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Cyclopentadienyl tricarbonyl complexes of ^{99m}Tc for the in vivo imaging of the serotonin 5-HT_{1A} receptor in the brain

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Abstract

Technetium and rhenium tricarbonyl complexes with derivatized cyclopentadienyl ligands were prepared starting from pertechnetate and an appropriate ferrocene ligand. Furthermore, the complexes ($M(CO)_3$cp-$COOC_5H_9N$-R, M = Tc, Re; R = Me, isopropyl) could be obtained starting from the precursor complexes $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and $[Re(CO)_3Br_3]^{2-}$. Their chemical identity was confirmed by chromatographic methods and electron spray mass spectrometry. The biodistribution of the ^{99m}Tc complexes (cytectrene I and cytectrene II) in Wistar rats was studied. Both compounds show high uptake in the brain and fast blood clearance. The pattern of regional distribution in the brain demonstrated in autoradiographic studies indicates binding to the 5-HT_{1A} and α_1 adrenergic receptors.

Keywords: Cyclopentadienyl tricarbonyl complexes; Receptor affinity; Brain uptake

1. Introduction

Due to the optimal radiation properties of ^{99m}Tc (pure γ-emitter, $E_\gamma = 140$ keV, half-life 6 h) there is a considerable interest in the development of ^{99m}Tc radiopharmaceuticals for imaging serotonergic CNS receptors using single photon emission tomography (SPET). The wide interest in the 5-HT_{1A} receptor is due to its implicated role in several major neuropsychiatric disorders such as depression, eating disorders or anxiety. For the diagnosis of these pathophysiological processes it is important to develop radioligands for the binding on the 5-HT_{1A} receptor in vivo [1].

To date most Tc compounds assigned as CNS receptor-targeted agents are square-pyramidal complexes of the oxo core $[Tc{=}O]^{3+}$ [2,3]. However, the in vivo behaviour of such complexes may be influenced by the Tc=O unit offering a free position *trans* to the oxo ligand for further reaction in vivo as observed especially for the '3+1' complexes [4,5]. To reduce this in vivo reactivity, we consider oxo-free Tc complexes containing the metal in lower oxidation states to be interesting alternatives. In fact, bioorganometallic Tc(I) and Tc(III) complexes described so far show excellent affinities to the target receptor but suffer from insufficient brain uptake [6–8].

We have recently described new Tc cyclopentadienyl derivatives (cytectrene I and cytectrene II) which show high affinity to the target receptor as well as high brain uptake in rats 20 min after i.v. application [9,10]. The goal of the present work was to elucidate the structure of the cytectrene complexes prepared by the method of

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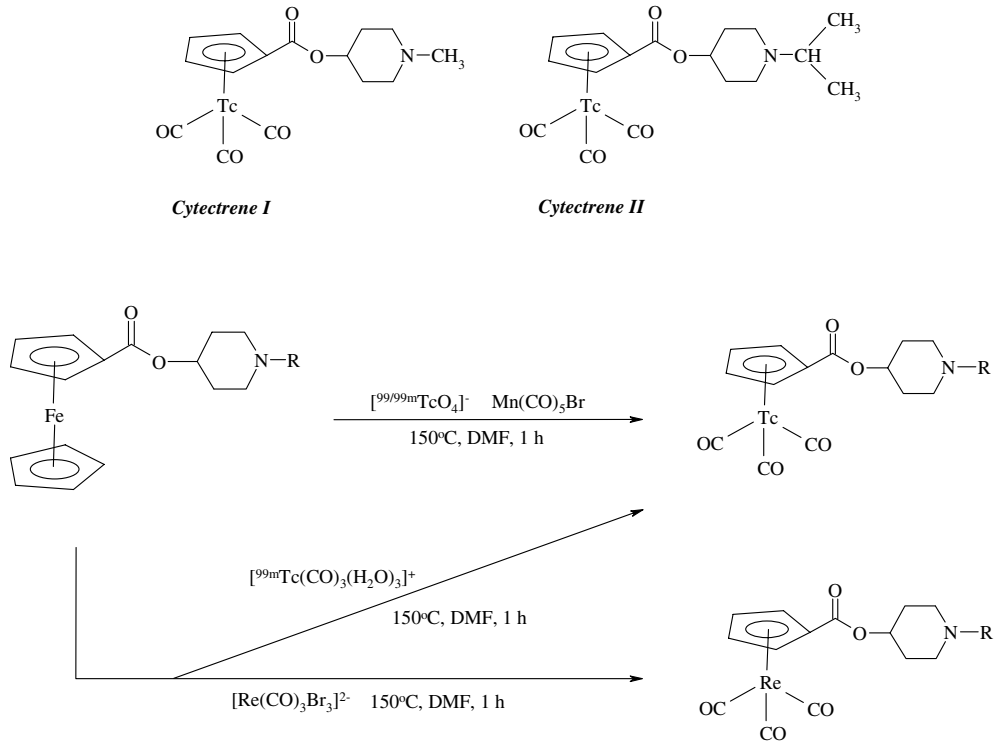

