Preparation and Preliminary Biological Evaluation of Al18F-Labeled Albumin-Binding Agent-Modified PARP-Targeted Imaging Probe

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Date: 2025-06-18T00:00:00+00:00

Abstract

Poly (ADP-ribose) polymerase (PARP) is an important target in cancer therapy, and dynamic assessment of its expression level is a necessary prerequisite for achieving precision medicine. In this study, based on the core structure of the PARP inhibitor Olaparib, we designed and synthesized a novel 18F-labeled PARP-targeted PET imaging probe [18F]AlF-NOTA-LA conjugated with an albumin binder, and evaluated its imaging performance through in vitro experiments and in vivo microPET imaging in tumor-bearing mice. The study showed that the labeling time of [18F]AlF-NOTA-LA was approximately 15 min, with a labeling yield of 49.2 ± 2.5%, a specific activity of 3.14 GBq/ mol, a radiochemical purity greater than 99%, a lipophilicity partition coefficient log P of -1.1 ± 0.013, and good in vitro stability. At 60 min, the uptake of [18F]AlF-NOTA-LA in MDA-MB-453 cells was 3.83 ± 0.26% AD, significantly higher than the uptake in A549 cells of 1.89 ± 0.12% AD. microPET imaging results showed that the tumor uptake of the probe in MDA-MB-453 tumor-bearing mice reached a maximum value of 5.31 ± 0.20% ID/g at 10 min, while the maximum uptake in A549 tumor-bearing mice and the blocking group was 3.04 ± 0.03% ID/g and 2.23 ± 0.11% ID/g, respectively. Preclinical study results preliminarily indicate that this probe has the potential to serve as a PARP-targeted PET imaging probe.

Full Text

Preparation and Preliminary Biological Evaluation of an [18F]AlF-Labeled Albumin-Binding Agent Modified PARP-Targeted Imaging Probe

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Abstract

Poly(ADP-ribose) polymerase (PARP) represents an important therapeutic target in cancer treatment, and dynamic assessment of its expression level is essential for achieving precision therapy. This study designed and synthesized a novel 18F-labeled PARP-targeted PET imaging probe by conjugating an albumin-binding moiety to the core structure of the PARP inhibitor olaparib. The resulting probe, [18F]AlF-NOTA-LA, was evaluated through in vitro experiments and in vivo microPET imaging in tumor-bearing mice to assess its imaging performance. The radiolabeling of [18F]AlF-NOTA-LA was completed in approximately 15 minutes with a radiolabeling yield of 49.2 ± 2.5%, a molar activity of 3.14 GBq/ mol, and radiochemical purity exceeding 99%. The probe exhibited a log P value of -1.1 ± 0.013 and demonstrated excellent in vitro stability. After 60 minutes, cellular uptake of [18F]AlF-NOTA-LA in MDA-MB-453 cells reached 3.83 ± 0.26%AD, significantly higher than the 1.89 ± 0.12%AD observed in A549 cells. MicroPET imaging revealed that tumor uptake in MDA-MB-453 tumor-bearing mice peaked at 10 minutes post-injection, achieving a maximum value of 5.31 ± 0.20%ID/g, while maximum uptake in A549 tumor-bearing mice and the blocking group was significantly lower at 3.04 ± 0.03%ID/g and 2.23 ± 0.11%ID/g, respectively. Preclinical results indicate that while the probe exhibited suboptimal pharmacokinetic properties in vivo, structural modifications may enhance its potential as a PARP-targeted PET imaging probe.

Keywords: PARP, olaparib, albumin binding, [18F]AlF, PET, breast cancer

Introduction

Poly(ADP-ribose) polymerase (PARP) is a class of enzymes with diverse cellular functions, primarily involving DNA repair, maintenance of genomic stability, and regulation of cell death [1,2]. The PARP family comprises at least 17 isoforms, with PARP1 being the most critical member responsible for over 90% of PARP activity in cells and playing a pivotal role in DNA damage repair. PARP1 binds to DNA damage sites (typically single-strand DNA breaks) and catalyzes the synthesis of poly(ADP-ribose) chains on protein substrates, thereby recruiting DNA repair proteins to the damage sites to facilitate repair [3,4]. Since tumor cells exhibit abnormally activated DNA damage repair pathways leading

to significantly enhanced PARP1 activity, PARP1 has become an important therapeutic target in cancer treatment [5,6].

