obtained with Mode A (30 mGy of pre-γ-irradiation) and Mode B (8.5 mGy of post-γ-irradiation) are shown in Tables 1 and 2, respectively. The NHEJ repair of DSB was little influenced by either modes of low-dose, low dose-rate γ-irradiation. DSB repair by HR, in contrast, was enhanced by ~50% and ~80% in Mode A and Mode B, respectively. This might implicate that both pre-γ-irradiation (Mode A) and post-γ-irradiation (Mode B) induce a radioadaptation, although both modes of irradiations, especially Mode B, are different from the original concept of radioadaptation. In fact, DSBs are generated during the γ-irradiation in Mode B, because I-SceI expression lasts for 3 days incubation as previously mentioned.

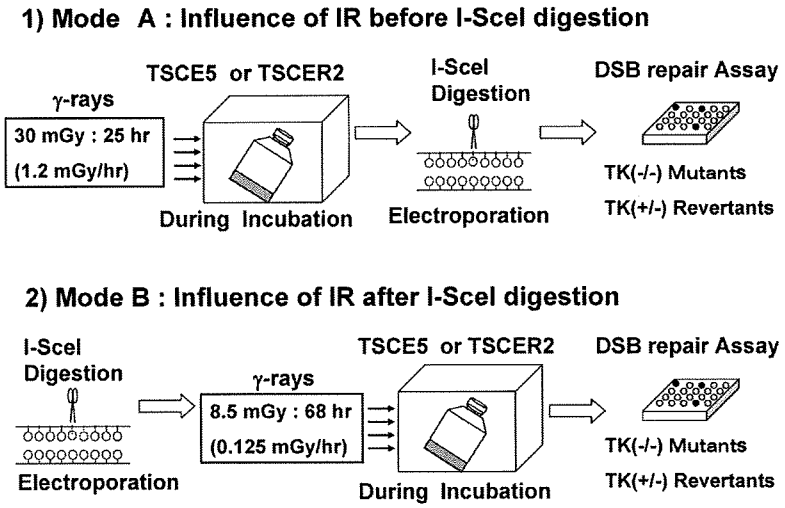

Fig. 4. Influence of low-dose IR exposure on DSB repair. Experimental schemes of radiation exposure and I-SceI expression are illustrated. Mode A: cells were exposed to low-dose, low-dose-rate γ-rays and then transfected with the I-SceI vector by electroporation (see text). 2) Mode B: cells were transfected with the I-SceI vector and then exposed to γ-rays at a much lower dose and dose-rate (see text).

Table 1. Effect of pre-IR exposure on DSB repair (Mode A).

a) NHEJ efficiency in TSCE5 cells

Exp.	Mutant Frequency, MF ($\times 10^{-6}$)				Effect of IR (Relative MF*)
	Control	γ-rays	I-SceI	γ-rays + I-SceI	
1	3.5	6.1	8600	8500	0.99
2	1.8	3.2	2900	3200	1.1
Average	2.7	4.7	5800	5900	1.0 (P = 0.82)

*Relative MF was calculated as MF (γ-rays + I-SceI)/MF (I-SceI).

b) HR efficiency in TSCER2 cells

Exp.	Revertant Frequency, RF ($\times 10^{-6}$)				Effect of IR (Relative RF*)
	Control	γ-rays	I-SceI	γ-rays + I-SceI	
1	–	–	90	114	1.3
2	–	–	62	96	1.5
3	–	–	25	45	1.8
Average	–	–	59	85	1.5 (P = 0.021)

*Relative RF was calculated as RF (γ-rays + I-SceI)/RF (I-SceI).

Table 2. Effect of post-IR exposure on DSB repair (Mode B).

a) NHEJ efficiency in TSCE5 cells

Exp.	Mutant Frequency, MF ($\times 10^{-6}$)				Effect of IR (Relative MF*)
	Control	γ-rays	I-SceI	γ-rays + I-SceI	
1	2.8	1.3	3400	4500	1.3
2	3.1	2.8	12000	17000	1.4
3	–	–	11000	11000	1.0
Average	3.0	2.1	8800	10800	1.2 (P = 0.12)

*Relative MF was calculated as MF (γ-rays + I-SceI)/MF (I-SceI).

b) HR efficiency in TSCER2 cells

Exp.	Revertant Frequency, RF ($\times 10^{-6}$)				Effect of IR (Relative RF*)
	Control	γ-rays	I-SceI	γ-rays + I-SceI	
1	–	–	82	160	2.0
2	–	–	160	270	1.7
3	–	–	110	190	1.7
Average	–	–	120	210	1.8 (P = 0.0013)

*Relative RF was calculated as RF (γ-rays + I-SceI)/RF (I-SceI).

