IN VITRO NUCLEAR MEDICINE

Tc-99m Human Serum Albumin: A Suitable Agent for Plasma Volume Measurements in Man

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In 16 patients we have carried out simultaneous plasma-volume measurements with human serum albumins tagged with Tc-99m (Tc-99m HSA) and I-131 (I-131 HSA). The correlation coefficient was 0.987. Tc-99m HSA, prepared from kits that predictably yield high labeling efficiency (and thereby negligible amounts of TcO_4^-), is clearly a superior agent for repeat plasma volume determinations, because of its shorter half-life and the reduced radiation dose to the subject.

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For many years, radioiodinated albumin has been the established radiopharmaceutical for measurement of plasma volume (*1*). The physical half-life of I-131 is 8 days and that of I-125 is 60 days. There are a number of clinical situations in which it would be desirable to make repeated serial measurements of plasma volume (*2–5*). A gamma-emitting nuclide with short half-life would be much more useful and practical in these situations (*1*).

Recently it has been possible to prepare Tc-99m HSA by a simple and reproducible method with a consistently high labeling efficiency, and therefore with less free pertechnetate. This study was undertaken to evaluate this Tc-99m HSA as a tracer for plasma-volume determinations, using I-131 HSA as a reference.

MATERIALS AND METHODS

Seven patients with documented alcoholic liver disease and portal hypertension, and nine patients following coronary artery bypass surgery were studied*.

Plasma volumes were determined after the simultaneous injection of I-131 human serum albumin† and Tc-99m human serum albumin‡. The latter kit consists of a reaction vial containing a lyophilized mixture of 21 mg HSA and 0.23 mg stannous tartrate, with hydrochloric acid added for pH adjustment. Each kit vial of Tc-99m HSA was freshly prepared within 1 hr before use in this study. A total of 24 μCi I-131 HSA and 100 μCi Tc-99m HSA were well mixed in a total volume of 24 ml sterile saline. Ten milliters were injected into each patient and the remainder was used for preparation of the standard, made by diluting 10 ml of the original solution with distilled water to a volume of 500 ml. Precisely timed heparinized blood samples were taken at 10, 20, and 30 min. One ml of plasma from each sample, as well as 1 ml of diluted standard solution, were counted in a NaI(Tl) well using a dual-channel spectrometer set at 135–145 keV and 320–400 keV. The results were plotted on semilog paper with time on the linear scale and counts/minute on the log scale, and the best-fit straight line was extrapolated to time zero. Extrapolation was performed to correct for leakage of albumin from the vascular space. The extrapolated value was used to calculate the plasma volume, from the following formula:

$$\text{Plasma volume} = \frac{\text{cpm of 1 ml standard}}{\text{cpm of 1 ml patient plasma}} \times \text{dilution factor.}$$

Spillover of I-131 counts into the Tc-99m channel was found to be negligible (less than 2%) and was

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TABLE 1. PLASMA VOLUME MEASUREMENTS USING Tc-99m HSA AND I-131 HSA

Patient No.	Tc-99m HSA (ml/kg)	I-131 HSA (ml/kg)	Percentage difference	Percentage bound Tc-99m HSA by TLC
1	36.4	36.1	+0.8	98.9
2	52.1	53.7	−3.0	98.9
3	47.4	44.1	+7.5	99.0
4	64.9	66.7	−2.7	99.8
5	42.7	43.4	−1.6	99.8
6	26.8	27.4	−2.2	99.1
7	66.5	68.6	−3.1	99.0
8	44.5	48.1	−7.5	98.4
9	38.7	41.5	−6.7	97.0
10	40.8	38.2	+6.8	97.8
11	51.2	54.2	−5.5	97.0
12	37.6	38.2	−1.6	98.7
13	65.9	63.5	+3.8	96.5
14	57.0	55.4	+2.9	98.0
15	45.4	43.9	+3.4	99.4
16	75.0	74.1	+1.2	99.4
Mean ± s.d.	49.6 ± 13.2	49.8 ± 13.2	3.8 ± 2.3	98.6 ± 1.0

