STABILITY STUDIES AND TUMOR UPTAKE OF A TECHNETIUM-TETRACYCLINE COMPLEX

Kenneth Breslow, Samuel E. Halpern, Frederick C. Schwartz, Naomi P. Alazraki, and William L. Ashburn

University of California and Veterans Administration Hospital, San Diego, California

The ^{99m}Tc-tetracycline complex (^{99m}TTC), a radiopharmaceutical under investigation as a tumor- and myocardial infarct-scanning agent, was studied in reference to its radiopharmacology and biologic distribution. The effects of pH as well as $SnCl_2$ and tetracycline concentrations were the parameters researched. Within the limits investigated, pH was found to have a profound effect on the compound regarding the formation of what appeared to be a colloid. The biologic distribution of the ^{99m}TTC was affected by the colloid formation resulting in an increased liver uptake of the radiopharmaceutical. Increasing $SnCl_2$ concentration decreased the dissolution of the compound to $^{99m}TcO_4^-$. The most favorable biologic distribution noted was from the compound with a pH of 7.5, 5 mg of tetracycline/cc, and 2 mg of $SnCl_2$/5 cc of solution. The uptake of ^{99m}TTC by a transplanted rat hepatoma suggested that this radiopharmaceutical might be useful as a tumor-scanning agent.

There is at the present time no outstanding radiopharmaceutical for the imaging of dead (or dying) myocardium or neoplastic tissue. A need exists for such compound(s). Recently, a ^{99m}Tc-labeled tetracycline compound has been studied at Harvard University (*1–3*) and the University of California at Los Angeles as both a tumor- and myocardial infarct-scanning agent. Studies in our laboratory quickly revealed problems with the radiopharmaceutical which we have chosen to call ^{99m}Tc–tetracycline complex (^{99m}TTC). Included in those problems were the marked effects that pH, $SnCl_2$ concentration, tetracycline concentration, and time had upon the compound and upon its biologic distribution in normal and tumor-bearing rats. The following data are the results of these investigations.

MATERIALS AND METHODS

Numerous ^{99m}TTC preparations were prepared utilizing the stannous chloride reduction method with variances in the quantity of $SnCl_2$, tetracycline, and pH of the final product. The formulation of the various preparations is indicated in Table 1. The general preparation was as follows: dry tetracycline hydrochloride (Cal. Biochem.) was dissolved in sterile water, then a 1 or 2% solution of $SnCl_2$ was prepared by dissolving $SnCl_2 \cdot 2\ H_2O$ in sufficient concentrated HCl so as to result in a 0.75 *N* solution when brought to volume with water. An appropriate aliquot of the tetracycline was pipetted into a suitable vessel and the $SnCl_2$ solution was added and vortex mixed for 15 sec. The $^{99m}TcO_4^-$ eluate (New England Nuclear Corp.) was then added together with normal saline to produce a final volume of 5 ml and this mixture was again vortex mixed for 15 sec. The resulting pH of these reactions ranged from 1.8 to 2.2. The pH was then titrated to the desired level with NaOH. This solution was passed through a 0.22-micron Millipore filter. The filtered product and the filter membrane were then calibrated for total activity utilizing a RADX dose calibrator. Chromatography was performed in 100% butanol on Gelman ITL strips and the percent free $^{99m}TcO_4^-$ determined. Under the conditions described, the Rf for $^{99m}TcO_4^-$ is I.O. and ^{99m}TTC is O.O. Biologic distribution studies were performed in normal Sprague-Dawley rats. The affinity of the radiopharmaceutical preparation for tumor was studied in Buffalo rats (Simonsen Laboratories) bearing hepatomas in their thighs. The biologic distribution of the substance that remained on the filters was studied in both normal and tumor-bearing rats. This substance was removed from the membrane of the

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For reprints contact: Samuel E. Halpern, Div. of Nuclear Medicine, University Hospital, 225 W. Dickinson St., San Diego, Calif. 92103.

