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Development of a novel radiobromine-labeled sigma-1 receptor imaging probe

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ABSTRACT

Introduction: Sigma-1 receptor is a target for tumor imaging. In a previous study, we synthesized a vesamicol analog, (+)-2-[4-(4-bromophenyl)piperidino]cyclohexanol [(+)-*p*BrV], with a high affinity for sigma-1 receptor, and synthesized radiobrominated (+)-*p*BrV. This radiobrominated (+)-*p*BrV showed high tumor uptake in tumor-bearing mice; however, radioactivity accumulation in normal tissues, such as the liver, was high. We assumed that the accumulation of (+)-*p*BrV in the non-target tissues was partially derived from its high lipophilicity; therefore, we synthesized and evaluated (+)-4-[1-(2-hydroxycyclohexyl)piperidine-4-yl]-2-bromophenol [(+)-BrV-OH], which is a more hydrophilic compound. Although we aimed to develop a PET tracer using ^{76}Br, in these initial studies, we used ^{77}Br because of its longer half-life.

Methods: (+)-[^{77}Br]BrV-OH was synthesized using the chloramine-T method with a radiochemical purity of 95%. Lipophilicity and affinity for sigma-1 receptor of (+)-[^{77}Br]BrV-OH were determined, and biodistribution experiments were performed. We also performed an in vivo blocking study by co-injecting excess amounts of the sigma-1 receptor ligand, SA4503, into mice.

Results: The lipophilicity and affinity for sigma-1 receptor of (+)-[^{77}Br]BrV-OH were lower than those of (+)-[^{77}Br]*p*BrV. (+)-[^{77}Br]BrV-OH also showed high tumor uptake in biodistribution experiments in DU-145 tumor-bearing mice,. Although (+)-[^{77}Br]*p*BrV was retained in most tissues, (+)-[^{77}Br]BrV-OH was cleared from these tissues. In blocking studies, the co-injection of SA4503 significantly decreased the tumor uptake of (+)-[^{77}Br]BrV-OH.

Conclusion: These results indicate that (+)-[^{76}Br]BrV-OH has potential as a PET probe for sigma-1 receptor imaging.

1. Introduction

Sigma receptors were originally reported in the late 1970s as a new subtype of the opioid receptor family due to the cross-reactivity of some opioid receptor ligands [1]. Later, they were reclassified as original and different from the other opioid receptors because of their unique characteristics. There are two independent subtypes of sigma receptors, namely sigma-1 and sigma-2 [2]. Reports have shown that sigma-1 receptor, with molecular weight is 25.3 kDa, contains 223 amino acids and is mainly located on the endoplasmic reticulum (ER) membrane [3]. Sigma-1 receptor acts as a chaperone molecule to other proteins with physiologically important functions, regulates physiological functions such as the release of signaling substances, and is related to critical functions in the central nervous system, including learning, memory, movement, emotion, stress, and pain [4,5]. Therefore, it is anticipated that ligands for sigma-1 receptor possess the potential for therapeutic applications in the field of neuropsychiatry, which deals with depression, drug addiction, schizophrenia, and neurodegenerative disorders [6].

The molecular identity of sigma-2 receptor had been obscure [7], and elucidating the biological functions of sigma-2 receptor had been disturbed by the lack of a known amino acid sequence. In 2011, it was reported that the sigma-2 receptor binding site resides within the progesterone receptor membrane component 1 (PGRMC1) protein complex [8]. On the other hand, it was reported that sigma-2 receptor and PGRMC1 are different binding sites derived from independent genes [9]. Alon et al. eventually reported that sigma-2 receptor was identified as TMEM97, and revealed its amino acid sequence and structural

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analysis [10]. Because the biological functions of sigma-2 receptor have not been completely known, this report is a turning point in elucidating these functions and roles. The high expression of both sigma receptor subtypes has been elucidated in various human tumors, such as prostate cancer, breast cancer, malignant melanoma, renal carcinoma, colon cancer, glioma, neuroblastoma, small cell lung carcinoma, and non-small cell lung carcinoma [11–13]. Moreover, these sigma receptors could be potential targets for tumor imaging because of their high expression in rapidly proliferating cells [14,15], and much research has focused on the development of tumor imaging agents [16].

Our research group has previously reported several vesamicol analogs with iodine for sigma receptor imaging and determined sigma receptor binding affinities of these analogs [17,18]. The (+)-enantiomer of 2-[4-(4-iodophenyl)piperidino] cyclohexanol [(+)-*p*IV, Fig. 1A], which showed the highest affinity for sigma receptors of the vesamicol analogs, was radiolabeled using radioiodine and evaluated as a tumor-imaging agent. (+)-[^{125}I]*p*IV not only showed a high uptake and a long residence in the tumor but also showed a high uptake in non-target tissues, such as in the liver, during biodistribution experiments in tumor-bearing mice [19]. Further, in addition to radioiodines (^{123}I for SPECT, ^{124}I for PET, ^{125}I for basic research, and ^{131}I for therapy), radiobromine (^{76}Br) is a promising radioisotope because it is a positron emitter, has a relatively long half-life ($t_{1/2}$ = 16.1 h), and has chemical properties similar to those of iodine. This relatively long half-life of ^{76}Br makes it possible to deliver the probes to distant places and to take delayed PET scan, such as at 1 day postinjection, which are impossible for ^{18}F-labeled probes. However, although several ^{18}F-labeled PET probes have been reported in sigma-1 receptor imaging [20–22], radiobromine-labeled sigma-1 receptor imaging probes have been barely developed except in our previous research, in which 2-[4-(4-bromophenyl)piperidino] cyclohexanol (*p*BrV, Fig. 1B), a bromine-substituted derivative, instead of iodine in *p*IV, was synthesized and its binding affinity of *p*BrV for sigma receptors was evaluated [23]. In addition, in vitro and in vivo experiments of radiobromine-labeled *p*BrV were performed. Although radiobromine-labeled *p*BrV was also highly accumulated in the tumor, its biodistribution did not sufficiently reveal specific accumulation in the tumor. In the present study, we designed a new brominated vesamicol derivative, (+)-4-[1-(2-hydroxycyclohexyl) piperidine-4-yl]-2-bromophenol [(+)-BrV-OH, Fig. 1C], which is a more hydrophilic compound than (+)-*p*BrV, by the introduction of a hydroxyl group because we assumed that (+)-*p*BrV accumulation in the non-target tissues could be partially derived from its high lipophilicity. We synthesized and evaluated radiobromine-labeled (+)-BrV-OH as a sigma receptor imaging agent. Although we aimed to develop ^{76}Br-labeled sigma receptor imaging agents for PET, we used ^{77}Br in these initial studies because of its longer half-life ($t_{1/2}$ = 57.0 h).

