Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1771–1775

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and In Vivo Imaging Properties of [^{11}C]Befloxatone: A Novel Highly Potent Positron Emission Tomography Ligand for Mono-Amine Oxidase-A

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Received 14 January 2003; revised 13 February 2003; accepted 21 February 2003

Abstract—Befloxatone (**1**, (5*R*)-5-(methoxymethyl)-3-[4-[(3*R*)-4,4,4-trifluoro-3-hydroxybutoxy]phenyl]-2-oxazolidinone) is an oxazolidinone derivative belonging to a new generation of reversible and selective mono-amine oxidase-A (MAO-A) inhibitors. In vitro and ex vivo studies have demonstrated that befloxatone is a potent, reversible and competitive MAO-A inhibitor with potential antidepressant properties. Befloxatone (**1**) was labelled with carbon-11 ($t_{1/2}$: 20.4 min) using [^{11}C]phosgene as reagent. Typically, starting from a 1.2 Ci (44.4 GBq) cyclotron-produced [^{11}C]CH_4 batch, 150–300 mCi (5.55–11.10 GBq) of [^{11}C]befloxatone ([^{11}C]-**1**) with a radiochemical- and chemical purity of more than 99% were routinely obtained within 20 min of radiosynthesis (including HPLC purification) with specific radioactivities of 500–2000 mCi/μmol (18.5–74.0 GBq/μmol). The results obtained in vivo with carbon-11-labelled befloxatone not only confirm the biochemical and pharmacological profile of befloxatone found in rodent and in human tissues but also point out [^{11}C]befloxatone as an excellent tool for the assessment of MAO-A binding sites using positron emission tomography, a high-resolution, sensitive, non-invasive and quantitative imaging technique.

MAO [amine: oxygen oxido-reductase (deaminating) (flavin containing) EC 1.4.3.4] is localized mainly in the outer mitochondrial membrane of neurons, glial- and other cells[1,2] and catalyses the oxidative deamination of neurotransmitters and xenobiotic amines. Two isoforms of MAO termed MAO-A and MAO-B (70% homology between the amino acid sequences) have been described, each consisting of two subunits[3,4] coded by different genes and with molecular weights of about 59,700 and 58,800 Da, respectively.[5] They differ in substrate specificity and inhibitor sensitivity. MAO-A preferentially deaminates 5-hydroxytryptamine (serotonin), norepinephrine and epinephrine and is selectively inactivated by low concentrations of clorgyline.[6] MAO-B preferentially deaminates phenylethylamine and benzylamine and is selectively inhibited by low concentrations of deprenyl.[7] Dopamine and tyramine are metabolized by both forms (for review, see refs [8–10]). Nevertheless this specificity is relative and the deamination of a given substrate by MAO-A or MAO-B depends not only on the substrate itself, but also on the relative concentration of each form of MAO. Histochemical, immunohistochemical and autoradiographic studies have revealed that the two isoforms of MAO also have a distinct regional distribution. In the brain, MAO-A is found primarily in catecholaminergic neurones whereas MAO-B is localised in serotoninergic neurones and glial cells.

Positron emission tomography (PET), a high-resolution, sensitive, non-invasive and quantitative imaging technique that can be used in humans, is the most advanced technology currently available for studying in vivo molecular interactions, and as such represents the method of choice for assessing the pharmacokinetics of new therapeutic agents, such as enzyme inhibitors. Few

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0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00215-4

radiotracers have been developed for MAO-A PET studies such as [^{11}C]clorgyline, [^{11}C]harmine and [^{11}C]brofaromine.[11–17] These radiotracers all present some drawbacks. [^{11}C]clorgyline present an unexplained species difference : [^{11}C]clorgyline was not retained in baboon brain in contrast to results in human.[15] [^{11}C]harmine is extensively metabolized in plasma.[13] Concerning [^{11}C]brofaromine, the first reversible MAO-A inhibitor labelled for PET, the brain binding could not be saturated by preloading MAO-A inhibitors.[11]

