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ScienceDirect
Nuclear Medicine and Biology 35 (2008) 861–867
NUCLEAR MEDICINE — AND — BIOLOGY
www.elsevier.com/locate/nucmedbio

In vivo evaluation in rodents of [^{123}I]-3-I-CO as a potential SPECT tracer for the serotonin 5-HT$_{2A}$ receptor

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Received 22 May 2008; received in revised form 5 September 2008; accepted 8 September 2008

Abstract

Introduction: [^{123}I]-(4-fluorophenyl)[1-(3-iodophenethyl)piperidin-4-yl]methanone ([^{123}I]-3-I-CO) is a potential single photon emission computed tomography tracer with high affinity for the serotonin 5-HT$_{2A}$ receptor (K_i=0.51 nM) and good selectivity over other receptor (sub) types. To determine the potential of the radioligand as a 5-HT$_{2A}$ tracer, regional brain biodistribution and displacement studies will be performed. The influence of P-glycoprotein blocking on the brain uptake of the radioligand will also be investigated.

Methods: A regional brain biodistribution study and a displacement study with ketanserin were performed with [^{123}I]-3-I-CO. Also, the influence of cyclosporin A (50 mg/kg) on the brain distribution of the radioligand was investigated. For the displacement study, ketanserin (1 mg/kg) was administered 30 min after injection of [^{123}I]-3-I-CO.

Results: The initial brain uptake of [^{123}I]-3-I-CO was quite high, but a rapid wash-out of radioactivity was observed. Cortex-to-cerebellum binding index ratios were low (1.1 – 1.7), indicating considerable aspecific binding and a low specific 'signal' of the radioligand. Tracer uptake was reduced to the levels in cerebellum (a 60% reduction) after ketanserin displacement. Administration of cyclosporin A resulted in a doubling of the brain radioactivity concentration.

Conclusions: Although [^{123}I]-3-I-CO showed adequate brain uptake and could be displaced by ketanserin, high aspecific binding to brain tissue was responsible for very low cortex-to-cerebellum binding index ratios, possibly limiting the potential of the radioligand as a serotonin 5-HT$_{2A}$ receptor tracer. We also demonstrated that [^{123}I]-3-I-CO is probably a weak substrate for the P-glycoprotein efflux transporter.

Keywords: Serotonin; P-glycoprotein; 5-HT$_{2A}$ receptor; Brain tracer

1. Introduction

Serotonin is a well-known neurotransmitter, regulating important functions such as sleep, mood and appetite [1]. It was demonstrated that serotonergic neurotransmission malfunctioning is responsible for several psychiatric conditions, for example depression. A subtype of the serotonin receptor family, the serotonin 5-HT$_{2A}$ receptor, has been implicated in pathologies such as schizophrenia, depression, anorexia and anxiety, both in humans [2–6] and in animals [7–9]. Imaging of the 5-HT$_{2A}$ receptor with SPECT is a valuable tool to aid psychiatrists in the diagnosis of these pathologies. Several radioligands have already been used to study the 5-HT$_{2A}$ receptor in the brain, mostly PET tracers. [^{11}C]-MDL100907 is the most frequently used tracer for imaging of the 5-HT$_{2A}$ receptor with PET [10]. [^{18}F]-Altanserin [11] and [^{18}F]-setoperone [12] have also been used. Currently, only one SPECT tracer ([^{123}I]-R91150), has been used clinically for imaging of the 5-HT$_{2A}$ receptor [13,14]. Since this radioligand shows high aspecific binding *in vitro*, we decided to develop a new potential SPECT tracer for imaging of the 5-HT$_{2A}$ receptor.

[^{123}I]-(4-fluorophenyl)[1-(3-iodophenethyl)piperidin-4-yl]methanone ([^{123}I]-3-I-CO) [15] was synthesized from the corresponding tributylstannylprecursor (**1**) using chloramine-T in acetic acid (Fig. 1).

The radioligand is an antagonist with high affinity for the 5-HT$_{2A}$ receptor (K_i=0.51 nM) and selectivity of at least a factor 20 over other receptor (sub)types, including the

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doi:10.1016/j.nucmedbio.2008.09.004

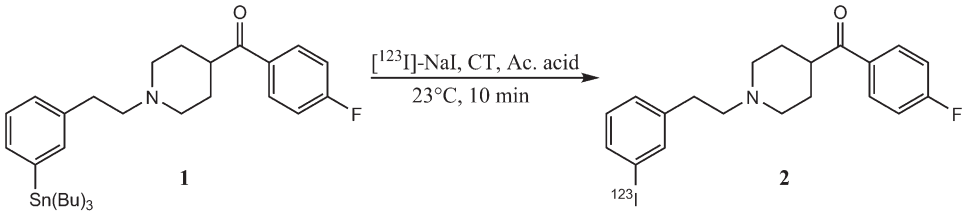

Fig. 1. Radiosynthesis of [^{123}I]-3-I-CO.