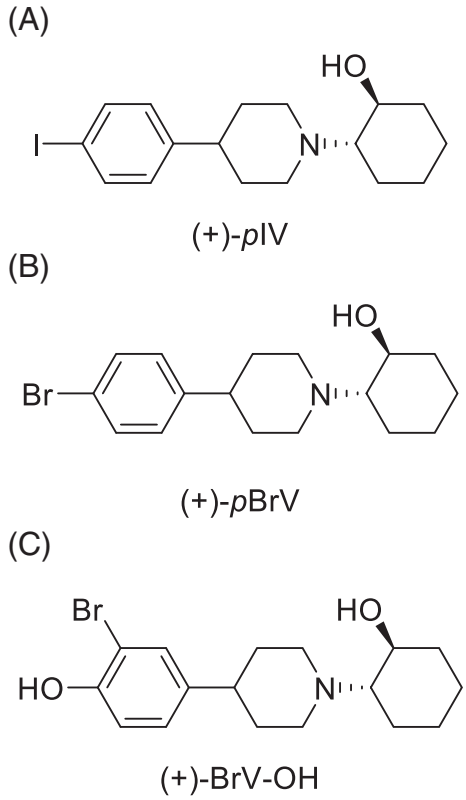

Fig. 1. Chemical structures of (A) (+)-*p*IV, (B) (+)-*p*BrV, and (C) (+)-BrV-OH.

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 a JEOL JMS-T100TD (JEOL Ltd). Optical rotations were measured by a model SEPA-300 high-sensitive polarimeter (HORIBA, Kyoto, Japan). [^{3}H]1,3-*o*-Di-tolylguanidine ([^{3}H]DTG) (1.1 TBq/mmol) and [^{3}H]pentazocine (1.0 TBq/mmol) 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-Aldrich Co. (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 from a ^{77}Se(p,n)^{77}Br reaction on an isotopically enriched $Cu_2^{77}Se$ coated tungsten target with 8 mA/11 MeV proton beam on a RDS Eclipse HP/RD cyclotron (Siemens, Knoxville, TN, USA) [23–25].

2.3. Chemical synthesis

2.3.1. Preparation of (+)-enantiomer of 4-[1-(2-hydroxycyclohexyl) piperidine-4-yl]-2-bromophenol [(+)-BrV-OH, (+)-(**2**)]

The (+)-enantiomer of 2-(4-(4-aminophenyl)piperidino) cychlohexanol was prepared in 4 steps from 4-phenylpiperidine using a method described previously [26,27]. Next reaction was performed according to the method described previously [28], and (+)-4-[1-(2-hydroxycyclohexyl)piperidine-4-yl]phenol [(+)-Ves-OH, (+)-(**1**)] was used in the next reaction without purification. Crude (+)-(**1**) (69 mg) was dissolved in 500 μL of acetic acid and 1 mL of water was added to the solution. Then, a mixture solution of bromine (13.3 μL) and acetic acid (26.7 μL) was added to the reaction mixture. After being stirred for 30 min at room temperature, the reaction mixture was adjusted to pH 12 with 2 M NaOH and was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and the solvent was removed in vacuo. The residue was purified by chromatography on silica gel using chloroform-methanol (10:1) as the eluent to obtain (+)-BrV-OH [(+)-(**2**)] [10.4 mg, 10% from (+)-2-(4-(4-aminophenyl) piperidino)cychlohexanol] as a yellow powder. At the same time, unreacted purified (+)-compound **1** (7.5 mg) was collected as a yellow powder. The purified (+)-(**1**) was used for radiolabeling.

^{1}H NMR ($CDCl_3$): δ 1.25 (4H, m), 1.73–1.84 (8H, m), 2.13 (1H, m), 2.24–2.26 (2H, m), 2.42 (1H, m), 2.75 (2H, m), 2.96 (1H, d), 3.40 (1H, s), 6.94–6.96 (1H, d), 7.07–7.08 (1H, d), 7.31 (1H, s)

ESI-MS (*m/z* calcd for $C_{17}H_{24}BrNO_2$ ($[M + H]^+$): 354.1, 356.1 found 354.0, 356.0).

Specific rotation: $[\alpha]_D^{22} = +19.7°$ (c = 0.14, methanol).