Befloxatone (**1**, (5*R*)-5-(methoxymethyl)-3-[4-[(3*R*)-4,4,4-trifluoro-3-hydroxybutoxy]phenyl]-2-oxazolidinone) is an oxazolidinone derivative belonging to a new generation of reversible and selective mono-amine oxidase-A (MAO-A) inhibitors.[18–23] In vitro and ex vivo studies have demonstrated that befloxatone is a potent, reversible and competitive MAO-A inhibitor with potential antidepressant properties. Befloxatone inhibited selectively and competitively MAO-A in human- and rat brain, heart, liver and duodenum homogenates with R_i values ranging from 1.9 to 3.6 nM for MAO-A and from 270 to 900 nM for MAO-B. In vitro, befloxatone was more potent at inhibiting MAO-A activity than reference compounds such as moclobemide, brofaromine or harmaline.[20] The specificity of befloxatone for MAO-A was tested against other neurotransmitters and transporter systems. In vivo, befloxatone increases brain levels of norepinephrine, dopamine and serotonin and decreases the levels of corresponding deaminated metabolites (3,4-dihydroxyphenylacetic acid and 5-hydroxyindolacetic acid).[20] Befloxatone is much more potent (10–500-fold) than other reversible or irreversible MAO-A inhibitors in classical anti-depressant tests in rodents. Befloxatone displays a higher activity in tests involving MAO-A (with ED_{50} of about 0.1–0.2 mg/kg po or 0.07 mg/kg iv) than in tests involving MAO-B (ED_{50} of about 58 mg/kg po).[21]

Based on the biochemical and pharmacological characteristics of this drug, befloxatone labelled with a positron-emitter appears to be an excellent candidate for the in vivo imaging of MAO-A density using PET and could therefore be used in the evaluation of MAO-A activity fluctuation associated with human diseases such as Parkinson's and Alzheimer's disease, depression and certain psychiatric disorders.[24]

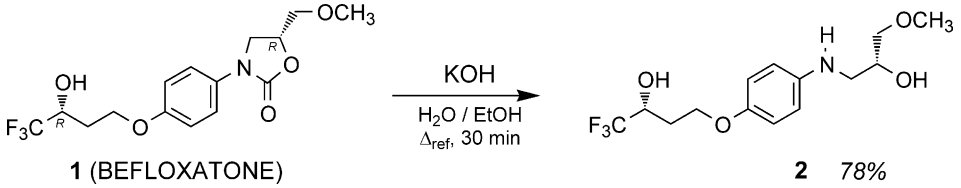
In this paper, we present an efficient synthesis of the carbon-11-labelled ligand befloxatone ([^{11}C]-**1**), and some preliminary results on the in vivo properties of this radiotracer in baboons, obtained by whole body PET study (including brain) and metabolite analysis.

Chemistry

Befloxatone[23] presents in its chemical structure one methoxy group, which could have been the site for carbon-11 labelling using either [^{11}C]methyl iodide or triflate- as reagent. Metabolism studies performed in both rats and dogs clearly showed that desmethyl-befloxatone and its glucuronic acids conjugate were the main metabolites. We therefore chose to label befloxatone on its oxazolidinone ring in order to prevent early loss of radioactivity by metabolisation. The precursor for carbon-11 labelling, compound **2** ((*R*)-1-methoxy-3-[[4-[(3*R*)-4,4,4-trifluoro-3-hydroxybutoxy]phenyl]amino]-2-propanol), was prepared in 78% yield from befloxatone (**1**) using 10 equiv of KOH in a refluxing mixture of water and EtOH (Scheme 1). All analytical data (^{1}H NMR, IR and mass spectrometry) were in accordance with the structure and both the chemical and enantiomeric purities were greater than 99%. The absence of any residual traces of befloxatone (**1**), which would give raise to unwanted carrier in the carbon-11 preparation, was verified by HPLC analysis.