Fig. 1. Structures and reaction scheme for the preparation of complexes ^{99m}Tc cytectrene I and ^{99m}Tc cytectrene II.

Wenzel and Saidi [11,12]. For that reason, carrier added Tc preparations were performed and the molecular mass of the product was determined. The composition of the Tc preparations was confirmed by comparison of their chromatographic data with those of ^{99m}Tc and cold Re preparations following the tricarbonyl concept of Alberto et al. [13] (Fig. 1).

2. Experimental

2.1. Synthesis

Caution. ^{99}Tc is a weak β-emitter (E_{max} = 292 keV, $T_{1/2} = 2.12 \times 10^5$ a). Shielding is not necessary when small amounts are used as applied for this work, since the β-particles do not penetrate glass walls and secondary X-rays (*bremsstrahlung*) do not play a role using that small amounts of ^{99}Tc. However, special care in the manipulation of ^{99}Tc samples is required to avoid contamination and incorporation.

$[Et_4N]_2[Re(CO)_3Br_3]$ was prepared as described in [14]. The ligand *N*-methylpiperidino-4[(bispentahaptocyclopentadienyl)iron] carboxylate was synthesized according to the reported procedure [9]. $Mn(CO)_5Br$ was prepared by brominating $Mn_2(CO)_{10}$ in hexane [15]. Sodium pertechnetate was obtained from a commercial ^{99}Mo/^{99m}Tc generator (Mallinckrodt). All other chemicals and solvents were of reagent grade and used in unmodified form.

2.1.1. $[^{99m}Tc(CO)_3(H_2O)_3]^+$

To the 10 ml sealed vial containing the following lyophilized formulation under nitrogen: (8.5 mg of sodium tartrate, 2.85 mg of sodium tetraborate, 7.15 mg of sodium carbonate and 4.5 mg of sodium boranocarbonate), 1ml of $^{99m}TcO_4^-$ from a commercial generator (200 MBq) was added. The vial was placed in a boiling water bath for 30 min. Yield >95%.

2.1.2. $[^{99/99m}Tc(CO)_3(L\ I)]$ (cytectrene I)

(a) The ligand L I (*N*-methylpiperidino-4[(bispentahaptocyclopentadienyl)iron] carboxylate) (1 mg) and $Mn(CO)_5Br$ (1 mg) were dissolved in 0.15 ml of dimethyl formamide in a 10 ml glass tube. 0.1 ml $^{99m}TcO_4^-$ (20 MBq) in saline and 20 μl 0.005M $^{99}TcO_4^-$ for carrier added (c.a.) preparations were added. The reaction mixture was purged with nitrogen and the tube was closed and heated at 150 °C in an oil bath. After 1 h, the reaction mixture was cooled to room temperature. The sample was passed through a C_{18} Sep-Pak column. The first fraction eluted with a mixture of 20% ethanol and 80% H_2O was discarded and the second fraction eluted with absolute ethanol was collected. Quality control was performed by TLC on silica gel in ether/diethylamine 95:5 v/v. R_f = 0.59. Yield: 85–90%.

(b) 100 μl (20 MBq) of the precursor complex $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was acidified with 50 μl of 1 N HCl and than added to a vial containing 1 mg of *N*-methylpiperidino-4[(bispentahaptocyclopentadienyl)-iron] carboxylate dissolved in 150 μl of dimethyl forma-

mide. The mixture was purged with nitrogen and the vial was closed and heated in an oil bath at 150 °C for 1 h. Yield: 60–70%.