PARP inhibitors exert antitumor effects by inducing "synthetic lethality" in BRCA1/2-mutated or homologous recombination deficiency (HRD)-positive tumor cells [7]. Since 2014, the U.S. Food and Drug Administration (FDA) has successively approved several PARP inhibitors—including olaparib, niraparib, talazoparib, and rucaparib—for clinical treatment of various solid tumors such as pancreatic [8], breast [9], prostate [10], and ovarian cancers [9,11]. Despite the important role of targeted drug therapy in cancer treatment, clinical applications still face challenges in effectively detecting tumor biomarkers. Real-time and accurate monitoring and evaluation of tumor PARP1 expression levels can help identify patients who may benefit from PARP inhibitor therapy and provide guidance for chemotherapy regimen selection and prognosis assessment.

In recent years, multiple domestic and international research teams have reported the clinical potential of PARP imaging. However, PARP imaging still faces several challenges. For instance, existing probes exhibit relatively low tumor uptake [12-14] and short retention times within tumors [14-16], leading to rapid decay of radioactive signals at tumor sites. These issues limit the imaging window, affect diagnostic accuracy, and hinder widespread application of radiolabeled PARP inhibitors. Previous studies have demonstrated that albumin-binding agents (ALB) incorporated as functional components of radioligands can effectively enhance tumor uptake of radiopharmaceuticals [17,18]. Binding of ALB to albumin can prolong the blood half-life of radioligands, thereby increasing tumor uptake and improving probe retention at tumor sites. This study designed and synthesized [18F]AlF-NOTA-LA by coupling an albumin-binding agent (4-(4-iodophenyl)butanoic acid) and a chelator (1,4,7-triazacyclononane-1,4,7-triacetic acid, NOTA) to the core structure of the PARP1 inhibitor olaparib, followed by 18F-Al labeling (Figure 1 [Figure 1: see original paper]). The feasibility of using this probe for PARP1-specific PET imaging was evaluated through in vitro and in vivo experiments.

1. Materials and Methods

1.1 Experimental Instruments and Materials

The following equipment was used: cyclotron (Sichuan Jiuyiyuan Particle Technology Co., Ltd.), high-performance liquid chromatography (HPLC) system (Model 1525, Waters Corporation, USA) with UV detector (Model 2487, Waters Corporation, USA), radioactive detector (PerkinElmer, USA), Sep-Pak C18 column (4.6 mm × 250 mm, PerkinElmer, USA), radioactivity calibrator (CRC-55tR, Beijing Pet Biotechnology Co., Ltd.), microPET scanner (Siemens, Germany), and small animal anesthesia machine (SAR-830/P, CWE, USA). Chemical reagents including 5-[(3,4-dihydro-4-oxo-1-phthalazinyl)methyl]-2-fluorobenzoic acid, 1-Boc-piperazine, 4-(4-iodophenyl)butanoic acid, thionyl chloride, N' -tert-butoxycarbonyl-L-2,4-diaminobutyric acid methyl ester, and

2,2-dimethyl-4-oxo-3,8,11,14,17-pentaoxa-5-azanonadecan-19-oic acid were purchased from Shanghai Bide Pharmaceutical Technology Co., Ltd. NHS-NOTA was obtained from Ganzhou Tanzhen Biomedical Co., Ltd., and other chemical reagents were purchased from Sinopharm Group Co., Ltd.

