Experimental Evaluation of Tc-99m Sulfur Colloid as a Potential Imaging Agent in Thromboembolic Disease: Concise Communication

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Based on literature reports suggesting the possible incorporation of Tc-99m sulfur colloid (Tc-SC) into fibrin deposits, this study was undertaken to evaluate the potential of this radiopharmaceutical as an imaging agent in thromboembolic disease. Animal models of deep-vein thrombosis and pulmonary embolism were used. The mean thrombus-to-blood (T/B) uptake ratios were comparable for fresh and older thrombi (up to 72 hr). Thrombus uptake was significantly lower in a group of five control dogs that received pertechnetate instead of Tc-SC. Intravenous heparin administration (5,000 IU) 2 hr before injection of Tc-SC caused a depression in T/B ratios but did not totally block Tc-SC uptake. Gamma imaging with Tc-SC allowed demonstration of deep-vein thrombi, but imaging of pulmonary emboli as areas of increased activity was not satisfactory. This study supports the concept of thrombus detection with radiolabeled particles but not the extension of this principle to the imaging of pulmonary emboli.

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The possible incorporation of Tc-99m sulfur colloid (Tc-SC) into sites of thrombosis has been suggested in several reports (*1–4*). Freeman et al. (*1*) and Helbig (*2*) reported on the accumulation of Tc-SC in clots caused by indwelling venous catheters. George et al. observed increased accumulation of Tc-SC in rejecting renal transplants (*3,4*) and used this radiopharmaceutical as an aid in the differentiation of renal transplant rejection from acute tubular necrosis. Using autoradiographic studies in experimental renal transplants, these authors demonstrated Tc-SC radioactivity to be trapped in areas of intravascular and glomerular fibrin thrombosis (*4*). Klingensmith et al. (*5*) demonstrated increased renal uptake of Tc-SC in experimental endotoxemia and postulated entrapment in fibrin deposits in the renal capillaries as the most likely mechanism.

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Based on these observations suggesting the possible incorporation of Tc-SC into fibrin deposits, we decided to investigate the potential of Tc-SC as an imaging agent in thromboembolic disease.

METHODS AND MATERIALS

Deep-vein thrombosis. This condition was experimentally induced in beagle dogs under intravenous sodium pentobarbital (30 mg/kg) by alteration of the intima with an electric current. The method induces formation of thrombi that are histologically and pathogenetically similar to human thrombi (*6–8*).

Eleven groups of animals were used in this portion of the study. Ten groups underwent tissue-counting studies and one group underwent scintigraphy as well.

Deep-vein thrombosis was induced in the inferior vena cava (IVC) for the tissue-counting studies. This vessel is easily accessible for placement of an i.v. electrode, which facilitated the performance of the anticipated large number of experiments. A vinyl-coated wire with bare tip, serving as the anode, was passed under fluoroscopic control through a femoral vein into the inferior vena cava. The cathode was connected to a skin clamp

positioned on the abdominal wall at the approximate level of the intravenous electrode. Both electrodes were connected to a current regulator, which in turn was connected to a 12-volt power supply. A direct 5-mA current was applied to the intima of the vessel for 1 hr and the electrodes were then removed. After thrombosis induction, a 2- to 3-mCi (74-111 MBq) dose of Tc-SC was injected intravenously in one rear extremity, and the animal was later killed.

To evaluate the effects of thrombus age on Tc-SC uptake, the tracer was injected in four groups of animals at various time intervals after induction of thrombus formation, and the animals were killed 1 hr after injection (Groups 1-4). To evaluate the persistence of Tc-SC labeling of thrombi, four groups of animals received Tc-SC injections at 24 hr after thrombus induction, and these were killed at various intervals after injection (Groups 2, 5, 6, 7). In all other animals, both the time interval between induction of thrombus formation and the time from injection of tracer to death were kept constant at 24 and 1 hr, respectively. To demonstrate that thrombus uptake was associated with Tc-SC and not with unbound Tc-99m, some animals received pertechnetate instead of Tc-SC (Group 8), also into a hind limb. A group of animals received i.v. heparin (5,000 IU) 2 hr before injection of Tc-SC to determine whether this would inhibit Tc-SC incorporation into the thrombus (Group 9). In another group, injections of Tc-SC were made proximally through a jugular vein instead of a rear extremity to determine whether optimal thrombus uptake of Tc-SC depends on delivery of a compact bolus to the area of thrombosis as opposed to a more gradual delivery through systemic recirculation (Group 10).

