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Supplementary Information

Development of a diagnostic and drug evaluation system for acute inflammation using a novel [^{89}Zr]DTPA-sorbitol probe

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Experimental section

Materials

All chemical reagents, including sorbitol, were purchased from Sigma-Aldrich (St. Louis, MO, USA). 0.5M HEPES buffer (pH 7.4) purchased from Boston Bioproducts. ^{89}Zr-oxalate was provided by Korea Atomic Energy Research Institute (KAERI, Jeongeup, Korea).

Animals

Specific pathogen-free 6-week-old C57BL/6 mice were obtained from SLC, Inc. (Shizuoka, Japan). All experimental procedures were approved by the KRIBB Institutional Animal Care and Use Committee (permit number: KRIBB-AEC-24177).

Preparation of DTPA-sorbitol

Amino-sorbitol (0.5mmol), N-chlorosuccinimide-DTPA (0.6 mmol), and Dichloromethane (DCM) were added to a 1 ml pressure vial. The solution was stirred at 40^{o}C for 4 h, dried over anhydrous MgSO4, and filtered; the filtrate was concentrated in vacuo. DTPA-sorbitol was obtained by preparative HPLC (ACN:H_2O) with a 95.9% yield (white solid, 50 mg). The NMR data are as follows: ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.30-7.39 (d, J = 8.23 Hz, 6H), 4.02-4.05 (m, 1H), 4.00 (s, 1H), 3.93-3.97 (d,J=4.3 Hz, 6H), 3.88(s,1H), 3.87 (s,1H), 3.84 (s,2H). 3.80-3.83 (m,5H), 3.74-3.79(m,5H), 3.70-3.73 (m, 6H), 3.62-3.68 (m, 1H), 3.51-3.56 (m, 1H), 3.44-3.48 (m, 1H), 3.13`3.25 (m, 5H), 2.81`2.85 (m, 1H) ; ^{13}C NMR (100 MHz, MeOD-d4) δ 179.9, 172.0, 171.9, 170.1, 134.7, 130.4, 126.4, 115.3, 71.5, 71.0, 70.9, 70.1, 62.8, 62.1, 56.1, 54.3, 54.1, 52.9, 51.9, 49.3, 47.7, 31.9; HRMS (ESI) calculated for $C_{28}H_{43}N_5O_{15}S^{+}$721.7, found 722.25.

Cytotoxicity assay

Raw 264.7 cells (ATCC, USA) were seeded in a 96 well plate at a density of 1x10^{6} cells/well and treated with the indicated DTPA-sorbitol concentration for 1, 3, 6, and 9 hours. Cells were cultured in 500 µL of Dulbecco's modified Eagle medium (Welgene Inc., South Korea) containing 10 % fetal bovine serum (Gibco, Thermo Fisher Scientific) and 100 U/mL penicillin–streptomycin (Gibco) at 37°C in a 5% CO_2 incubator overnight. After incubation with DTPA-

sorbitol for 9 hours, the cell medium was discarded and replaced with 100 µl of WST-1 reagent (Takara Bio, Ohtsu, Japan) as indicated by the supplier. The cells then were incubated for 1.5 hours, and viability was calculated from absorbance measurement at 450 nm.

Radiolabeling of [^{89}Zr]DTPA-sorbitol and Radiochemical yield

[^{89}Zr]oxalate was obtained from the Korea Atomic Energy Research Institute (KAERI, Jeongeup, Korea) and produced using RFT-30 (30 MeV cyclotron). [^{89}Zr]DTPA-sorbitol was prepared through DTPA chelation. Synthesized DTPA-sorbitol was dissolved in 1 mL of 0.5 M HEPES buffer, followed by the addition of 37MBq of [^{89}Zr]oxalate. The pH was adjusted to 7.4 using HCl then was gently mixed using a Thermomixer at 37.5 °C at 350rpm for 60 minutes. Radiochemical yield (RCY) of [^{89}Zr]DTPA-sorbitol was analyzed by Radio-thin-layer chromatography (Radio-TLC) using a Bioscan AR-2000 scanner (Eckert & Ziegler, Berlin, Germany).

Statistical analysis

All data are expressed as the mean ± standard deviation (SD) from at least three independent experiments, and statistical significance of the difference was determined by unpaired Student's tests using GraphPad Prism 5. P values less than 0.05 were considered statistically significant.

