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Note

In vitro and in vivo studies with sodium pertechnetate and technetium-labelled methylene diphosphonate

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The isotope technetium-99m is a conveniently available generator-produced radionuclide which, together with the introduction of new chelating agents has become widely used for labelling a variety of compounds. It has favourable nuclear properties for diagnostic imaging [1]. These include a convenient γ-ray energy of 140 keV, absence of β-decay, and a short half-life of 6 h. It is eluted as sodium pertechnetate ($Na^{99m}TcO_4$) from its parent with saline from a generator consisting of ^{99}Mo adsorbed on to an alumina column.

The pertechnetate ion ($^{99m}TcO_4^-$) is considered to be the most stable chemical form [2], and it does not readily bind to chelating agents such as ethane-1-hydroxy-1,1-diphosphonate (EHPD) and methylene diphosphonate (MDP). In order to prepare a bone imaging agent, a less stable reduced state of ^{99m}Tc is usually desirable. A number of reducing substances are available, and some of these include ferrous ion [3], ferric chloride and ascorbic acid [4], sodium borohydride [5], stannous ion [6] and concentrated hydrochloric acid [7]. Stannous ion is the most popular, and it is considered to be the most suitable reducing agent for bone seeking compounds. It causes a reduction of technetium from Tc(VII) to Tc(IV) oxidation state [8].

Apart from the diphosphonates, other ^{99m}Tc-labelled radiopharmaceuticals can similarly be prepared by the stannous reduction of pertechnetate. Some of these include diethylenetriaminepentacetic acid [9], gluconate [10] and dimethylglyoxime [11]. It should be pointed out that the chemical forms of many of these labelled complexes are still unknown. In the case of dimethylglyoxime Deutsch et al. [11] have shown, using X-ray analysis, that reduction of $^{99m}TcO_4^-$ by tin(II) in the presence of this agent yields a ^{99m}Tc-Sn-di-

methylglyoxime complex in which the tin and technetium are intimately connected by a triple bridging arrangement.

Reduced technetium can be reoxidised to pertechnetate by presence of oxygen:

$$TcO_2 + 2H_2O \rightarrow TcO_4^- + 4H^+ + 3e$$

The stability of the labelled complex (^{99m}Tc—MDP) is also affected by presence of oxygen, so it is important that sufficient stannous ions are added to ensure complete reduction of the technetium. Free $^{99m}TcO_4^-$ has occasionally been detected in our ^{99m}Tc—MDP preparation in such quantities which were sufficient to produce localisation in non-osseous structures, thus decreasing the quality of the bone scan.

These experiments were carried out (i) to determine the efficiency of labelling stannous methylene diphosphonate (Sn-MDP) with free $^{99m}TcO_4^-$ by radiochromatography (ii) to demonstrate the presence of both the labelled complex and any unreduced $^{99m}TcO_4^-$ by autoradiography (iii) to compare the whole body distribution of $^{99m}TcO_4^-$ and ^{99m}Tc-labelled MDP by nuclear imaging with a γ camera and (iv) to quantitate the distribution of each agent in bone and various types of soft tissues.

EXPERIMENTAL

The preparation of Sn-MDP in the form of a freeze-dried kit and its use for labelling red blood cells have already been described [12, 13]. Each kit contains 5 mg MDP + 0.3 mg SnF_2. For labelling purposes 5 ml of eluted, pyrogen-free $^{99m}TcO_4^-$ solution containing the required amount of radioactivity were added to the stannous diphosphonate mixture in the kit vial. The mixture was shaken vigorously for 2 min to ensure complete and uniform labelling, and the solution filtered through a 0.22 μm membrane filter before use.

The radiochemical purity, i.e. the fraction of the total activity present in the stated chemical form, was determined by thin-layer chromatography (TLC) using 85% methanol as the solvent system. Pre-coated silica gel (0.25 mm thick) plastic sheets (Camlab., Hornchurch, Great Britain) were used as adsorbent. A 5-μl volume of the labelled preparation containing 5 μg MDP was spotted at a distance of 1 cm from one end of each TLC strip. This end was placed lowermost in a chromatographic tank containing a depth of 0.5 cm of the solvent. The strips were allowed to develop until the solvent front migrated to a distance of 1 cm from the upper end. The process was repeated for $^{99m}TcO_4^-$. At the end of the development the strips were dried in air. Some were cut into 5-mm sections for counting in a well-type crystal scintillation counter to determine their radioactivity content. The remainder were held in contact with Industrial "C" Kodak film by surgical tape, and the films left to expose in a dark box for 3 h. They were then developed in Kodak D. 19 developer, fixed in Kodak FX 40 fixer, and dried in an oven at 40°.

The "in vivo" distribution of $^{99m}TcO_4^-$ and ^{99m}Tc—MDP was tested in New Zealand albino rabbits weighing 2.3—2.9 kg. Each animal was injected with a dose of 150—200 μCi ^{99m}Tc activity in a marginal ear vin, and 2 h later it was placed under a Toshiba γ-camera and an image of the isotope

distribution recorded for both $^{99m}TcO_4^-$ and ^{99m}Tc—MDP. This period of 2 h from injection was chosen as it corresponds to the interval when most bone scans are performed in patients undergoing a skeletal survey.

