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Research Article

Preparation of [^{99m}Tc]TRODAT-1 involving a simple purification method

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Summary

Direct preparation of the novel dopamine transporter imaging agent [^{99m}Tc]TRODAT-1 results in uncertainty concerning the labelling efficiency. Without purification of the tracer, the statistically inefficient labelling yield becomes apparent in an inadequate radiochemical purity. The aim of the present work was to improve the radiochemical purity of this tracer by including a facile, on-the-spot purification step in the labelling process.

Kits containing 100 μg free ligand resulted in an average labelling efficiency of 91.6% (S.D. = 5.15%, n = 32), while an average of 73.0% (S.D. = 6.76%, n = 22) was obtained for the 50 μg ligand series. Introduction of solid-phase extraction into the preparation process allowed the quantitative removal of hydrophilic species from both series of reaction mixtures. An average radiochemical purity of 98% was achieved for the purified tracer.

Key Words: technetium-99 m; TRODAT-1 solid-phase extraction; kit preparation dopamine transporters; SPECT

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Introduction

The novel ^{99m}Tc-labelled tropane derivative [^{99m}Tc]TRODAT-1 was introduced and characterized by Meegalla *et al.*[1] This safe radiotracer for the imaging of presynaptic dopamine transporters[2–5] first applied clinically was obtained in a multistep preparation.[6] The labelling was recently improved by the development of a kit formulation containing the lyophilized 'cold' components in a single vial.[7] One way to obtain a reliably high radiochemical purity is to subject the complexes formed to purification on a reverse-phase, semipreparative HPLC column, as recommended by Meegalla *et al.*[1] While this purification step provided the complex of [^{99m}Tc]TRODAT-1 free from significant radiochemical impurities, it was necessary to reformulate the purified product so as to satisfy the pharmaceutical requirements.[1]

The aim of the present work was to combine the favourable kit labelling procedure with a simple purification technique of solid-phase extraction ensuring high ($>95\%$) and reliable radiochemical purity and eliminating the need for complicated and time-consuming pharmaceutical reformulation.

Results and discussion

All of the unpurified reaction mixtures resulted in three different peaks in the recorded HPLC radiochromatograms. The retention times were around 0.6 min for peak 1, 1.0 min for peak 2 and 4.0 min for peak 3. The correlation between the ^{99m}Tc activity used for labelling and the ratios of the different peaks was analysed for both series of kits (50 μg of ligand: $n = 30$, 100 μg of ligand $n = 38$; Figure 1).

If data exceeding the experienced critical activities (1.5 GBq for 50 μg, and 2.5 GBq for 100 μg) were omitted, the main peak (peak 3) still exhibited a high statistical fluctuation for both series (peak 3 = 72.99% ± 6.76%: 50 μg of ligand, $n = 22$; peak 3 = 91.58% ± 5.15%: 100 μg of ligand, $n = 32$). Even for the higher ligand-containing series of kits, where the average of peak 3 was greater than 90%, these fluctuations resulted in a ratio of peak 3 fall of 90% in 10 cases out of 32.

Peak 1 is supposed to be co-elution of different hydrophilic compounds (free pertechnetate, and Tc complexes with glucoheptonate and EDTA), while peak 2 might be due to a decomposition product of