For purification the reaction mixtures were loaded onto a semi-preparative PRP-1 column and eluted in a linear gradient system of acetonitrile with 0.1%TFA (A) and water with 0.1%TFA (B) in 20 min from 20% to 50% (A) at a flow rate of 2 ml/min. The fraction corresponding to the desired complex ($R_t = 10$ min) was collected, the solvent was removed by vacuum evaporation and the sample was reanalysed by HPLC. ESI^+-MS: *m/z* 389.9 $[^{99}Tc(CO)_3(L\ I)]^+$.

2.1.3. [Re(CO)$_3$(L I)]

$[Et_4N]_2[Re(CO)_3Br_3]$ (3 mg) and *N*-methylpiperidino-4 [(bispentahaptocyclopentadienyl)iron] carboxylate (3 mg) were dissolved in 0.3 ml of dimethyl formamide in a 10-ml glass tube and 0.2 ml of 0.1 N HCl was added. The reaction mixture was purged with nitrogen and the vial was closed and heated at 150 °C in an oil bath. After 1 h, the reaction mixture was cooled to room temperature and the reaction product was characterized by HPLC.

For separation of by-products and ligand excess, the entire reaction mixture was diluted in 0.5 ml of 0.1% TFA, filtered, loaded onto a semi-preparative PRP-1 column and eluted in the same linear gradient system as above. ESI^+-MS: *m/z* 476, 478 $[Re(CO)_3(L\ I)]^+$.

2.2. Analytical methods

HPLC and TLC analyses were performed to determine the radiochemical purity and stability of the preparations and to confirm the identity of non carrier added (n.c.a.) preparations with c.a. and rhenium preparations. For HPLC studies a Perkin–Elmer device was used consisting of a Turbo LC System with a quaternary pump (Series 200 LC Pump), a Programmable Absorbance Detector Model 785A and a γ-ray detector (Bohrloch, NaI(Tl) crystal). HPLC analyses were carried out with a PRP-1 column (250×4.1 mm) using a gradient eluant of acetonitrile (A) with 0.1% TFA/water (B) with 0.1% TFA, gradient elution: 0–5 min 20–50% A, 5–15 min 50% A, 15–17 min 50–20% A and a flow rate of 1.0 ml/min. The effluent from the columns was monitored by UV absorbance at 254 nm for ^{99}Tc or Re reference complexes or γ-ray detection for the ^{99m}Tc complexes. For separation of 0.5 ml samples of Tc/Re complexes a semi-preparative PRP-1 column (305×7 mm, 10 μm, flow rate 2 ml/min) was used. After separation of the desired complex fraction, the acetonitrile was removed by vacuum evaporation. TLC analyses were performed on silica gel in ether/diethylamine 95:5 v/v. Mass spectrometric analyses were carried out on a Micromass Tandem Quadrupole Mass Spectrometer (Quattro LC) operating in the MS mode. Mass spectral data were recorded at the negative and positive ESI mode using a cone voltage of 20 or 60 V.

2.3. Biological studies

The biodistribution studies were carried out according to the relevant national regulations using male Wistar rats (5–6 weeks old). 0.5 ml of the ^{99m}Tc complex solution (0.5 MBq) was injected into the tail vein under slight ether anesthesia. After the injection, the rats were sacrificed by heart puncture 5, 20, 40 and 60 min post injection (p.i.). Selected organs were isolated for weighing and counting. The activity concentration in the tissue of organs was calculated in terms of percentage of injected total activity (dose) per organ.