For the imaging studies, thrombosis formation was induced in the femoral vein. The IVC model was not suitable for imaging studies because, in the anesthetized beagle, the spleen occupies a considerable portion of the abdominal cavity and overlies the IVC. For these studies, the intravenous electrode was passed caudad through a jugular vein into a femoral vein, and the skin electrode was placed on the ipsilateral extremity. Within 24 hr of thrombus formation, the dogs were injected with 5 mCi (185 MBq) of Tc-SC through a distal vein in the ipsilateral hind limb, and serial imaging was begun immediately; the animals were killed 1 hr after injection.

In all instances, postmortem examination of the area of thrombosis was performed by a veterinary pathologist (GAP). Various tissue samples were collected, weighed, and counted in an NaI(Tl) scintillation counter; these included the entire thrombus, a segment of normal vein, and a blood sample.

Pulmonary emboli. The potential usefulness of Tc-SC for imaging pulmonary emboli as areas of increased activity was evaluated using an experimental model of pulmonary embolism. Autologous experimental pulmonary emboli were produced in seven dogs using a modified Wessler technique (*9*) as described previously by Alderson et al. (*10*). The technique involves (a) isolation of a segment of a jugular vein, (b) injection of 50-100 NIH units of human thrombin into the isolated vein segment, (c) subsequent release of the thrombi thus formed into the lungs, and (d) i.v. injection of india ink before sacrifice to identify embolized areas on gross examination of the resected lungs. After pulmonary embolization, the dogs received a 1- to 3-miCi (37-111 MBq) i.v. dose of Tc-SC, and imaging of the chest was performed serially during 1 hr. At 1 hr after injection, the dogs were killed, the lungs excised and inspected, and dissection of the arterial tree was performed for removal of clots. Samples of lung, clots, and blood were obtained, weighed, and counted on a scintillation counter. Tissue distribution data were also obtained from seven control dogs.

Imaging of the lungs and sites of deep-vein thromboses was performed using a standard-size gamma camera fitted with a low-energy, all-purpose, parallel-hole collimator, or a large-field camera fitted with a converging collimator. Statistical comparisons among groups of data were made using the Kruskal-Wallis Rank Sums (*11*) or Student's t-test. Quality control of the radiopharmaceutical was obtained by thin layer chromatography.

RESULTS

Deep-vein thrombosis. Results of tissue-counting

TABLE 1. THROMBUS UPTAKE OF Tc-SC AS A FUNCTION OF THROMBUS AGE

Group	Thrombus age at time of Tc-SC injection (hr)	Time from injection to sacrifice (hr)	N	Weight of thrombi (mg)*	Thrombus-to-blood ratio*
1	4	1	6	251 ± 80	23.84 ± 10.43
2	24	1	12	129 ± 21	11.38 ± 2.33
3	48	1	7	65 ± 62	14.79 ± 6.31
4	72	1	7	44 ± 40	16.46 ± 5.70

* $\bar{X}$ ± s.e.m.