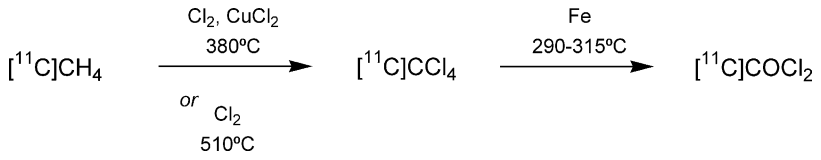
Befloxatone (**1**) was labelled with carbon-11 ($t_{1/2}$: 20.4 min) using [^{11}C]phosgene as reagent. [^{11}C]Phosgene ([^{11}C]$COCl_2$) is synthesized from cyclotron-produced [^{11}C]methane ([^{11}C]CH_4) in 9–10 min, via [^{11}C]carbon tetrachloride ([^{11}C]CCl_4) using two different processes (Scheme 2). [^{11}C]CH_4 was mixed with chlorine and the mixture passed through a glass U-tube containing pumice stone impregnated with $CuCl_2$ at a temperature of 380 °C (process A).[25] In process B, the mixture of [^{11}C]CH_4 and chlorine was simply passed through an empty linear glass-tube at a temperature of 510 °C.[26] Then, in both processes, the on-line synthesized [^{11}C]CCl_4 was passed through a glass U-tube containing iron filings at 290–310 °C, giving [^{11}C]$COCl_2$. The decay-corrected yield, based on starting [^{11}C]CH_4 was 31–54%.

The cyclization reaction of [^{11}C]$COCl_2$ and the amino-alcohol **2** in dichloromethane at room temperature was almost quantitative and fast (30–60 s) and afforded radiochemically, chemically and enantiomerically pure [^{11}C]befloxatone ([^{11}C]-**1**) in 90–95% radiochemical yield (Scheme 3). Typically, starting from a 1.2 Ci (44.4 GBq) [^{11}C]CH_4 production batch, 150–300 mCi (5.55–11.10 GBq) of [^{11}C]befloxatone ([^{11}C]-**1**) with a radiochemical- and chemical purity of more than 99% were routinely obtained within 20 min of radiosynthesis, including HPLC purification (column: Lichrosphere Si_{60}, 7 μm, Merck, 10 × 250 mm ; solvents and conditions: CH_2Cl_2/EtOAc: 70/30 [v:v], 8 mL/min, t_R [^{11}C]-**1**: 6.0–6.5 min). The specific radioactivities measured at the

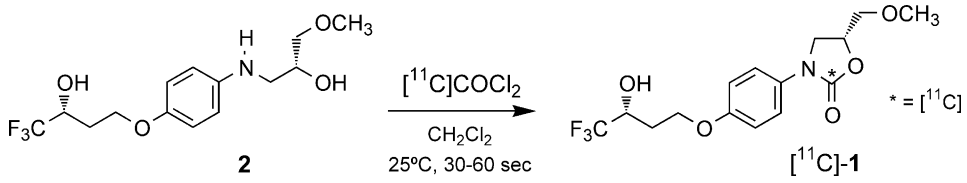


1 (BEFLOXATONE) **2** *78%*

Scheme 1.



Scheme 2.



Scheme 3.

end of the radiosynthesis were 500–2000 mCi/μmol (18.5–74.0 GBq/μmol). The total decay-corrected radiochemical yield of [^{11}C]befloxatone ([^{11}C]-**1**), based on starting [^{11}C]CH_4, was 27–50%. Whichever the synthetic process of [^{11}C]phosgene employed, no significant differences could be observed neither in terms of amount of [^{11}C]befloxatone produced nor in terms of associated specific radioactivities obtained.

