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A PET study comparing receptor occupancy by five selective cannabinoid 1 receptor antagonists in non-human primates☆

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ABSTRACT

There is a medical need for safe and efficacious anti-obesity drugs with acceptable side effect profiles. To mitigate the challenge posed by translating target interaction across species and balancing beneficial *vs.* adverse effects, a positron emission tomography (PET) approach could help guide clinical dose optimization. Thus, as part of a compound differentiation effort, three novel selective CB1 receptor (CB_1R) antagonists, developed by AstraZeneca (AZ) for the treatment of obesity, were compared with two clinically tested reference compounds, rimonabant and taranabant, with regard to receptor occupancy relative to dose and exposure. A total of 42 PET measurements were performed in 6 non-human primates using the novel CB_1R antagonist radioligand [^{11}C]SD5024. The AZ CB_1R antagonists bound in a saturable manner to brain CB_1R with *in vivo* affinities similar to that of rimonabant and taranabant, compounds with proven weight loss efficacy in clinical trials. Interestingly, it was found that exposures corresponding to those needed for optimal clinical efficacy of rimonabant and taranabant resulted in a CB_1R occupancy typically around ~20–30%, thus much lower than what would be expected for classical G-protein coupled receptor (GPCR) antagonists in other therapeutic contexts. These findings are also discussed in relation to emerging literature on the potential usefulness of 'neutral' *vs.* 'classical' CB_1R (inverse agonist) antagonists. The study additionally highlighted the usefulness of the radioligand [^{11}C]SD5024 as a specific tracer for CB_1R in the primate brain, though an arterial input function would ideally be required in future studies to further assure accurate quantitative analysis of specific binding.

1. Introduction

Cannabinoid 1 receptors (CB_1R) are among the most abundantly expressed G protein-coupled receptors in brain (Mackie, 2005) and implicated in the control of appetite and motivation to eat (Kirkham et al., 2002). In addition, CB_1R are expressed in metabolically active peripheral organs (adipose tissue, skeletal muscles and liver; Nogueiras et al., 2009) stressing their role in the overall regulation of energy balance (Wierzbicki, 2006).

Much effort has been put into the discovery and development of CB_1R ligands able to block the effects of endogenous cannabinoids (endocannabinoids, eCBs), given the potential for therapeutic effect of such agents in a variety of somatic and psychiatric disorders.

Abbreviations: AZ, AstraZeneca; AUC, area under the curve; BBB, blood–brain barrier; CB_1R, cannabinoid 1 receptor; CD, candidate drug; eCB, endocannabinoid; CNS, central nervous system; GPCR, G-protein coupled receptor; PET, positron emission tomography; RO, receptor occupancy; ROI, region of interest; SUV, standardised uptake value.

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Whereas a number of therapeutic areas have been proposed (*e.g.*, smoking cessation, cognition-promoting), only the anti-obesity indication has been clinically validated thus far, and has been the main focus of major Pharma programmes with CB_1R blocking agents over the last decade. Such compounds have interchangeably been referred to as antagonists or inverse agonists at the CB_1R, the latter denomination reflecting their ability *in vitro* to reverse constitutive G-protein activity in the absence of an agonist (Howlett et al., 2011). For simplicity, we have chosen to refer to the CB_1R ligands in the present context as CB_1R antagonists (*cf.* further Discussion section).

In rodents, administration of CB_1R antagonists results in decreased food intake and body weight, and may also stimulate adiponectin production and secretion, inhibit lipogenesis and increase glucose uptake (Wu et al., 2011). The preclinical observations have been validated in humans by extensive clinical studies with rimonabant, confirming weight loss and improved lipid and glucose metabolism in treated obese humans (Van Gaal et al., 2005; Després et al., 2005; Pi-Sunyer et al., 2006). Similar findings have also been reported with taranabant and otenabant (Aronne et al., 2010, 2011). The clinical trials have, however, additionally revealed adverse psychiatric side effects, particularly depression, which has led to withdrawal of the drugs from clinical use (Beyer et al., 2010).