Figures of experimental section

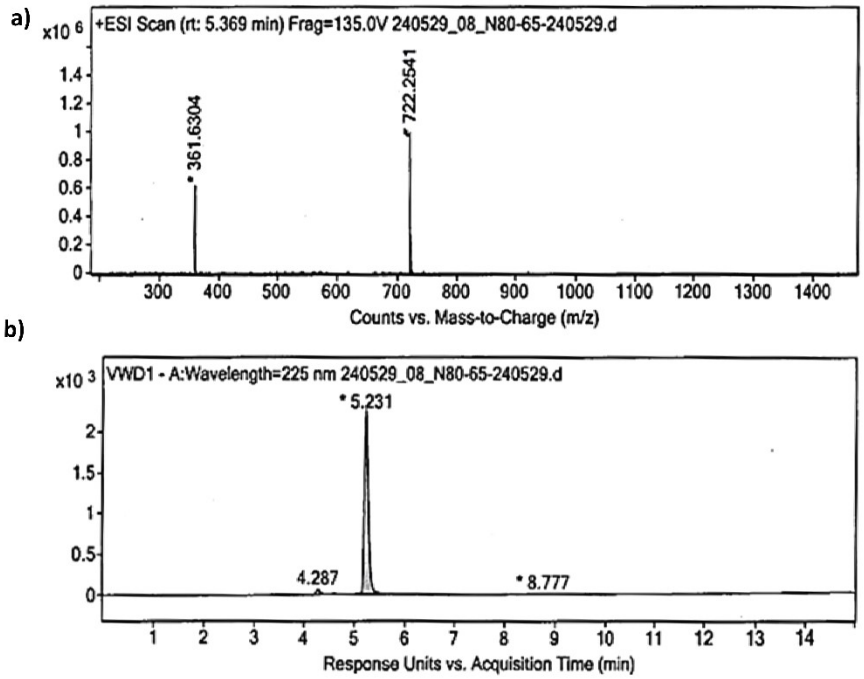

Figure S1. a) LC-Mass spectrum and b) HPLC spectrum of DTPA-sorbitol

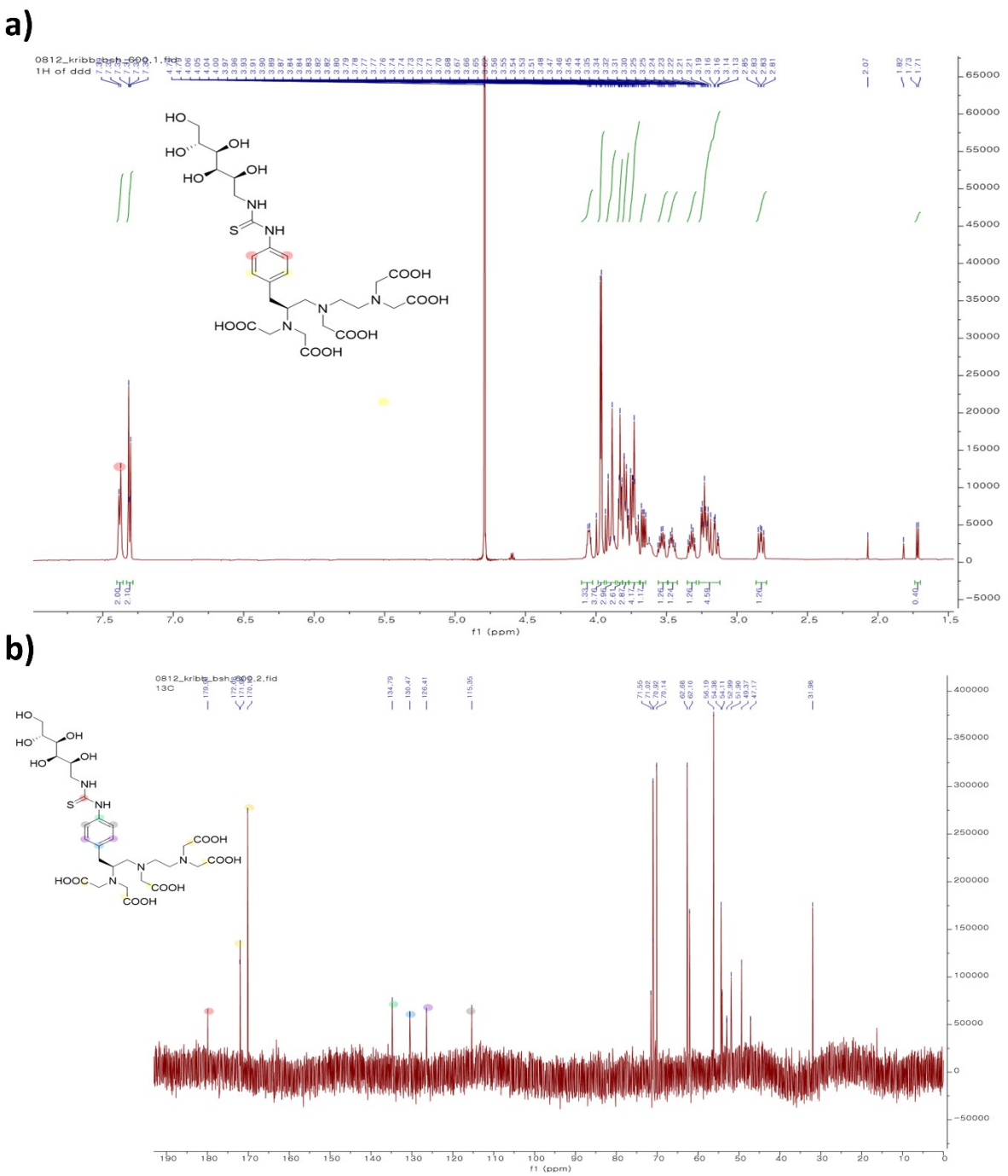

Figure S2. a) ^{1}H and (b) ^{13}C NMR spectra of DTPA-sorbitol