For ex vivo autoradiography studies male rats (weight 118–134 g) were injected into the tail vein with 0.5 ml of a saline solution containing 24–30 MBq ^{99m}Tc cytectrene. Twenty minutes after injection the animals were sacrificed by ether anesthesia. The brains were rapidly removed and immediately frozen by immersion in isopentane/dry ice solution at −50 °C. Before cutting, the frozen brains were weighed and the radioactivity concentration was determined using an automated gamma counter. Then the stage mounted (embedded in tissue freezing medium, Reichert/Jung) brains were cutted in a cryocut microtome (CM 1850, Leica) into 20 μm horizontal sections, thaw mounted on microscope slides and dried under a continous air stream for about 2 min. The blocking studies were carried out by pre-treating the rat with (*R*-(+)-8-OH-DPAT (5 mg/kg i.v.) or prazosin hydrochloride (5 mg/kg i.v.) 10 min prior to the tracer injection. For autoradiography the slides containing the brain sections were apposed to imaging plates in an autoradiographic cassette for approximately 20 h and scanned in the bio-imaging analyser BAS 2000 and BAS 5000 (Fuji Photo Film Co., Tokyo). The quantitative analysis of the in vivo distribution was carried out with the AIDA program (Raytest, Straubenhardt).

3. Results and discussion

In an effort to develop new organometallic precursors for radiopharmaceutical applications, Alberto and coworkers have found a convenient route to prepare the important intermediate $[^{99m}Tc(CO)_3(H_2O)_3]^+$ which is water soluble and undergoes ligand exchange reactions with nitrogen and oxygen donor atoms-containing ligands as well as isonitriles or the cyclopentadienyl moiety. To elucidate the structure of the ^{99m}Tc cytectrene complexes, c.a. technetium preparations following the preparation route of Wenzel and n.c.a. preparations using the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor complex were performed with the methyl derivative of the ferrocene ligand. Additionally, cold rhenium preparations were

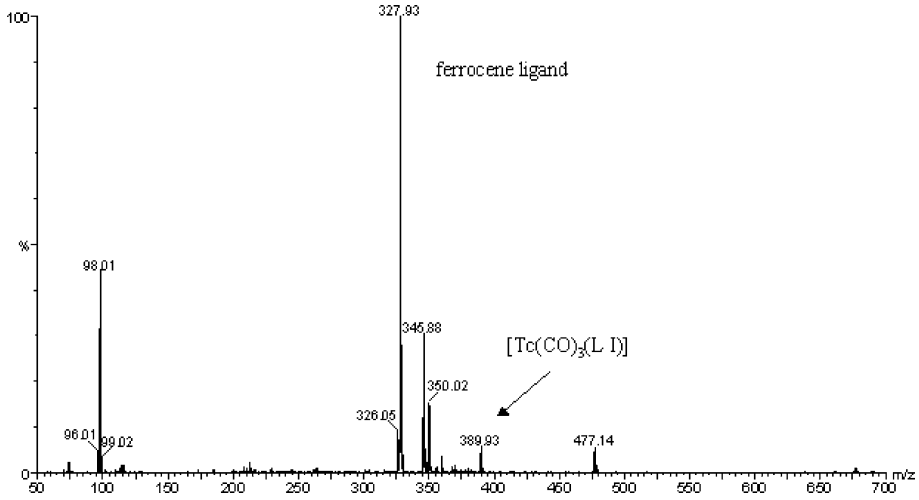

Fig. 2. ESI$^+$-MS of the c.a. $^{99/99m}$Tc preparation.

performed starting from the precursor complex $(NEt_4)_2[Re(CO)_3Br_3]$.

The chromatographic behaviour of preparation products was compared and electron spray mass spectrometry (ES-MS) analysis was carried out to determine the composition of final products. The preparation of the cytectrene I complex following the method of Wenzel leads to yields between 70% and 80% determined by TLC. The radiochemical purity of the Tc complex preparation following HPLC separation was >98%. The yield of the isolated fraction was 40–60% of the starting activity. The cytectrene complex fraction is well separated from non-radioactive substances using the described HPLC conditions. That means, that the specific activity and the receptor binding of the separated ^{99m}Tc complex is not influenced by other substances. It is possible to prepare the c.a. $^{99/99m}$Tc complex in similar yields adding 10^{-7} mol $^{99}TcO_4^-$ to the reaction mixture. The mass spectrum shows the expected molecular mass of 390 (Fig. 2). It could be shown by comparison of the chromatographic data that the same tricarbonyl complex is formed when the precursor complex $[^{99m}Tc(CO)_3(H_2O)_3]^+$, prepared by using a tricarbonyl kit formulation, reacts in acidic solution with 1.0 mg of the ferrocene ligand.