Influence of low-dose X-ray irradiation on DSB repair

We have extensively studied the effects of low-dose IR by using a loss of heterozygosity (LOH) analysis system.[40–42] The thymidine kinase deficient (TK^-) mutants induced in TK6 cells can be classified as LOH type and non-LOH type by this system. The LOH mutants were further classified as homozygous-type and hemizygous-type, and the replaced or deleted part of the chromosome was identified by so-called chromosome mapping. In addition to this kind of analysis at the chromosome level, non-LOH mutants were further characterized at the DNA sequence level to confirm that the mutation occurs in the *TK* gene or not. Recently we could establish the optimum condition for mutagenic radioadaptation in TK6 cells.[43] Under such condition as shown in Fig. 5, the greatest reduction in *TK* mutation frequency was observed in TK6 cells exposed to a challenging X-ray irradiation (2 Gy), and the TK^- mutants so obtained were analyzed by the LOH system.[43]

The TK^- mutation frequency (MF) obtained after the challenging X-ray (2 Gy) exposure, 18.3×10^{-6} was reduced to 11.4×10^{-6} (62% of the original level) by inducing the radioadaptation (50 mGy of pre-X-irradiation at 6 hr before the above challenging X-irradiation; Fig. 6). LOH analysis could classify the TK^- mutational events as non-LOH (mostly mutations in the *TK* gene), hemizygous LOH (deletion of chromosome) and homozygous LOH (homologous recombination [HR] between chromosomes), as mentioned above.[40–42] Non-LOH events are, in theory, classified as chromosomal alterations, but most of non-LOH mutants obtained in this experiment were confirmed to be small mutations in the *TK* gene by DNA base sequencing of mRNA obtained from the mutants.[43] The pre-irradiation decreased the frequencies of non-LOH events and homozygous LOH events to 27% and 60% of the original levels, respectively. The frequency of hemizygous LOH events, however, was not significantly altered by the pre-irradiation. Since LOH events are most likely the consequence of DSB repair, we tried to investigate the influence of priming X-ray irradiation on DSB repair efficiency under the optimum condition for radioadaptation.

The repair efficiency of DSB *via* NHEJ was hardly influenced by the pre-irradiation of 50 mGy X-rays (Table 3). On the other hand, a ~70% enhancement in HR repair of DSB was observed after this treatment. The enhanced activity of HR observed in this experiment could reflect the activity of error-free DSB repair, providing a reduction in genetic alterations at the chromosome level. In fact, we observed a ~60% reduction in the induction of homozygous LOH as mentioned above. The chromosome-mapping analysis demon-

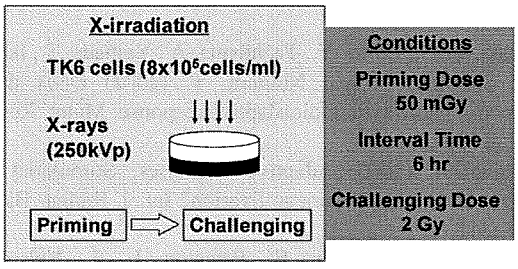

Fig. 5. An experimental scheme for mutagenic radioadaptation. The optimum conditions providing the greatest reduction in the frequency of *TK* mutations induced after a challenging X-ray (2 Gy) irradiation of TK6 cells, are shown in the right panel of this figure. The details have already been described in our previous work.[43]

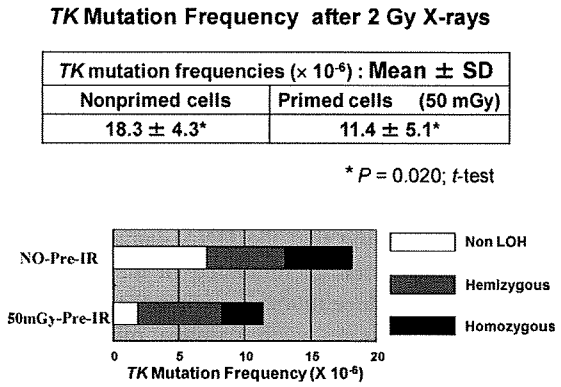

Fig. 6. Genetic analysis of radioadaptation induced by low-dose X-rays. Results of the *TK* mutation assay performed under the optimum condition for radioadaptation (Fig. 5) are summarized in the table, and the classification of the isolated TK^- mutants was made by LOH analysis and the results are shown in the histograms (see text).