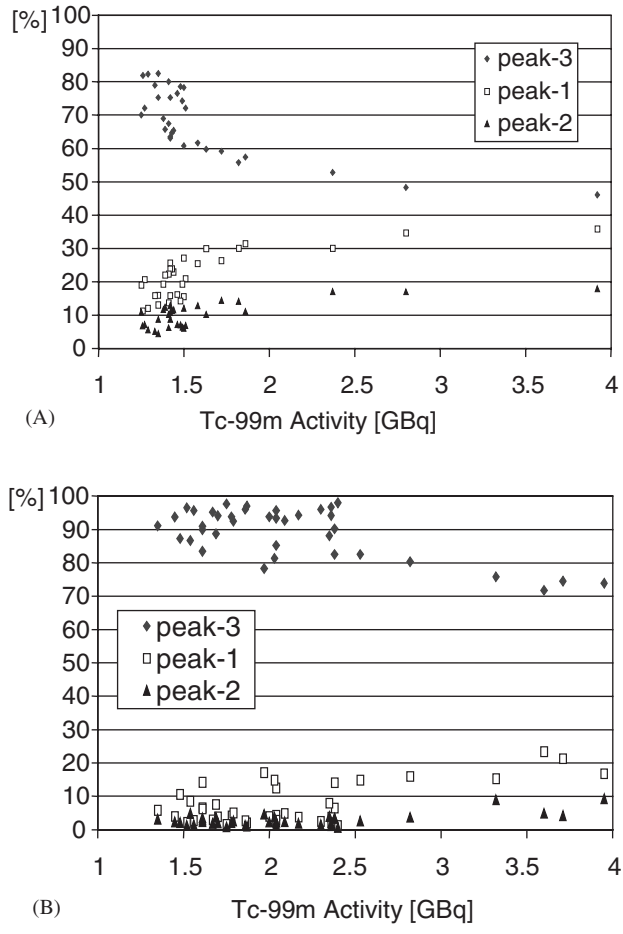

Figure 1. Ratios of different radiochemical species identified by HPLC in the crude reaction mixtures formed after incubation of two series of kits with [^{99m}Tc]pertechnetate. A: 50 µg of ligand, B: 100 µg of ligand. Peak 1: hydrophilic species of ^{99m}Tc; peak 2: decomposed complex; peak 3: complex of [^{99m}Tc]TRODAT-1

[^{99m}Tc]TRODAT-1 in which the $^{99m}TcO^{3+}$ core is presumably still in bisaminebistiol N_2S_2 co-ordination. Peak 3 is considered to be a mixture of two diastereomers formed during the co-ordination of $^{99m}TcO^{3+}$ by the ligand TRODAT-1.[8]

The efficiency of formation of the complex [^{99m}Tc]TRODAT-1 was extremely sensitive to the specific activity of the starting [^{99m}Tc]pertechnetate solution used for labelling. The molar ratio of ^{99}Tc should be kept as low as achievable (utilizing a frequently eluted generator), but

the total amount of pertechnetate species ($^{99m}Tc + {}^{99}Tc$) in the reaction mixture was also found to influence efficient complex formation. This observation could be explained in that the molar ratio of the available complex-forming ligand (which is limited by avoiding any undesirable pharmacological effect of the free ligand TRODAT-1 itself) was far lower ($< 10^{-6}$ M) than is common in conventional ^{99m}Tc-kits (10^{-3}–10^{-5} M). Based on our results high labelling efficiency can be reached only, if TRODAT-1 is present in an excess of at least 600 times as compared to the molarity of pertechnetate (3.5×10^{-10} M: calculated from the maximal applicable ^{99m}Tc activity of 2.5 GBq, eluted daily basis from generator) in the reaction mixture.

The next set of experiments was aimed at purification of the crude complex on a C-18 SepPak Light cartridge. When the reaction mixture was applied onto a small, C-18 reverse-phase cartridge, the bulk of the activity was retained by the solid phase. After washing with saline, 83.7–98.8% of the applied activity (< 2.5 GBq, $n = 32$) was stacked on the solid phase for the higher ligand-containing series, and 76.5–89.7% (< 1.5 GBq, $n = 22$) for the lower ligand-containing series. On average, 19.7% and 18.2% of the activity was eluted by the first fraction of the 1:1 ethanol/saline mixture for the higher and for the lower ligand-containing series, respectively. Approximately 50% of the total activity was removed by the second fraction of the 1:1 ethanol/saline mixture for both series. The HPLC analysis of the different fractions indicated that $> 95\%$ of the activity of the reaction mixture collected after passage through the C-18 SepPak cartridge was associated with peak 1, while the first fraction of the 1:1 ethanol/saline mixture showed the presence of peak 2. In the second fraction of the 1:1 ethanol/saline mixture eluted from the cartridge, $> 95\%$ of the activity corresponded to peak 3. The ratio of impurity peak 2 lay in the range from 1–4% (the purer the reaction mixture was, the lower the amount of peak 2 measured in this fraction). Peak 1 was removed from all these samples almost completely ($< 1\%$). A simple saline dilution and sterile filtration resulted in the tracer in a ready-to-inject form, offering the advantage that these steps are easy to perform under aseptic conditions.

