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Electrolytically Labeled [^{99m}Tc]MDP: Chromatographic Pattern, Stability and Biodistribution in Rats

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The labeling of methylene diphosphonate with ^{99m}Tc is possible after reduction of pertechnetate by means of controlled potential electrolysis. This results in a [^{99m}Tc]MDP complex which differs slightly from ^{99m}Tc(Sn)-MDP in paper and gel chromatography. The scintigraphic images of both preparations are comparable in quality. Biodistribution in rats shows a higher bone uptake for the ^{99m}Tc(Sn)-MDP complex, whereas [^{99m}Tc]MDP shows a higher uptake in the gastric wall.

Introduction

Since the labeling of diphosphonates with ^{99m}Tc,[1-3] skeletal scintigraphy is a routine procedure in the evaluation of bone lesions. The most widely used agent is [^{99m}Tc]methylenediphosphonate ([^{99m}Tc]-MDP).[4] For the labeling procedure it is necessary to reduce pertechnetate to a lower oxidation state, Tc(IV), in the presence of excess MDP. As a reducing agent, stannous chloride is commonly used. However, the role of tin in the *in vivo* distribution, the mechanism of uptake and the localization of technetium-methylenediphosphonate is not clear. For this reason other reducing agents were tried. Using concentrated HCl and vacuum evaporation, it was possible to prepare technetium complexes with the anti-oxidants ascorbic acid and gentisic acid.[5] Autoclaving $^{99m}TcO_4^-$ with HCl for 30 min at 394 K resulted in a reduction, as controlled with paper chromatography. A technetium, hydroxyethylidene-diphosphonate ([^{99m}Tc]HEDP) complex could be formed after reduction of $^{99m}TcO_4^-$ with concentrated HBr adding HEDP after evaporation.[7] Reduction with NaBH4 and subsequent complexing with HEDP, led to a mixture of different ^{99m}Tc(NaBH4)-HEDP complexes.[7] To avoid contamination of the radiopharmaceutical with any reducing agent electrolytical reduction of $^{99m}TcO_4^-$ in presence of the desired ligand might be possible. Russel[9] labeled tetracycline and EDTA with ^{99m}Tc in this way. In an electrochemical cell, consisting of two compartments connected by a salt bridge, Steigman *et al.*[10] prepared several ^{99m}Tc radiopharmaceuticals with reduction of $^{99m}TcO_4^-$ by hydrogen evolved at the cathode. As ^{99m}Tc(Sn)-MDP is the most widely used complex, we studied the possibility of preparing [^{99m}Tc]MDP after electrolytical reduction of $^{99m}TcO_4^-$ in presence of excess MDP. The notation ^{99m}Tc(Sn)-MDP refers to the ^{99m}Tc-complex prepared by Sn(II) as the reductant and [^{99m}Tc]MDP refers to the electrochemically prepared complex. To evaluate the role of tin with respect to the bone uptake and stability of the complex, this [^{99m}Tc]MDP complex has been compared with ^{99m}Tc(Sn)-MDP. Stability and composition of the complexes prepared were investigated by paper and gel chromatography. The *in vivo* distribution of both preparations has been studied in rats.

Materials and Methods

Equipment

Controlled potential electrolysis was carried out with a home-made potentiostat. A 5 mL polarographic cell (Metrohm EA 880-T-5) was employed with a mercury pool electrode (area 7.1 cm^2) an Ag/AgCl-reference electrode (Ingold 373-90), and a platinum wire auxilliary electrode. The potential of the working electrode was −1.25 V vs Ag/AgCl-reference electrode.

Ascending paper chromatography was performed on Whatman 3 MM with 0.5 M acetate buffer (pH 5.0) as the eluant. The radioactivity was detected by means of a thin-layer scanner (Berthold Scanner

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II Lb 2722-2) with a solid scintillation (NaI) detector. Gel chromatography was performed on Biogel P4 (Biorad) 100–200 mesh with normal saline as the eluant in 0.9 × 28 cm and 0.9 × 13 cm glass columns.