TABLE 1. IN VITRO STABILITY AND BINDING EFFICIENCY AS A FUNCTION OF $SnCl_2$ CONCENTRATION (MG/5 ML OF SOLUTION)

$SnCl_2$ (mg)	0 hr (%)	1 hr (%)	2 hr (%)	3 hr (%)	4 hr (%)	5 hr (%)
¼	89.0	48.7	—	—	—	—
	86.8	64.0	—	—	—	—
½	89.9	66.1	—	46.1	—	—
1	93.0	70.8	—	—	—	—
	94.0	76.9	—	—	—	—
	96.3	77.4	—	—	—	—
	95.0	89.0	65.8	—	—	—
	94.0	91.0	65.7	—	—	—
2	97.0	95.5	94.0	90.7	—	—
	95.0	95.0	93.0	92.0	—	—
	98.6	96.2	—	89.5	—	—
	96.5	93.8	—	—	—	—
	97.0	94.2	—	—	—	—
	96.8	84.6	—	—	—	—
	96.2	95.8	95.5	92.3	80.7	73.2
	96.7	95.8	96.0	87.5	73.1	63.2
	98.3	80.7	—	—	—	—
3	96.6	94.2	94.0	—	—	—
	95.4	94.5	93.8	—	—	—
	98.9	—	—	98.2	—	76.9
	98.3	—	—	98.1	—	97.8
5	97.9	98.0	97.7	—	—	—
	97.9	97.0	98.0	—	—	—
	99.0	—	—	98.8	—	99.0
	99.3	—	—	99.0	—	97.8

Constants: 5 mg/ml tetracycline HCl, pH 7.4–7.8, 5 ml final volume.

filter by ultrasonification using a minimal amount of fluid of the same pH and ionic concentration as the filtered material. Rats were studied at a variety of time periods (10 min–24 hr postinjection). In vitro stability studies were performed by repeat chromatography of the original compounds at appropriate intervals (see Tables 1 and 2). Initial binding efficiency was computed on the basis of the chromatography. The percent of the total radioactivity removed by the filter was studied by direct counting of filter and filtrate. All imaging was performed on a gamma scintillation camera using pinhole collimation.

RESULTS

Initial binding efficiency. Table 1 shows clearly that the initial binding efficiency was relatively high (about 88%) with as little as 0.25 mg $SnCl_2$. A progressive rise in this initial binding efficiency occurred as the quantity of $SnCl_2$ was increased. It should be noted, however, that little significant increase in binding efficiency occurred above 2 mg of $SnCl_2$/5 ml of solution.

Stability as a function of stannous chloride. Table 1 also shows that the increasing amounts of tin in the solution reduced the breakdown of the ^{99m}TTC to $^{99m}TcO_4^-$. The data suggest that within the limits studied, this was a direct relationship. For practical purposes 2 mg of $SnCl_2$ appeared adequate for stabilization of the radiopharmaceutical.

Filter retention. Table 2 shows the average percent of activity removed by the filter as a function of the three variables under investigation. It is obvious from these data that the most important but not the only parameter related to filter retention within the limits studied was the pH of the solution. At a low pH a large percent of the radioactivity was removed from the original solution. As the pH was raised, the quantity remaining on the filter decreased to negligible amounts. There was some indication that the total quantity of tetracycline used was also important in this process but with the formulations used in this study it was less important than the pH. When very large quantities of $SnCl_2$ were combined with very low concentrations of tetracycline, filter retention increased and the murky color of the solution suggested colloid formation.

Biologic distribution. Biologic distribution studies were performed in both normal and tumor-bearing rats. Numerous preparations were studied containing various concentrations of the constituents. Figure 1 shows the results of injecting five different preparations (see figure legends) into five tumor-bearing animals and imaging 4 hr postinjection. In

TABLE 2. AVERAGE PERCENT ACTIVITY RETENTION ON 0.22-μm FINAL FILTER AS A FUNCTION OF pH, $SnCl_2$ CONCENTRATION, AND ^{99m}Tc-TETRACYCLINE CONCENTRATION

