Supporting information

Rectal Delivery of ⁸⁹Zr-Labeled Infliximab-Loaded Nanoparticles Enables PET Imaging-guided Localized Therapy of Inflammatory Bowel Disease

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Materials and Methods Materials

Infliximab was procured from MedChem Express (Monmouth Junction, NJ). p-Isothiocyanatobenzyl desferrioxamine (p-NCS-Bn-DFO) was sourced from Macrocyclics (Dallas, TX). ⁸⁹Zr-oxalate was purchased from Gangwon Andyco Positron Research and Development Co., Ltd. Tannic acid (TA) and poloxamer F-68 were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Dextran sodium sulfate (DSS, Mw: 36–50 kDa) was procured from Yeasen Biotech Co., Ltd. (Shanghai, China).

Synthesis and radiolabeling of ⁸⁹Zr-INF

The synthesis of DFO- infliximab and radiolabeling of DFO-infliximab with ⁸⁹Zr was performed as previous studies.¹ Infliximab was dissolved in a 0.1 M Na₂CO₃/NaHCO₃ buffer solution at a concentration of 2 mg/mL and mixed with a volume of p-NCS-Bn-DFO dissolved in DMSO at a concentration of 5 mg/mL. The molar ratio of the bifunctional chelator to antibody was maintained at 10:1. The pH of the reaction solution was adjusted to 9 using 0.1 M Na₂CO₃. The mixture was allowed to react at 37 °C for 75 minutes. Excess chelator was subsequently removed using a prepacked PD-10 desalting column. Following purification, the DFO-infliximab conjugate was concentrated using a centrifugal filter unit with a molecular weight cutoff of 30 kDa. The final concentration of infliximab was determined using the Enhanced BCA Protein Assay Kit. The pH of ⁸⁹Zr-oxalate was neutralized to 7 using 0.5 M HEPES and 1 M Na₂CO₃ prior to radiolabeling. Roughly 1 mg of DFO-infliximab was incubated with 3 mCi of neutralized ⁸⁹Zr-oxalate at 37 °C for 15 minutes. The radiochemical purity and stability of the ⁸⁹Zr-labeled antibodies were evaluated using radio thin layer chromatography (TLC, BioScan, USA) with a mobile solvent of 0.5 M citrate buffer (pH 5).

Preparation and characterization of INF@PPNP

To prepare the INF loaded nanoparticle, an aqueous TA solution (1.7 mg/mL in PBS) and an aqueous INF solution (1 mg/mL in PBS) were mixed in a 1:1 ratio, and the mixture was transferred to a 30 K ultrafiltration tube and centrifuged at 4000 rpm for 10 minutes to remove unbound TA. Next, the inner residual liquid of the ultrafiltration tube was resuspended in 1 mL of PBS, and a solution of F-68 (9.53 mg/mL in PBS) with the same volume as TA and INF was added. After thorough mixing, the mixture was centrifuged again in the 30 K ultrafiltration tube at 4000 rpm for 10 minutes. This washing step was repeated three times, each time adding 1 mL of PBS. After the final centrifugation, 2 mL of PBS was added to resuspend the solution. The Hydrodynamic size and zeta potential of the INF@PPNP were measured using dynamic light scattering (DLS) performed with a Zetasizer Nano ZSE equipped with MPT-2 autotitrator (Malvern Instruments, Ltd.). The drug release was performed by suspending INF@PPNP in SIF with various pH value (7.2, 7.6, and 8.0) for 12 h. The INF@PPNP were dispersed in 10 mL of SIF and incubated at 37 °C with mild stirring (100 rpm). 1 mL of samples were collected at predetermined time intervals and replaced with prewarmed fresh release media. The collected aliquots were centrifuged (80,000 g for 10 min, 4 $^{\circ}$ C) and the supernatant was analyzed using HLPC to quantify the antibody released from the nanoparticles.

Preparation of ⁸⁹Zr-INF@PPNP

Following the above-mentioned procedure, INF was labeled with ⁸⁹Zr to obtain ⁸⁹Zr-INF. Subsequently, using the same method as described above, the nanoparticles were prepared to obtain ⁸⁹Zr-INF@PPNP. Stability studies of ⁸⁹Zr-INF@PPNP were conducted in simulated intestinal fluid (SIF). One liter of SIF was prepared as follows: 10 g of pancreatin and 6.8 g of potassium dihydrogen phosphate. The solution pH was adjusted to 7.5 \pm 0.1 using a 0.1 M NaOH solution.