Radioactivity was counted in a γ-counter with a well-type NaI crystal (Tri Gamma Baird Atomic). Activities injected into the rats were measured in a dose calibrator. Scintigrams of rats were made with a Pho IV γ camera (Searle). Tissue radioactivities were counted in the γ counter.

Chemicals

Methylenediphosphonate was obtained commercially (Sigma Chemical Company). $^{99m}TcO_4^-$ was eluted with normal saline from a generator and diluted as needed. All other reagents were of normal analytical reagent grade.

Animals

Male Wistar rats weighing 180–230 g were used.

Procedures

[^{99m}Tc]MDP was prepared as follows. MDP (acid form) was dissolved in normal saline. The pH was adjusted to 7.0 by adding NaOH. $^{99m}TcO_4^-$ was added to this solution. The MDP concentration in the working solution was 0.4%. Of this solution 1.5 mL was put in the electrolysis vessel. The solution was deaerated by a stream of nitrogen for about 15 min. This was continued during the electrochemical experiment. Samples were taken at $t = 0$, $t = 30$, $t = 60$ and $t = 90$ min. The samples were analyzed by paper and gel chromatography. ^{99m}Tc(Sn)-MDP was prepared by dissolving 200 mg MDP and 50 mg $Sn(II)Cl_2 \cdot 2H_2O$ in 90 mL normal saline. The pH was adjusted to 7.0 by NaOH and normal saline was added to a total volume of 100 mL. One milliliter of this solution was mixed with 2 mL $^{99m}TcO_4^-$.

Of both preparations chromatography on paper and Biogel was performed. Gel chromatography of the peak fraction, considered as the [^{99m}Tc]MDP complex was repeated on another column of the same gel phase. Both preparations were diluted with normal saline (1:100 and 1:500) and samples were analyzed by paper chromatography.

A scintigram was performed on four rats 2 h after injection of 0.5 mL of the radiopharmaceuticals (500 μCi; 18.5 MBq) in the tail vein. Tissue distributions, at $1\frac{1}{2}$ and 3 h after injection were performed in 23 rats injected with 1.59 ± 0.03 MBq of either radiopharmaceutical.

Results

During the electrolysis, at certain time intervals 5 μL aliquots were analyzed by paper chromatography. The results at $t = 0$, $t = 60$ and $t = 90$ min are given in Fig. 1. At $t = 0$ all radioactivity was detected at $R_f = 0.6$–0.7, the R_f value of $^{99m}TcO_4^-$. At $t = 60$ min about 40% of the radioactivity was detected at R_f 0.6–0.7, and 60% at R_f 0.9. At $t = 90$ min only 20% was found at R_f 0.6–0.7 and 80% at R_f 0.9. In Table 1 the R_f values of the different ^{99m}Tc compounds are given with the peak radioactivity fractions obtained from gel chromatography. $^{99m}TcO_2$ ($R_f = 0$) was not detected.

In Figs 2 and 3 the gel chromatographic patterns of [^{99m}Tc]MDP are given at $t = 15$ and $t = 90$ min. They are identical on both columns. The peak fractions are again analyzed on paper and gel chromatography (Figs 4a and b).

Nearly all activity was detected in the same fractions (Nos 13 and 17). Dilutions of both preparations gave different results: in a dilution of 1:100, ^{99m}Tc(Sn)-MDP showed a significant amount of a hydrolyzed form of technetium. When diluted 1:500 nearly all ^{99m}Tc(Sn)-MDP disappeared and the amount of hydrolyzed ^{99m}Tc increased. In contrast [^{99m}Tc]MDP remains stable even in a dilution of 1:500.

The scintigraphic images of both preparations are comparable in quality (Figs 5a and b). A clear uptake of radioactivity in the skeleton was obtained with little uptake in the soft tissues.