$SnCl_2$ (mg/5 cc)	pH	Tetracycline HCl (mg/1.0 cc)	Percent activity retention
⅛	3.0	3	40
	5.5	3	21
	7.5	5	12
¼	3.0	3	57
	5.5	3	28
	7.5	1.2	11
	7.5	5	9
½	5.5	3	29
	7.5	5	12
1	5.5	3	32
	5.5	5	18
	7.5	3	12
	7.5	5	6
2	5.5	3	28
	5.5	5	24
	7.5	1.2	23
	7.5	2	16
	7.5	5	4
3	7.5	5	4
5	7.5	5	4

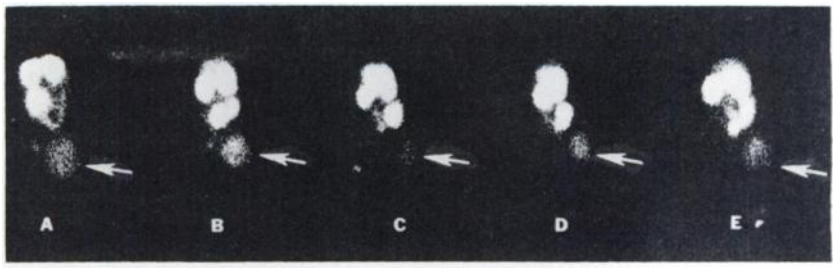

FIG. 1. Biologic distributions of ^{99m}TTC in rats bearing 15-gm tumor in right thigh. Preparations as follows: (A) 0.5 mg $SnCl_2$/5 cc, 5 mg tetracycline/cc pH 7.5; (B) 1.0 mg $SnCl_2$/5 cc, 5 mg tetracycline/cc pH 7.5; (C) 2.0 mg $SnCl_2$/5 cc, 1.0 mg tetracycline/cc pH 7.5; (D) 2.0 mg $SnCl_2$/5 cc, 2.0 mg tetracycline/cc pH 7.5; (E) 2.0 mg $SnCl_2$/5 cc, 5.0 mg tetracycline/cc pH 7.5.

every case, the organ with the greatest uptake of the radiopharmaceutical was the kidney followed next by the liver. Tumor uptake (arrow) was approximately the same with all preparations as was the quantity of the radiopharmaceutical excreted into the intestine. Liver uptake appeared to be less for the Fig. 1E preparation than for any other formulation.

Figure 2 shows the results of injecting the filtered material (A and B) into a normal animal and the residue remaining on the filter into another (C and D). Both animals were imaged at 10 and 60 min postinjection. The distribution of the filtered material is as described under Fig. 1 whereas the material from the filter distributes mostly in the liver and spleen and to some extent in the kidneys and lungs.

Within 10 min (Fig. 3) following the injection of what was found to be the best combination for ^{99m}TTC (pH, 7.5, 5 mg/cc tetracycline; 2 mg/5 cc $SnCl_2$), the kidneys were intensely radioactive and the urinary bladder was visible. Considerable activity was also noted in the liver. Within an hour, activity was visible in the stomach and to a lesser extent in the gastrointestinal tract. By 12 hr some of the soft-tissue background had diminished. The kidneys remained intensely radioactive at this time period whereas the quantity of tracer within the liver appeared to diminish somewhat but not entirely. The stomach continued to show evidence of radioactivity and this was presumed (but not proven) to be $^{99m}TcO_4^-$. No bladder activity was noted at 12 hr postinjection. The tumor which was visible within the first few minutes remained quite radioactive throughout the study. The resolution of the tumor appeared to be enhanced as the time between injection and imaging increased.

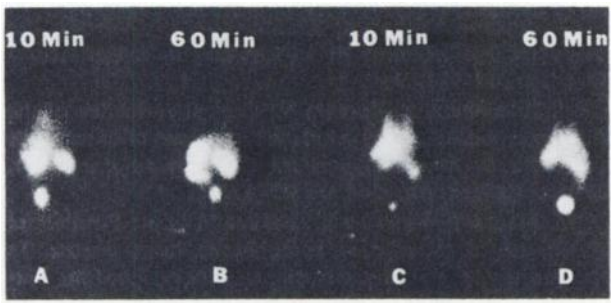

FIG. 2. Distribution of ^{99m}TTC following filtration (A and B) and of material remaining on filter (C and D).