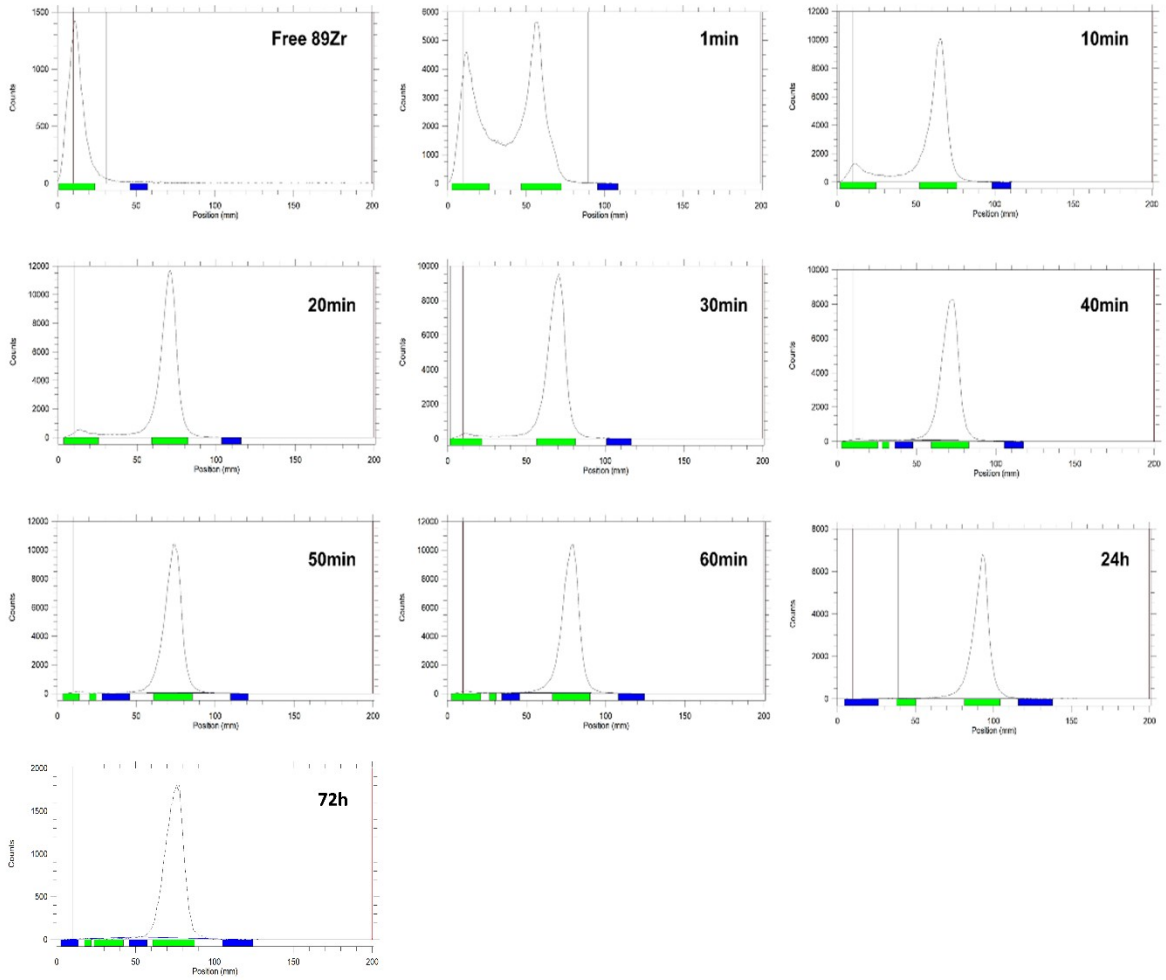

Figure S3. Radiochemical yield of [^{89}Zr]DTPA-sorbitol labeling monitored by Radio-TLC. Chelation of ^{89}Zr with DTPA-sorbitol was completed within 40 minutes, and the binding remained stable for up to 72 hours.

