On the Nature of Technetium Compounds

1. A Semiquantitative Biological Model System to Assay 99mTc-Complexes/Radiopharmaceuticals

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Abstract. Eleven different 99mTc compounds having some relevance in clinical nuclear medicine have been classified according to their localizing properties. The following classes of 99mTc compounds have been compared in a semiquantitative manner, using a Wistar strain rat model: liver and hepatobiliary agents such as Tc-phytate, Tc-sulfur colloid, reduced (SnII) TcO4−, and 99mTc-LIDA (dimethyl-IDC); bone agents like Tc-pyrophosphate, Tc-HEDP, Tc-EDTMP; kidney and bladder agents comprising Tc-DTPA, Tc-glucoheptonate, Tc-citrate, and TcO4−. The in vivo pharmacokinetic behavior lends itself to a unified approach with respect to the nature of 99mTc compounds. Most of the 99mTc complexes display an excretory type of pathway. These compounds were found to be relatively stable in aqueous solutions at room temperature (25°–28° C) for at least 2 h without any additives. Both paper chromatographic and biologic (tissue distribution) criteria, commonly used for quality assurance and control of 99mTc compounds, were employed to study these compounds.

Introduction

There are several methods that have been adopted to assay the purity of 99mTc compounds commonly designated as radiopharmaceuticals1. Of these, chromatographic procedures and the biologic method of tissue distribution criteria, commonly used for quality assurance and control of 99mTc compounds, were employed to study these compounds.

layer-column-gel chromatography, and electrophoresis, although useful in many respects to determine the level of impurity present in radiopharmaceuticals, have not been found to be very versatile to predict with absolute certainty the predisposition of Tc-compounds in an in vivo system. Theoretically, an a priori knowledge of the chemical configuration of each constituent present in a Tc radiopharmaceutical, be it the required radiopharmaceutical or impurities, could only be derived from sound principles of analytic chemical techniques like chromatography. So far, however, no absolute correlation between chromatographic patterns and biologic assays has been established. Furthermore, it is necessary to distinguish between closely related chemical species, e.g., Tc present in compounds in different oxidation states, ligand exchange products, and labile complexes. It is possible that such species are sometimes present in radiopharmaceuticals when they elicit certain biologic responses responsible for the change in the blood levels or target-to-nontarget ratios, especially when slightly degraded ‘kits’ are used [15]. In addition, the presence of various endogenous donors or acceptors like amino acids, proteins and inorganic ions in an in vivo system could result in further mobilization or attenuation of a Tc compound. This is reflected in its tissue distribution but is not apparent in chromatograms. In these circumstances the biologic method of assay is a better index of the biologic status of a Tc radiopharmaceutical.

Many investigators have employed different animal model systems to study the absorption, distribution, and excretion patterns of Tc radiopharmaceuticals. A large body of such data has been reviewed [15], and the lack of standardization and/or disparity of some of the results reported therein are obvious. The reason for some of the inconsistent values obtained, apart from differences existing between animal species, is the very nature of the Tc radiopharmaceuti-

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1 In this article the term Tc radiopharmaceutical is loosely applied to all Tc compounds including Tc complexes employed in diagnostic nuclear medicine. The symbol Tc represents the radionuclide 99mTc.
cals, which in most cases is excretory; hence quantification becomes difficult. Since a large amount of the Tc activity is excreted (and possibly lost), anomalous results could be obtained unless specific methods are devised to measure the fraction of the excreted activity. One method that could be used with advantage is to house the animals in metabolic cages and to monitor the excretion. Urine is often found on the body fur of the experimental animals, and this presents difficulties in quantification. To overcome some of the above handicaps a simple type of animal model has been studied by ligating the penis of male rats. Such a model could have practical application in quantifying the urine of the excretory activity in the bladder, despite the limitations imposed by the technique. This would also aid in quality assurance and control of Tc radiopharmaceuticals formulated from diagnostic precursor agents such as Sn(II) complexes (designated as ‘kits’ and used in ‘instant labelling’ with TC(O2)4).

A semiquantitative method for the analysis (bioradiosay) of Tc radiopharmaceuticals has been standardized in normal male rats by ligating the penis. The method is a more reliable index of the performance characteristics of some of the common Tc agents currently used in diagnostic nuclear medicine.

Materials and Methods

Sodium Pertechnetate. This was obtained by the solvent extraction procedure [13]. This solution in physiological saline (0.145 M NaCl) had a neutral pH and was colorless. The solution was filtered down through a 0.22 µm membrane (Millipore Corp.) to remove particulate and colloidal matter, which might remain as reduced, but uncomplexed Tc in the TC(O2)4 and be localized in the reticuloendothelial system (RES).

