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Preparation and evaluation of an astatine-211-labeled sigma receptor ligand for alpha radionuclide therapy

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ABSTRACT

Introduction: Sigma receptors are overexpressed in a variety of human tumors, making them potential targets for radionuclide receptor therapy. We have previously synthesized and evaluated ^{131}I-labeled (+)-2-[4-(4-iodophenyl)piperidino]cyclohexanol [(+)-[^{131}I]*p*IV], which has a high affinity for sigma receptors. Therefore, (+)-[^{131}I]*p*IV significantly inhibited tumor cell proliferation in tumor-bearing mice. In the present study, we report the synthesis and the *in vitro* and *in vivo* characterization of (+)-[^{211}At]*p*AtV, an ^{211}At-labeled sigma receptor ligand, that has potential use in alpha-radionuclide receptor therapy.
Methods: The radiolabeled sigma receptor ligand (+)-[^{211}At]*p*AtV was prepared using a standard halogenation reaction generating a 91% radiochemical yield with 98% purity after HPLC purification. The partition coefficient of (+)-[^{211}At]*p*AtV was measured. Cellular uptake experiments and *in vivo* biodistribution experiments were performed using a mixed solution of (+)-[^{211}At]*p*AtV and (+)-[^{125}I]*p*IV; the human prostate cancer cell line DU-145, which expresses high levels of the sigma receptors, and DU-145 tumor-bearing mice.
Results: The lipophilicity of (+)-[^{211}At]*p*AtV was similar to that of (+)-[^{125}I]*p*IV. DU-145 cellular uptake and the biodistribution patterns in DU-145 tumor-bearing mice at 1 h post-injection were also similar between (+)-[^{211}At]*p*AtV and (+)-[^{125}I]*p*IV. Namely, (+)-[^{211}At]*p*AtV demonstrated high uptake and retention in tumor via binding to sigma receptors.
Conclusion: These results indicate that (+)-[^{211}At]*p*AtV could function as an new agent for alpha-radionuclide receptor therapy.

1. Introduction

The sigma receptors were discovered in the 1970s and categorized as an opioid receptor subtype [1]. Because of their unique characteristics, the sigma receptors were subsequently reclassified as a unique receptor type that was distinct from the opioid receptors. There are at least two subtypes; a 25.3-kD protein designated sigma-1 and a 21.5-kD protein designated sigma-2 [2]. The sigma-1 receptor comprises 223 amino acids and is predominantly localized to the endoplasmic reticulum (ER) membrane [3]. Although the identity of the sigma-2 receptor subtype has been unknown, recently, the sigma-2 receptor was identified as potentially being identical to the progesterone receptor membrane component 1 [4].

The sigma-1 receptors regulate a number of critical functions in the central nervous system, including neurotransmitter release, neurotransmitter receptor function, learning, memory, and movement [5]. Thus, their activity has been linked to neurological conditions, such as anxiety, depression, schizophrenia, and drug addiction [6]. Meanwhile, it has been reported that the sigma-1 and sigma-2 receptors are highly expressed in many kinds of human tumor and in malignant cells at higher levels than in non-malignant cells from the same tissue [7,8]. The high expression of sigma receptors in tumors induces the development of compounds labeled with radionuclides that target sigma receptors for tumor diagnosis. In fact, a number of radiolabeled sigma receptor ligands already have been developed for use as positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging agents [9,10].

We have previously developed several vesamicol derivatives introduced with iodine into the vesamicol benzene ring and determined their sigma receptor-binding affinities [11,12]. Iodine introduction increased the affinity of vesamicol derivatives for the sigma receptors. Moreover, the (+)-enantiomers of the vesamicol derivatives showed

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the higher affinity for the sigma receptors than the corresponding (−)-enantiomers. Among these vesamicol analogs, the (+)-enantiomer of 2-[4-(4-iodophenyl)piperidino] cyclohexanol [(+)-*p*IV] demonstrated the highest sigma receptor affinity [12]. Therefore, (+)-[^{125}I]*p*IV was prepared and evaluated in the biodistribution experiments using DU-145 tumor-bearing mice, which overexpresses the sigma receptors [13,14]. Because (+)-[^{125}I]*p*IV demonstrated high uptake and long residence in the tumor, we synthesized (+)-[^{131}I]*p*IV, which was labeled with ^{131}I as a beta particle emitter instead of ^{125}I and evaluated its utility for radionuclide therapy [15]. Results revealed that a single injection of (+)-[^{131}I]*p*IV significantly inhibited tumor growth in DU-145 tumor-bearing mice.