Colitis Models

All animal experiments were conducted following the National Institutes of Health guidelines for the care and use of laboratory animals and approved by the Animal Management and Ethics Committee of Jiangsu Institute of Nuclear Medicine (Wuxi, China). Colitis was induced by administering 5% DSS (dextran sulfate sodium) to female Kunming mice (8-10 weeks old, 20±3g, SPF grade, Changzhou Kewei Animals Co., Ltd) for 7 days.

In Vivo Pharmacokinetics

The DSS-induced mouse model of ulcerative colitis was divided into three groups: intravenous injection of ⁸⁹Zr-INF, rectal administration of free ⁸⁹Zr-INF, and rectal administration of ⁸⁹Zr-INF@PPNP (n=3-5). For intravenous injection, each mouse received 100 μ Ci of ⁸⁹Zr-labeled free antibody at a dosage of 3 mg/kg. Blood samples were collected from the tail vein at 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 10, 24, 48, and 72 hours after administration. Wet weights of the blood samples were measured, and the radioactive dose was counted using a γ counter. For rectal administration, each mouse received 10 μ Ci of ⁸⁹Zr-INF or ⁸⁹Zr-INF@PPNP with a volume of 100 μ L and a dosage of 3 mg/kg. Blood samples were collected at various time points after administration for wet weight measurement, γ counting, and PET imaging to compare the pharmacokinetics of the two administration routes.

Colitis treatment

Sixteen mice with similar disease activity index induced by DSS were divided into three groups: intravenous infusion of INF treatment group, rectal administration of INF@PPNP treatment group, and positive control group (n≥4). An additional 5 healthy KM mice from the same batch were used as the healthy negative control group. All groups of KM mice were fasted for 10 hours before administration. The doses for the intravenous infusion of INF treatment group and the rectal administration of INF@PPNP treatment group were both 5 mg/kg (100 μ L), and the treatments were administered continuously for 3 days. The severity of colitis in mice was evaluated using the disease activity index at the same time each day. At the end of the treatment, mice were euthanized on the 4th day. The entire colon of the mice was collected, from the lower end of the cecum to the tip of the anus, and the length was measured.

Enzyme-linked immunosorbent assay (ELISA) methods

Colons were weighed and homogenized in cold PBS (w/v = 1:9) with a protease inhibitor cocktail for 20 minutes. The homogenates were then centrifuged at 10000 g at 4°C, and the resulting supernatants were collected and stored at -80°C. The levels of TNF- α , IL-1 β , and IL-6 cytokines were determined using ELISA kits (Multisciences (Lianke) Biotech, Co., Ltd, Hangzhou, China) following the manufacturer's protocols.

Myeloperoxidase (MPO) detection

Homogenates were prepared using pre-chilled PBS (pH 6.0) containing 0.5% hexadecyltrimethylammonium hydroxide (HTAB) at a ratio of 9:1 (buffer volume to colon weight). 10 mg of *o*-dianisidine hydrochloride was dissolved in 60 mL of 50 mM phosphate-citrate buffer at pH 5.0. Immediately before use, 12 μ L of fresh 30% H₂O₂ was added. 10 μ L of homogenate supernatant was added to each well of a 96-well plate, followed by the addition of 200 μ L of *o*-dianisidine hydrochloride solution containing H₂O₂. The change in absorbance due to MPO activity was monitored immediately after addition, with readings taken every 1 minute at 405 nm using a microplate reader, continuously measuring for 10 minutes.

Hematoxylin and Eosin (H&E) and Fluorescent Immunohistochemistry (IHC) Staining

Upon harvesting, the tissues from the mice were promptly fixed in formalin for 48 hours at 4 °C. The collected tissues were embedded in paraffin, sectioned to approximately 5 μ m thickness, and subsequently stained following standard H&E protocols. For fluorescent immunohistochemical (IHC) analysis, a TNF- α antibody (ABclonal, A0277) was used at a dilution of 1:200. Visualization of stained nuclei was achieved through DAB staining. The slides with both H&E and fluorescent IHC staining were digitally scanned using an Olympus IX51 Microscopy system.

Statistical Analysis

Unless otherwise specified, all data in this study are presented as mean \pm standard deviation (mean \pm SD). Differences between multiple groups were analyzed using one-way analysis of variance (ANOVA). A value of P < 0.05 was considered statistically significant.



Figure S1. STEM-EDS elemental mapping of Zr-INF@PPNP. Scale bar is 50 nm.



Figure S2. IL-1 β and IL-6 levels in the colon tissues of mice using ELISA.

1. G. Yan, X. Wang, Y. Fan, J. Lin, J. Yan, L. Wang, D. Pan, Y. Xu and M. Yang, *Mol. Pharm.*, 2022, **19**, 3632-3639.