Formulation of labelled product for in vivo injection was effected as follows: (1) HPLC solvent removal by evaporation; (2) taking up the residue in 5 mL of physiological saline; (3) sterile filtration. The solution for injection was a clear and colourless solution and its pH was between 4.5 and 8.5. The radiosynthesized [^{11}C]befloxatone co-elutes with an authentic sample of befloxatone. The preparation was found to be >99% chemically and radiochemically pure, as demonstrated by HPLC analysis (column:symmetry-M® C-18, 5 μm, waters, 4.6×50 mm; solvents and conditions: A/B 30:70 [v:v], 2 mL/min, t_R [^{11}C]-**1**:2.20 min [A:H_2O containing 2% low-UV PIC® B7 reagent; B:H_2O:CH_3CN:50:50 [v:v] containing 2% low-UV PIC® B7 reagent]). It was free from starting labelling precursor and was shown to be chemically and radiochemically stable for at least 120 min in physiological saline. Administration to animals were done within 15 min after end of synthesis. Post-release control results (sterility- and endotoxine tests for example) were in accordance with our in-house radiopharmaceutical quality assurance standards.

PET studies in anaesthetised adult Papio anubis baboons

The present paper describes two whole-body dynamic PET scans, performed in baboons after iv injection of [^{11}C]befloxatone as well as two controls PET experiments in order to determine exclusively brain pharmacokinetics of the radiotracer. Finally, two blocking experiments (administration of a loading dose of cold befloxatone 30 min before the tracer) were also performed. For all PET scans, arterial blood samples were withdrawn at designated time points to follow plasma [^{11}C]befloxatone kinetics and metabolites.

The whole body dynamic PET scans obtained after iv injection of 9.76±3.6 mCi (or 361±133 MBq, SA >700 mCi/μmol at time of injection) of [^{11}C]befloxatone provided activity time courses for the organs presented in Figure 1. [^{11}C]befloxatone rapidly distributed in the plasma (alpha-phase of about 5 min) and then slowly to the rest of the body (calculated half-life for the beta-phase of 57 min). At 30 min pi, plasma radioactive concentration was very low: 0.23±0.19 ID / 100 mL. During the first 10 min pi, highest radioactivity uptake was found in the kidneys and the lungs (about 0.11% and 0.06% ID/mL of tissue, respectively). Liver displayed lower uptake (about 0.02% ID/mL of tissue) and very low uptake was found in muscle and bladder (about 0.004 and 0.012% ID/mL of tissue, respectively). The pharmacokinetics of [^{11}C]befloxatone in kidney showed a slow decrease during the 2 h of the experiment, while the bladder displayed a constant and high increase. This indicates a high rate of urinary excretion. Lung radioactivity is maximal at the first frame and shows a relatively rapid washout. No accumulation was found during the first 10 min. This is indicative of no- or low-specific binding in the lung. Liver uptake is low at the beginning of the experiment and raises during the experiment. Muscle uptake is very low and stays very low during the experiment (about 0.002% ID/mL).

The distribution of [^{11}C]befloxatone in the peripheral organs reflects the distribution of the MAO-A.[27,28] The shape of the kinetics of the radioactivity in the liver and the bladder suggests that in baboons befloxatone is mainly metabolised in the liver and that metabolites are mainly excreted by the urinary tract. Metabolite analysis showed that [^{11}C]befloxatone at tracer dose was relatively stable in vivo, since 78% of the radioactivity in plasma at 30 min pi represented unchanged befloxatone. The only HPLC detectable metabolite was a hydrophilic compound. No peak was detectable at the retention time of the desmethylbefloxatone, the main metabolite in human.

Kinetics of the tracer in cerebral structures and in plasma (uncorrected for metabolites), following iv injection of 15.47±2.4 mCi (or 572±89 MBq, SA >700 mCi/μmol at time of injection) are shown in Figure 2A (filled symbols). [^{11}C]befloxatone crosses the blood–brain barrier. In all brain regions, radioactivity was detected early and increased rapidly. The highest uptake was found in striatum, with a maximum value at 30 min pi (1.65±0.05% ID/100 mL), then radioactivity decreased slowly until the end of the experiment.