PET-imaging in non-human primates using a suitable radioligand may confirm binding to CB_1R in brain and serve for translation of dose- and exposure-response relationships to humans. Several PET radioligands have been exploited for measuring CB_1R interactions in non-human primates and human subjects: [^{18}F]MK9470 (Burns et al., 2007; Sanabria-Bohórquez et al., 2010), [^{11}C]OMAR (Wong et al., 2010), [^{11}C]MePPEP (Terry et al., 2009) and [^{18}F]FMPEP-d_2 (Terry et al., 2010). Promising characteristics have been demonstrated for [^{18}F]FMPEP-d_2, which shows a high imaging signal to noise ratio, but slow washout from brain. In search for improved radioligands [^{11}C]SD5024 has been developed (Donohue et al., 2008a,b). This radioligand has been shown to bind with high-affinity (K_i = 0.5 nM; IC_{50} = 2.8 nM) and selectivity to CB_1R, and display high brain uptake and a distribution pattern in line with the known CB_1R distribution in the non-human primate and human brain (Herkenham et al., 1990; Glass et al., 1997; Tsujikawa et al., 2014) and should, thus, be useful for examination of drug effects on CB_1R binding by PET *in vivo*.

The present study in non-human primates was part of a compound differentiation effort and aimed to examine CB_1R occupancy of three novel selective CB_1R antagonists that were considered for development by AstraZeneca (AZ) as anti-obesity therapeutics. These AZ compounds exhibit promising body weight reductions and metabolic improvements in rodent models of obesity and metabolic derangement (Table 1 and AZ unpublished data). The PET evaluation in non-human primates was thus conducted to search for optimal dosing towards development studies, and to explore dose/exposure *vs*. occupancy in relation to expected clinical effects with these agents. For the purpose of comparison, CB_1R occupancy of rimonabant and taranabant was therefore also examined. This study also extends the pharmacological characterization of [^{11}C]SD5024 binding *per se* by using CB_1R antagonists of distinct chemical classes.

2. Materials and methods

The study was performed according to "The Guidelines for Planning, Conducting, and Documenting Experimental Research" (Dnr 4820/06-600) of Karolinska Institutet as well as conforming to the guidelines in the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985). The study was approved by the Animal Ethics Committee at Stockholms Norra Djurförsöksetiska Nämnd, and was consistent with applicable regulatory requirements and the AstraZeneca policy on Bioethics. The study was carried out from June 2007 to January 2008.

2.1. Animals—non-human primates

Six female normal-weight cynomolgus monkeys (Macaca fascicularis; weight from 3.4 to 5.4 kg) were included in the study. The monkeys were housed at the Astrid Fagraeus Laboratory at Karolinska Institutet, Solna, Sweden. Anaesthesia was induced and maintained for the duration of each measurement using repeated intramuscular injection of a mixture of ketamine (3–4 mg/kg/h; Ketalar®, Parke-Davis) and xylazine hydrochloride (1–2 mg/kg/h; Rompun® Vet. Bayer of Sweden). A head fixation system was used to secure a fixed position of the monkey's head during the PET measurements (Karlsson et al., 1993). Body temperature was maintained by a Bair Hugger model 505 (Arizant Healthcare, Eden Prairie, MN, USA) and monitored by a rectal thermometer (Precision Thermometer, Harvard Apparatus). Heart and respiration rates were measured every 20 min throughout the experiment. Changes in the vital parameters were recorded if they were 25% different compared to the baseline values.

2.2. Study design

The study included 14 experimental sessions, two to four *per* tested drug. Each experimental session consisted of three PET-measurements in the same monkey: one at baseline (in the morning) and two after pretreatment with different drugs doses (Fig. 1). The study design was adaptive, *i.e.* doses and the numbers of doses to be tested were chosen depending on the results of the preceding session.

