Preparation and Preliminary Biological Evaluation of Al18F-Labeled PARP-Targeted Imaging Probes Modified with Albumin-Binding Agents

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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 precision treatment. This study designed and synthesized a novel 18F-labeled PARP-targeted PET imaging probe, [18F]AlF-NOTA-LA, conjugated with an albumin-binding moiety based on the core structure of the PARP inhibitor olaparib, and evaluated its imaging performance through in vitro experiments and in vivo microPET imaging in tumor-bearing mice. The results demonstrated that [18F]AlF-NOTA-LA had a labeling time of approximately 15 min, a labeling yield of 49.2 ± 2.5%, a specific activity of 3.14 GBq/ mol, a radiochemical purity >99%, a log P value 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 that in A549 cells (1.89 ± 0.12% AD). microPET imaging revealed that tumor uptake in MDA-MB-453 tumor-bearing mice peaked at 10 min with a value of 5.31 ± 0.20% ID/g, 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. Preliminary preclinical results indicate that this probe shows potential as a PARP-targeted PET imaging agent.

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 a crucial therapeutic target in cancer treatment, and dynamic assessment of its expression level is essential for precision therapy. This study designed and synthesized a novel ^{18}F-labeled PARP-targeted PET imaging probe, [^{18}F]AlF-NOTA-LA, by conjugating an albumin-binding agent to the core structure of the PARP inhibitor olaparib. The imaging performance of this probe was evaluated through a combination of in vitro experiments and microPET imaging in tumor-bearing mice. The results demonstrated that [^{18}F]AlF-NOTA-LA could be labeled within approximately 15 minutes, with a labeling yield of 49.2 ± 2.5%, a specific activity of 3.14 GBq/ mol, radiochemical purity exceeding 99%, and a log P value of -1.1 ± 0.013, indicating excellent in vitro stability. At 60 minutes, cellular uptake of [^{18}F]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 only 3.04 ± 0.03%ID/g and 2.23 ± 0.11%ID/g, respectively. These preclinical findings suggest that this probe holds promise as a PARP-targeted PET imaging agent.

Keywords: PARP, olaparib, albumin binding, [^{18}F]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. 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 key 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 DNA repair. Due to the aberrant activation of DNA damage repair pathways in tumor cells, PARP1 activity is significantly enhanced, making PARP1 an important therapeutic target in cancer treatment.

PARP inhibitors exert antitumor effects by suppressing PARP enzyme activity and inducing “synthetic lethality” in BRCA1/2-mutated or homologous

recombination deficiency (HRD)-positive tumor cells. Since 2014, the U.S. Food and Drug Administration (FDA) has successively approved several PARP inhibitors—including olaparib, niraparib, talazoparib, and rucaparib—for clinical use in various solid tumors such as pancreatic cancer, breast cancer, prostate cancer, and ovarian cancer. Despite the importance of targeted therapy in cancer treatment, clinical application still faces challenges in effectively detecting tumor biomarkers. Real-time and accurate monitoring of tumor PARP1 expression levels can help identify patients who will benefit from PARP inhibitor therapy and guide chemotherapy selection and prognostic evaluation.

In recent years, multiple research teams have reported the clinical potential of PARP imaging. However, several challenges remain. For instance, existing probes exhibit relatively low tumor uptake and short retention times within tumors, leading to rapid decay of radioactive signals at tumor sites. These limitations restrict the imaging window, compromise diagnostic accuracy, and hinder widespread application of radiolabeled PARP inhibitors. Previous studies have demonstrated that albumin-binding agents (ALB) can effectively enhance tumor uptake of radiopharmaceuticals. Binding of ALB to albumin can prolong the blood half-life of radioligands, thereby increasing tumor uptake and improving probe retention at tumor sites. In this study, based on the core structure of the PARP1 inhibitor olaparib, we conjugated an ALB (4-(p-iodophenyl)butanoic acid) and a chelator (1,4,7-triazacyclononane-1,4,7-triacetic acid, NOTA), and obtained [^{18}F]AlF-NOTA-LA via ^{18}F-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.

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1. Materials and Methods

1.1 Experimental Instruments and Materials

Instruments included a 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), radioactivity detector (PerkinElmer, USA), Sep-Pak C18 column (4.6 mm × 250 mm, PerkinElmer, USA), radioactivity calibrator (CRC-55tR, Beijing Pate Biotechnology Co., Ltd.), microPET scanner (Siemens, Germany), and small animal anesthesia machine (SAR-830/P model, CWE, USA). Chemicals 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-aza-nonadecan-19-oic acid were purchased from Shanghai Bide Pharmaceutical Technology Co., Ltd. NHS-NOTA was obtained from Ganzhou Tanzhuo Biomedical Co., Ltd., and all other chemical reagents were purchased from Sinopharm Group Co., Ltd.

