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LABELING AND TESTING OF ^{99m}Tc-STREPTOKINASE FOR THE DIAGNOSIS OF DEEP VEIN THROMBOSIS

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A detailed study has been made of the method for labeling streptokinase with ^{99m}Tc in order to get a radioactive indicator for rapid scintigraphic visualization of thrombi and emboli. The best method found for preparing ^{99m}Tc-streptokinase was by reducing ^{99m}Tc-pertechnetate with 2 μmole $SnCl_2$ at pH 0.7 and then adding 50,000–75,000 IU of streptokinase at a final pH of about 2. After 1 hr of equilibration the labeling efficiency was 75–80% as determined by the method of gel chromatography column scanning. The dynamic behavior and distribution of ^{99m}Tc-streptokinase in different organs was studied in rabbits where a high uptake was found in liver. In a clinical investigation using ^{99m}Tc-streptokinase prepared according to this work, however, the liver uptake in man was only 10–20%.

The best agent discovered thus far for locating deep vein thrombosis is ^{125}I-labeled fibrinogen. This technique, however, requires an enlarging clot and the accumulation in the thrombus must be followed daily during several days (*1,2*). The risk of transmitting serum hepatitis has also been considered, and in the United States the use of labeled fibrinogen in routine clinical use is prohibited (*3*). Many investigators have therefore attempted to visualize thrombi by using other radioactive indicators. Methods of venography using ^{99m}Tc-pertechnetate and labeled albumin macroaggregates and microspheres have recently proved to be useful techniques but require the availability of a scintillation camera (*3–5*). Various clot-lysing systems like labeled plasmin or thrombolytic agents such as urokinase and streptokinase have been used with varying success for the external imaging of both thrombi and emboli (*3,6–10*).

One of the most promising agents for visualizing thrombi and emboli is ^{99m}Tc-labeled streptokinase. This substance, however, is difficult to label to a high yield with ^{99m}Tc (*11*). In this work, the labeling of streptokinase with ^{99m}Tc has been studied in detail in order to get a labeling procedure that results in a reproducible high labeling yield. This preparation has also been tested for clinical use (*12*).

MATERIALS AND METHODS

Streptokinase was supplied by AB KABI (Stockholm, Sweden) in a dry ampule containing 600,000 IU of streptokinase (about 50,000 MW) and 27.5 mg of phosphate buffer (23 mg $NaH_2PO_4 \cdot H_2O$, 4.5 mg $Na_2HPO_4 \cdot 2H_2O$). The labeling technique was in principle based on a reduction of ^{99m}Tc-pertechnetate with stannous tin, $SnCl_2 \cdot 2H_2O$ (Merck AG, Darmstadt, Germany) with streptokinase present or incorporated by adding the reduced technetium to a solution of streptokinase.

The method of gel chromatography scanning (GCS) was used for analyzing the influence of pH, $SnCl_2$-concentration, streptokinase concentration, and time on the labeling yield (*13–15*). A 0.1-ml aliquot of the ^{99m}Tc-streptokinase preparation in question was applied at the top of a gel chromatography column followed by 15.0 ml of 0.9% NaCl/HCl buffer with the same pH as the sample. The columns, which had an inner diameter of 15 mm, were filled to a height of 30 cm with Sephadex® G-25-fine (Pharmacia Fine Chemicals AB, Uppsala, Sweden), previously saturated with the NaCl/HCl buffer in question. The column was sealed and scanned with a slit (1 mm) collimated NaI(Tl) crystal. The GCS profiles of preparations at pH 2 with different $SnCl_2$ concentrations thus obtained are shown in Fig. 1. The peak with a maximum at 23 cm in the GCS profiles indicates the presence of ^{99m}Tc-streptokinase. The peak at 5 cm is the ^{99m}Tc-pertechnetate and between these two peaks ^{99m}Tc-labeled phosphates of various molecular weights are present (*14,15*).