Currently, astatine-211 (^{211}At) has gained much attention as an alpha particle emitting radionuclide for its use in radionuclide therapy. ^{211}At ($t_{1/2}$ = 7.21 h) decays to ^{207}Bi (41.8%) with emission of α-particles (5.9 MeV) or ^{211}Po (58.2%) via electron capture. Subsequently, ^{207}Bi ($t_{1/2}$ = 33.9 years) decays to ^{207}Pb via electron capture and ^{211}Po ($t_{1/2}$ = 516 ms) decays to ^{207}Pb with an emission of α-particles (7.5 MeV). Moreover, ^{211}At, the 85th element in the periodic table, is one of halogen elements. This study evaluated the feasibility of using a sigma receptor ligand labeled with ^{211}At instead of ^{131}I for radionuclide therapy. The (+)-enantiomer of 2-[4-(4-astatinophenyl)piperidino] cyclohexanol [(+)-[^{211}At]*p*AtV] was synthesized and its utility was evaluated both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

[^{125}I]Sodium iodide (644 GBq/mg) was purchased from PerkinElmer (Waltham, MA, USA). Haloperidol was purchased from Sigma Chemical (St. Louis, MO, USA). SA4503 was kindly supplied by M's Science (Kobe, Japan). TLC analyses were performed with silica plates (Art 5553, Merck, Darmstadt, Germany). Other reagents were of reagent grade and used as received.

2.2. Production of astatine-211

Astatine-211 was produced according to the ^{209}Bi(α, 2n)^{211}At reaction using AVF cyclotrons at the Research Center for Nuclear Physics (RCNP), Osaka University, and at the research facility Takasaki Ion Accelerators for Advanced Radiation Application (TIARA), the Japan Atomic Energy Agency. Both cyclotrons have several external beam lines, and our targets were placed on the irradiation system connected to the end of beam line. In the RCNP, 40 MeV $^{4}He^{2+}$ beam were degraded to 28 MeV by inserting 0.28 mm of aluminum plate attached directly to the target to avoid the production of ^{210}At, and the degraded beam were bombarded to 0.1 mm thickness of Bi layer on a 1 mm of aluminum backing for 1.5 h with 2 μA. In the TIARA, 50 MeV ^{4}He2+ beam were degraded to 28 MeV by 0.4 mm of aluminum plate insertion, and the degraded beam were bombarded to 0.25 mm thickness of Bi metal plate for 2.5 h with 2 μA. The thickness of aluminum degrader was calculated using SRIM code [16]. The backing of aluminum was cooled with circulating water during irradiations.

The irradiated targets were transported to the Central Institute of Radioisotope Science, Kanazawa University for 4 h. Then, ^{211}At was isolated from the irradiated target materials using dry distillation method [17] under argon gas flow in a quartz tube in a 650 °C oven. Distillated ^{211}At was deposited outside the oven in a PEEK tube connected from the quartz tube. After dry-distillation, ^{211}At was recovered using a 2 mL of methanol washed from the PEEK tube. The resulting solution was placed in a glass reaction vial, and the solvent was removed using heat and a stream of N_2 gas. Finally, ^{211}At was prepared as an aqueous solution using distillated water.

The activity of ^{211}At was measured by a dose calibrator (curiemeter IGC-3R, Hitachi Aloka Medical, Ltd., Tokyo, Japan) before and after dry-distillation. Gamma-ray spectrometry was also performed using Ge-detector (GEM-18180P, ORTEC, Oak Ridge, TN, USA) to assign the radionuclide produced in the target and the final solution of ^{211}At.