1.2 Cell Lines and Experimental Animals

Human breast cancer cell line MDA-MB-453 (high PARP1 expression) was purchased from Wuhan Boster Biological Technology Co., Ltd. and cultured in L-15 medium (containing 15% fetal bovine serum and 1% penicillin-streptomycin) at 37 °C with 5% CO_2. Human non-small cell lung cancer cell line A549 (low PARP1 expression) was obtained from the Chinese Academy of Sciences Cell Bank and cultured in DMEM medium (containing 10% fetal bovine serum) under similar conditions (37 °C, 5% CO_2). Female BALB/c nude mice (18-20 g) were purchased from Changzhou Cavens Experimental Animal Company. MDA-MB-453 or A549 cells (1$×10^{7}$ cells in 0.1 mL saline) were subcutaneously inoculated into the right axilla of mice. MicroPET imaging and biodistribution studies were performed when tumor diameter reached 6-10 mm. All animal experiments were approved by the Ethics Committee of Jiangsu Institute of Nuclear Medicine (JSINM-2024-114) and conducted in accordance with institutional and national regulations for laboratory animal management and use.

1.3 Chemical Synthesis

The synthetic route for the labeling precursor is shown in Figure 1. The scheme involves multiple steps using HBTU, TEA, DCM at room temperature, followed by deprotection with TFA, hydrolysis with LiOH, reaction with $SOCl_2$ in DCE at 80 °C, and final conjugation with NHS-NOTA in DMF with DIPEA at room temperature to yield NOTA-LA, which is then labeled with [18F]AlF using H18F, $AlCl_3$, and CH_3COOH.

1.3.1 Synthesis of tert-butyl (14-(4-{2-fluoro-5-[(4-oxo-3,4-dihydrophthalazin-1-yl)methyl]benzoyl}piperazin-1-yl)-14-oxo-3,6,9,12-tetraoxatetradecyl)carbamate

4-(4-fluoro-3-(piperazine-1-carbonyl)benzyl)phthalazin-1(2H)-one (10 mmol), HBTU (15 mmol), and triethylamine (30 mmol) were dissolved in 100 mL dichloromethane. 2,2-dimethyl-4-oxo-3,8,11,14,17-pentaoxa-5-azanonadecan-19-oic acid (12 mmol) was added, and the mixture was stirred overnight at room temperature. The mixture was washed with 10% hydrochloric acid, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography [eluent: dichloromethane:methanol = 200~50:1 (V/V)] to obtain a white solid (3.2 g, 45.7%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (s, 1H), 8.27 (dd, J=7.7, 1.5 Hz, 1H), 8.00–7.77 (m, 3H), 7.48–7.32 (m, 2H), 7.24 (t, J=9.0 Hz, 1H), 6.74 (t, J=5.8 Hz, 1H), 4.34 (s, 2H), 4.16 (d, J=23.1 Hz, 2H), 3.58 (dd, J=35.5, 13.6 Hz, 8H), 3.44 (s, 2H), 3.33 (s, 8H), 3.19 (d, J=11.3 Hz, 2H), 3.05 (q, J=6.1 Hz, 2H), 1.36 (s, 9H). ESI-MS (m/z): 700.67 $[M + H]^+$; 722.68 $[M + Na]^+$.

1.3.2 Synthesis of 4-[3-(4-{14-amino-3,6,9,12-tetraoxatetradecanoyl}piperazine-1-carbonyl)-4-fluorobenzyl]-1(2H)-phthalazinone (2) tert-butyl (14-(4-{2-fluoro-5-[(4-oxo-3,4-dihydrophthalazin-1-yl)methyl]benzoyl}piperazin-1-yl)-14-oxo-3,6,9,12-tetraoxatetradecyl)carbamate (5 mmol) was dissolved in 35 mL dichloromethane, and 10 mL of 4 mol/L HCl in ethyl acetate was added. The mixture was stirred at room temperature for 30 minutes. The reaction mixture was filtered, and the filter cake was adjusted to pH=12 with dilute ammonia water. After dichloromethane extraction, concentration, and drying, a white solid was obtained (2.67 g, 89.1%). ESI-MS (m/z): 601.03 $[M + H]^+$; 622.59 $[M + Na]^+$.