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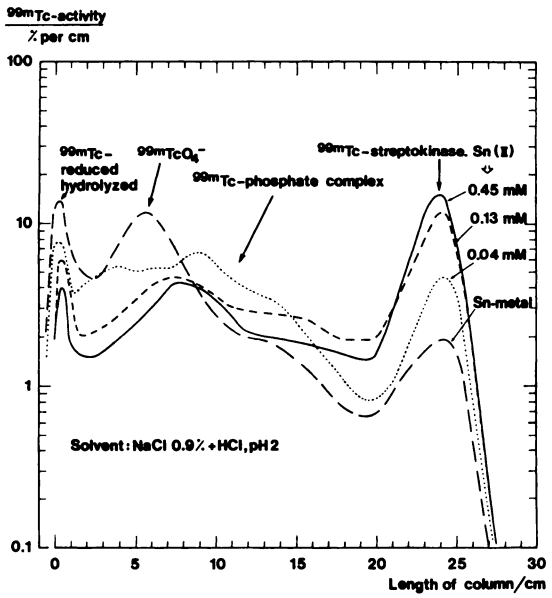

FIG. 1. Gel chromatography column-scanning profiles obtained from labeling streptokinase with ^{99m}Tc at pH 2 with various Sn(II) concentrations.

The in vivo dynamic behavior of the ^{99m}Tc-streptokinase and distribution in different organs was studied in rabbits lying supine under a scintillation camera.

Sequential scintigrams were registered every 5 sec after intravenous injection of the ^{99m}Tc-labeled compound. The information thus obtained was stored on magnetic tape and different regions of interest were later given detailed analysis on a computer (*16*). The uptake and elimination of the ^{99m}Tc activity were studied with special attention to the kidneys, liver, heart (blood), and bladder.

RESULTS AND DISCUSSION

The fraction of ^{99m}Tc activity between 20 and 30 cm in the GCS profiles which corresponds to ^{99m}Tc-streptokinase was studied as a function of $SnCl_2$ concentration at various pH values. These experiments were performed with 50,000 IU of streptokinase dissolved in 2–3 ml ^{99m}Tc-pertechnetate and different amounts of $SnCl_2$ were added in the presence of a piece of pure metallic tin. Adjustments of pH were performed with 0.1 *M* NaOH and 0.1 *M* HCl.

Examples of GCS profiles obtained from experiments carried out at pH 2 are given in Fig. 1. Similar experiments that were carried out at pH 1.0, 2.0, 4.0, 5.2, and 7.4 indicate that the labeling yield of ^{99m}Tc-streptokinase decreases at pH values higher than 4 and at pH 7.4, no ^{99m}Tc-streptokinase appears to be present but a broad peak in the GCS profiles at 10–20 cm indicates the presence of ^{99m}Tc-labeled phosphates (*14*). The best labeling yield of ^{99m}Tc-labeled streptokinase was thus obtained at pH values between 1 and 2 and a $SnCl_2$ concentration of about 0.5 m*M*.

The labeling method described by Dugan, et al included the use of a phosphate buffer to raise the pH to about 12 (*6*). We reproduced their method of labeling and with the use of the GCS method we registered a labeling yield of only about 10% for ^{99m}Tc-streptokinase.

The variation of the labeling yield with different amounts of streptokinase was studied by adding a mixture of 2.5 ml ^{99m}Tc-pertechnetate and 0.5 ml $SnCl_2$ 4 m*M* to various amounts of streptokinase at pH 1.6–1.9. After 1 hr of equilibration, samples were analyzed with the GCS method. The fractions of ^{99m}Tc-pertechnetate (4–10 cm), ^{99m}Tc-streptokinase, ^{99m}Tc-phosphate complex, and reduced hydrolyzed ^{99m}Tc (0–3 cm) are displayed in Fig. 2 as a fraction of the amounts of streptokinase used in the preparation. This figure indicates that an amount of about 50,000–75,000 IU of streptokinase gives the best labeling yield.

The temporal variation of the ^{99m}Tc-streptokinase labeling was studied by adding a mixture of 2.5 ml ^{99m}Tc-pertechnetate in 0.9% NaCl and 0.5 ml $SnCl_2$ solution (4 m*M* $SnCl_2$, 0.2 *M* HCl, and 0.12 *M* NaCl) into a vial with 50,000 IU of streptokinase. Samples were taken after different periods of time and analyzed with the GCS method. The fractions of ^{99m}Tc-activity representing ^{99m}Tc-streptokinase, ^{99m}Tc-phosphate complex, ^{99m}Tc-pertechnetate, and reduced hydrolyzed ^{99m}Tc at different times after the addition of the ^{99m}Tc-$SnCl_2$ solution to the streptokinase are shown in Fig. 3. This figure indicates that the labeling process is a rather slow reaction between reduced hydrolyzed ^{99m}Tc and streptokinase with a first order rate constant of about 0.1 min^{-1}.