The biodistribution in rats for both preparations are given in Table 2. For both preparations a clear bone uptake is observed. The uptake of ^{99m}Tc(Sn)-MDP is higher than that of [^{99m}Tc]MDP. The uptake of ^{99m}Tc(Sn)-MDP is higher in liver and spleen.

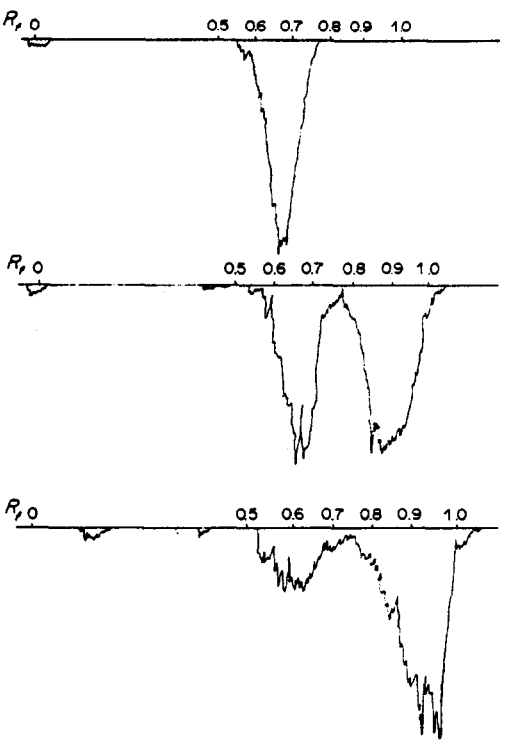

Fig. 1. Radioactivity peaks of [^{99m}Tc]MDP on scanned paper chromatograms at $t = 0$, $t = 60$ and $t = 90$ min from top to bottom respectively.

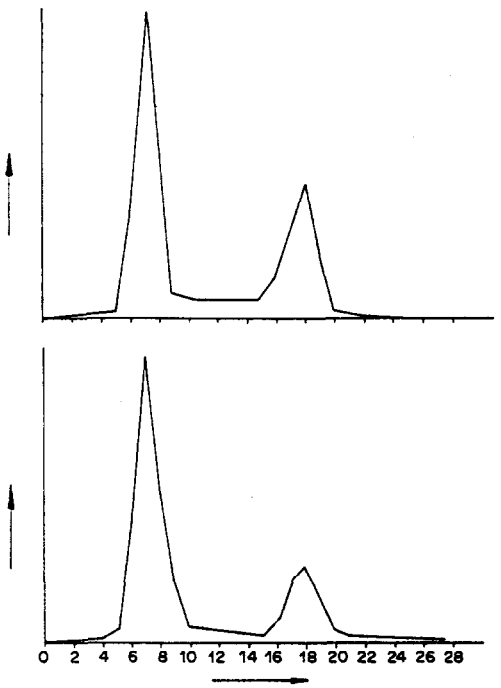

Fig. 2. Gel chromatography of [^{99m}Tc]MDP after 15 min (top) and 90 min electrolysis (bottom); column 13 × 0.9 cm, peak fraction 7 = [^{99m}Tc]MDP and fraction 18 = $^{99m}TcO_4^-$.

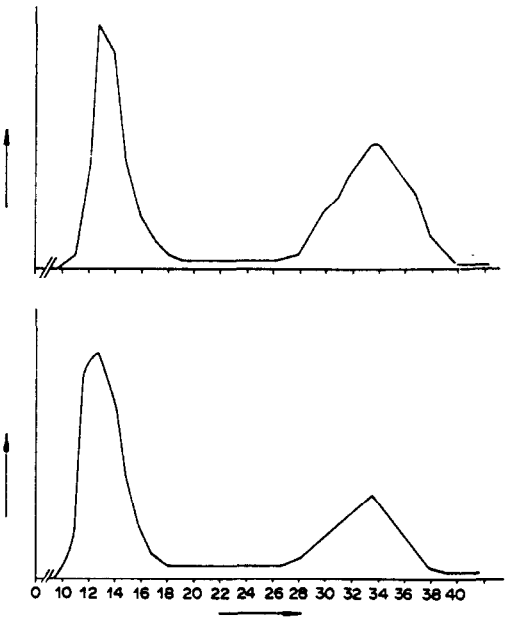

Fig. 3. Gel chromatography of [^{99m}Tc]MDP after 15 min (top) and 90 min electrolysis (bottom); column 28 × 0.9 cm, peak fraction 13 = [^{99m}Tc]MDP and fractions 32–34 = $^{99m}TcO_4^-$.