1.2 Cell Lines and Experimental Animals

The 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. The human non-small cell lung cancer cell line A549 (low PARP1 expression) was obtained from the Chinese Academy of Sciences Cell Bank and cultured under similar conditions (37°C, 5% CO_2) in DMEM medium (containing 10% fetal bovine serum). Female BALB/c nude mice (18–20 g) were purchased from Changzhou Cavens Experimental Animal Company. Tumors were established by subcutaneous inoculation of MDA-MB-453 or A549 cells (1$×10^{7}$ cells in 0.1 mL saline) 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 Jiangsu Institute of Nuclear Medicine Animal Ethics Committee (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 precursor is shown in Figure 1. The final product [^{18}F]AlF-NOTA-LA was obtained through a series of steps involving conjugation of the olaparib core with an albumin-binding moiety and NOTA chelator, followed by ^{18}F-Al radiolabeling.

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, followed by addition of 2,2-dimethyl-4-oxo-3,8,11,14,17-pentaoxa-5-aza-nonadecan-19-oic acid (12 mmol). The mixture was stirred at room temperature overnight, 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 afford 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 M HCl in ethyl acetate was added.

The reaction mixture was stirred at room temperature for 30 minutes, filtered, and the filter cake was adjusted to pH=12 with dilute ammonia water. After extraction with dichloromethane, 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 under reduced pressure, and the residue was diluted with 30 mL dichloromethane for subsequent 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 prepared 4-(4-iodophenyl)butanoyl chloride in dichloromethane was slowly added 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 from 70% ethanol to afford 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, 7.2 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 M lithium hydroxide solution was added. The mixture was stirred at room temperature overnight. The pH was adjusted to 4 by slow addition of 2 M dilute hydrochloric acid. After filtration and drying, a white solid was obtained (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, followed by addition of compound 2 (3.6 mmol). The mixture was stirred at room temperature overnight, 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 afford 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 M HCl in ethyl acetate was added. The mixture was stirred at room temperature for 15 minutes. The reaction solution was filtered, and the filter cake was adjusted to pH=12 with dilute ammonia water. After extraction with dichloromethane, 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 NOTA-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 at room temperature overnight. After semi-preparative purification, a white solid was obtained (11.45 mg, 34.3%). ESI-MS (m/z): 1123.21 [M + Na]$^+$, HPLC purity >98%.

1.4 [^{18}F]AlF Radiolabeling

NOTA-LA (1 mg/mL, 30 µL) was reacted with ^{18}F target water (1.85 GBq, 10 µL), acetic acid (5 µL), and aluminum chloride (2 mmol/L, 6 µL) 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 the product was eluted with 300 µL anhydrous ethanol to obtain [^{18}F]AlF-NOTA-LA. The product was diluted with acetonitrile to 3.7 MBq/mL, and 10 µL was injected for analytical radio-HPLC to determine radiochemical purity.

1.5 Partition Coefficient Measurement

The probe was added to an equal volume mixture of n-octanol and water, vortexed, and centrifuged (15,000 r/min, 5 min). One hundred microliters of the upper and lower phases were collected separately, and radioactivity was measured using a γ-counter to calculate the partition coefficient log P.

1.6 In Vitro Stability Study

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

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 PVDF 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). Finally, species-matched horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5000) were applied for 1 hour at room temperature. Bands were visualized using BeyoECL Plus Western blot detection system (Shanghai Beyotime Biotechnology Co., Ltd.) and quantified using ImageJ software.

1.8 Cell Uptake Study

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 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 the same 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

MDA-MB-453 tumor-bearing mice were anesthetized (1.5% isoflurane in oxygen mixture) and injected intravenously with [^{18}F]AlF-NOTA-LA (3.7–7.4 MBq) diluted in 200 L saline via the tail vein. 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. Radioactive uptake in various organs was quantified by drawing regions of interest and expressed as %ID/g.

1.10 Biodistribution Study

After intravenous injection of [^{18}F]AlF-NOTA-LA, MDA-MB-453 tumor-bearing mice were dissected at 10, 30, and 60 minutes to collect major organs (heart, liver, spleen, lung, stomach, small intestine, muscle, bone, pancreas, brain, blood, tumor, and ovary). Organs were weighed and radioactivity was 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 at 10 minutes post-injection for organ collection.

1.11 Statistical Analysis

Data analysis was performed using GraphPad Prism 8.0 software. Comparisons between two groups were conducted using independent 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 the product purity exceeded 98% (Figure 2 [Figure 2: see original paper]A).