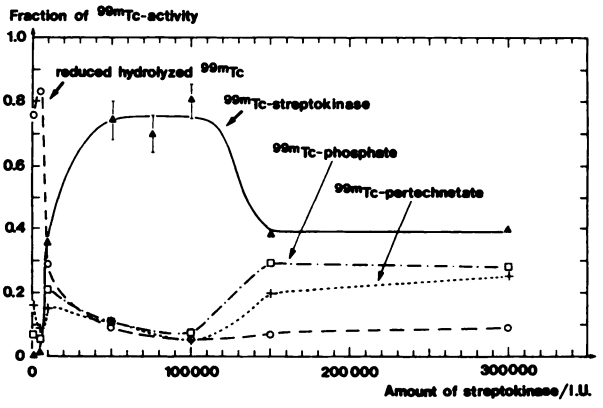

FIG. 2. Fraction of ^{99m}Tc-activity in different zones of gel chromatography column-scanning profile that represents reduced hydrolyzed ^{99m}Tc (top–3 cm), ^{99m}Tc-pertechnetate (4–10 cm), ^{99m}Tc-phosphate complex (10–20 cm), and ^{99m}Tc-streptokinase (20–30 cm) when amount of streptokinase in labeling procedure was varied.

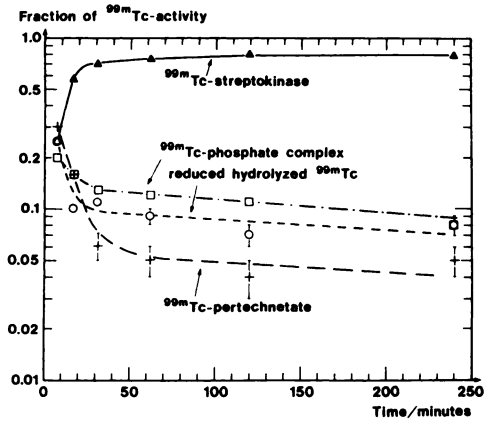

FIG. 3. Fractions of ^{99m}Tc-activity in different zones of gel chromatography scanning profile that represent reduced hydrolyzed ^{99m}Tc (top–3 cm), ^{99m}Tc-pertechnetate (4–10 cm) ^{99m}Tc-phosphate complex (10–20 cm), and ^{99m}Tc-pertechnetate reduced with $SnCl_2$ was added to 50,000 IU of streptokinase at pH 2.

The preparation is thus ready to use, preferably 30 min after preparation. The ^{99m}Tc-streptokinase, however, seems to be stable for at least 4 hr and no increase in the fraction of ^{99m}Tc-pertechnetate was found which would indicate any oxidation of the reduced ^{99m}Tc.

The in vivo dynamic behavior of various preparations of ^{99m}Tc-streptokinase was studied in rabbits after intravenous administration of about 5,000 IU of ^{99m}Tc-labeled streptokinase per kilogram of body weight. The maximum liver uptake and biologic half-times for liver and blood disappearance for these preparations are given in Table 1. The first preparation, Case A, corresponds to the labeling method that we found gave the best labeling yield. The main part (70%) of the administered ^{99m}Tc activity in this case is accumulated in the liver, which is in agreement with the findings of Gross (*8*), who reported uptake in liver after administration of ^{131}I-labeled streptokinase to patients. Only slight differences in the liver uptake and the blood disappearance were registered in Case B where the preparation was purified on a Sephadex G-25 column before administration to the rabbit or in Case C where the rabbit was pretreated with 75,000 IU of streptokinase 18 hr before the experiment. The use of streptokinase containing glutamate instead of phosphate in Case D also resulted in about the same accumulation in the liver. With this type of streptokinase, however, precipitation of tin also occurred very easily. Streptokinase containing phosphate therefore seems to be preferable to streptokinase with glutamate. The last type of preparation is seen in Case E where the pH value was adjusted to pH 7.4 with a phosphate buffer. In this case, no special accumulation took place in the liver but accumulation of ^{99m}Tc activity in the growing zones of the skeleton was registered which indicates the presence of phosphate complex labeled with ^{99m}Tc. The presence of ^{99m}Tc-labeled complexes in this preparation was also confirmed with the GCS method. Dugan, et al reported that the tissue distribution of the ^{99m}Tc compound obtained from their labeling method indicated an elimination pattern similar to that of ^{99m}Tc-pertechnetate (*6*). This shows that they also might have obtained ^{99m}Tc-labeled phosphate complex and reduced hydrolyzed ^{99m}Tc in their ^{99m}Tc-streptokinase preparation at high pH, which we also registered with the GCS method when reproducing their method.