Table 3. The effect of a priming X-ray exposure on DSB repair (X-ray - X-ray adaptive experiment).

a) NHEJ efficiency in TSCE5 cells

Exp.	Effect of IR (Relative MF*)
1	0.98
2	0.76
3	0.99
Average	0.91

*Relative MF was calculated as MF (X-rays + I-SceI)/MF (I-SceI).

b) HR efficiency in TSCER2 cells

Exp.	Effect of IR (Relative RF*)
1	2.2
2	1.2
3	1.7
Average	1.7

*Relative RF was calculated as RF (X-rays + I-SceI)/RF (I-SceI).

strated that the observed homozygous LOH events were mostly of the crossing-over type.[23] In contrast, the analysis of TK (+/–) revertants observed with our DSB repair assay suggests that HR in this I-SceI system mostly reflects a gene conversion activity, with a relatively small proportion of non-crossing-over events (data not shown). More supporting evidence is required to determine if an enhanced HR activity is reflected by the reduction in homozygous LOH events.

Further applications and perspectives

It is of theoretical and practical importance to estimate human health risks from low-doses of ionizing radiation. One example is the risk for astronauts exposed to space radiation, because the background radiation in space is, at least, more than 100-fold higher than the background level found on earth. Currently, we have the opportunity to study the influence of space radiation in TK6 cells, which were recently brought back to earth after preservation for more than four months, mostly in a frozen state, in the International Space Station. Assuming that the DNA damage caused by space radiation has been accumulated in the frozen cells, such damage could induce mutations when the cells begin to grow again. Furthermore, such damage might have the potential ability to induce radioadaptation and this radioadaptation might be detected as an enhancement in DSB repair in the I-SceI digestion system in the recovered cells.

The following points involved in our I-SceI digestion system merit discussion. Because our I-SceI system does not uncover all NHEJ and HR events, it is difficult to evaluate accurately the extent of DSB repair *via* both HR and NHEJ pathways. For example, our system does not monitor sister-chromatid HR, which is probably the major HR pathway in mammalian cells. Small gene conversion events, which do not extend into the exon 5 region, can also not be detected by this system. Although the I-SceI system may over-estimate the repair efficiency of NHEJ compared with HR, this methodology can still be considered to contribute to elucidating the DSB repair associated with low-dose IR exposure.

Finally, we would like to emphasize that the present evaluation of DSB repair using the I-SceI system, may contribute to our overall understanding of radioadaptation. Other types of studies regarding gene expression, epigenetic changes *etc.*, are also required for a more complete understanding.

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PET and Macro- and Microautoradiographic Studies Combined with Immunohistochemistry for Monitoring Rat Intestinal Ulceration and Healing Processes

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^{18}F-FDG PET is used mainly in clinical settings for imaging focal cancer sites, but the usefulness of the modality in imaging gastrointestinal ulcers has not been established. We investigated whether PET can be used for noninvasive monitoring of indomethacin-induced small-intestine ulceration. **Methods:** Intestinal ulcers were induced in rats by subcutaneous administration of indomethacin. An ^{18}F-FDG PET scan was obtained at 1, 2, and 7 d after indomethacin administration. ^{18}F-FDG uptake in the small intestine was quantified by γ-counting, and macro- and microautoradiographic studies were performed to determine the site of ^{18}F-FDG uptake in tissue and at the cellular level. **Results:** Ulcers observed in the intestine (mainly in the ileum) 1–4 d after indomethacin administration were most severe at 1 d after administration and were almost healed at day 7. The PET study showed increased ^{18}F-FDG uptake in the intestine correlating to the severity of ulceration, returning to the basal level on day 7. Ex vivo imaging and γ-counting showed that these regions of high uptake corresponded to regions of ulceration. A microautoradiographic study combined with immunohistochemistry revealed heavy accumulation of ^{18}F-FDG in inflammatory cells containing peroxidase on day 1 and in cells forming granulation tissue (α-smooth muscle actin–positive myofibroblasts and ED2-positive macrophages) on days 2–4 in and around ulcers. Proliferating (Ki67-immunopositive) intestinal crypt cells were also densely labeled with ^{18}F-FDG in intact intestinal tissue taken from the indomethacin-treated and the control animals. **Conclusion:** Our experimental data suggest that ^{18}F-FDG PET may be useful for evaluating the occurrence of small-intestine ulcers. Ulceration could be visualized early by the prominent uptake of ^{18}F-FDG by inflammatory cells and by the formation of granulation tissue by cells in and around ulcers.

