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Biological evaluation of a technetium-99m-labeled integrated tropane-BAT and its piperidine congener as potential dopamine transporter imaging agents

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Abstract

Introduction: Recently, we have reported modification of ^{99m}Tc-TRODAT-1 by integrating the N2S2 metal chelating unit and the tropane skeleton. Results of a preliminary biodistribution study in rats were promising with respect to brain uptake. The present report deals with the further biological characterization of the ^{99m}Tc-labelled integrated TRODAT derivatives (^{99m}Tc-TropaBAT and ^{99m}Tc-norchloro-TropaBAT) and with the synthesis and biological evaluation of a novel ^{99m}Tc-labelled piperidine-based derivative (^{99m}Tc-PipBAT).

Methods: Biodistribution of all radiolabelled complexes was studied in normal mice. A more detailed ex vivo intracerebral distribution study of the two ^{99m}Tc-TropaBAT complexes was additionally performed in normal rats. Autoradiography of brain sections of normal mice (with or without pretreatment with FP-β-CIT or haloperidol) and rats was performed. Affinity for the dopamine transporter (DAT) was also assessed in vitro in the presence or absence of cocaine.

Results: Both ^{99m}Tc-TropaBAT complexes show a slightly higher brain uptake than ^{99m}Tc-TRODAT-1, but the striatum/cerebellum activity ratio is less favourable. Nevertheless, significant striatal uptake was detected after ex vivo autoradiography, but this uptake was also observed after pretreatment with FP-β-CIT. Unexpectedly, no striatal uptake was detected after in vitro incubation of mouse brain sections with the tracer agents. For ^{99m}Tc-PipBAT, neither brain uptake nor in vitro striatal uptake was found.

Conclusion: Both ^{99m}Tc-TropaBAT complexes exhibit similar diffusion into brain as ^{99m}Tc-TRODAT-1, and ex vivo autoradiography shows significant striatal uptake. However, the inferior striatum/cerebellum activity ratio, the striatal uptake in mice pretreated with FP-β-CIT or haloperidol, and the lack of striatal uptake during in vitro incubation prove that the DAT is not targeted. Brain uptake disappears when the tropane skeleton is replaced by a piperidine ring, and also in this case no striatal uptake is found in vitro.

Keywords: Technetium-99m-BAT; Tropanes; Dopamine transporter; Piperidine; Radio-LC-MS

1. Introduction

Over the last decade, many efforts have been made in nuclear medicine to visualise the dopamine transporter (DAT) in order to map presynaptic dopaminergic neurons. Depletion of these neurons in the substantia nigra is seen in a number of neurodegenerative diseases, especially in Parkinson's disease (PD). A decreased specific striatal uptake of a radiolabelled DAT ligand could indicate a pathological state in an early stage and allow differential diagnosis with nondegenerative movement disorders, and follow-up of such uptake would allow prognosis of PD [1–5].

Several useful tropane radioligands such as [^{11}C]cocaine [6–9], [^{11}C]CFT (2β-carbomethoxy-3β-(4-fluorophenyl) tropane [10,11], [^{11}C]FE-β-CIT [12,13] and [^{11}C]- and [^{18}F]FP-β-CIT [13–15] have been developed as DAT radiotracers for positron emission tomography (PET). In addition, several iodine-123-labelled tropane derivatives have been evaluated for single photon emission tomography

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(SPET) [6,16–23], of which the most useful agent so far is ^{123}I-Ioflupane, which recently has become commercially available in Europe (DaTSCAN, GE Healthcare, Little Chalfont, UK) [24–27].

Despite the usefulness and relative success of the mentioned tracer agents, they suffer from the fact that the respective radionuclides have to be produced in a cyclotron, which limits their availability and increases the cost. The nearly optimal nuclear-physical properties of technetium-99m, its continuous availability and relatively low cost have stimulated an intensive search for a ^{99m}Tc-labelled alternative.

For labelling of a DAT-binding ligand with ^{99m}Tc, generally, a metal complexing unit is coupled via a spacer to a tropane derivative. Examples are ^{99m}Tc-TRODAT-1 [27–37] (Fig. 1) and ^{99m}Tc-TECHNEPINE [38,39]. However, both tracer agents suffer from low initial brain uptake and suboptimal specific-to-nonspecific uptake ratio. One of the possible reasons might be the pendent approach, which involves a serious change in structure and increase in molecular mass as compared to labelling with ^{11}C or ^{18}F.