Patients with symptoms indicating possible deep venous thrombosis in the legs have been investigated by Kempi, et al with both ^{99m}Tc-streptokinase pre-

TABLE 1. MAXIMUM LIVER UPTAKE AND BIOLOGIC HALF-TIMES FOR LIVER AND BLOOD STUDIED IN RABBITS AFTER I.V. ADMINISTRATION OF VARIOUS ^{99m}Tc-STREPTOKINASE PREPARATIONS

^{99m}Tc-streptokinase preparation	Labeling yield of ^{99m}Tc-Sk* (%)	Maximum liver uptake % of admin. activity	Biologic half-times of disappearance from Liver (min)	Blood T_1 (min)	Blood T_2 (min)	Blood T_3 (min)
A: ^{99m}Tc-streptokinase (phosphate) no buffer, pH 2, Admin. Sk: 7,400 IU/kg	80	70	712	0.42	3.2	212
B: ^{99m}Tc-streptokinase (phosphate) no buffer pH 2, purified on Sephadex G-25 column, Admin. Sk: 5,700 IU/kg	75	69	4,870	0.43	1.9	153
C: Pretreatment of rabbit 18 hr before studies with Sk 75,000 IU/kg ^{99m}Tc-streptokinase (phosphate) no buffer, pH 2, Admin. Sk: 5,800 IU/kg	66	78	4,440	0.61	—	135
D: ^{99m}Tc-streptokinase (glutamate) no buffer pH 1, Admin. Sk: 11,300 IU/kg	60	68	934	0.05	—	145
E: ^{99m}Tc-streptokinase (phosphate) phosphate buffer, pH 7.4 Admin. Ek: 5,000 IU/kg	5	10	121	0.64	3.0	86

* Sk is streptokinase.

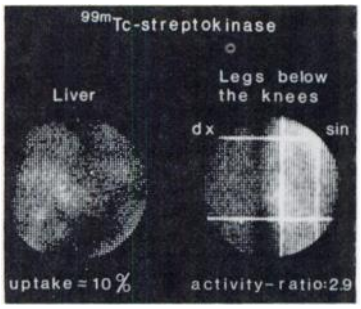

FIG. 4. A scintillation camera picture of liver and of thrombus in left tibial vein at 60 min after i.v. injection of 50,000 IU of ^{99m}Tc-streptokinase in man.

pared at pH 2 in accord with this work and phlebography (*12*). Nineteen patients were examined and an activity ratio between the two legs that exceeded or was equal to 1.1 was found in all 11 patients in whom phlebography showed a thrombus. An example of a thrombus in the left tibial vein 60 min after the administration of ^{99m}Tc-streptokinase is shown in Fig. 4. The activity ratio between the left and the right leg in this case was 2.9. In the other eight patients the activity ratios were normal although in two of these patients phlebography suggested a thrombus (*12*). A scintillation camera view of the liver 60 min after the administration of ^{99m}Tc-streptokinase is also shown in Fig. 4. The liver uptake in man was only about 10–20%, which is much lower than the uptake in the liver of rabbits.

CONCLUSION

The best labeling efficiency and the best reproducibility in the preparation of ^{99m}Tc-streptokinase were obtained by adding 2.5 ml ^{99m}Tc-pertechnetate to a vial containing 0.5 ml of freshly prepared $SnCl_2$ solution (4 m*M* $SnCl_2$; 0.12 *M* HCl; and 0.12 *M* NaCl). After 1 min this mixture of reduced ^{99m}Tc at pH 1.3 was transferred to a vial containing 50,000–75,000 IU of streptokinase (phosphate) dissolved in 0.5 ml 0.9% NaCl. After about 30 min this preparation, which has a pH of about 1.9–2.0, can be used for visualization of thrombi and theoretically also of emboli. The liver uptake in rabbits is about 70% and the biologic half-time of the disappearance from the liver is about 700 min.

In a clinical investigation ^{99m}Tc-streptokinase, prepared at pH 2 according to this work, showed a high correlation to phlebography in the diagnosis of vein thrombosis (*12*).

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