Tissue distribution studies were carried out in Wistar rats, 150—200 g in weight. Each rat was anaesthetised with pentobarbitone sodium (30 mg/kg) intraperitoneally, and a dose (40—60 μCi) of $^{99m}TcO_4^-$ and ^{99m}Tc—MDP was injected into a tail vein in separate rats. The animals were killed 2 h later, and samples from the following organs were removed for counting with a NaI(T1) counter: bone, blood, liver, kidney, muscle, thyroid and spleen.

RESULTS AND DISCUSSION

Radiochromatograms and the corresponding autoradiographs are shown for $^{99m}TcO_4^-$ and ^{99m}Tc—MDP in Fig. 1. In Fig. 1A the peak activity occurs at some distance from the point of application of the sample on the TLC strip, and this suggests that $^{99m}TcO_4^-$ moves with the solvent front with an R_F value which was found to be 0.86. In Fig. 1B the peak appears at the origin, and this indicates that the ^{99m}Tc—MDP complex remains fixed and does not move with the solvent front during development.

The radiochemical purity of the preparation gives a measure of the yield of the product formed after labelling, and this can be referred to as the labelling efficiency where

$$\text{labelling yield} = \frac{\text{peak activity}}{\text{total activity}} \times 100\%$$

The percentage labelling yield of the ^{99m}Tc—MDP complex was found to be 96.3 ± 2.1 (mean ± S.D.; n = 10). A high labelling yield is usually desirable as this would lead to an increase in the target/non-target ratio.

The nature of the labelled complex which remains at the origin on the TLC

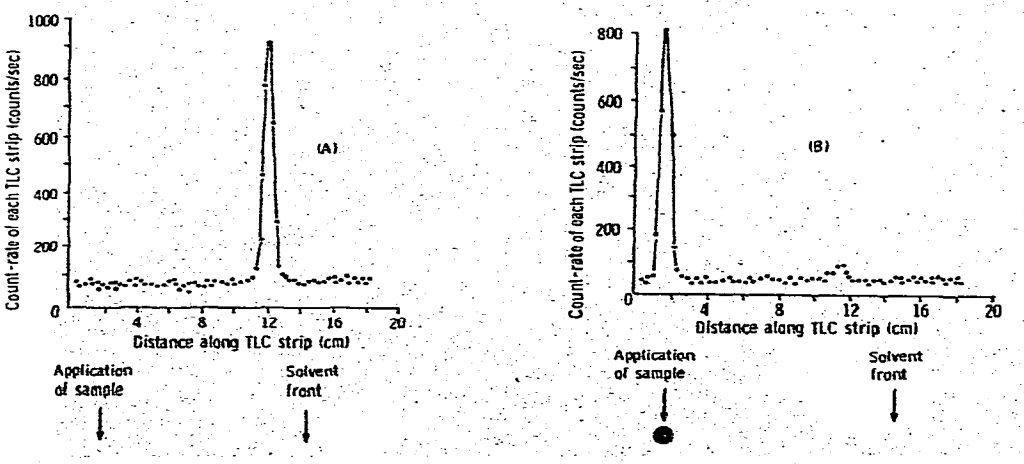

Fig. 1. Radiochromatogram and autoradiographs of (A) pertechnetate, $^{99m}TcO_4^-$, and (B) technetium-labelled methylene diphosphonate, ^{99m}Tc—MDP.

strip is not fully understood. It has been suggested that this complex might consist of some unbound or reduced hydrolysed ^{99m}Tc [14, 15]. Billinghurst [16] has suggested a method for separating free $^{99m}TcO_4^-$ and reduced hydrolysed ^{99m}Tc using a combination of acetone and saline as solvents.

Several analytical techniques are available which can be used for assessment of radiochemical impurities in technetium-labelled compounds. Among these, paper chromatography (PC) and TLC are considered to be relatively simple to perform, and they provide an estimate of the free $^{99m}TcO_4^-$ yield. For a more detailed quantitative measurement of radiochemical impurities, PC and TLC may be combined with paper electrophoresis and gel filtration. The latter techniques are not suitable for a day-to-day quality control program because of their complexity, and due to the fact that they are too time-consuming to perform.

PC was not considered in this work because of the slow development of this system which results in oxidation and subsequent streaking of the radiopharmaceutical on the paper strip. The amount of streaking on TLC silica gel is less than that on the paper as would be expected from the shorter development time.