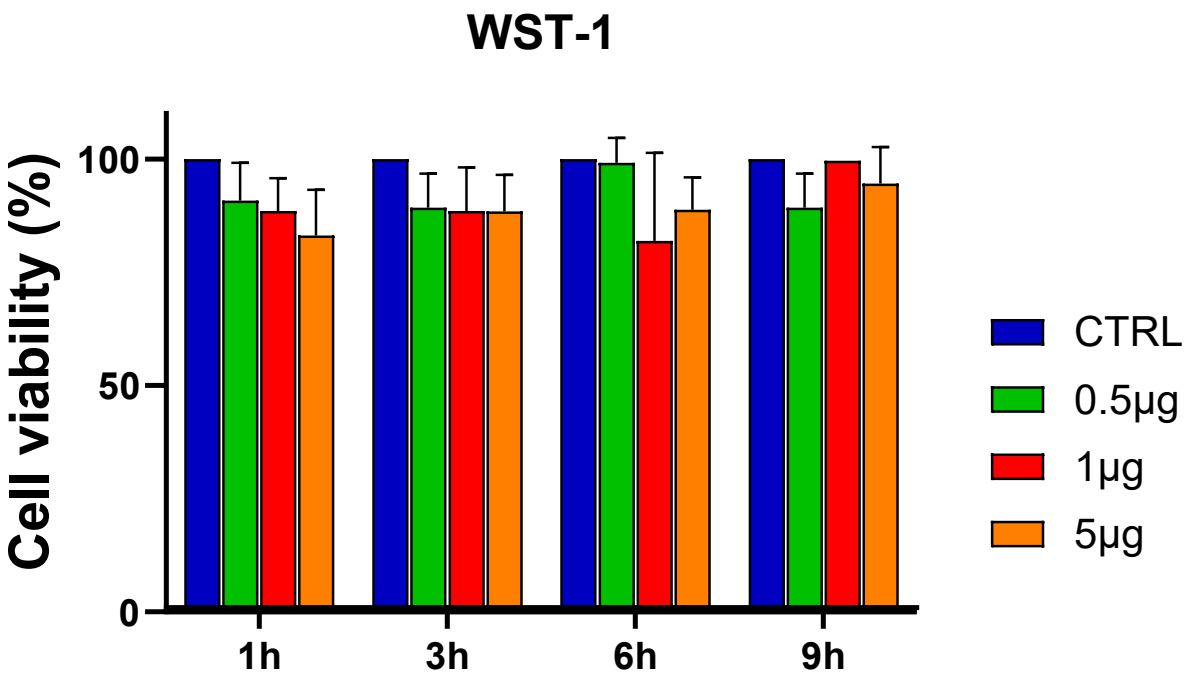

Figure S4. Cytotoxicity of DTPA-sorbitol. Viability of RAW 264.7 cells was assessed after incubation with various concentrations of DTPA-sorbitol at 1, 3, 6, and 9 hours. The data are presented as mean ± SD of three independent experiments.