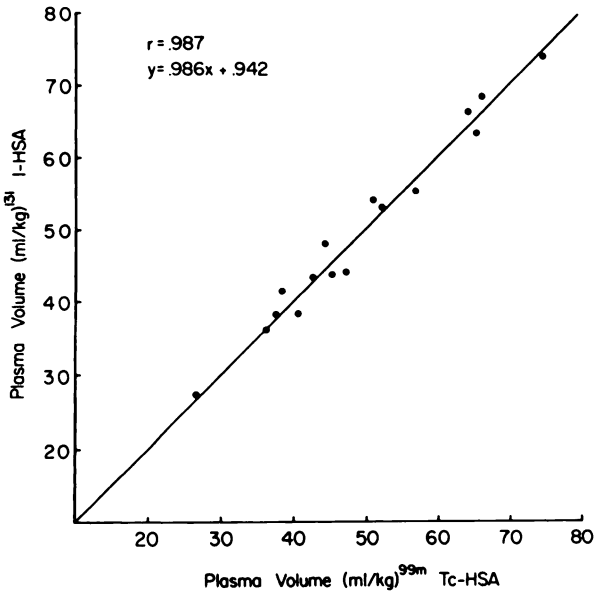

FIG. 1. Comparison of plasma volumes measured simultaneously in the same patients with I-131 HSA and Tc-99m HSA.

therefore not taken into account in the calculations.

Thin-layer chromatography was performed on all preparations of Tc-99m HSA, using 10-cm|| silica gel plates and with acetone as the developing solvent. After chromatography was complete, the plates were dried and cut into 1-cm pieces, which were counted in the gamma counter at 135–145 keV. Bound Tc-99m HSA was found at the origin, with free pertechnetate at the solvent front.

RESULTS

The results are presented in Table 1 and Figs. 1 and 2. The mean plasma volume per kilogram of body weight measured with Tc-99m HSA was 49.6 ± 13.2 ml/kg, compared with 49.8 ± 13.2 ml/kg as determined with I-131 HSA. The correlation coefficient was 0.987 (Fig. 1). The mean percentage difference in plasma volume between the two (using I-131 HSA as the reference) was 3.8 ± 2.3%. The two agents gave comparable results over the entire range of plasma volumes present in these 16 patients (26.8 to 75.0 ml/kg) despite the fact that the Tc-

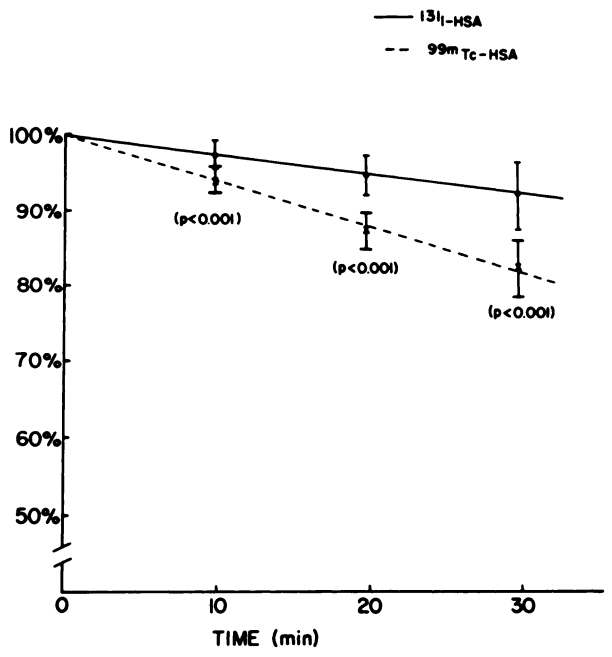

FIG. 2. Rates of loss of Tc-99m HSA and I-131 HSA labels from circulation of patients. In each instance extrapolated (t_0) value was normalized to 100% to permit this interpatient analysis of these two radiopharmaceuticals. Actual recorded counts at 10, 20, and 30 min were expressed relative to t_0 100% value. Shown are mean ± 1 s.d.

99m left the circulation somewhat faster than did the I-131 (Fig. 2). In addition to the kits used in this study, serial chromatographic studies of different batches prepared from three lots of Tc-99m HSA over a 9-mo period showed that the amount of Tc-99m-tagged HSA was never less than 96.5%, with a mean of 98.6 ± 1.0% (n = 31).

