrolysis time was varied over 42–150 sec. An incubation time of 30 min was employed before buffering. The preparation corresponding to the shortest electrolysis time required to produce a BE greater than 90% was passed through a 0.22-μm membrane filter (previously treated with a 5.0% solution of HSA) to test for a filterable colloid.

RESULTS

1. The BE of ^{99m}Tc-HSA exceeds 90% up to the point where 3 $\times$ 10^{15} ^{99}Tc atoms are added (Fig. 1). Any additional increase in the number of ^{99}Tc atoms produces a striking decline in BE. At 10^{16} added ^{99}Tc atoms, the BE fell to 50.8%, at 10^{17} ^{99}Tc atoms, it fell to 10.8%.

2. Figure 2 shows the results obtained when ^{99m}Tc-HSA preparations containing 10^{16} ^{99}Tc atoms were electrolyzed for varying periods of time. An electrolysis time of 80 sec yielded a BE above 90%. In addition, 99.4% of the activity in this preparation passed through the 0.22-μm membrane filter.

DISCUSSION

Reagent deterioration or compounding errors may yield ^{99m}Tc radiopharmaceuticals of unacceptable radiochemical purity. Low binding efficiencies in which the above factors cannot be implicated usually remain unexplained. In the past we have occasionally encountered unacceptably low BEs when preparing ^{99m}Tc-HSA. In each of the four unacceptable ^{99m}Tc-HSA preparations encountered in the past year, the radiopharmaceutical had been compounded for use

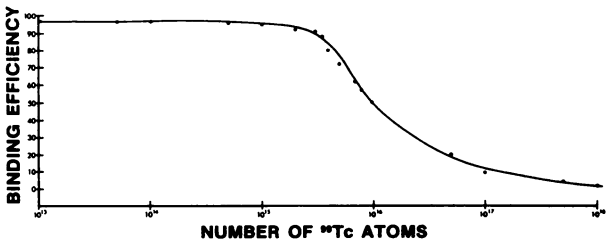

FIG. 1. Binding efficiency of ^{99m}Tc to HSA is plotted as function of number of ^{99}Tc atoms (log scale) added to each kit. Duplicate determinations were run from 5 $\times$ 10^{14} to 5 $\times$ 10^{16} added ^{99}Tc atoms, and average binding efficiency is shown. "Break point" in resulting curve is at approximately 3 $\times$ 10^{15} added ^{99}Tc atoms.

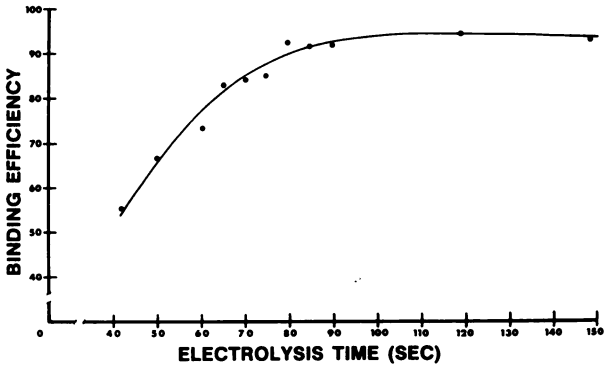

FIG. 2. Binding efficiency of ^{99m}Tc to HSA is shown as function of electrolysis time for 10^{16} added ^{99}Tc atoms. Each data point is mean of two determinations. Binding efficiency above 90% is first achieved at 80 sec.

in a cardiac flow study: in these instances 120–160 mCi of sodium pertechnetate was added to the electrolysis kit. On other occasions, however, up to 350 mCi of $Na^{99m}TcO_4$ has been added with no loss in radiochemical purity (BE > 95%). Thus, radioactivity alone did not appear to be the limiting factor. Further inspection of our quality control records revealed that in each of these four preparations the pertechnetate used was from the initial elution of a 500-mCi ^{99}Mo–^{99m}Tc generator.