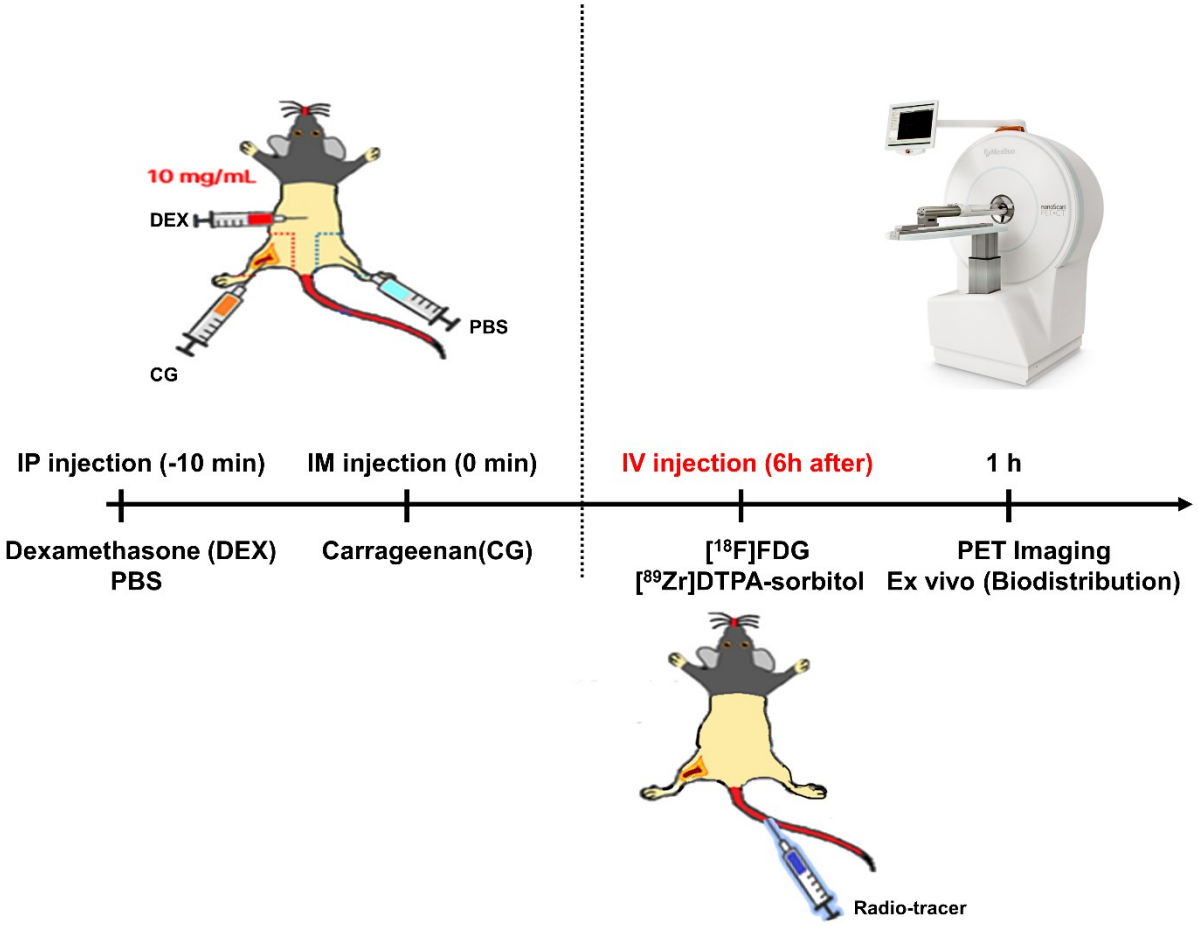

Figure S5. Experimental protocol for in vivo study. Mice received dexamethasone (20 mg/kg) via intraperitoneal injection, followed by the administration of 4% carrageenan and PBS into the left and right thighs, respectively, after 10 minutes. Six hours later, radiotracers were administered via the tail vein. PET imaging was conducted 1 hour after radiotracer administration. A biodistribution study was performed immediately following in vivo imaging.