DISCUSSION

The incorporation of tetracycline into tumor tissue was first described by Rall, et al (*4,5*). Since then, the localization of tetracycline fluorescence in a variety of human tumors has been reported (*6–8*). This has been used clinically as a cytologic test for gastric carcinoma (*9–11*). Similar work has been done with other human malignancies (*12,13*). Soon it became obvious that difficulties were arising in the detection of tumor tissue with tetracycline. Ackerman (*14*) demonstrated that tetracycline localization in malignant tissues was erratic and undependable. Phillips, et al (*15*) found that if there were any necrotic elements in the malignancy, little if any incorporation of the antibiotic occurred in the necrotic parts of the tumor. Kocandrle, et al (*16*) noted that tetracycline was not fixed in the actual undamaged tumor cell but only in those regressively changed parts of the tumor. He also concluded that the amount of tetracycline fixed bore no relation to the degree of malignancy of the tumor. Malek, et al (*17*) found that the tetracycline accumulation in the heart myofibriles damaged by ischemia was a regular occurrence and suggested the use of a labeled, gamma-emitting tetracycline for further research in this area. Finally, experi-

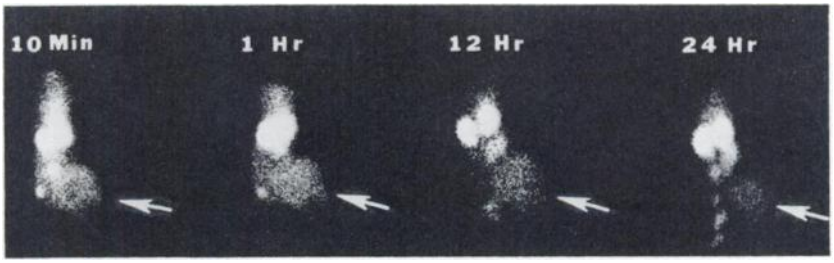

FIG. 3. Results shown of injection of ^{99m}TTC into Buffalo rat, bearing 15-gm hepatoma in his right thigh (arrow).

ments by Dewanjee, et al (*18*) indicated that ^{119}Sn (Sn^{2+}) was strongly chelated by the tetracycline molecule. Further experiments utilizing double-labeled ^{99m}Tc-^{119m}Sn tetracycline indicated that in addition to the tin acting as a reducing agent for the technetium, the Sn^{2+} is essential in the binding of the technetium to the tetracycline molecule. Free technetium was liberated after removal of the Sn^{2+} by precipitation or complexation. Our data strongly support the results of these experiments with initial binding efficiency and stability enhanced by an increasing Sn^{2+} concentration. Although our chromatographic method does not prove that all the reduced technetium detected is bound to tetracycline, it does indicate that free $^{99m}TcO_4^-$, which would degrade the image because of its slow clearance from the body, can be eliminated from the solution. Our experiments further suggest that the material removed by Millipore filtration acts as a colloid biologically since it is extracted by the liver and spleen. The formation of this substance can be diminished by using a pH of 7.4–7.8 as a final pH of the solution and by having the appropriate ratios of tin and tetracycline. It would appear from body-distribution studies that the preparation formulated with 2 mg of $SnCl_2$/5 cc of solution, 5 mg of tetracycline/1.0 cc of solution, and a final pH of 7.5 would be the most appropriate compound for future study.

Imaging, using the rat-tumor model that we have described, suggests that ^{99m}TTC might be useful as an agent for imaging malignant tissue. Certain problems are apparent in the proposed use of ^{99m}TTC for tumor scanning, however, mostly due to the large amount of background radioactivity in the kidneys, liver, and intestines. Only carefully performed clinical studies using ^{67}Ga as a benchmark will determine its true value as a tumor-scanning agent.

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