Blood Clearance Rates of Technetium-99m Albumin Preparations: Concise Communication

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Technetium-labeled human serum albumin (HSA) is extensively used as a cardiac imaging agent. An evaluation of the blood-clearance rates of electrolytically reduced HSA (EHSA) and four stannous-reduced HSA (SnHSA) preparations was conducted in dogs, and was compared with that of radioiodinated HSA (IHSA). The EHSA was found to have a clearance rate only about 1.5 times that of IHSA, whereas the SnHSA agents were cleared at two to five times the rate of IHSA. Thus, EHSA has definite advantages over SnHSA preparations for the purposes of blood-volume determinations required in quantitative cardiac studies and for the reduction of extravascular background in the accurate delineation of cardiac boundaries.

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The estimation of cardiac output by the Stewart-Hamilton principle (external monitoring of the first cardiac passage of a radiolabeled blood component) requires determination of the blood volume (*1*). Results using radioiodinated human serum albumin (HSA) have correlated well with those of other techniques (*2–4*). Furthermore, determination of ejection fraction under equilibrium conditions optimally requires that the tracer employed remain within the circulation blood volume and must not diffuse significantly during the procedure. HSA preparations labeled with iodine-131 and iodine-125 are not satisfactory radiopharmaceuticals for use with the modern imaging methods that permit delineation of individual cardiac chambers, and thus Tc-99m HSA preparations have been used in cardiac flow studies (*5,6*). These applications require nondiffusible tracers, but some diffusion from the blood does occur with these preparations. Corrections have been applied to the tracer concentrations obtained at various times after injection to compute a zero-time concentration—i.e., the concentration that would have obtained if diffusion had not occurred—from which blood volume may be calculated. Customary corrections usually assume that blood clearance takes the form of a single exponential during the study period.

The present investigation was undertaken to answer, with regard to technetium-labeled HSA as prepared by a number of different methods, the following questions:

1. What are the blood-clearance characteristics of technetium-labeled HSA, as compared with iodinated HSA, during the first 15 min after injection?
2. Are there systematic differences in the disappearance rates of these various HSA preparations, and if so, why?
3. Can such differences, if significant, be reduced?

METHODS

Five different human serum albumin (HSA) preparations were used in this study: iodine-125-labeled human serum albumin (IHSA)*; Tc-99m-labeled, electrolytically reduced human serum albumin (EHSA)†; Tc-99m-labeled, stannous-reduced human serum albumin (SnHSA-NEN)‡; Tc-99m-labeled, stannous-reduced human serum albumin (SnHSA-UC)||; and a Tc-99m-labeled stannous human serum albumin (SnHSA-WBAMC), made here by the method of Dunson (personal communication). This last was compounded as follows: Dilute 5 ml of 25% salt-poor HSA to 40 ml with water for injection, and acidify to pH < 4.5 with

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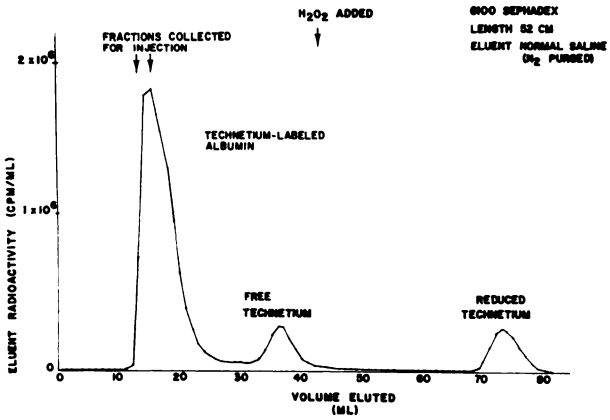

FIG. 1. Elution pattern of a Tc-99m-labeled human serum albumin preparation.

1 *N* HCl. Add this to 25 mg $SnCl_2 \cdot 2H_2O$ in 5 ml HCl solution. Adjust to pH 3.5 with NaOH, sterilize through a Millipore filter®, and divide into 1-ml aliquots. For labeling, add 30–100 mCi [^{99m}Tc] pertechnetate in 1–4 ml. This method is similar to the low-pH procedure of Eckelman et al. (*8*).

Each commercial SnHSA kit was reconstituted according to the manufacturer's instructions in the package insert. Aliquots were taken from each of the five HSA solutions and were subjected to gel-filtration chromatography on a 52-cm G-100 Sephadex® column eluted with 0.9% NaCl immediately before injection. The reduced technetium was released from the column by the addition of dilute H_2O_2 (*8*). The fractions collected between the mid portion of the upslope and the peak of the initial activity curve (Fig. 1) were injected as a "chromatographed" agent. These fractions characteristically followed closely on the void volume.