5-HT_{2C} receptor [15]. The potential of the ligand as a single photon emission computed tomography (SPECT) tracer for the serotonin 5-HT_{2A} receptor tracer was determined by performing a regional brain biodistribution study with [^{123}I]-3-I-CO in Sprague–Dawley rats. To demonstrate specific binding of the radioligand to the 5-HT_{2A} receptor, a displacement study using ketanserin as the displacing agent was performed. Also, a metabolite analysis was executed to exclude the presence of radiolabelled metabolites in brain. Precursor synthesis, radiosynthesis and biodistribution studies in mice have already been published elsewhere [16].

P-glycoprotein is an adenosine triphosphate-driven efflux protein located amongst others in the blood-brain barrier. It is also over expressed in different tumour types [17]. It was demonstrated recently that blocking of P-glycoprotein function with cyclosporin A had a profound effect on the brain uptake of several radioligands [18–20], resulting in a strong increase in brain radioactivity concentration.

P-glycoprotein modulation could prove useful for increasing drug concentrations (a.o. chemotherapeutics) and in oncology where P-glycoprotein (Pgp) plays a role in multidrug-resistance [21]. Also, several central nervous system-active drugs such as phenytoin (antiepileptic), clomipramine (antidepressant) and chlorpromazine (neuroleptic) are substrates for Pgp. Possibly, their brain concentration (and, hence, their therapeutic effect) can be altered by modulation of Pgp [17]. Radioligands with demonstrated Pgp affinity could be used for monitoring Pgp activity in the blood-brain barrier. Also, Pgp efflux can reduce brain uptake of radiotracers and thus hamper successful imaging [22]. For these reasons, the influence of Pgp blocking with cyclosporin A on the regional brain biodistribution of [^{123}I]-3-I-CO in rodents was also investigated.

2. Materials and methods

2.1. Chemicals and radiochemicals

All chemicals and reagents were purchased from Acros Organics (Beerse, Belgium) and were used without further purification unless described otherwise. Solvents used were of high-performance liquid chromatography (HPLC) quality and were purchased from Chemlab (Belgium). No carried added [^{123}I]-sodium iodide (in 0.05M NaOH) was purchased from GE Healthcare. Ketanserin was used as the tartrate salt and was obtained from Tocris Cookson (Bristol, UK). It was dissolved in 0.9% NaCl solution containing 10% ethanol (v/v). Cyclosporin A was obtained from Sigma–Aldrich and was dissolved in a mixture of ethanol and polyethoxylated castor oil and diluted with 0.9% NaCl before injection. A concentration of 50 mg/kg was used.

2.2. Chemistry

Synthesis and radiosynthesis of [^{123}I]-3-I-CO were performed as described previously [16]. Briefly, [^{123}I]-3-I-CO was synthesized starting from the corresponding tributylstannylprecursor. The precursor was iodinated using chloramine-T in the presence of glacial acetic acid, nca [^{123}I]-NaI and ethanol as solvent. Radiosynthesis was terminated by the addition of sodiummetabisulphite solution. The radioligand was purified on HPLC, using a reversed-phase C_{18} column (Alltech Apollo C_{18} 7×250 mm, 5 μm) and a 50/50 mixture of acetonitrile and phosphate buffer (0.02 M, pH 7) as the eluent. Solvent was removed with a C_{18} Sep-PAK cartridge, and the radioligand was formulated for injection in 0.9% NaCl containing 10% ethanol after sterile filtration. A radiochemical yield of 75±5% (n=3) was obtained. Radiochemical purity was always higher then 95%.

2.3. Regional brain biodistribution study in rodents

All animal experiments were conducted according to the regulations of the Belgian law and the Ghent University local ethical committee (ECP 05/14). [^{123}I]-3-I-CO (3–5 MBq) was injected through the penile vein in male Sprague–Dawley rats (n=3 animals per time point, 200–250 g bodyweight). At predefined time points after injection of the radioligand (20 min, 40 min, 1 h, 2 h and 4 h), the animals were sacrificed by decapitation under isoflurane anaesthesia. Blood was collected from the heart and the brain was rapidly removed and dissected into different regions: the cortex (which was further dissected into the frontal cortex, parietal cortex, occipital cortex and temporal cortex), cerebellum and a subcortical region. The different brain and blood samples were weighed and counted for radioactivity with an automated gamma counter [Cobra, Packard Canberra, equipped with five 1"×1" NaI(Tl) crystals]. Aliquots of the injected tracer solution (n=3) were weighed and counted for radioactivity to determine the injected radioactivity dose received by the animals. Results were corrected for decay and tissue radioactivity concentrations