Discussion

Electrolysis of $^{99m}TcO_4^-$ solutions in a mercury pool gave only sufficient yield at electrode potentials of −1.2 V vs Ag/AgCl reference electrodes, or more negative. As already found by Russel,[9,11] the potential in this tracer experiment is 0.3–0.5 V more negative than found in most polarographic experiments at carrier concentration range. Russel found a half wave potential of 0.7 vs SCE at pH 7.8, in a solution of 0.1 M EDTA. Claessens[12] found −0.84 V vs SCE at pH 7.3 in a solution of $^{99}TcO_4^-$ and MDP (1:4). These and other data on polarographic properties of $^{99}TcO_4^-$ were obtained by use of mercury drop electrodes, with drop times of 1–5 s, with carrier concentrations of $^{99}TcO_4^-$ in the same order of magnitude as the complexing agents. Electrochemical reduction of compounds with a mercury pool electrode differs from that with a mercury drop electrode in surface area accessibility, time window, refreshment of the accessible electrode surface and adsorption of parent compounds and reaction products.

Table 1. R_f values of the different ^{99m}Tc-compounds by paper chromatography. The peak fractions eluated from the gel columns (13 × 0.9 cm and 28 × 0.9 cm) are also given; hydrolyzed ^{99m}Tc is not eluated from these columns

Components	R_f value	Peak fraction 13 × 0.9 cm	Peak fraction 28 × 0.9 cm
Hydrolyzed ^{99m}Tc	0	—	—
$^{99m}TcO_4^-$	0.6–0.7	18	34
^{99m}Tc(Sn)-MDP	1.0	6	10
[^{99m}Tc]MDP	0.9	7	13

Furthermore, during the prolonged coulometric experiments, traces of oxygen have more time to interfere with the electrode-process and may influence the reversibility of the redox reactions. This may occasionally lead to high activation energies, expressed as half-wave potentials, as shown by the TcO_4^- reduction.

The evaluation of the electrolysis products is possible with paper chromatography. Using sodium acetate (pH 5) as the solvent, a good separation between $^{99m}TcO_4^-$ and [^{99m}Tc]MDP can be achieved with hydrolyzed technetium remaining at the start position.[13] The R_f value of [^{99m}Tc]MDP differs slightly from the R_f value of ^{99m}Tc(Sn)-MDP (0.9 vs 1.0). After 90 min of electrolysis about 80% of the radioactivity is concentrated at R_f 0.9. No hydrolyzed technetium is detected by then.

With gel chromatography $^{99m}TcO_4^-$ and [^{99m}Tc]MDP are very well separated.[14–16] There is only one obvious [^{99m}Tc]MDP peak with both columns used. Repeated gel chromatography of the peak revealed no obvious pertechnetate or hydrolyzed technetium (as established by paper chromatography). Again the peaks of ^{99m}Tc(Sn)-MDP differ slightly from the [^{99m}Tc]MDP peaks (fraction 6 vs fraction 7 and fraction 10 vs fraction 13 on the respective columns). The [^{99m}Tc]MDP complex was *in vitro* more stable to dilution than the ^{99m}Tc(Sn)-MDP complex. Schümichen[17,18] established a fast transformation in a hydrolyzed technetium compound after dilution of [^{99m}Tc]pyrophosphate and [^{99m}Tc]-HEDP with neutral saline, especially with concerning [^{99m}Tc]pyrophosphate. For ^{99m}Tc-complexes prepared