A catheter was inserted into the jugular vein of a standard beagle and the dog was anesthetized using halothane and oxygen. Animals used were handled in strict adherence to the Guide for the Care and Use of Laboratory Animals, DHEW (NIH)74-23. After obtaining a baseline blood sample before injection, the chromatographed HSA agent was injected through the catheter and the catheter was thoroughly flushed with a rapid saline drip. Blood samples were collected at 5, 10, and 15 min post-injection.

After a 16-min delay, a baseline blood sample was collected and the unchromatographed agent (original solution), with an activity seven to ten times that of the chromatographed agent, was injected. Blood samples were collected at 5, 10, and 15 min post-injection, following which the dog was allowed to recover. The blood samples were centrifuged for 10 min at 2400 RPM; 1-ml aliquots of plasma were removed for counting.

This procedure was repeated on four standard beagles, each of which received all five of the HSA agent-pairs (chromatographed and unchromatographed materials) during the course of approximately 2 mo. At least 1 wk elapsed between two successive experiments on a particular dog in order to allow full recovery from the effects of the procedure.

For the four dogs, and for each radiopharmaceutical, the equation of the line of least-squares fit was determined through the natural logarithm of plasma concentrations obtained at 5, 10, and 15 min. The coefficient of the independent variable is the slope of the line and is the clearance rate of the albumin product from the blood.

Results from the different HSA agents were compared using means and ranges.

TABLE 1.

(A) BLOOD-CLEARANCE RATES OF VARIOUS UNCHROMATOGRAPHED HSA AGENTS (%/MIN)

Dog No.	IHSA	EHSA	SnHSA-WBAMC	SnHSA-NEN	SnHSA-UC
1	0.9	1.0	1.4	3.3	4.9
2	0.1	0.6	1.9	3.5	4.4
3	0.7	3.6	2.2	0.7	4.5
4	1.9	0.3	2.5	2.0	4.4
Mean	0.9	1.4	2.0	2.4	4.6
Minimum	0.1	0.3	1.4	0.7	4.4
Maximum	1.9	3.6	2.5	3.5	4.9

(B) BLOOD-CLEARANCE RATES OF VARIOUS CHROMATOGRAPHED HSA AGENTS (%/MIN)

Dog No.	IHSA	EHSA	SnHSA-WBAMC	SnHSA-NEN	SnHSA-UC
1	0.5	0.1	0.6	6.6	7.1
2	0.0	0.2	0.6	4.7	6.2
3	0.0	0.2	1.6	3.6	7.8
4	0.1	0.2	1.5	6.0	5.3
Mean	0.1	0.2	1.1	5.2	6.6
Minimum	0.0	0.1	0.6	3.6	5.3
Maximum	0.5	0.2	1.6	6.6	7.8

RESULTS

Table 1A shows the clearance rates, considered as single exponentials, for each of the unchromatographed products for times between 5 and 15 min. The clearance rates are tabulated in ascending order, though not in the order in which the agents were administered. The IHSA clearance rate was slowest (0.9%/min) and EHSA clearance rates were only slightly faster (1.4%/min). The others follow in the order listed, with SnHSA-UC most rapid (4.6%).

The blood-clearance rates for the chromatographed fractions are given in Table 1B. Note that IHSA, EHSA, and SnHSA-WBAMC all exhibited improvement, whereas the other two products became worse. The clearance rates of IHSA and EHSA were very low, that of SnHSA-WBAMC was somewhat higher, and clearance rates of the two commercial stannous-reduced agents were much higher.

The gel-filtration procedure had a moderate effect in reducing IHSA or EHSA clearance rates (Table 2), and improvement was noted in each of the animals tested with each agent. The SnHSA-WBAMC clearance rate was also improved, but the SnHSA-NEN and SnHSA-UC rates were significantly degraded. Table 3 shows the mean values of the bound, unbound, and reduced fractions of each of the HSA agents as determined by gel-filtration chromatography.

Immediately before the second injection (the unchromatographed agent) a baseline blood sample was drawn, with a total elapsed time of 31 min from the first injection (chromatographed agent). The regression equations based on the 5-, 10-, and 15-min data predicted the blood concentrations at 31 min postinjection in excellent agreement with the 31-min baseline blood samples, thus supporting the use of a single exponential to describe clearance rates in the time range 5–30 min postinjection.