TABLE 2. THROMBUS UPTAKE AS A FUNCTION OF TIME AFTER INJECTION OF Tc-SC

Group	Thrombus age at time of Tc-SC injection (hr)	Time from injection to sacrifice (hr)	N	Thrombus-to-blood ratio*
2	24	1	12	11.38 ± 2.33
5	24	2	6	12.0 ± 2.37
6	24	4	5	6.92 ± 1.53
7	24	6	5	5.14 ± 0.60

$\bar{X}$ ± s.e.m.

studies are primarily expressed using thrombus-to-blood (T/B) ratios as an indication of target-to-background ratios. Table 1 presents the relationship between the thrombus age, thrombus size, and the T/B ratio (cpm/g of thrombus:cpm/g of blood). There was a wide spectrum of thrombus sizes, but only a few of them (6%) resulted in total occlusion of the vessel lumen. The T/B ratios are fairly high in all groups. Although the mean T/B ratio is higher for the group injected at 4 hr after induction of thrombus formation, there is considerable variation around the mean, and the differences among groups are not found to be statistically significant. Linear regression analysis of thrombus weight against T/B ratio was done using the data summarized in Table 1, and no correlation was found between them ($r = 0.003$, $n = 32$). The activity per unit weight in the thrombi was found to be significantly higher than that found in blood and normal vein samples ($p < 0.01$). The mean percent of injected dose/g in the thrombi of Group 2 dogs was <0.01%.

Table 2 presents the effect on T/B ratio of the time interval between the injection of Tc-SC and the sacrifice of the animal, for clots that were 24 hr old at the time of radiocolloid injection. A decline on the thrombus-to-blood ratio is suggested as the time interval increases beyond 2 hr; at 6 hr, the difference in T/B ratios is significant at the 5% level ($p < 0.05$).

Table 3 summarizes the results of thrombus uptake under different experimental conditions. The control dogs, which received [^{99m}Tc] pertechnetate, had significantly lower T/B ratios ($p < 0.01$) than those given Tc-SC, and the cpm/g in these thrombi were significantly lower than in Group 2 ($p < 0.01$). The mean T/B ratio for the group of dogs receiving proximal injections of Tc-SC was lower than that of the group receiving distal injections ($p < 0.005$). Heparin caused a depression of T/B ratio that was significant at the 5% level ($p = 0.05$).

The imaging studies demonstrated the feasibility of locating deep-vein thrombi by scintigraphy with Tc-SC. The results of these studies are summarized in Table 4, with scintigraphic visualization of clots rated on a scale of 0–4, based on intensity of clot uptake relative to surrounding background activity. The T/B ratios in the femoral thrombi were generally higher ($\bar{X}$ = 56.40 ± 22.70 s.e.m.) than in those formed on the IVC, a reflection of hemodynamic differences among those ves-

TABLE 3. THROMBUS UPTAKE OF Tc-SC IN DIFFERENT EXPERIMENTAL CONDITIONS*

Group	Experimental condition	N	Thrombus-to-blood ratio ($\bar{X}$ ± s.e.m.)
2	Tc-SC injected in a hind extremity†	12	11.38 ± 2.33
8	$^{99m}TcO_4^-$ given instead of Tc-SC	5	1.35 ± 0.24
9	Heparin given before Tc-SC	6	4.90 ± 1.27
10	Proximal injection of Tc-SC	5	3.3 ± 1.07

* All thrombi were 24 hr old at time of tracer injection; 1 hr between injection and sacrifice.

† This group also included in Tables 1 and 2.

TABLE 4. RESULTS OF COUNTING AND IMAGING STUDIES IN FEMORAL VEIN THROMBI*

Dog no.	Weight of thrombi (mg)	Thrombus-to-blood ratio	Scintigraphic score
1	58	53.6	3+
2	82	6.6	0
3	115	4.6	2+
4	80	33.8	4+
5	361	33.1	4+
6	98	16.5	0
7	112	72.6	0
8	23	35.9	1+
9	509	56.2	4+
10	175	251.1	4+

* Imaging begun immediately after administration of Tc-99m sulfur colloid.