In an attempt to overcome these problems, our group has proposed an integrated approach in which a ^{99m}Tc-complexing bis-amino dithiol (BAT) moiety is partially integrated in the tropane skeleton (Fig. 1). Preliminary biodistribution study results in rats of two ^{99m}Tc-integrated tropane-BAT conjugates (^{99m}Tc-TropaBAT and ^{99m}Tc-norchloro-TropaBAT) showed a slightly higher brain uptake in normal rats, as compared to ^{99m}Tc-TRODAT-1 [40]. In this paper, we report the further biological evaluation of the ^{99m}Tc-TropaBAT complexes.

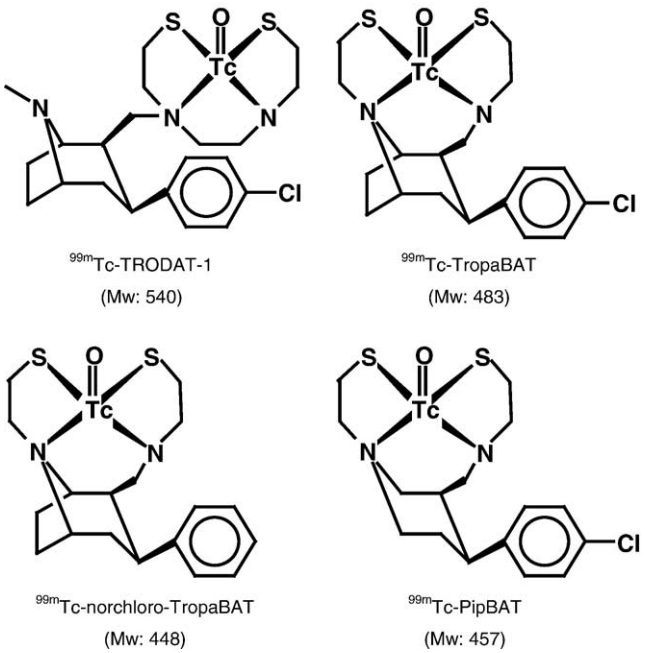

Fig. 1. Proposed structures of ^{99m}Tc-TRODAT-1, both ^{99m}Tc-TropaBAT derivatives (^{99m}Tc-norchloro-TropaBAT and ^{99m}Tc-TropaBAT) and ^{99m}Tc-PipBAT and their molecular mass.

A common characteristic of all compounds described above is the presence of a tropane skeleton as in the parent compound cocaine. However, Kosikowski et al. [41–44] have shown that the presence of a tropane skeleton is not a prerequisite for DAT binding affinity. Therefore, we have also synthesised an integrated piperidine–BAT technetium complex resembling the newly described integrated tropane-BAT conjugates but lacking the 2-carbon bridge (Fig. 1), resulting in a lower molecular mass than ^{99m}Tc-TRODAT-1. We also studied its binding affinity for the DAT and brain uptake.

2. Experimental

2.1. Materials

$Na^{99m}TcO_4$ was obtained by elution of a ^{99}Mo/^{99m}Tc generator (Ultratechnekow FM, Tyco Healthcare, Petten, The Netherlands). ^{123}I-Ioflupane (DaTSCAN) was obtained commercially from GE Healthcare (Little Chalfont, UK). All other reagents were ACS or HPLC grade and were purchased from conventional sources.

^{1}H NMR spectra were recorded using a Varian 200-MHz spectrometer (Varian, Palo Alto, CA, USA). Chemical shifts are reported in parts per million relative to the internal standard tetramethylsilane (TMS, $\delta=0$).

Reversed-phase HPLC was carried out using a system consisting of a Merck Hitachi L-7100 separation module (Merck, Overijse, Belgium) connected to a RP C18 column (XTerra RP18, 5 μm, 4.6×250 mm, Waters, Milford, MA, USA). The column eluate was analysed using a radiometric detector [2-in. NaI(Tl) detector connected to a radiation analyser module, Canberra Packard, Meriden, CT, USA] and a RaChel analysis program (version 1.40, Lablogic, Sheffield, UK).

2.2. Radio-LC-MS analysis

The radio-LC-MS system consisted of a Waters 2690 separation module (Waters) connected to an RP C18 column (XTerra MS C18, 3.5 μm, 2.1×50 mm, Waters). The column eluate was monitored for radioactivity using a radiometric detector [3-in. NaI(Tl) detector connected to a radiation analyser module, The Nucleus, Oak Ridge, USA]. The column eluate was then directed to a time-of-flight mass spectrometer (Micromass LCT, Manchester, UK) equipped with an orthogonal electrospray ionization (ESI) probe. Acquisition and processing of data were performed using Masslynx software, version 3.5.

Electrospray ionization was performed in positive mode (ES+). In order to enable accurate mass calculations, the column eluate was mixed with an internal calibration mass solution (Kryptofix 222, 0.01 mg/ml in CH_3CN–H_2O 50:50 v/v) infused at a flow rate of 1 μl/min. The mass difference between the theoretical mass and the measured accurate