In accordance with this result, the reaction of the rhenium tricarbonyl precursor complex $[Et_4N]_2[Re(CO)_3Br_3]$ with the ferrocene ligand also leads to the tricarbonyl cyclopentadienyl complex as demonstrated by the mass spectrum in Fig. 3 (m/z = 476/478).

After confirmation of the composition of the cytectrene complexes their biodistribution in Wistar rats was studied. Moreover, the distribution of cytectrene I in rat brain regions was investigated autoradiographically and compared with that of cytectrene II. The biodistribution of both cytectrene complexes was determined some years ago [9]. At this time the structure of complexes was not confirmed. Now we controlled the brain uptake of cytectrene I prepared according to Wenzel and from a carbonyl kit. The biodistribution of cytect-

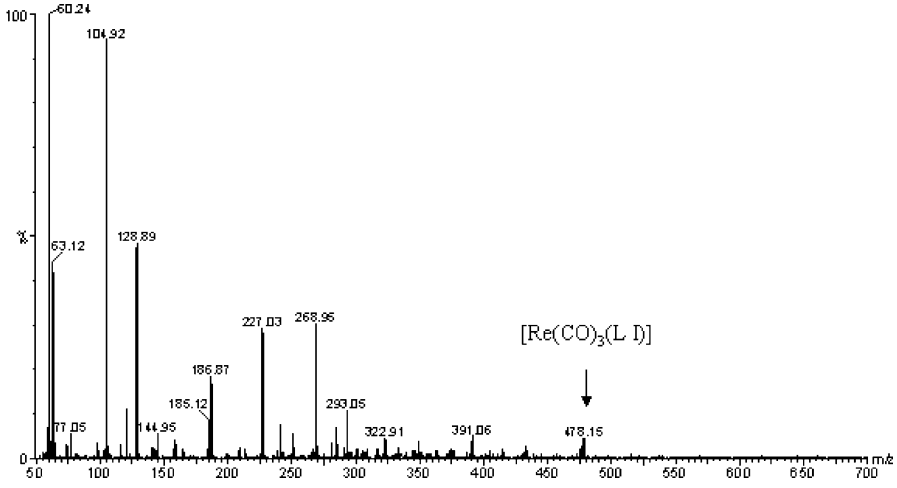

Fig. 3. ESI$^+$-MS of the Re preparation.

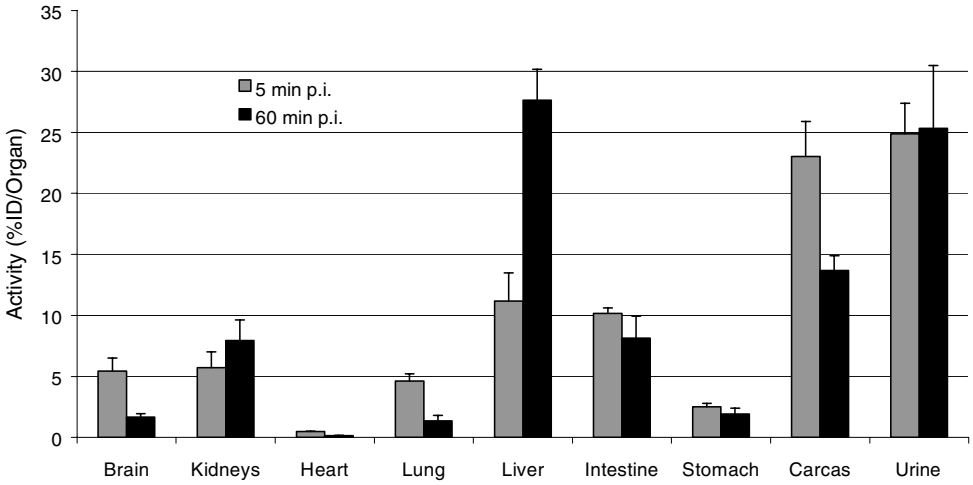

Fig. 4. Biodistribution and elimination of Tc cytectrene I in the rat (mean ± SD; $n = 4$).