In order to elute approximately 70% of the trapped [^{99m}Tc]TRODAT-1 from the SPE cartridge, 1 ml of the 1:1 ethanol/saline mixture was sufficient. In a typical experiment (starting with 2.0 GBq of [^{99m}Tc]pertechnetate), 800 MBq of [^{99m}Tc]TRODAT-1 was collected. Investigation of the stability of such samples during 24 h indicated a slow degradation of [^{99m}Tc]TRODAT-1 into a complex with moderate

lipophilicity, but no conversion into hydrophilic species. Dilution of [^{99m}Tc]TRODAT-1 with saline resulted in a solution stable during 6 h in a pharmacologically safe form containing $<5\%$ ethanol.

The results of the biodistribution studies revealed a moderate brain uptake ($0.12\% \pm 0.05\%$ dose/organ) 60 min after injection. The striatal region exhibited the highest uptake within the brain ($0.092\% \pm 0.038\%$ dose per gram of striatum) with a striatum to cerebellum ratio of 2.21. All of the uptakes by the different organs in rats were in agreement with the published data.[1]

Experimental

The synthesis of free TRODAT-1 was performed in-house by a combination of different literature methods.[1, 9–11] The purity of the last intermediate 2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3.2.1]-oct-2-yl]methyl][[*S*-(4-methoxybenzyl)thio]ethyl]amino]ethyl]amino]-*S*-(4-methoxybenzyl)ethanethiol was checked by ^{1}H-NMR (recorded in $CDCl_3$ on a Bruker Avance DRX 400 spectrometer at 400 MHz), IR and MS spectroscopy: all the analytical and physical data were similar to those for the literature compound.

Two series of one-vial kits were prepared aseptically by using 100 or 50 μg of free TRODAT-1 as ligand (0.224 and 0.112 μM respectively). The other ingredients of each vial for both series were the same: 0.136 mg of tin(II) chloride dihydrate, 0.20 mg of sodium glucoheptonate, 0.93 mg of EDTA disodium salt, 1.79 mg of sodium hydrogenphosphate, 1.87 mg of sodium dihydrogenphosphate monohydrate, 3.5 mg of sodium chloride and 20 mg of D-mannitol. The preparation of the solutions, and the filling and freeze-drying of the vials for the experimental kits were carried out in a clean environment. The chemical forms of ^{99m}Tc in the crude reaction mixture obtained after incubation were analysed by reverse-phase HPLC.[1] In 22 cases for the 50-μg TRODAT-1 series and in 32 cases for the 100-μg series, the reaction mixtures were applied to a preconditioned C18-SepPak Light cartridge (Waters Corporation). In these experiments, the solid phase was washed with 15 ml of saline followed by 0.3–0.5 ml of 1:1 ethanol/saline. The bulk of the radioactivity was eluted from the cartridge with 1 ml of 1:1 ethanol/saline and was diluted with 10 ml of sterile saline, followed by sterile filtration (Millipore, MILX GP 33 mm) into a sterile evacuated vial.

For *in vivo* biodistribution studies, male Wistar rats (280 g ± 13 g, $n = 9$) were used. Under urethane anaesthesia, 2–4 MBq of [^{99m}Tc]TRODAT-1 solution was injected directly into the femoral vein of rats, and 60 min later the animals were sacrificed. The organs of interest were removed, weighed and homogenized in hot 40% sodium hydroxide solution. The percentage dose per organ was calculated via a comparison of the tissue counts to those on suitably diluted aliquots of the injected material.

Conclusions

Utilization of a 'one-vial cold kit' containing 100 μg of ligand and fast SPE cartridge purification eliminates the uncertainty in labelling efficiency and ensures high and reliable radiochemical purity for the [^{99m}Tc]TRODAT-1. The facile nature of the extra purification steps in an aseptic environment with the aid of single-use items favours the applicability of this method in routine nuclear medicine practice.

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