Sn(II) Ligands. The following chemicals were used as the ligands: Phy (phytate, i.e., inositol hexaphosphoric acid, sodium salt; Sigma Chemical Co., St. Louis, Mo.); LIDA (lidocaine iminodiacetic acid i.e., N-(2,6-dimethylphenylcarbamoylmethyl) iminodiacetic acid, also known as HIDA or dimethyl-IDA in literature; PyP (tetrasodium pyrophosphate, B.D.H., Poole, England); HEDP (1-hydroxyethylidiphosphonic acid, disodium salt); EDTMP (ethylenediamine N,N',N'-tetramethyleneephosphonic acid, sodium salt, Monsanto Chemical Co., U.S.A.); DTPA (diethylenetriaminepentaaacetic acid, Koch Light Labs Ltd., Coinbrook Bucks, England); GHA, (glucoheptonic acid as Ca salt); Cis (trisodium citrate, B.D.H., Bombay, India). The appropriate amount (~200-300 mg) of the respective chemical reagent was dissolved in physiological saline (12-18 ml); sometimes initial dissolution was aided by adding a few ml of dil. NaOH dropwise in the case of LIDA, EDTMP, and DTPA, and a solution of 20-30 mg of SnCl2·2H2O (E. Merck, Darmstadt, FRG) in ~0.33 N HCl was added to it while stirring (10-15 mg in the case of GHA). The resulting solution was neutralized with drop-by-drop addition of ~0.5 N NaOH until the pH was 5-7. Up to 20-30 ml of the solution was made to give a ratio of the ligand to SnCl2·2H2O of about 10:1 per ‘kit’ vial (1 ml each); the ratio was 20:1 in the case of GHA. This solution was filtered with a 0.22-µm membrane and dispensed (1 ml each) into sterile 10 ml-capacity multidose vials. The contents of the vials were lyophilized (16–20 h), and finally the vials were sealed under vacuum and resealed with aluminum caps. The vials were stored at 0–5°C in a refrigerator. (Whenever the kits were to be used for clinical diagnostic investigational work, appropriate precautions were taken and the entire procedure was carried out in the sterile area of a laminar clean air-flow bench).

Tc-Complex Radiopharmaceutical. The required radiopharmaceuticals were prepared by adding 4.0 ml of the above TC(O2)4 (about 1.0 mCi) to the respective decapped kit vial, and the contents were mixed. The vials were subsequently closed with rubber closures only.

Controls. TC(O2)4, reduced TC(O2)4 (i.e., Tc in a lower oxidation state) and TC-sulfur colloid served as the controls. Reduced TC(O2)4 was freshly prepared by the addition of 1.0 ml of SnCl2 solution (containing 1.0 mg SnCl2·2H2O in approx. 0.05 N HCl, pH 1.7) to 3 ml of TC(O2)4 (1.0 mCi) in physiological saline and the contents were mixed. (The same amount of Sn (II) was used in each vial as that obtained in the other kit vials to simulate the reaction conditions, although the radiopharmaceutical was different agent was added). TC-sulfur colloid was formulated using slightly modified thiosulphate procedure reported earlier [15]. In this method 2.0 ml of TC(O2)4 (1.0 mCi) in saline was added to a mixture containing 1.5 ml mannitol (5%) and 0.3 ml of Na3S2O3 (1%) in a vial, followed by 0.5 ml of 1.0 N HCl. The vial was quickly closed with a rubber closure, sealed with an aluminum cap, and placed in a boiling hot water bath for 5 min. The vial was cooled and finally 1 ml of sodium citrate (1.0 M) was added with thorough mixing.

Assay. Both chromatographic assay and bioradiosay were performed within minutes (<25 min) after the reconstitution of the respective Tc radiopharmaceutical. Ascending paper chromatography was done on Whatman Grade 1 paper strips. A single spot containing a few µCi of the radiopharmaceutical was applied to the paper by means of a glass capillary, air dried, and then developed (~15 cm) in the solvent systems contained in tall jars (closed system): 85–15 MeOH: H2O: 0.85% NaCl solution (0.145 M) and 1.0 M sodium acetate-acetic acid buffer solution (pH 4.8). The developed paper strips were dried under a heat lamp, cut into 5-mm strips, and counted in a well-type γ-scintillation counter.

Each compound was biologically screened for the levels of distribution and localization in isolated whole organs and tissues of male Wistar strain rats weighing 230-550 g. About 50 µCi/0.2 ml was injected (I.V, penis vein) under diethyl ether anesthesia, and then developed (~15 cm) in the solvent systems contained in tall jars (closed system): 85–15 MeOH: H2O: 0.85% NaCl solution (0.145 M) and 1.0 M sodium acetate-acetic acid buffer solution (pH 4.8). The developed paper strips were dried under a heat lamp, cut into 5-mm strips, and counted in a well-type γ-scintillation counter.

Each compound was biologically screened for the levels of distribution and localization in isolated whole organs and tissues of male Wistar strain rats weighing 230–550 g. About 50 µCi/0.2 ml was injected (I.V, penis vein) under diethyl ether anesthesia, and the penis ligated. The rats were killed serially at 5, 15–20, 60±3, 120±3, and 180±3 min postinjection by the withdrawal of 3–8 ml of blood (subsequently weighed, or else the density assumed = 1) in a graduated syringe (thinner coated with a solution of an anticoagulant such as EDTA solution) by cardiac puncture under anesthesia, and then the animal was dissected and the heart removed. Whole organs were excised: spleen, stomach and its contents, gut with its contents, liver, kidneys, lungs, thyroid; also excised were weighed representative samples of the thigh muscle (~3.0–4 g) and two bone (tibia and fibula) specimens of the two hind legs with marrow (~0.7–1.10 g) but no overlying tissue, intact bladder with all its contents (ligated), and the site of the injection (to exclude any unabsorbed activity remaining at that site). Any blood adhering to the tissues was removed by blotting with absorbent paper. The weighed samples of the bone were washed with water prior to counting. The carcass containing the remaining tissues not included in the above was wrapped up in absorbent paper. The organs and carcass were placed about 40 cm away from the surface of the crystal NaI (Tl) and assayed for radioactivity in a lead-shielded γ-scintillation counter at the photoplane for a present time period of 20 s each. The sum of the activity present in