2.2 Radiochemistry

Compared with previously reported ^{18}F-labeled PARP probes ([^{18}F]F-PARPi: 90 min, radiochemical yield (RCY) 10%; [^{18}F]FTT: 90 min, RCY=45%; [^{18}F]Olaparib: 135 min, RCY=18%; [^{18}F]Talazoparib: 120 min, RCY=13%; [^{18}F]Rucaparib: 150 min, RCY=11%), [^{18}F]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 specific activity of 3.14 GBq/ mol.

2.3 Partition Coefficient and In Vitro Stability

The partition coefficient log P of [^{18}F]AlF-NOTA-LA was -1.1 ± 0.013, indicating good hydrophilicity. In vitro studies demonstrated that after 4 hours of co-incubation with PBS or mouse plasma, the radiochemical purity of [^{18}F]AlF-NOTA-LA remained above 99%, confirming excellent in vitro stability (Figure 2C and D).

2.4 Western Blot Analysis and Cell 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 ($t = 4.606$, $p < 0.01$). MDA-MB-453 cells showed significantly higher probe uptake at all time points compared to A549 cells (Figure 3C). At 60 minutes, [^{18}F]AlF-NOTA-LA uptake in MDA-MB-453 cells reached a plateau of 3.83 ± 0.26%AD, approximately 2-fold higher than in A549 cells (1.89 ± 0.12%AD) ($t = 11.45$, $p < 0.001$), 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 exhibited significant tumor uptake of [^{18}F]AlF-NOTA-LA at 10 minutes post-injection, reaching a maximum of 5.31 ± 0.20%ID/g, which

gradually decreased 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, the negative A549 tumor-bearing model showed 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 (Figure 4E). By comparison, tumor-to-muscle ratios at 120 minutes in A549 tumor-bearing mice and the blocking group were only 1.66 ± 0.40 ($t = 15.39$, $p < 0.0001$) and 8.38 ± 1.84 ($t = 10.15$, $p < 0.001$), respectively.

Tao et al. developed a ^{64}Cu-labeled PARP small molecule probe, [^{64}Cu]Cu-DOTA-olaparib, which showed maximum tumor uptake of 3.45 ± 0.47%ID/g in MSTO-211H tumor-bearing mice. Wang et al. reported a ^{68}Ga-labeled PARP small molecule probe, [^{68}Ga]Ga-DOTA-Olaparib, with maximum tumor uptake of 2.83 ± 0.32%ID/g in SKOV3 human ovarian cancer xenograft mice. Compared with these chelator-conjugated probes, [^{18}F]AlF-NOTA-LA demonstrated higher tumor uptake (5.31 ± 0.20%ID/g), confirming the effectiveness of the albumin-binding strategy. Furthermore, compared with three other ^{18}F-labeled PARP inhibitors—[^{18}F]olaparib (3.16 ± 0.36%ID/g), [^{18}F]Rucaparib (5.49 ± 0.49%ID/g) in PSN1 tumor-bearing mice, and [^{18}F]Talazoparib (3.7 ± 0.7%ID/g) in HCC1937 tumor-bearing mice—[^{18}F]AlF-NOTA-LA showed superior performance. Compared with two clinically advanced probes, [^{18}F]F-PARPi and [^{18}F]FTT, which showed tumor-to-muscle ratios of 5.1 ± 0.9 and 1.9 at 120 minutes, respectively, [^{18}F]AlF-NOTA-LA achieved ratios of 6.49 ± 0.33 and 27.73 ± 4.12 at 60 and 120 minutes, respectively, demonstrating clear advantages.

2.6 Biodistribution Study

As shown in Figure 5 [Figure 5: see original paper]A, tumor uptake of [^{18}F]AlF-NOTA-LA was 5.11 ± 0.32%ID/g at 10 minutes post-injection, which was significantly reduced to 1.83 ± 0.85%ID/g in the blocking group ($t = 6.21$, $p < 0.01$), further confirming in vivo PARP targeting specificity. Tumor uptake gradually decreased to 3.10 ± 0.76 and 1.76 ± 0.26%ID/g at 30 and 60 minutes, consistent with microPET imaging results. Due to conjugation with an albumin-binding agent, blood uptake was relatively high, with 11.11 ± 0.76 and 13.27 ± 2.03%ID/g in experimental and blocking groups at 10 minutes, respectively (Figure 5B). 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 significant defluorination and good in vivo stability.

This study conjugated an albumin-binding agent to the olaparib core and prepared the PARP-targeted imaging agent [^{18}F]AlF-NOTA-LA using a "one-step" method. The probe features a simple labeling process, high labeling yield, and good in vitro stability, demonstrating potential as a novel PARP imaging probe.

Author Contributions

Wei Xu: Experimental design, chemical synthesis, animal experiments, data analysis, and manuscript writing. Yan Junjie: Chemical synthesis design and guidance. 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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Note: Figure translations are in progress. See original paper for figures.