2.3. Study compounds

Five CB_1R antagonists were tested. The compounds were administered as intravenous infusion over 10–20 min, 60 min before the start of PET measurements. The chemical names, structures and test doses are given in Tables 1 and 2. Test and reference compounds were manufactured at Department of Process R&D, AstraZeneca R&D, Södertälje, Sweden (AZ compound **1**), or at the Department of Medicinal Chemistry, AstraZeneca R&D, Mölndal, Sweden (AZ compounds **2**, **3** and taranabant; Chen et al., 2007). Rimonabant was sourced from Sungene GmbH, Gatersleben, Germany.

The compounds were formulated as a suspension of crystalline nanoparticles, with the exception of taranabant, which was formulated as a suspension of amorphous nanoparticles (Department of Pharmaceutical Development, AstraZeneca R&D, Mölndal, Sweden). The suspensions were diluted to appropriate concentrations with 5% mannitol solution and kept frozen until administration. Before intravenous administration to the animals, the suspensions were further diluted using 5% mannitol solution to suitable concentration according to the intended dose and dose volume.

2.4. PET measurements

2.4.1. Radiochemistry

Reference SD5024 ((−)-3-(4-chlorophenyl)-*N*′-[(4-cyanophenyl)sulfonyl]-4-phenyl-4,5-dihydro-1*H*-pyrazole-1-carboxamidine) and its corresponding iodo precursor for radiolabeling ((−)-3-(4-chlorophenyl)-*N*′-[(4-iodophenyl)sulfonyl]-4-phenyl-4,5-dihydro-1*H*-pyrazole-1-carboxamidine) were supplied by Department of Medicinal Chemistry, AstraZeneca R&D Mölndal, Sweden. All other chemicals were obtained from commercial sources and were of analytical grade.

Radiosynthesis of [^{11}C]SD5024 was performed in a custom-made semi-automated synthesis module (Airaksinen et al., 2008) via palladium-mediated [^{11}C] cyanation as previously reported (Donohue et al., 2008a). Briefly, [^{11}C]hydrogen cyanide was produced in accordance with the method of Airaksinen et al. (2008) and trapped in a solution of KH_2PO_4 (0.5 mg, 3.67 μmol) in DMSO (0.3 mL) to provide [^{11}C]potassium cyanide. Subsequently, this solution was transferred to a vial containing the iodo precursor to SD5024 (1 mg, 1.77 μmol) and $Pd(PPh_3)_4$ (5.5 mg, 4.76 μmol) in DMSO (0.3 mL). The mixture was heated at 135 °C for 5 min, and then cooled to room temperature after which HPLC mobile phase (MeCN-0.01 M H_3PO_4, 55: 45 v/v; 1 mL) was added. [^{11}C]SD5024 was separated from this solution by reversed phase HPLC on a μ-Bondapak C-18 column (300 × 7.8 mm, 10 μm; Waters) eluted at 6 mL/min with column effluent monitored in series for absorbance at 254 nm and for radioactivity with a GM tube. The fraction containing [^{11}C]SD5024 (t_R = 12.3 min) was collected and evaporated to dryness. The residue was dissolved in sterile physiological phosphate-buffered saline (pH = 7.4; 8 mL) and passed through a sterile filter (0.22 μm pore size; Millex GV; Millipore Corporation).

The radiochemical purity and identity of the product were determined with analytical reversed phase HPLC on a μ-Bondapak C-18 column (300 × 3.9 mm, 10 μm; Waters) eluted with MeCN-0.01 M-H_3PO_4 (55: 55 v/v) at 3 mL/min and the column effluent was monitored in series for absorbance at 254 nm and for radioactivity with a β-flow radio detector (Beckman). [^{11}C]SD5024 eluted with the same retention time as that for the reference standard of SD5024 (t_R = 3.8 min), thereby confirming the radiochemical identity of the product. The radiochemical purity of [^{11}C]SD5024 exceeded 99%.

In each PET-measurement a sterile physiological phosphate buffer (pH = 7.4) solution containing [^{11}C]SD5024 was injected as a bolus into a sural vein during 5 s