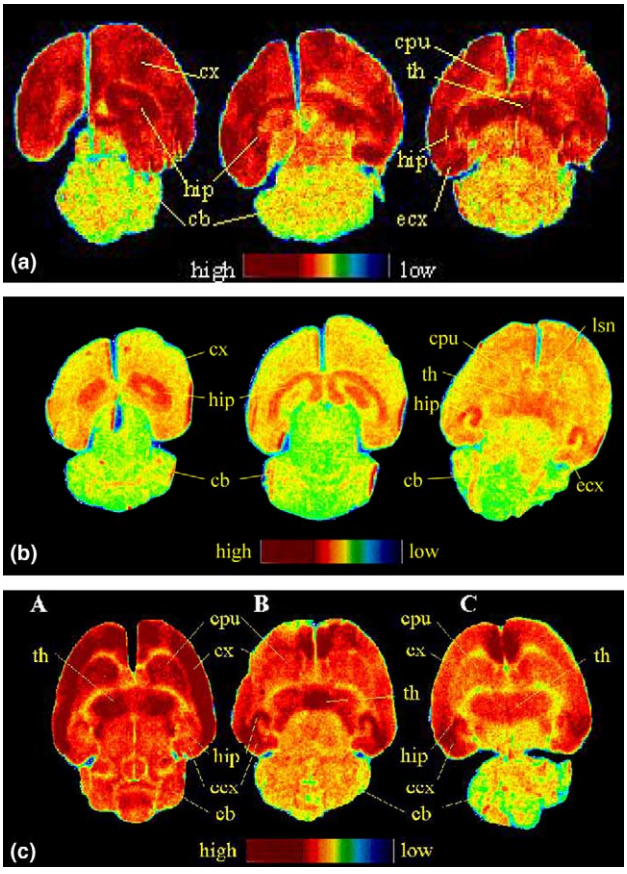

Fig. 5. (a) Ex vivo autoradiograms of rat brain in horizontal section levels and radioactivity standard 20 min after i.v. application of the ^{99m}Tc cytectrene I complex. Hip = hippocampus, cx = frontal cortex, th = thalamus, ecx = entorhinal cortex, cpu = caudate-putamen, cb = cerebellum. (b) and (c) Ex vivo autoradiograms of rat brain in horizontal section levels and radioactivity standards 20 min after i.v. application of the ^{99m}Tc cytectrene II complex illustrating distribution without pretreatment (b) and after pretreatment (c) with (*R*)-(+)-8-OH-DPAT (A) isotonic saline (B) and with prazosin hydrochloride (C). Hip = hippocampus, cx = frontal cortex, lsn = lateral septal nucleus, th = thalamus, ecx = entorhinal cortex, cpu = caudate-putamen, cb = cerebellum.

rene I is shown in Fig. 4. Cytectrene I is eliminated through the liver and kidneys as described in [9] and also the brain uptake is comparable.

The autoradiograms of rat brain (Figs. 5(a)–(c)) show an enrichment of activity in brain areas which are rich in 5-HT_{1A} receptors, such as hippocampus and entorhinal cortex as well as in regions for α_1 adrenergic receptors, such as thalamus, cortex and cerebellum. The ^{99m}Tc cytectrene I complex exhibits a higher nonspecific binding than cytectrene II. In contrast with cytectrene I, cytectrene II seems to be more differentiated. The ^{99m}Tc complex exhibits a high brain uptake (1.02% ID/g brain) 20 min after i.v. application. The activity is accumulated in brain areas which are rich in 5-HT_{1A} receptors such as hippocampus (1.27% ID/g) and entorhinal cortex (1.35% ID/g). The ratio hippocampus to cerebellum amounts to 1.5 (Figs. 5(b) and (c)) [10]. The enrichment can be blocked in brain by 5-HT_{1A} receptor agonist (*R*)-(+)-8-OH-DPAT. Besides the binding to the 5-HT_{1A} receptor, in the autoradiograms occurs a second binding site in the thalamus, cortex and cerebellum region. Prazosin hydrochloride, a selective α_1 adrenergic receptor antagonist, to some extent blocks this binding.

In the present study we could show that ^{99m}Tc tricarbonyl complexes with cyclopentadienyl derivatives fulfill the requirements of potential radiopharmaceuticals for imaging brain receptors. They are able to cross the blood–brain barrier and show a significant receptor affinity.

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