1.3.3 Synthesis of methyl N^6-(tert-butoxycarbonyl)-N^2-(4-(4-iodophenyl)butanoyl)-L-lysinate (4) 4-(4-iodophenyl)butanoic acid (10 mmol) was dissolved in 50 mL dichloroethane, followed by addition of thionyl chloride (35 mmol). The mixture was reacted at 80 °C for 4 hours. Thionyl chloride was removed by vacuum concentration, and the residue was diluted with 30 mL dichloromethane for later use. N' -tert-butoxycarbonyl-L-2,4-diaminobutyric acid methyl ester (10 mmol) and triethylamine (15 mmol) were dissolved in 30 mL dichloromethane, and the previously prepared 4-(4-iodophenyl)butanoyl chloride solution in dichloromethane was slowly added dropwise at 0 °C. After addition, the mixture was warmed to room temperature and stirred for 1 hour. The mixture was washed with water, concentrated, and the residue was recrystallized with 70% ethanol to obtain a white solid (3.92 g, 73.7%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60 (d, J=7.9 Hz, 2H), 6.94 (d, J=7.9 Hz, 2H), 6.06 (d, J=7.7 Hz, 1H), 4.58 (q, J=7.2, 6.7 Hz, 2H), 3.74 (s, 3H), 3.10 (q, J=6.7 Hz, 2H), 2.60 (t, J=7.6 Hz, 2H), 2.22 (t, J=7.5 Hz, 2H), 1.95 (hept, J=6.9 Hz, 2H), 1.69 (h, J=6.7 Hz, 2H), 1.49 (dt, J=14.3, 7.4 Hz, 2H).

1.3.4 Synthesis of N^6-(tert-butoxycarbonyl)-N^2-(4-(4-iodophenyl)butanoyl)-L-lysine (5) Compound 4 (5 mmol) was dissolved in 20 mL methanol, and 5 mL of 4 mol/L lithium hydroxide solution was added. The mixture was stirred overnight at room temperature. 2 mol/L dilute hydrochloric acid was slowly added dropwise to adjust the pH to 4. The mixture was filtered and dried to obtain a white solid (2.46 g, 95.3%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.42 (s, 1H), 8.02 (d, J=7.7 Hz, 1H), 7.62 (d, J=7.8 Hz, 2H), 7.02 (d, J=7.9 Hz, 2H), 6.75 (t, J=5.7 Hz, 1H), 4.13 (td, J=8.6, 4.8 Hz, 1H), 2.88 (qd, J=6.8, 3.4 Hz, 2H), 2.11 (t, J=7.4 Hz, 2H), 1.76 (p, J=7.5 Hz, 2H), 1.66 (ddt, J=14.0, 9.9, 5.7 Hz, 1H), 1.54 (dtd, J=14.1, 9.4, 5.1 Hz, 1H), 1.36 (s, 11H), 1.31–1.19 (m, 2H). ESI-MS (m/z): 541.47 $[M + Na]^+$.

1.3.5 Synthesis of LA-Boc Compound 5 (3 mmol), HBTU (4.5 mmol), and triethylamine (9 mmol) were dissolved in 20 mL dichloromethane. Compound 2 (3.6 mmol) was added, and the mixture was stirred overnight at room temperature. The mixture was washed with 10% hydrochloric acid, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column

chromatography [eluent: dichloromethane:methanol = 95:5 (V/V)] to obtain a white solid (1.59 g, 48.2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (s, 1H), 8.27 (d, J=7.7 Hz, 1H), 7.99-7.81 (m, 5H), 7.61 (d, J=7.8 Hz, 2H), 7.49-7.33 (m, 2H), 7.23 (t, J=9.0 Hz, 1H), 7.00 (d, J=7.8 Hz, 2H), 6.84-6.62 (m, 1H), 4.33 (s, 2H), 4.16 (d, J=23.0 Hz, 3H), 3.62 (d, J=16.0 Hz, 4H), 3.19 (d, J=9.0 Hz, 6H), 2.86 (q, J=6.9 Hz, 2H), 2.11 (t, J=7.3 Hz, 2H), 1.88 (s, 2H), 1.75 (p, J=7.2 Hz, 2H), 1.63-1.42 (m, 2H), 1.35 (s, 9H), 1.21 (s, 2H). ESI-MS (m/z): 1123.21 $[M + Na]^+$.