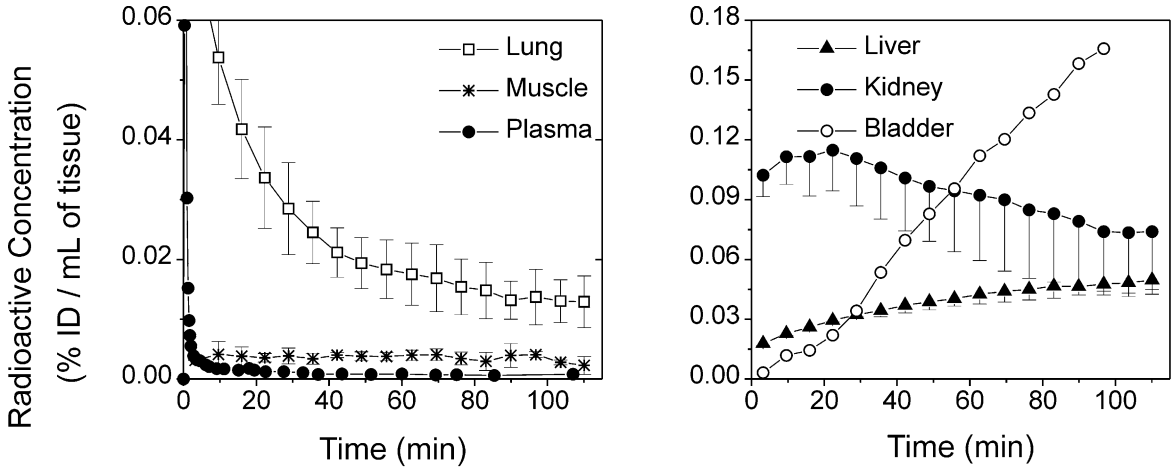


Figure 1. [^{11}C]Befloxatone pharmacokinetics in peripheral organs from whole-body dynamic PET scans ($n=2$) in the baboon. Values (mean ± S.D.) are expressed in % ID/mL of tissue. Experiments were performed with an ECAT EXACT HR+ (CTI/Siemens, Knoxville) tomograph in three-dimensional mode.[29] Scans were acquired from the top of the skull to the pelvis, requiring scanning in five bed positions. Emission scan started at the iv injection of 9.76 ± 3.6 mCi (or 361 ± 133 MBq, SA > 700 mCi/μmol at time of injection) of [^{11}C]befloxatone and acquisition time was 1 min for each bed position, allowing for each body segment, 17 dynamic scans separated by about 5 min, over the next 110 min. Arterial blood samples were withdrawn at designated time points to follow plasma [^{11}C]befloxatone kinetics and metabolites.