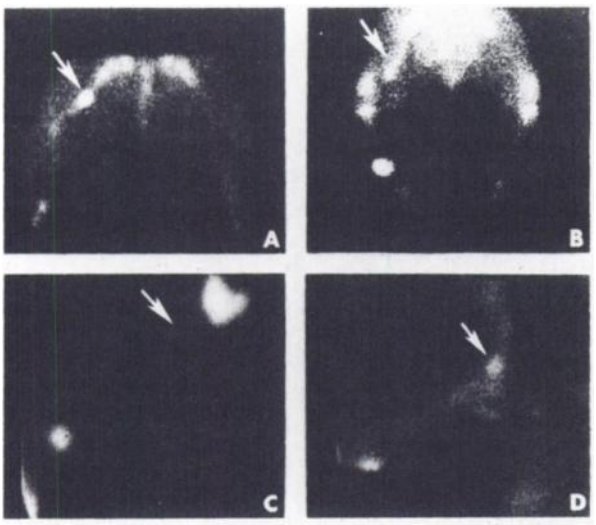

FIG. 1. Anterior scintigrams of three dogs with experimental deep-vein thrombi obtained after i.v. injection of Tc-SC. Arrows point to sites of thrombi. Focal activity in lower left of each panel indicates injection site. Images in upper panels [4+ (A) and 3+ (B)] were obtained with parallel-hole collimator. Images in lower panels were obtained with converging collimator in a single animal with clot in high ileofemoral area [2+ (D)]. Because of considerable caudal extension of spleen, lead shield was placed over its caudal part to enhance visualization of clot (D).

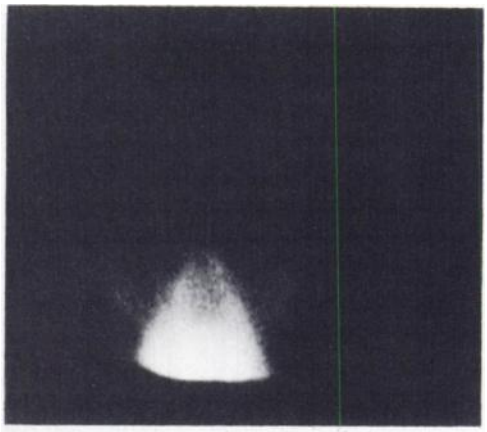

FIG. 2. Anterior scintigram of chest in dog with multiple experimental pulmonary emboli obtained after i.v. injection of Tc-SC. Faint outine of lungs is seen; emboli are not demonstrated as areas of increased activity. Liver activity obscures basal pulmonary regions.

sels. Figure 1 presents examples of scintigraphically demonstrated deep-vein thrombi. Clots were not located scintigraphically in three of ten animals; in one of the three (No. 7), the thrombus was located in the high ileofemoral region and was obscured by bone-marrow activity in the sacroiliac area.

Pulmonary emboli. A total of 21 clots were recovered from the lungs of the seven dogs with experimental emboli. Only one embolus was demonstrated in vivo by scintigraphic imaging (a large occlusive embolus in the apex of one lung). Figure 2 shows an uninformative scintigraphic image of a dog with experimental emboli. Results of tissue-counting studies in these and control animals are presented in Table 5. Animals with pulmonary emboli exhibited significantly higher lung-to-blood ratios ($p < 0.001$) and lung-to-liver ratios ($p < 0.01$) than the controls. This was not related to an increase in the amount of free TC-99m in lung, since there was no significant difference in the urine-to-blood ratios of the two groups ($p > 0.20$). There also was a poor correlation between the lung-to-blood and urine-to-blood ratios ($r = 0.37$) in the group of animals with emboli.

DISCUSSION

Results of this study extend our earlier observations concerning the hypothesis of Tc-SC as a potential radiopharmaceutical for clinical use in the detection of thrombosis (*12*). The T/B ratios found are of sufficient magnitude for external thrombus detection. The observation of high T/B ratios in fresh clots and older clots, as well as the lack of correlation between thrombus weight and uptake of Tc-SC, suggests that Tc-SC may be useful in detecting thrombi at different stages in their evolution. This would be an advantage over other labeled agents such as iodinated fibrinogen. In a study of the effect of thrombus age on the uptake of I-125-labeled fibrinogen, Coleman et al. (*13*) reported that the highest T/B ratios were obtained when fibrinogen was injected at 4 hr after thrombosis induction. They also observed that the T/B ratios were lower when fibrinogen was injected later after induction of thrombus formation. When fibrinogen was injected at 24 hr, the T/B ratios were lower (mean = 4.6) than the ratios observed with Tc-SC in the present study (mean = 11.4).