1.3.6 Synthesis of LA LA-Boc (1 mmol) was dissolved in 10 mL dichloromethane, and 5 mL of 4 mol/L HCl in ethyl acetate was added. The mixture was stirred at room temperature for 15 minutes. The reaction mixture was filtered, and the filter cake was adjusted to pH=12 with dilute ammonia water. After dichloromethane extraction, concentration, and drying, a white solid was obtained (2.67 g, 89.1%). ESI-MS (m/z): 1001.17 $[M + H]^+$.

1.3.7 Synthesis of NOTA-LA LA (26.05 nmol) and NHS-NOTA (31.25 nmol) were dissolved in 1 mL DMF, followed by addition of 25 L N,N-diisopropylethylamine. The mixture was stirred overnight at room temperature. After purification by semi-preparative separation, a white solid was obtained (11.45 mg, 34.3%). ^{1}H NMR (400 MHz, DMSO) δ 12.62 (s, 1H), 8.39 (dd, J = 13.5, 6.7 Hz, 3H), 8.26 (dd, J = 7.8, 1.5 Hz, 1H), 7.97 (d, J = 7.9 Hz, 1H), 7.93 -7.79 (m, 2H), 7.61 (dd, J = 9.2, 3.1 Hz, 2H), 7.45 (d, J = 7.8 Hz, 1H), 7.39 - 7.33 (m, 1H), 7.23 (t, J = 9.0 Hz, 1H), 7.00 (d, J = 8.1 Hz, 2H), 4.33 (s, 2H), 4.21 -4.10 (m, 3H), 3.49 (dd, J = 24.6, 14.0 Hz, 28H), 3.17 -2.87 (m, 14H), 2.59 (d, J = 22.3 Hz, 6H), 2.48 (d, J = 6.9 Hz, 2H), 2.12 (td, J = 7.3, 2.6 Hz, 2H), 1.91 (s, 2H), 1.74 (p, J = 7.5 Hz, 2H), 1.60 -1.23 (m, 6H). ESI-MS (m/z): 1123.21 $[M + Na]^+$, HPLC > 98%.

1.4 [18F]AlF Labeling

NOTA-LA (1 mg/mL, 30 µL) was mixed with 18F target water (1.85 GBq, 10 µL), acetonitrile (200 µL), aluminum chloride (2 mmol/L, 6 µL), and acetic acid (5 µL) to adjust pH to 4. The reaction was carried out at 90 °C for 8 minutes. After reaction, the mixture was diluted with 20 mL pure water and passed through a C18 reversed-phase Sep-Pak cartridge. The cartridge was washed with 20 mL pure water, and [18F]AlF-NOTA-LA was eluted with 300 µL anhydrous ethanol. The product was diluted with acetonitrile to 3.7 MBq/mL, and 10 µL was injected into analytical radioactive HPLC to determine radiochemical purity.

1.5 Determination of Lipophilicity (log P)

The probe was added to an equal volume mixture of n-octanol and water. After shaking and centrifugation (15,000 r/min, 5 min), 100 L aliquots were taken

from the upper and lower layers. Radioactivity was measured using a γ-counter, and the lipophilicity (log P) was calculated.

1.6 In Vitro Stability Studies

[18F]AlF-NOTA-LA (1.48 MBq, 50 µL) was mixed with phosphate-buffered saline (PBS, 0.01 M, pH 7.4, 450 µL) or mouse plasma (450 µL) and incubated at 37 °C for 0, 2, and 4 hours. At each time point, 25 µL samples were taken for stability analysis by radio-HPLC.