DISCUSSION

For plasma-volume measurements, many tracers other than radiolabeled albumin have previously been evaluated. In 1934 Culbertson used homologous anticrystallized egg albumin; Gregerseren (1935) introduced Evans blue dye (dye T-1824); and Rodman (1956) used I-131-labeled globulin (*6–9*). Radiolabeled macroglobulins give smaller estimates of plasma volume, presumably because they do not leave the vascular space at the same rate as albumin (*1*). However, globulins are easily heat-damaged, whereas albumin can be heat-treated to minimize the risks of transmission of serum hepatitis (*1*). Hosain et al. (1969) (*10*) compared both Tc-99m ($t_{1/2}$ = 6 hr, 140 keV) and In-113m ($t_{1/2}$ = 1.7 hr, 393 keV) as short-lived protein labels for the measurement of plasma volume. The results indicated that labeling Tc-99m HSA by the method then in use was tedious and that both tracers overestimated plasma volume by an average of 5%. In-113m chloride, citrate, and transferrin have been evaluated by several investigators and found to result consistently in overestimation of plasma volumes as compared with radioiodinated proteins (*1,11–13*).

Scheffel et al. (unpublished study) had previously compared Tc-99m HSA prepared by a different method and I-125 HSA for the measurement of plasma volume in 27 subjects. The amount of free pertechnetate occasionally varied in an unpredictable fashion. As would be expected, the higher the amount of free TcO_4^-, the faster the Tc-99m label left the circulation, and the greater the overestimation of the plasma volume.

Ongoing quality control has shown that the present kits allow rapid preparation of Tc-99m HSA with consistently less than 4% free TcO_4^- present. The studies have also demonstrated that these two radionuclides do disappear from the vascular compartment at different rates. As shown in Fig. 2, at 30 min after the injection, the mean residual activity of Tc-99m was only about 82.2 ± 4.1% of that obtained by zero-time extrapolation, whereas 91.9 ± 4.7% of I-131 HSA was still retained within the vascular space at 30 min. The data presented in Fig. 2 reemphasize the point made by many previous authors (*1,11*), namely the importance of obtaining multiple postinjection samples for accurate zero-time extrapolation in order to correct for the leakage of labeled albumin from the vascular compartment.

The radiation dose to the critical organ (thyroid) with the use of I-131 HSA is enormously higher (approx. × 190) than that associated with Tc-99m HSA. The radiation dose with the more frequently used I-125 HSA is also higher by at least a factor of 45 (Table 2) (*14*). Because of its shorter half-life, Tc-99m HSA will be especially useful in repeated measurements of plasma volume in individual patients.

FOOTNOTES

* Written informed consent conforming to current NIH guidelines was obtained from each subject.

† Mallinckrodt Nuclear, St. Louis, Mo.

‡ Union Carbide Corp., Rye, N.Y.

|| Eastman 13181.

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TABLE 2. RADIATION DOSIMETRY OF Tc-99m-, I-131-, AND I-125 HSA

	Dose (mrad/μCi)			Dose (mrad/examination)		
	Tc-99m HSA	I-131 HSA	I-125 HSA	Tc-99m HSA (40 μCi)	(10 μCi) I-125 HSA	I-131 HSA (10 μCi)
Blood (whole body)	<0.02	1.37	0.41	0.67	13.69	4.13
Bone marrow	0.02	1.40	0.58	0.97	14.07	5.77
Gonads (ovaries)	0.02	1.37	0.37	0.80	13.74	3.74
Thyroid	0.40	306.61	73.66	16.17	3,066.09	736.63

1. Assumes the effective half-life of Tc-99m HSA = 5.9 hr; I-131 HSA = 96.2 hr; and I-125 HSA = 169.4 hr.
2. Assumes the fraction of dose delivered to blood is about 98% from Tc-99m HSA and 90% from I-131- or I-125 HSA.
3. Assumes thyroidal uptake is not blocked and the fraction of dose delivered to the thyroid gland is approximately 2% from Tc-99m HSA and 10% from I-131- or I-125 HSA.

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