Although the detailed chemistry of the electrolytic method of preparation is a matter of some debate, there is little question that an equivalency exists between the amount of zirconium ion produced at the anode and the number of ^{99}Tc and ^{99m}Tc atoms reduced. When a ^{99m}Tc-HSA kit is prepared according to the manufacturer's instructions, approximately 1,000 μg of zirconium is dissolved at the anode.* In this system the technetium must first be reduced by zirconium to label the HSA efficiently (*2*). A con-

* From Faraday's law, the amount of Zr dissolved may be calculated as follows:

$$\frac{22.805\text{gm/gm equivalent wt. Zr} \times 0.1\text{ A} \times 42\text{ sec}}{96{,}483\text{ coulombs/gm equivalent wt.}} = 993\ \mu\text{g}.$$

taminant present in the initial generator eluate was incorrectly suspected as the cause of the observed low BEs. If one assumes that a generator delivered for use on Monday has not been eluted for up to 96 hr, then, as Lamson et al. (*3*) have recently shown, approximately 95% of the technetium contained in the initial eluate will be ^{99}Tc carrier. The effects of such a high proportion of carrier ^{99}Tc can readily be shown in any system requiring the reduction of ^{99m}Tc in the presence of a limited amount of reducing agent (*4*). We estimate that in using the initial eluate of a 500-mCi generator, up to 10^{16} ^{99}Tc atoms could be added to a ^{99m}Tc-HSA kit. As Fig. 1 shows, a BE of only 50.8% would then be expected. In this situation much of the reducing capacity of the zirconium ions is wasted on ^{99}Tc atoms, and to this extent zirconium is not available for reducing the ^{99m}Tc. Each milligram of zirconium appears to be capable of reducing about 3×10^{15} technetium atoms.

Our observations suggest that when the manufacturer's instructions for the preparation of ^{99m}Tc-HSA are followed, no more than 10^{15} technetium atoms (both ^{99}Tc and ^{99m}Tc) should be added to any kit. Binding efficiencies in excess of 90% can be anticipated if this recommendation is followed. The following formula can be utilized to calculate the volume of eluate (V_{max}) that will contain a total of 10^{15} technetium atoms:

$$V_{max} = \frac{10^{15}\,(V)}{(A_0 - A_t)(1.282 \times 10^{16})},$$

where V is the total volume (ml) of eluate; A_0 is the ^{99}Mo activity (Ci) at the time of last elution; A_t is the ^{99}Mo activity (Ci) at the time of present elution; and 1.282×10^{16} is the number of technetium atoms formed per curie of ^{99}Mo decay. Of course, V_{max} should never exceed 7 ml, the maximum volume of eluate compatible with the design of the ^{99m}Tc-HSA kit (New England Nuclear Corp.).

CONCLUSIONS

Four solutions to the observed ^{99}Tc carrier problem are suggested, all of which limit the amount of ^{99}Tc added to a kit:

1. When preparing ^{99m}Tc-HSA, avoid using the initial eluate from ^{99}Mo–^{99m}Tc generators.

2. If the initial eluate is used, the maximum recommended volume (V_{max}) should be calculated. For a 500-mCi generator with an initial eluate of 1,000 mCi/20.0 ml, V_{max} would be only 0.69 ml or 34.5 mCi, assuming that 92 hr had elapsed since the manufacturer last eluted the generator. The calculated V_{max} would be exceeded if the maximum of 100 mCi of ^{99m}Tc-pertechnetate, suggested on the package insert, were used. A ^{99m}Tc-HSA kit prepared 6 hr after elution with 100 mCi of this eluate would exhibit a BE below 60% (Fig. 1, 5.8×10^{15} ^{99}Tc atoms).

3. Pre-elute the generator to remove the accumulated ^{99}Tc atoms.

4. Electrolyze the ^{99m}Tc-HSA kit for 80 sec, which will obviate the carrier problem under the worst possible conditions. Note, however, that the possibility of colloid formation could limit this approach.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Jack Hill and Jeri McBride.

REFERENCES

1. DWORKIN HJ, GUTKOWSKI RF: Rapid closed-system production of ^{99m}Tc-albumin using electrolysis. *J Nucl Med* 12: 562–565, 1971

2. STEIGMAN J, ECKELMAN WC, MEINKEN G, et al.: The chemistry of technetium labeling of radiopharmaceuticals by electrolysis. *J Nucl Med* 15: 75–80, 1974

3. LAMSON ML, KIRSCHNER AS, HOTTE CE, et al.: Generator-produced $^{99m}TcO_4^-$: Carrier free? *J Nucl Med* 16: 639–641, 1975

4. SMITH TD, STEIMERS JR, RICHARDS P: Chemical effect of Tc-99 on Tc-99m labeled radiopharmaceuticals. *J Nucl Med* 16: 570–571, 1975