1.7 Western Blot Analysis

Proteins were extracted and quantified from MDA-MB-453 and A549 cells, then loaded onto 10% SDS-polyacrylamide gels for electrophoretic separation. Proteins were transferred to hydrophilic polyvinylidene fluoride membranes for antibody incubation. After blocking, membranes were incubated overnight at 4 °C with rabbit monoclonal anti-PARP1 antibody (1:5000, Cell Signaling Technology) and mouse monoclonal anti-β-Tubulin antibody (1:2000, Cell Signaling Technology). Subsequently, species-matched horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5000) were applied for 1 hour at room temperature. Bands were visualized using the BeyoECL Plus Western blot detection system (Beyotime Biotechnology, Shanghai), and grayscale values were quantified using ImageJ software.

1.8 Cellular Uptake Studies

MDA-MB-453 and A549 cells were seeded in 24-well plates at a density of 5$×10^{5}$ cells/mL. Radioactive probe diluted in DMEM (74 KBq/mL) was added to each well, and cells were incubated at 37 °C for four time points (30, 60, 120, and 240 minutes). In the blocking group, MDA-MB-453 cells were pre-incubated with 0.5 M olaparib for 30 minutes to inhibit probe uptake before adding the radioactive probe (74 KBq/mL) under identical conditions. At each time point, cells were washed twice with PBS and lysed with 0.1 M sodium hydroxide. Radioactivity in the lysate was measured, and cellular uptake was calculated and expressed as percentage of absorbed dose (%AD).

1.9 MicroPET Imaging

Anesthetized MDA-MB-453 tumor-bearing mice (anesthetized with 1.5% isoflurane in oxygen) were injected intravenously via the tail vein with [18F]AlF-NOTA-LA (3.7–7.4 MBq) diluted in 200 L saline. Static microPET scans were performed at 10, 30, 60, 90, and 120 minutes post-injection, with a scan duration of 10 minutes each. In the blocking group, mice were pre-injected with olaparib (5 mg/kg body weight) dissolved in 100 L mixed solvent (95% saline + 5% DMSO) 30 minutes before probe injection to evaluate competitive inhibition. Regions of interest were drawn to quantitatively analyze radioactive uptake in various organs, expressed as %ID/g.

1.10 Biodistribution Studies

At 10, 30, and 60 minutes after intravenous injection of [18F]AlF-NOTA-LA, MDA-MB-453 tumor-bearing mice were dissected to collect major organs (heart, liver, spleen, lung, stomach, small intestine, muscle, bone, pancreas, brain, blood, tumor, and ovary). Each organ was weighed and its radioactivity measured, with values expressed as %ID/g. In the blocking group, MDA-MB-453 tumor-bearing mice were pre-injected with olaparib (5 mg/kg body weight) 30 minutes before probe injection, followed by dissection and organ collection at 10 minutes post-injection.

1.11 Immunohistochemistry

To verify PARP-1 expression levels in MDA-MB-453 and A549 cells, immunohistochemical staining was performed. Tumor tissues from xenograft-bearing nude mice were fixed, routinely paraffin-embedded, and sectioned continuously at 5 m thickness for PARP-1 immunohistochemical staining. After deparaffinization, sections were treated with peroxidase blocker for 5 minutes. PARP-1 monoclonal antibody was applied at a dilution of 1:200 overnight at 4 °C, followed by ready-to-use secondary antibody and DAB (3,3' -diaminobenzidine) chromogenic development. After counterstaining, dehydration, and clearing, sections were observed under an optical microscope.

1.12 Statistical Analysis

Data analysis was performed using GraphPad Prism 8.0 software. Comparisons between two groups were conducted using independent paired t-tests, with $P < 0.05$ considered statistically significant.

2. Results

2.1 Chemical Synthesis

The molecular weight of NOTA-LA was determined to be 1219.96, consistent with the theoretical calculated value (1196.42). HPLC analysis showed product purity greater than 98% (Figure 2 [Figure 2: see original paper]A).