2.3. Preparation of (+)-[^{211}At]*p*AtV (2)

(+)-[^{211}At]*p*AtV (2) was prepared by a standard halogenation reaction of the corresponding tributylstannyl precursor (Fig. 1). Briefly, 10 μL of the aqueous solution of [^{211}At]astatine solution (3.0 MBq/mL) was added to a reaction vial. Then, 50 μL of (+)-2-[4-(4-tributylstannylphenyl)piperidino] cyclohexanol [(+)-*p*-tributylstannylvesamicol] (1) (1 mg/mL in methanol), synthesized as described previously [18], 1 μL of acetic acid, and 10 μL of *N*-chlorosuccinimide solution (2 mg/mL in methanol) were added to the reaction vial containing the ^{211}At. After shaking the reaction mixture at room temperature for 30 min, the reaction mixture was purified by reversed phase (RP)-HPLC performed with a Cosmosil 5C_{18}-MS-II column (4.6 × 150 mm; Nacalai Tesque, Kyoto, Japan) at the flow rate of 1 mL/min with a gradient mobile phase of 75% acetonitrile in water with 0.05% triethylamine to 95% acetonitrile in water with 0.05% triethylamine for 20 min. The column temperature was maintained at 40 °C.

2.4. In vitro stability of (+)-[^{211}At]*p*AtV

To evaluate the stability of (+)-[^{211}At]*p*AtV *in vitro*, (+)-[^{211}At]*p*AtV was diluted 10-fold with 0.1 M phosphate buffer pH 7.4 or freshly prepared mouse plasma, and the solutions were incubated at 37 °C for 18 h. After 18 h incubation, in the case of the buffer solutions, the samples were drawn and analyzed by TLC with chloroform and methanol (5:1) as a developing solvent. In the case of the serum solutions, 10 μL of the samples were drawn and mixed with the same volume of acetonitrile. After centrifugation, the supernatants were analyzed by TLC.

2.5. Determination of partition coefficient

The partition coefficient of (+)-[^{211}At]*p*AtV was measured as described previously [15]. The partition coefficient was determined by calculating the ratio of cpm/mL in 1-octanol to that in the buffer, and expressed as a common logarithm (log *P*).

2.6. Cellular uptake experiments in vitro

Radiotracer uptake experiments in tumor cells were performed as described previously using DU-145 prostate cancer cell lines (ATCC, Manassas, VA) [19,20]. To compare the uptake between (+)-[^{211}At]*p*AtV and the corresponding ^{125}I labeled compound, the cellular uptake experiments were performed by a double tracer method. Briefly, the cells were pre-incubated for 24 h, and incubated at 37 °C in the culture medium without fetal bovine serum containing (+)-[^{211}At]*p*AtV (7.4 kBq/well) and (+)-[^{125}I]*p*IV (3.7 kBq/well), which was prepared by a method used in a previous study [15], for different time intervals (15, 30, 60, and 120 min). The cell uptakes of (+)-[^{211}At]*p*AtV and (+)-[^{125}I]*p*IV were also examined by incubation with 10 μM of haloperidol. Haloperidol is a non-selective sigma ligand, and it was used as a blocking agent could inhibit both sigma-1 and sigma-2 interactions. After incubation, the cells were washed twice with ice-cold PBS and were resolved by adding 0.5 mL of 1 M NaOH. The solutions were then collected, and the radioactivity was determined with an auto well gamma counter (ARC-380; Hitachi Aloka Medical, Ltd.) and corrected for background radiation. A window from 16 to 71 keV was used for counting ^{125}I and a window from 71 to 110 keV was used for counting for ^{211}At. Correlation factors to eliminate any crossover of ^{125}I activity into ^{211}At were determined by measuring the ^{125}I standard in both windows. More than two weeks after the experiments, ^{125}I activity was determined because the radioactivity of ^{211}At was negligible at that time. The radioactivity of each sample was normalized for the protein level,