Nuclear Medicine and Biology 40 (2013) 445–450

Contents lists available at SciVerse ScienceDirect

Nuclear Medicine and Biology

journal homepage: www.elsevier.com/locate/nucmedbio

Development and evaluation of a radiobromine-labeled sigma ligand for tumor imaging

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ARTICLE INFO

Article history:
Received 7 January 2013
Received in revised form 5 February 2013
Accepted 13 February 2013

Keywords:
Sigma receptor
Tumor
Radiobromine

ABSTRACT

Introduction: Sigma receptors are appropriate targets for tumor imaging because they are highly expressed in a variety of human tumors. Previously, we synthesized a vesamicol analog, (+)-2-[4-(4-iodophenyl) piperidino]cyclohexanol ((+)-*p*IV), with high affinity for sigma receptors, and prepared radioiodinated (+)-*p*IV. In this study, to develop a radiobromine-labeled vesamicol analog as a sigma receptor imaging agent for PET, nonradioactive and radiobromine-labeled (+)-2-[4-(4-bromophenyl)piperidino]cyclohexanol ((+)-*p*BrV) was prepared and evaluated *in vitro* and *in vivo*. In these initial studies, ^{77}Br was used because of its longer half-life.
Methods: (+)-[^{77}Br]*p*BrV was prepared by a bromodestannylation reaction with radiochemical purity of 98.8% after HPLC purification. The partition coefficient of (+)-[^{77}Br]*p*BrV was measured. *In vitro* binding characteristics of (+)-*p*BrV to sigma receptors were assayed. Biodistribution experiments were performed by intravenous administration of a mixed solution of (+)-[^{77}Br]*p*BrV and (+)-[^{125}I]*p*IV into DU-145 tumor-bearing mice.
Results: The lipophilicity of (+)-[^{77}Br]*p*BrV was lower than that of (+)-[^{125}I]*p*IV. As a result of *in vitro* binding assay to sigma receptors, the affinities of (+)-*p*BrV to sigma receptors were competitive to those of (+)-*p*IV. In biodistribution experiments, (+)-[^{77}Br]*p*BrV and (+)-[^{125}I]*p*IV showed high uptake in tumor via sigma receptors. The biodistributions of both radiotracers showed similar patterns. However, the accumulation of radioactivity in liver after injection of (+)-[^{77}Br]*p*BrV was significantly lower compared to that of (+)-[^{125}I]*p*IV.
Conclusion: These results indicate that radiobromine-labeled *p*BrV possesses great potential as a sigma receptor imaging agent for PET.

1. Introduction

Although the sigma receptor was originally discovered in the late 1970s as a new subtype of opioid receptor [1], it was subsequently reclassified as an original, different type of receptor from the opioid receptors. There are at least two subtypes of sigma receptors, designated 25.3 kD sigma-1 and 21.5 kD sigma-2 [2]. In the central nervous system, these receptors have been shown to be involved in the regulation of neurotransmitter release, modulation of neurotransmitter receptor function, learning and memory processes, and regulation of movement and posture [3]. Although they are expressed in peripheral tissues such as liver, kidney, and endocrine organs, their functions in these tissues have been much less understood. At the same time, it has been reported that both sigma receptor subtypes are highly expressed in a variety of human tumors such as prostate cancer, breast cancer, malignant melanoma, renal carcinoma, colon carcinoma, glioma, neuroblastoma, small cell lung carcinoma, and non-small cell lung carcinoma [4–6]. Malignant cells show higher receptor expression than non-malignant cells originating from the same tissue [7]. Although the molecular functions of the sigma receptors are not yet fully defined, there is increasing evidence that sigma receptors could play a significant role in cancer biology [6]. The sigma receptors could be potential biomarkers of tumor proliferation because they are highly expressed in rapidly proliferating cells and are down-regulated when cells become quiescent [8–10]. Sigma receptor ligands could also be candidate drugs for cancer therapy because some ligands have been reported to affect cell growth and apoptosis [11,12]. Thus, imaging sigma receptors might have potential in the prognosis and early diagnosis of the therapeutic

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http://dx.doi.org/10.1016/j.nucmedbio.2013.02.008

effects of drugs by determining the expression level of sigma receptors and drug development by determining receptor occupancy. For now, it was also reported that radiolabeled sigma ligands should be useful for monitoring the early effects of chemotherapy before morphologic changes are observed [13].