Images recorded with the γ-camera are shown in Fig. 2. It can be seen that free $^{99m}TcO_4^-$ distributes generally in soft tissues (Fig. 2A) whereas ^{99m}Tc—MDP localises predominantly in the skeleton (Fig. 2B). In the case of the latter agent it is important that complete reduction of technetium occurs as any which remains in the unreduced state will simply be taken up by soft tissues such as the thyroid, stomach and salivary glands. This reduction is brought about by adding the optimum amount of stannous ions, as excess of reductant

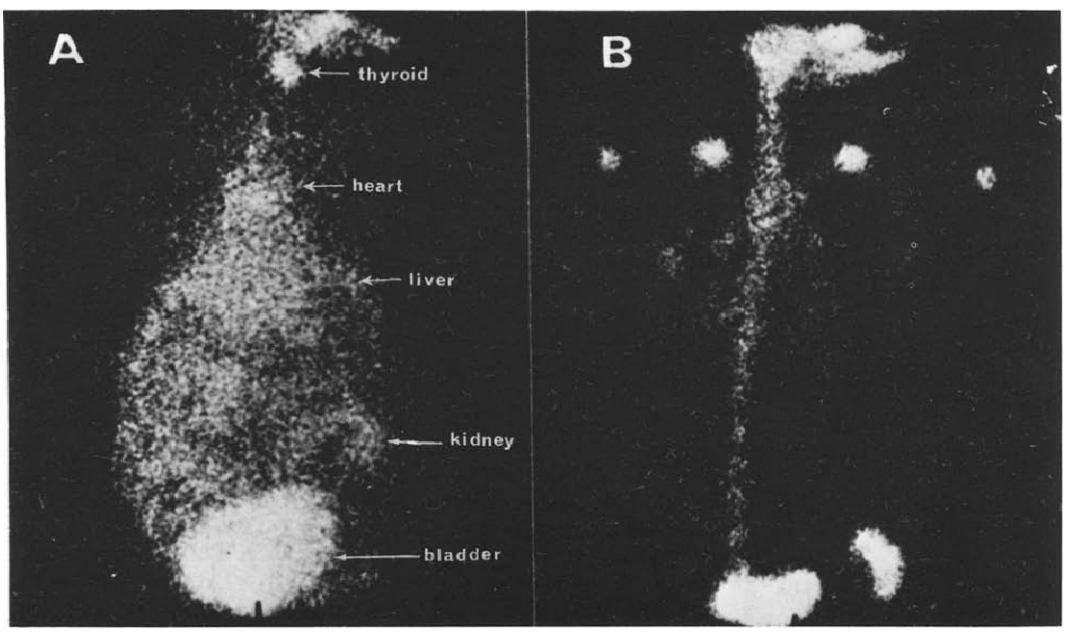

Fig. 2. γ-camera images of the rabbit taken 2 h after injection of a dose of (A) pertechnetate and (B) technetium-labelled methylene diphosphonate.

can lead to formation of technetium and stannous colloids which would then be taken up selectively by the liver and spleen:

$$2TcO_4^- + 3SnF_2 + 2H_2O \rightarrow 2TcO_2 \downarrow + 3SnO_2 \downarrow + 4HF + F_2$$

The results of the tissue distribution study are shown in Table I. In the case of free $^{99m}TcO_4^-$ a high concentration occurs in soft tissues and there is comparatively less uptake in bone. On the other hand the ^{99m}Tc—MDP combination gave the highest concentration in bone with rapid clearance from the non-osseous compartments. This is in agreement with the high labelling yield of the bone complex (96.3 ± 2.1%) as shown by radiochromatography, and with the γ-camera images shown in Fig. 2.

TABLE I

TISSUE DISTRIBUTION OF $^{99m}TcO_4^-$ AND ^{99m}Tc—MDP IN RATS WHICH HAVE BEEN KILLED 2 h AFTER INJECTION

Tissue	Percentage of dose per gram of tissue (mean ± S.D.; n = 5)	
	$^{99m}TcO_4^-$	^{99m}Tc—MDP
Bone	0.58 ± 0.12	2.54 ± 0.81
Blood	0.81 ± 0.23	0.03 ± 0.01
Liver	1.35 ± 0.44	0.04 ± 0.01
Kidney	1.29 ± 0.37	0.83 ± 0.26
Thyroid	2.71 ± 0.92	0.02 ± 0.01
Muscle	0.19 ± 0.06	0.01 ± 0.005
Spleen	0.84 ± 0.25	0.03 ± 0.01

TLC is widely accepted as a reliable "in vitro" method of testing for presence of radiochemical impurities in bone seeking radiopharmaceuticals. It is simple, rapid, inexpensive and provides complete separation of the ^{99m}Tc—MDP complex from any unreduced free $^{99m}TcO_4^-$. These impurities are likely to degrade the quality of the bone scan, increase the absorbed radiation dose to the patient, or localise in non-target areas, thus giving incomplete or misleading information to the user.

It might be argued that in vitro testing of a radiopharmaceutical is probably a poor reflection of its biological distribution. For this reason in vitro assay by radiochromatography should occasionally be checked by in vivo scintigraphy or by quantitative assessment in small laboratory animals. The combination of these techniques has been shown to yield sufficient data for routine quality control and provides useful information about the radiochemical purity of the technetium-labelled compound.

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