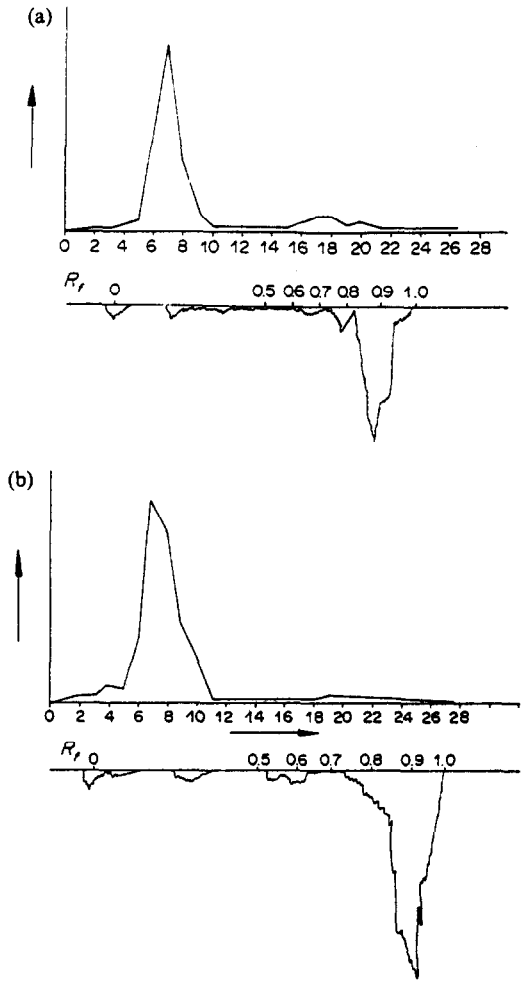

Fig. 4. Gel and paper chromatograms of peak fraction 7 as described (a) in Fig. 2 and of peak fraction 13 as described (b) in Fig. 3; column 13 × 0.9 cm, there is only one obvious peak, considered as [^{99m}Tc]MDP.

without reducing agents a higher stability against air oxidation has been suggested.[10] The *in vivo* uptake as evaluated on the scintigrams is comparable in quality for both preparations. However, the biodistributions show more bone uptake for the ^{99m}Tc(Sn)-MDP complex, whereas [^{99m}Tc]MDP shows more uptake in the gastric wall. This may be caused by a reoxidation to $^{99m}TcO_4^-$ *in vivo*. Russel[9,11] proved by means of reversed pulse polarography under certain conditions that a reoxidation of reduced technetium is possible *in vitro*. The observations of Russel in his *in vitro* experiment and our *in vivo* results are in agreement so far.

In conclusion, it is possible to prepare a [^{99m}Tc]MDP complex by electrolytical reduction of technetium pertechnetate in presence of excess MDP. The chromatographic properties of this complex on paper and gel chromatography are slightly different from ^{99m}Tc(Sn)-MDP. The bone-seeking properties of both complexes are comparable. Tin is not necessary for bone uptake. The stability of [^{99m}Tc]MDP to dilution *in vitro* is higher, but *in vivo* a reoxidation to pertechnetate may occur to some extent.

