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LOCALIZATION OF DEEP VEIN THROMBOSIS USING RADIOACTIVE STREPTOKINASE

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Animal investigations have been performed which suggest the use of ^{99m}Tc-streptokinase as an agent for the detection of preformed, deep vein thrombosis in man.

The use of radionuclides as tracers for localization and identification of deep vein thrombosis has been a subject of much interest in the past several years (*1–3*). The success of using ^{125}I-fibrinogen and ^{131}I-fibrinogen for detecting actively forming thrombi has been well established (*2–4*). Unfortunately, iodinated fibrinogen may not be incorporated or attracted to a preformed thrombus, i.e., fibrin deposition is minimal once a thrombus is formed.

Streptokinase, a secretory protein of hemolytic streptococci, has been found to induce, in vivo, dissolution of already formed venous and arterial thrombi. The mechanism of thrombolysis produced by streptokinase involves a series of reactions where streptokinase adsorbs to and penetrates in and around the thrombus; it activates plasminogen located within the thrombus, and yields sufficient plasmin for fibrin dissolution and thrombus lysis (*5*). Because the therapeutic use of streptokinase has been reported in the treatment of venous and arterial thrombosis (*5,6*), we decided to investigate the usefulness of ^{99m}Tc-labeled streptokinase (^{99m}Tc-Sk) as a possible agent for localization of an already formed thrombus. Siegel, et al (*7*) have reported using ^{131}I-streptokinase in animals. However, using ^{99m}Tc as the tagging agent allows for increased photon flux at the thrombus site, with better resolution, while maintaining patient radiation absorbed dose within acceptable limits. As with all enzyme-labeling procedures, the tagging procedure used must be gentle enough to maintain the integrity of the protein molecule, yet effective enough to bring about the labeling reaction. The following procedure is used to prepare ^{99m}Tc-Sk:

1. To a vial containing 100,000 iu of streptokinase, add 2 ml of $^{99m}TcO_4^-$ (approximately 15–20 mCi).
2. Add 0.1 mg of $SnCl_2$ (1 mg/ml) freshly prepared in 0.2 *N* HCl.
3. Add 1 ml phosphate buffer*, pH 12.
4. Let stand for 5 min.
5. Pass through a Dowex 1-X8 anion exchange column and collect ^{99m}Tc-Sk.

The enzymatic activity of ^{99m}Tc-Sk, activating plasminogen to plasmin, was found to have been similar to unlabeled streptokinase when using the Gross method (*6*).

Deep vein thrombosis was surgically produced in the right femoral vein of six female mongrel dogs by damaging the endothelial lining of the femoral vein. Equivalent sham procedures were performed in the left femoral vein without damaging the vein as a control procedure. The animals were injected with 3 mCi of ^{99m}Tc-Sk through the jugular vein from 1–4 days after completing the surgical procedure. Scintillation scanning was initiated 10 min after administration of ^{99m}Tc-Sk with repetitive scans performed every 30 min up to 4 hr postadministration. Positive scans indicating uptake of radioactivity were noted as early as 10 min after administration of ^{99m}Tc-Sk, and optimum visualization occurred at 1 hr. After scintillation scanning was completed, the surgical areas—thrombus and sham areas—were removed and counted for radioactivity concentration. Ratios of radioactivity per milligram clot to radioactivity per milligram sham site were determined and

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* Phosphate buffer, pH 12—12.508 Na_2HPO_4, 2.768 $Na_2H_2PO_4$, 2.348 NaCl, 12.008 NaOH; qs to 1,000 ml with sterile water for injection.

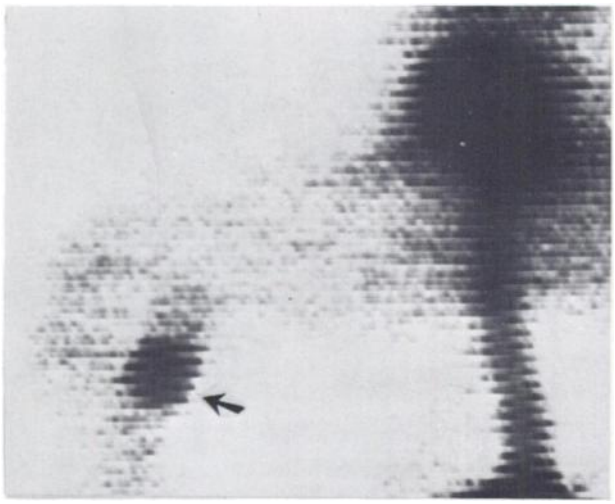

FIG. 1. Increased radioactivity in right femoral vein of dog 60 min after injection of 300 μCi of ^{99m}Tc-streptokinase.

found to have been greater than 25:1 at 4 hr. Ratios of radioactivity per milligram clot to radioactivity per milligram whole blood were also determined and found to be greater than 20:1 at 4 hr. The percent of radioactivity incorporated into the thrombus at 1 hr was estimated at 2.3% by comparing counts in the thrombus with radioactivity in a tissue equivalent phantom correcting for tissue absorption and volume. Blood clearance of ^{99m}Tc-Sk was determined by sampling venous blood every hour up to 4 hr after injection of ^{99m}Tc-Sk.

Because streptokinase can be precipitated from plasma by trichloroacetic acid (TCA), the plasma fractions were mixed with 10% TCA and the precipitate counted for radioactivity. Such determinations indicate the biological elimination pattern of the administered radioactive streptokinase. A biexponential elimination pattern was determined. Eighty-two percent of injected ^{99m}Tc-Sk has an effective half-life of 12 min whereas the remaining 18% has an 85-min half-life. Tissue distribution studies indicated a similar elimination pattern of $^{99m}TcO_4^-$ (*8*) with the largest percentage of the radioactivity located in the kidneys 1 hr after administration of ^{99m}Tc-Sk.

Figure 1 shows the visualization by scintiscan of a surgically produced thrombus (see arrow) in the right femoral vein of a dog 60 min after the administration of ^{99m}Tc-Sk. This anterior scan of the lower abdomen also shows a large amount of radioactivity in the bladder and extending catheter. Bladder uptake is concomitant with the short, effective half-life of ^{99m}Tc-Sk.

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