2.2 Radiochemistry

Compared with previously reported 18F-labeled PARP probes ([18F]F-PARPi: 90 min, radiochemical yield (RCY) 10% [12]; [18F]FTT: 90 min, RCY = 45% [19]; [18F]Olaparib: 135 min, RCY = 18% [20]; [18F]Talazoparib: 120 min, RCY = 13% [13]; [18F]Rucaparib: 150 min, RCY = 11% [21]), [18F]AlF-NOTA-LA demonstrated excellent labeling efficiency, completing radiolabeling within 15 minutes with an uncorrected yield of 49.2 ± 2.5%, radiochemical purity exceeding 99% (Figure 2B), and molar activity of 3.14 GBq/ mol.

2.3 Lipophilicity and In Vitro Stability

[18F]AlF-NOTA-LA exhibited a log P value of -1.1 ± 0.013, indicating good hydrophilicity. In vitro studies demonstrated that after 4 hours of co-incubation with PBS or mouse plasma, the probe maintained radiochemical purity greater than 99%, confirming excellent in vitro stability (Figure 2C and D).

2.4 Western Blot Analysis and Cellular Uptake

As shown in Figure 3 [Figure 3: see original paper]A, MDA-MB-453 cells exhibited higher PARP1 protein expression levels compared to A549 cells, with relative expression normalized to β-Tubulin of 1.07 ± 0.21 versus 0.58 ± 0.05 in A549 cells ($t = 4.606$, $p < 0.01$). Cellular uptake of [18F]AlF-NOTA-LA in MDA-MB-453 cells was significantly higher than in A549 cells at all time points (Figure 3C). At 60 minutes, uptake in MDA-MB-453 cells reached a plateau of 3.83 ± 0.26%AD, approximately 2-fold higher than the 1.89 ± 0.12%AD observed in A549 cells ($t = 11.45$, $p < 0.001$), consistent with Western blot results (1.84-fold difference). In the blocking group, MDA-MB-453 cell uptake was markedly reduced to only 0.43 ± 0.06%AD at 60 minutes ($t = 21.54$, $p < 0.0001$).

2.5 MicroPET Imaging

As shown in Figure 4 [Figure 4: see original paper]A and D, MDA-MB-453 tumor-bearing mice demonstrated significant tumor uptake of [18F]AlF-NOTA-LA, peaking at 10 minutes post-injection with a maximum value of 5.31 ± 0.20%ID/g. Uptake gradually decreased thereafter to 2.88 ± 0.09, 2.36 ± 0.18, 1.86 ± 0.12, and 1.62 ± 0.05%ID/g at 30, 60, 90, and 120 minutes, respectively. In contrast, A549 tumor-bearing mice showed a maximum tumor uptake of only 3.04 ± 0.03%ID/g (Figure 4B). In the olaparib blocking group, tumor uptake in MDA-MB-453 tumor-bearing mice was significantly inhibited, with uptake of only 2.23 ± 0.11%ID/g at 10 minutes (Figure 4C), demonstrating strong PARP1 targeting specificity ($t = 23.04$, $p < 0.0001$). Probe uptake in muscle of MDA-MB-453 tumor-bearing mice was low, decreasing from 1.77 ± 0.15%ID/g at 10 minutes to 0.05 ± 0.01%ID/g at 120 minutes, resulting in tumor-to-muscle ratios increasing from 2.99 ± 0.13 to 27.73 ± 4.12 over the same period (Figure 4E). In comparison, tumor-to-muscle ratios at 120 minutes were only 1.66 ± 0.40 ($t = 15.39$, $p < 0.0001$) and 8.38 ± 1.84 ($t = 10.15$, $p < 0.001$) in A549 tumor-bearing mice and the blocking group, respectively.