Previously, we have developed several vesamicol analogs with iodine into the 4-phenylpiperidine moiety as sigma receptor imaging agents, and determined the binding affinities for the sigma receptors of the vesamicol analogs [14,15]. In these vesamicol analogs, the (+)-enantiomer of 2-[4-(4-iodophenyl)piperidino] cyclohexanol [(+)-*p*IV] showed the highest affinities for the sigma receptors [15]. Thus, to evaluate the potential of radioiodinated (+)-*p*IV for tumor imaging, biodistribution experiments of (+)-[^{125}I]*p*IV using tumor-bearing mice were performed. As a result, (+)-[^{125}I]*p*IV showed high uptake and long residence in the tumor. High tumor-to-blood and tumor-to-muscle ratios were achieved because the radioactivity levels of blood and muscle were low [16]. However, the accumulations of radioactivity in normal tissues, such as liver and kidney, were high. The clearances of radioactivity from these tissues were slow.

At present, bromine-76 is a promising radionuclide because it is a positron emitter and has a relatively long half-life ($t_{1/2}$ = 16.1 h) for PET imaging. Additionally, the chemical properties of bromine are similar to those of iodine. In this study, to develop a novel PET tracer for sigma receptor imaging, we designed 2-[4-(4-bromophenyl) piperidino] cyclohexanol (*p*BrV), which is a bromine-substituted derivative, instead of iodine in *p*IV, and evaluated the binding affinity of *p*BrV for sigma receptors, and performed *in vitro* and *in vivo* experiments of radiobromine-labeled(+)-*p*BrV. Although we are interested in developing ^{76}Br-labeled sigma receptor imaging agents for PET, in these initial studies, ^{77}Br was used because of its longer half-life ($t_{1/2}$ = 57.0 h).

2. Materials and methods

2.1. Materials

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL JNM-ECS400 spectrometer (JEOL Ltd., Tokyo, Japan), and the chemical shifts were reported in ppm downfield from an internal tetramethylsilane standard. Electrospray ionization mass spectra (ESI-MS) were obtained with an LCQ (Thermo Fisher Scientific, Waltham, MA, USA). Optical rotations were measured by a model SEPA-300 high-sensitive polarimeter (HORIBA, Kyoto, Japan). [^{3}H]1,3-Di-tolylguanidine ([^{3}H]DTG) (1.1 TBq/mmol), (+)-[^{3}H]pentazocine (1.0 TBq/mmol), and [^{125}I]sodium iodide (644 GBq/mg) were purchased from PerkinElmer (Waltham, MA, USA). TLC analyses were performed with silica plates (Art 5553, Merck, Darmstadt, Germany). SA4503 was kindly supplied by M's Science (Kobe, Japan). DTG, (+)-pentazocine, and haloperidol were purchased from Sigma Chemical (St. Louis, MO, USA). Other reagents were of reagent grade and used as received.

2.2. Production of bromine-77

^{77}Br was produced in University of Fukui. Radiosynthetic isolation and purification of ^{77}Br were performed according to a previously reported method with minor modification [17,18]. ^{77}Br was obtained from a ^{77}Se(p,n)^{77}Br reaction on an isotopically enriched $Cu_2^{77}Se$-coated tungsten target. The target was bombarded with an 8 mA/11 MeV proton beam on an RDS Eclipse HP/RD cyclotron (Siemens, Knoxville, TN, USA) for 2 h. The activity was dry-distilled from the target under argon gas flow in a quartz tube in a 1100 °C oven. ^{77}Br ion and a small amount of target material were deposited outside the oven in the quartz tube; the ^{77}Br ion was washed from the quartz tube with distilled water. The bromine-77-containing water was passed over a C18 Sep-Pak column (Waters, Milford, MA, USA). The resulting solution was placed in a glass reaction vial, and the solvent was removed using heat and a stream of N_2.