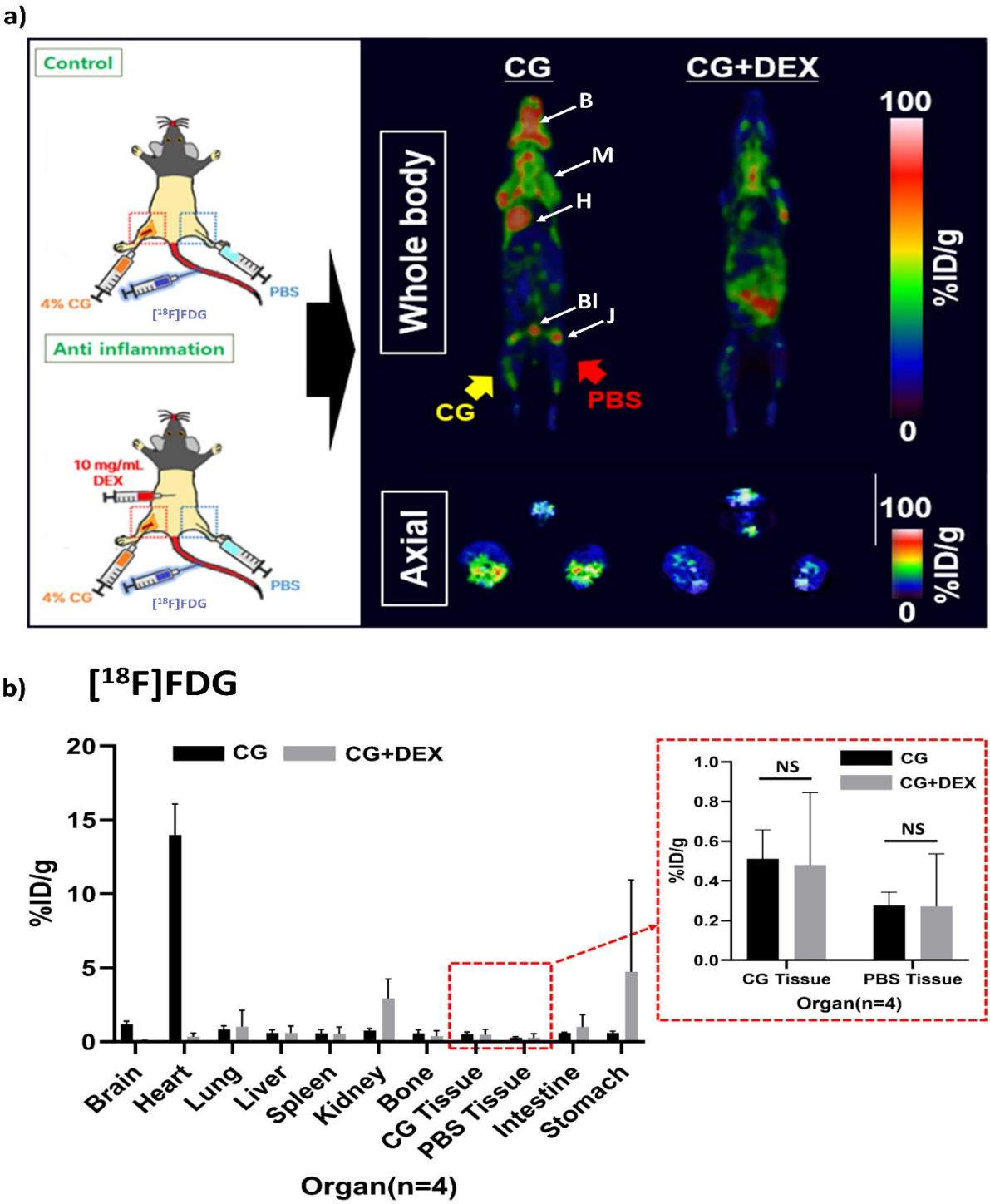

Figure S6. [^{18}F]FDG PET/CT imaging in carrageenan-induced inflammation mice and anti-inflammatory treated mice. Mice were administered same radioactivity of [^{18}F]FDG intravenously. Reconstructed PET/CT images are presented. B: Brain, M: Muscle, H: Heart, Bl: Bladder, J: Joint. CG: carrageenan, DEX: dexamethasone.