Compared to other PARP probes, Tao et al. [16] developed a 64Cu-labeled PARP probe [64Cu]Cu-DOTA-olaparib with maximum tumor uptake of 3.45 ± 0.47%ID/g in MSTO-211H tumor-bearing mice. Wang et al. [14] reported a 68Ga-labeled PARP probe [68Ga]Ga-DOTA-Olaparib with maximum uptake of 2.83 ± 0.32%ID/g in SKOV3 ovarian cancer xenografts. [18F]AlF-NOTA-LA demonstrated higher tumor uptake (5.31 ± 0.20%ID/g), confirming the effectiveness of the albumin-binding agent strategy. Additionally, compared to

three 18F-labeled PARP inhibitors—[^{18}F]olaparib [20] and [^{18}F]Rucaparib [15] showing maximum uptake of 3.16 ± 0.36%ID/g and 5.49 ± 0.49%ID/g, respectively, in PSN1 tumor-bearing mice, and [^{18}F]Talazoparib [13] showing 3.7 ± 0.7%ID/g in HCC1937 tumor-bearing mice—[18F]AlF-NOTA-LA exhibited superior performance. Similar to 18F-labeled PARP inhibitor imaging, this probe showed high non-specific uptake in the abdomen and suboptimal pharmacokinetic properties, requiring structural modifications to reduce lipophilicity and accelerate clearance from non-target regions. Compared to two clinically advanced probes, [18F]F-PARPi [12] and [18F]FTT [22] showed tumor-to-muscle ratios of 5.1 ± 0.9 and 1.9 at 120 minutes, respectively, while [18F]AlF-NOTA-LA achieved ratios of 6.49 ± 0.33 and 27.73 ± 4.12 at 60 and 120 minutes, respectively, demonstrating clear superiority.

2.6 Biodistribution Studies

As shown in Figure 5 [Figure 5: see original paper]A, tumor uptake of [18F]AlF-NOTA-LA in MDA-MB-453 tumor-bearing mice was 5.11 ± 0.32, 3.10 ± 0.76, and 1.76 ± 0.26%ID/g at 10, 30, and 60 minutes post-injection, respectively. In the blocking group, tumor uptake was significantly reduced to only 1.83 ± 0.85 and 0.46 ± 0.08%ID/g at 10 and 30 minutes (Figure 5C), further confirming in vivo PARP targeting specificity. Due to the albumin-binding moiety, [18F]AlF-NOTA-LA showed relatively high blood uptake of 11.11 ± 0.76 and 13.27 ± 2.03%ID/g at 10 minutes in the experimental and blocking groups, respectively (Figure 5A and C). As the primary metabolic organ, liver uptake was 20.63 ± 0.32 and 6.12 ± 1.24%ID/g at 10 and 30 minutes, respectively. Kidney uptake rapidly decreased from 9.12 ± 2.79%ID/g at 10 minutes to 1.97 ± 0.19%ID/g at 30 minutes. Bone uptake remained below 1%ID/g, indicating no in vivo defluorination and good stability. Immunohistochemistry results (Figure 5D) confirmed significantly higher PARP-1 expression in MDA-MB-453 xenograft tissues compared to A549.

Discussion

This study successfully synthesized a PARP-targeted imaging agent [18F]AlF-NOTA-LA by coupling an albumin-binding agent to the olaparib core structure using a "one-step" radiolabeling method. The probe demonstrated convenient labeling, high yield, and excellent in vitro stability. However, it exhibited high non-specific abdominal uptake and suboptimal in vivo pharmacokinetic properties. Following structural optimization, this probe shows promise as a potential PARP-targeted PET imaging agent.

Author Contributions

Wei Xu: Experimental design, chemical synthesis, animal experiments, data analysis, and manuscript writing
Yan Junjie: Design and guidance of chemical synthesis procedures

Pan Donghui: Experimental data analysis
Li Meng: Animal experiments
Wu Wei: Data compilation
Yang Min: Overall experimental guidance and manuscript revision

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Funding: This work was partially supported by the National Natural Science Foundation of China (3237143).

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