Key Words: gastroenterology; molecular imaging; animal imaging; ^{18}F-FDG; PET; indomethacin; intestinal ulceration; microautoradiography

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Nonsteroidal antiinflammatory drugs (NSAIDs) are the most commonly prescribed drugs worldwide; however, a side effect of conventional NSAIDs, such as indomethacin, is ulceration in the small intestine, caused by suppression of mucosal prostaglandin production via inhibition of cyclooxygenase activity (*1–3*). Capsule enteroscopy is a recently developed technique that enables direct examination of the entire length of the small intestine; this examination is considered the gold standard for the diagnosis of Crohn disease of the intestine (*4,5*). Capsule enteroscopy has provided additional information on NSAID-induced intestinal pathology other than Crohn disease. Graham et al. (*6*) reported that small-intestine mucosal injury was found in 71% of chronic NSAID users, such as patients with rheumatoid arthritis, and Laurence et al. (*7*) reported that 68%–75% of volunteers had tissue injury in the small intestine after a 2-wk period of ingesting diclofenac, an NSAID with omeprazole, a proton pump inhibitor. These studies indicate the high incidence of NSAID-induced enteropathy.

PET coupled with ^{18}F-FDG, a glucose tracer, has been used clinically in the diagnosis of cancer (*8,9*) and for the evaluation of anticancer therapeutics (*10*). The accumulation of ^{18}F-FDG in cancer tissue is thought to reflect enhanced glucose use by neoplastic cells (*11–13*). ^{18}F-FDG accumulation is observed in inflammatory lesions as well as in malignant tumors (*14–17*). Regarding the gastrointestinal tract, ^{18}F-FDG PET studies have been reported in Crohn disease (*18–21*). In a PET study, Bicik et al. (*18*) demonstrated higher ^{18}F-FDG uptake in patients in the clinically active state than in those in the inactive state. These clinical reports, however, have not clarified the origin of the ^{18}F-FDG signal in tissue or at the cellular level. More recently, genetic murine colitis models were demonstrated by ^{18}F-FDG PET with a flow cytometric study, which indicated that only CD4-positive T lymphocytes accumulated ^{18}F-FDG (*22*). However, no previous studies report using PET to clarify the

possibility of noninvasive imaging of the course and healing of small-intestine ulceration. In the present study, we noninvasively observed a newly created small-intestine ulceration and its healing using ^{18}F-FDG PET. We used macro- and microautoradiographic studies, combined with immunohistochemistry, to identify the origin of the ^{18}F-FDG signals, also revealing a transition in signal between the occurrence and healing of the ulcer.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Nippon Charles River; age, 8 wk) were used in all experiments. The rats were housed in a cage with a raised mesh base, under constant environmental conditions (room temperature, 22°C−24°C; relative humidity, 60%−70%) and a 12 h−12 h light–dark cycle. Food and water were provided ad libitum. The experimental procedures used in the present study were approved by the Animal Care Committee of the Osaka City University Graduate School of Medicine. All efforts were made to minimize animal suffering and the number of animals used for the studies.

Induction of Small-Intestine Ulceration and Determination of Myeloperoxidase Activity

Small-intestine ulceration was induced by subcutaneous administration of indomethacin (Sigma-Aldrich) suspended in saline with a drop of polysorbate 80 (ICN Biomedicals Inc.) at a dose of 10 mg/kg. At 1, 2, 4, and 7 d after indomethacin administration, the rats were euthanized under deep ether anesthesia. To visualize the intestinal lesions, 1 mL of Evans blue dye (Wako Pure Chemical Industries Ltd.; 1% w/v) was injected intravenously 30 min before euthanasia in 2–3 indomethacin-administered animals. The small intestine was removed and fixed with 2% formalin.