2.3. Preparation of (+)-enantiomer of 2-(4-(4-bromophenyl) piperidino)cyclohexanol [(+)-pBrV, (+)-**2**]

The (+)-enantiomer of 2-(4-(4-aminophenyl)piperidino)cyclohexanol (**1**) was prepared in 4 steps from 4-phenylpiperidine using a method described previously [19,20].

(+)-Compound **1** (274 mg, 1 mmol) was dissolved in 1 mL of 10% HBr, and $NaNO_2$ (138 mg, 2 mmol) in 1 mL of water was added to the solution by dropwise under shade at 0 °C. After the solution was stirred for 10 min at 0 °C, CuBr (574 mg, 4 mmol) in 1 mL of 47% HBr was added dropwise to the solution. After being stirred for 30 min at 0 °C, the reaction solution was stirred for 20 h at room temperature. The reaction solution was adjusted to pH 12 with 2 M NaOH, and was extracted with 5% methanol in dichloromethane. The organic layer was dried with Na_2SO_4, and the solvent was removed *in vacuo*. The residue was purified by chromatography on silica gel using chloroform–methanol (19:1) as the eluent to obtain (+)-2-(4-(4-bromophenyl)piperidino)cyclohexanol [(+)-*p*BrV] (**2**) (86 mg, 26%) as a brown powder.

^{1}H NMR ($CDCl_3$): δ 1.26 (4H, m), 1.64–1.95 (8H, m), 2.06–2.34 (3H, m), 2.46 (1H, m), 2.76 (2H, m), 2.95 (1H, d), 3.39 (1H, m), 7.11 (2H, d), 7.43 (2H, d)

MS (ESI) $C_{17}H_{24}{}^{79}BrNO$: *m/z* 338 $(M + H)^+$, $C_{17}H_{24}{}^{81}BrNO$: *m/z* 340 $(M + H)^+$

Specific rotation: $[\alpha]_D^{22} = +21.7°$ (c = 0.14, methanol)

2.4. Preparation of (−)-enantiomer of 2-(4-(4-bromophenyl) piperidino)cyclohexanol [(−)-pBrV, (−)-**2**]

(−)-*p*BrV was prepared by the same method as was (+)-*p*BrV using the (−)-enantiomer of vesamicol as a starting material instead of the (+)-enantiomer of vesamicol. (−)-*p*BrV was obtained in 29% yield from (−)-compound **1**.

^{1}H NMR ($CDCl_3$): δ 1.23 (4H, m), 1.59–1.92 (8H, m), 2.13–2.24 (3H, m), 2.46 (1H, m), 2.74 (2H, m), 2.93 (1H, d), 3.40 (1H, m), 7.10 (2H, d), 7.42 (2H, d)

MS (ESI) $C_{17}H_{24}{}^{79}BrNO$: *m/z* 338 $(M + H)^+$, $C_{17}H_{24}{}^{81}BrNO$: *m/z* 340 $(M + H)^+$

Specific rotation: $[\alpha]_D^{22} = -22.8°$ (c = 0.14, methanol)

2.5. In vitro competitive binding assay

Animal experimental protocols were approved by the Committee on Animal Experimentation of Kanazawa University. Experiments with animals were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Kanazawa University. The animals were housed with free access to food and water at 23 °C with a 12-h alternating light/dark schedule. Rat brain and liver membranes for binding experiments were prepared from rat brains without cerebellum and rat liver in male Sprague–Dawley rats (200 g, Japan SLC, Inc., Hamamatsu, Japan) using a method described previously [14,21].

A sigma-1 receptor binding assay and a sigma-2 receptor binding assay were performed using the same methods described previously [22].

2.6. Preparation of (+)-[^{77}Br]pBrV ((+)-**4**)

(+)-[^{77}Br]*p*BrV was prepared by a standard halogenation reaction of the corresponding tributylstannyl precursor under no-carrier-added conditions. Briefly, an aliquot of the aqueous solution of [^{77}Br] bromine solution (3.7 MBq/mL) was added to a reaction vial and the