TABLE 2. COMPARISON OF DIFFERENCES BETWEEN UNCHROMATOGRAPHED AND CHROMATOGRAPHED HSA AGENTS

	HSA Agents				
Dog No.	IHSA	EHSA	SnHSA-WBAMC	SnHSA-NEN	SnHSA-UC
1	—0.4	—0.9	—0.8	3.3	2.2
2	—0.1	—0.4	—1.3	1.2	1.8
3	—0.7	—3.4	—0.6	2.9	3.3
4	—1.8	—0.1	—1.0	4.0	0.9
Mean difference	—0.8	—1.2	—0.9	2.8	2.0
Minimum	—1.8	—3.4	—1.3	1.2	0.9
Maximum	—.1	—.1	—.6	4.0	3.3

TABLE 3. MEAN VALUES OF THE BOUND, FREE, AND REDUCED FRACTIONS AS DETERMINED BY GEL-FILTRATION CHROMATOGRAPHY

Product	Percentage bound	Percentage free	Percentage reduced
IHSA	97.3	2.7	—
EHSA	95.3	2.3	2.4
SnHSA-WBAMC	96.6	1.7	1.7
SnHSA-NEN	94.0	1.0	5.0
SnHSA-UC	91.0	5.7	3.3

DISCUSSION

The blood-clearance rates of all five unchromatographed HSA preparations may be described by a single exponential during the period from 5 to 15 min after injection. IHSA showed the slowest clearance rate, whereas the EHSA rate was about 50% faster and that of the SnHSA preparations two to five times as fast. The lowest clearance rate over the period of study is the most desirable, since this minimizes leakage of the tracer into extravascular fluid. Background radiation is thus reduced, which facilitates the outlining of cardiac chambers and blood pools in vascular studies. Furthermore, accuracy in the computation of blood volumes is enhanced, since errors in extrapolation to zero time are reduced. Thus, of the four Tc-99m-labeled HSA products studied, EHSA is by far preferable to the SnHSA preparations. Similar results were noted by Weber et al. (*5*). The clearance rate of the unchromatographed EHSA determined in this study predicts a blood level of 43% at 1 hr, a result in excellent agreement with that found using a similar preparation in man (*9*). The SnHSA-WBAMC, if unchromatographed, performs almost as well as EHSA (Table 1A), but the SnHSA-NEN clears considerably faster. The SnHSA-UC clears most rapidly of all and is thus much less desirable.

Gel filtration of IHSA results in an agent with an almost negligible clearance rate, and similar results are seen with EHSA (Table 1B). Gel filtration of the SnHSA-WBAMC resulted in a product considerably improved in clearance characteristics and almost equalling that of the unchromatographed IHSA.

Peculiarly, degradation in the clearance rates resulted from the gel filtration of the SnHSA-NEN and SnHSA-UC. The degradation was consistent in every animal tested and with both products. One possible explanation may be that the chromatographed agents contained a larger proportion of denatured albumin moieties, in the particular fraction collected, than the unchromatographed agents. Table 1B shows that both of these products are unsatisfactory for use in equilibrium studies.

The differences in blood clearance noted for the unchromatographed agents appear to correlate with the percentage of bound albumin. The IHSA, with the lowest percentage of free plus reduced anion, had the slowest clearance, whereas the clearance rates of the SnHSA preparation increased as the percentage of free plus reduced technetium increased (Table 3). Recently, Meinken et al. showed that

SnHSA preparations may contain unbound reduced technetium, dimers and monomers of albumin, and denatured albumin (*10*). Colloids may also be present (*8*). Thus, several mechanisms may account for the disappearance of the radioactivity of the HSA agents from the blood during the interval studied. Free and reduced anions and albumin fragments might be cleared by diffusion into extravascular spaces and filtration by the kidneys; albumin aggregates and colloids should be removed by phagocytic mechanisms. In the former instance, extravascular diffusion would result in increased background and subsequent cardiac-image degradation, a situation that would not occur in the latter case. In either event, equilibrium concentrations are affected. Thus it appears that altered albumin and unbound technetium moieties resulting from the reduction by tin might be more rapidly cleared from the blood than agents not containing those moieties, and thus is in agreement with our results.

We conclude that EHSA is the most suitable tracer, of those tested, for purposes of imaging blood pools and estimating blood volumes with a technetium-labeled albumin radiopharmaceutical. The SnHSA-WBAMC agent is not quite as satisfactory as EHSA unless subjected to gel filtration, with all of the difficulties and complications (sterility, apyrogenicity, etc.) inherent in the process. The SnHSA-NEN and SnHSA-UC are even less suitable for these applications. If I-123-labeled HSA, with the same characteristics as I-125 HSA, should become available, one would expect to have a radiopharmaceutical combining the advantages of slow blood disappearance rate (for accurate determination of blood volume) and appropriate energy and photon flux for imaging procedures.

We therefore recommend that when blood-volume determinations are required as part of quantitative cardiac-flow studies, the blood-clearance characteristics of the product employed must be carefully considered. Products with the slowest clearance rates are most desirable, and it appears that currently the EHSA has definite advantages over stannous-reduced preparations.

FOOTNOTES

* Mallinckrodt, Inc., St. Louis, Mo.
† Catalog No. NRP-175, New England Nuclear Radiopharmaceutical Div., North Billerica, Mass.
‡ Cardiolite®, New England Nuclear Corp., Boston, Mass.
|| Union Carbide, Tuxedo, N.Y.

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