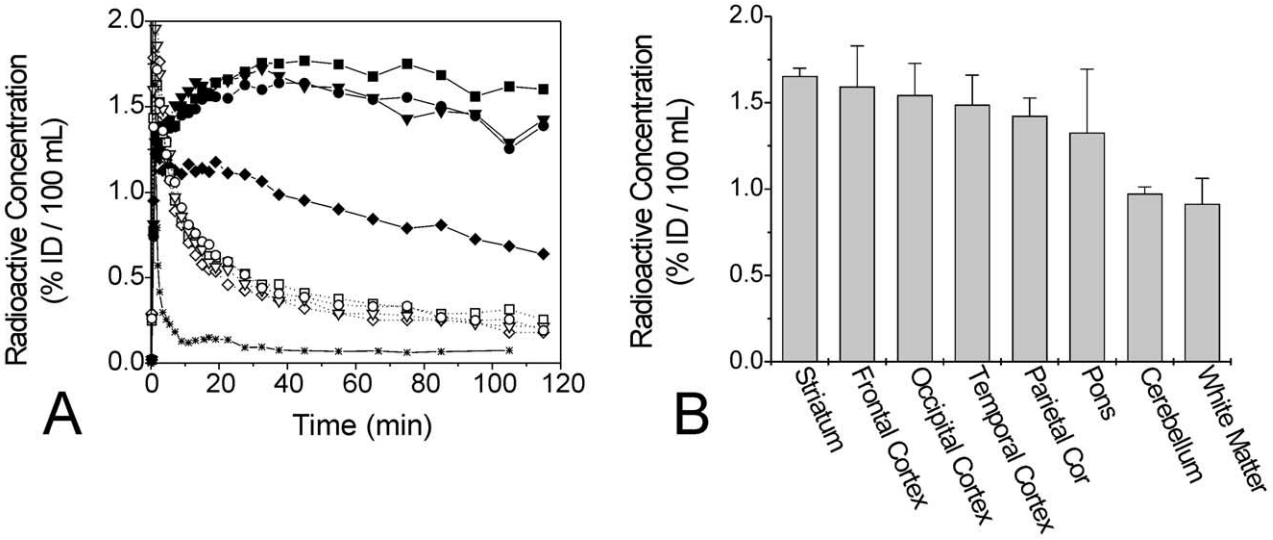


Figure 2. [^{11}C]Befloxatone pharmacokinetics in the baboon brain and plasma ($n=4$) : experiments were performed with an ECAT 953B/31 (CTI/Siemens, Knoxville) brain-dedicated tomograph.[30] Emission scan started at the iv injection of 15.47 ± 2.4 mCi (or 572 ± 89 MBq, SA > 700 mCi/μmol at time of injection) of [^{11}C]befloxatone and lasted 120 min. Thirty-three frames were acquired with scan duration starting from 30 s and increasing up to 10 min during the experiment. Arterial blood samples were withdrawn at designated time points to follow plasma [^{11}C]befloxatone kinetics and metabolites. Four experiments were conducted, two control experiments and two blocking experiments by administration of a loading dose of cold befloxatone (0.4 mg/kg) 30 min before the tracer. (A) Typical time-activity curves obtained in several brain structures (filled symbols, ■ : frontal cortex ; ▼ : occipital cortex ; ● : striatum ; ◆ : cerebellum ; ∗ : plasma) after iv administration of a tracer dose of [^{11}C]befloxatone (14.2 mCi ; i.e., 46 nmol) and in a blocking experiment (injection of 0.4 mg/kg of cold befloxatone 30 min before tracer injection) on the same animal (open symbols). Values are expressed in % ID / 100 mL of tissue. (B) [^{11}C]befloxatone uptake in differents brain structures in baboon (control experiments). Data (mean ± S.D.) are expressed in % ID / 100 mL of tissue obtained 30 min pi.

Several brain regions such as thalamus, pons and cortical structures present similar kinetics and a high uptake (Fig. 2B). Other regions such as cerebellum and white matter presented lower uptake and the pharmacokinetics was faster. In these regions, the radioactive peak was reached as early as 5 min pi and the clearance was faster. The in vivo brain distribution of [^{11}C]befloxatone reflects the MAO-A distribution reported by in vitro studies in primate brain either by histochemistry[27] or autoradiography.[28]

Administration of a pre-loading dose of unlabelled befloxatone (0.4 mg/kg) before the radiotracer prevents the tracer to accumulate in the brain (Fig. 2A; open symbols), indicating that the larger part of the radioactivity is specifically bound to MAO-A. In this case, the radioactive

concentrations were very low and identical in all structures ($0.32 \pm 0.05\%$ ID/100 mL at 60 min p.i.). This indicates that [^{11}C]befloxatone brain binding in vivo is saturable. The ratio of total binding to non-saturable binding increases with time and is higher than 5 after 1 h.

Conclusion

The present study shows that befloxatone can be efficiently labelled with the positron emitter carbon-11. In vivo, [^{11}C]befloxatone penetrates and accumulates rapidly in the brain, and furthermore displays a high specific binding. These results, obtained in vivo, not only confirm the biochemical and pharmacological profile of befloxatone found in rodent and in human tissues,[13,14] but also point out [^{11}C]befloxatone as an excellent tool for PET assessment of MAO-A binding sites.

Acknowledgements

The authors wish to thank cyclotron operators Mr. Daniel Gouel, Mr. Christophe Peronne and Mr Christophe Lechêne for performing the irradiations.

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