A decrease in T/B ratio with time after injection of Tc-SC was suggested by the results in Table 2. Although the observation was not highly significant statistically, some release of activity may indeed take place. Therefore, an injection-to-imaging time of up to 2 hr seems to be optimal.

Depression of thrombus accumulation of Tc-SC seen in animals given heparin, although of marginal statistical

TABLE 5. UPTAKE OF Tc-SC IN THE LUNGS

	N*	Uptake ratios ($\bar{X} \pm$ s.e.m.) Tissue-to blood	Lung-to liver
Animals with emboli			
Emboli	7	24.7 ± 6.8	—
Perfused lung	7	15.8 ± 2.4	0.15 ± 0.03
Lung from controls	7	3.2 ± 0.6	0.071 ± 0.009

* N = number of animals; multiple samples obtained from each animal.

significance, suggests that the mechanism of thrombus uptake of Tc-SC is related, to some extent, to the presence of fibrin deposits. This concept is supported by the observation made by George et al. (*14*) concerning instances of acute renal rejection without uptake of Tc-SC in patients on high-dose heparin therapy. However, thrombus uptake, although lower with heparin, is not totally blocked by it. This suggests that other mechanisms for thrombus uptake of Tc-SC must be present, such as (a) electrostatic attraction, since radiocolloids are charged particles; or (b) adhesion, a biophysical characteristic that platelets display irrespective of their ability to initiate clot formation, and postulated as the most satisfactory explanation of the thrombus uptake of labeled albumin particles (*15*). This concept is supported by the observation of low T/B ratios with proximal injections compared with injections distal to the site of thrombosis. Therefore, uptake of Tc-SC may occur through biophysical interaction with the clot surface, aided by the presence of a fibrin mesh in the periphery of the clot as described by Webber (*15*).

Results of the imaging studies using a model of femoral-vein thrombosis demonstrated the feasibility of locating deep-vein thrombi with Tc-SC. The series is relatively small to attempt drawing strong conclusions, but several observations seem in order. There are two primary limitations to the use of Tc-SC for thrombo-scintigraphy. (1) Uptake in bone marrow may obscure clots with low-intensity uptake. This may be overcome in the lower extremities by the use of lateral and oblique views, but in the pelvis it represents a more formidable limitation, which may be compounded by the presence of bladder activity. (2) Although T/B ratios are generally high (in part due to avid reticuloendothelial extraction of the colloid), total activity in the clots is small and counting rates are consequently low. Despite those limitations, a detection rate of 70% was observed in this study. To the extent that these experimental observations can be extrapolated to the clinical situation with humans, it is felt that radiolabeled particles represent a viable option in the diagnostic problem of thrombosis detection. Technetium-99m sulfur colloid might not, however, be an optimum choice of radiolabeled particle. Further comparative studies are needed to define optimal parameters concerning the relationship between physicochemical constitution (particle size, charge, shape, etc.) and biologic behavior.

Extension of the principle of thrombus uptake of Tc-SC to the imaging of pulmonary emboli as areas of increased activity did not prove satisfactory in this study. Even though, on a per-gram basis, the T/B ratios in pulmonary emboli are fairly high, the emboli are apparently obscured by the uptake of Tc-SC in the surrounding normal lung, containing many times the activity of a clot. Lung uptake was surprisingly high in the animals with experimental pulmonary emboli, and this observation was highly significant statistically. The poor correlation between urinary and lung activity suggests that the increased lung activity reflects an actual increase in Tc-SC uptake rather than in free Tc-99m. Increased lung uptake of Tc-SC has been reported in association with different clinical entities (*16–18*), but we have not found reports of its association with pulmonary embolism. We conjecture that pulmonary vascular occlusion may lead to a prolonged residence time of the radiocolloid through the lungs, making more of it available for uptake by the pulmonary reticuloendothelial cells in a manner similar to the increased splenic uptake seen in portal hypertension.

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