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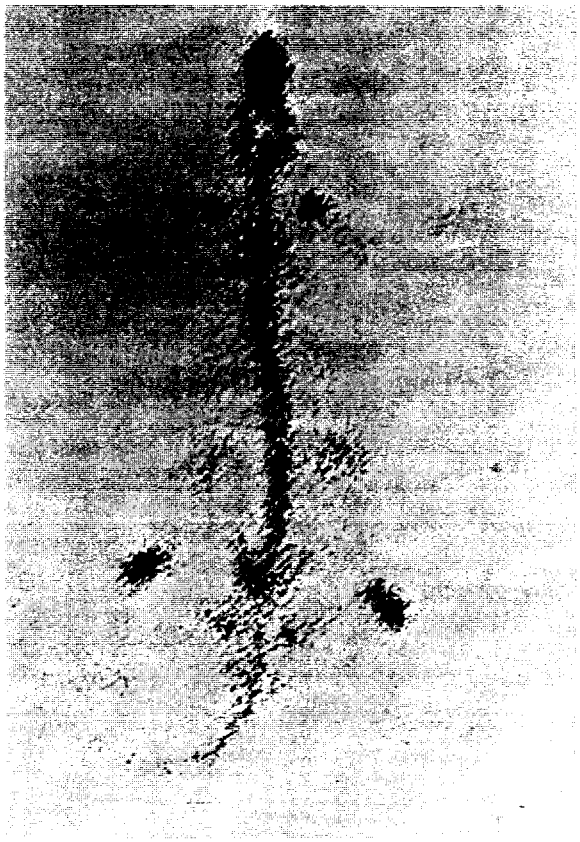
Table 2. Biological distribution of [^{99m}Tc]MDP and ^{99m}Tc(Sn)-MDP in rats

	[^{99m}Tc]MDP (peak fraction 13, Fig. 3) (% dose/g tissue)		^{99m}Tc(Sn)-MDP (% dose/g tissue)	
	1½ h after dose $N = 7$	3 h after dose $N = 6$	1½ h after dose $N = 5$	3 h after dose $N = 5$
Femur	1.99 ± 0.12	1.63 ± 0.31	3.49 ± 0.71	3.24 ± 0.50
Femur muscle	0.03 ± 0.00	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.02
Blood	1.16 ± 0.02	0.09 ± 0.01	0.04 ± 0.01	0.02 ± 0.00
Liver	0.25 ± 0.05	0.21 ± 0.07	0.51 ± 0.08	0.39 ± 0.10
Spleen	0.11 ± 0.04	0.06 ± 0.03	0.45 ± 0.10	0.52 ± 0.20
Kidney	0.35 ± 0.03	0.33 ± 0.04	0.50 ± 0.14	0.39 ± 0.04
Gastric wall	0.76 ± 0.25	0.34 ± 0.09	0.02 ± 0.01	0.02 ± 0.00

(a)



(b)

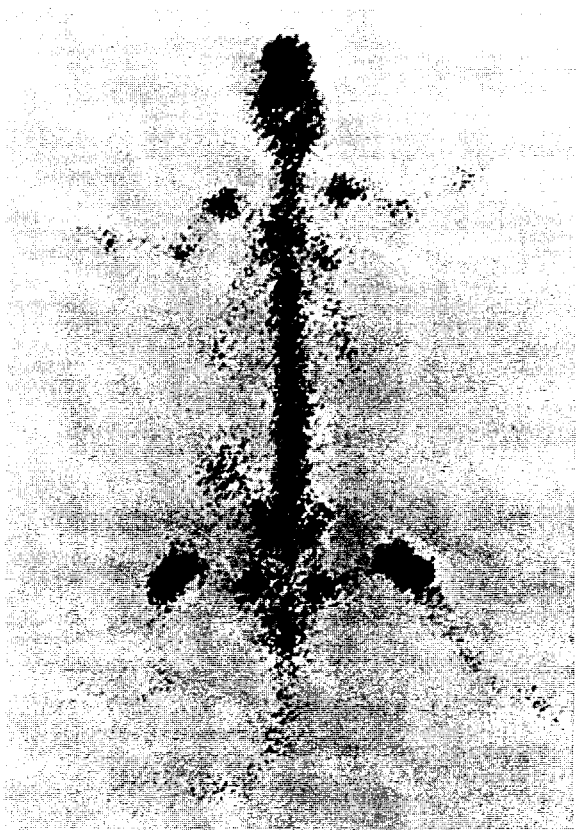


Fig. 5. Scintigram of a rat 2 h after injection of [^{99m}Tc]MDP, peak fraction 13 as described (a) in Fig. 3 and (b) after injection of ^{99m}Tc(Sn)-MDP.