Myeloperoxidase activity was measured to evaluate neutrophil infiltration into the intestinal mucosa according to the method reported by Krawisz et al. (*23*), with some modification. Four to five animals were used on each experimental day (days 1, 2, 4, and 7). The animals were euthanized under deep ether anesthesia at 1, 2, 4, and 7 d after indomethacin administration. Blood was then totally withdrawn by perfusion with ice-cold saline through the left ventricle. The small intestine was removed, and the wall along the opposite side of the mesenteric attachment was cut. After the tissue was rinsed with saline, the small intestine was homogenized with a 50 mmol/L concentration of phosphate buffer containing 0.5% (w/v) hexadecyl-trimethyl-ammonium bromide (Wako). The homogenized samples were subjected to freezing and thawing 3 times and centrifuged at 2,000 rpm for 10 min at 4°C. Supernatant (100 μL) was added to 1.9 mL of 10 mmol/L phosphate buffer (pH 6.0), to which 1 mL of 1.5 mol/L *o*-dianisine dihydrochloride (Sigma-Aldrich) containing 0.0005% (w/v) hydrogen peroxide (H_2O_2) was added. Absorbance at 450 nm of each sample was assessed using a spectrophotometer (Beckman Instruments). Protein content was measured using a modified bicinchoninic acid assay method (bicinchoninic acid protein assay reagent kit; Pierce). Myeloperoxidase activity was obtained from the slope of the reaction curve, on the basis of the following equation for specific activity:

$$\mu\text{mol } H_2O_2/\text{min/mg protein} = (\text{OD/min})/\text{OD}/\mu\text{mol } H_2O_2 \times \text{mg protein}.$$

^{18}F-FDG PET

^{18}F was produced by the ^{18}O(p, n)^{18}F nuclear reaction in a cyclotron at the Osaka City University, and ^{18}F-FDG was synthesized by the method reported by Hamacher et al. (*24*) using an automated ^{18}F-FDG synthesis system (JFE Steel Co.).

We used a small-animal PET scanner (microPET Focus220; Siemens Medical Solutions Inc.) with an animal port (diameter, 220 mm; axial extent, 78 mm; parallel slices, 95; and spatial resolution, 1.7 mm in full width at half maximum at the center of the field of view). The scanner had a detector system comprising 47 crystal rings made of lutetium oxyorthosilicate; 3-dimensional list-mode data were acquired. At 7 d before treatment and at 1, 2, and 7 d after indomethacin administration, PET scans were obtained on 4 rats. ^{18}F-FDG, at an activity of 55.5 MBq/kg in 0.3 mL of saline, was administered to each rat via the tail vein while the animal was conscious. At 45 min after the ^{18}F-FDG administration, the rats were injected intravenously with propofol (Diprivan; Zeneca) at a dose of 10 mg/kg, followed by continuous infusion at 40 mg/kg/h during the PET scan, as previously reported (*25*). Emission data were then acquired for 20 min, with an energy window of 400–650 keV and a coincidence-timing window of 6 ns. The scanned area was 7.8 cm, from the stomach to the bladder, including the entire intestinal area. From 50 to 70 min after the injection of ^{18}F-FDG, emission data were sorted into a single-frame sinogram, with a span of 3 and a ring difference of 47, and reconstructed using filtered backprojection and maximum a posteriori algorithms. To clearly visualize the intestinal ulceration, maximum-intensity-projection images of the maximum a posteriori–reconstructed images were displayed using ASIPro software (version 4.10; Concorde Microsystems).

In our model of indomethacin-induced small-intestine ulcers, the diameter of each ulcer was approximately 1.0–1.5 mm (data not shown). Consequently, circular regions of interest (ROIs) (diameter, 1 mm) were drawn on the abdominal area (except the kidney, bladder, or bone) in the filtered backprojection–reconstructed images, using PMOD (version 2.75; PMOD Technologies Ltd.). A total of 7–8 ROIs were selected from the most intense signal in 7 consecutive filtered backprojection–reconstructed images from each animal. The ROI values were averaged to produce mean standardized uptake values (SUVs) based on the following equation for quantitative comparison of ^{18}F-FDG uptake. The mean SUV obtained from 50 to 60 ROIs was used as the data for each animal. These data were plotted against time (days) after indomethacin administration:

$$\text{SUV} = \text{mean ROI value (MBq/mL)}/\{\text{injected activity (MBq)}/\text{body weight (g)}\}$$

γ-Counting Study

Healthy rats and those with ulcers (1 d after indomethacin administration) were injected with ^{18}F-FDG at a dose of 55.5 MBq/kg via the tail vein. Rats (n = 4–6 animals in each group) were euthanized at 5, 15, 30, 45, and 90 min after ^{18}F-FDG injection under deep ether anesthesia. A 5-cm length of ileum was taken from the region 5–20 cm above the colon, washed with

saline, and weighed. Radioactivity of ^{18}F-FDG in each sample was counted using an auto-well γ-counting system (ARC-2000; ALOKA Co. Ltd.). In addition, we evaluated radioactivity at 45 min after ^{18}F-FDG injection among the different time intervals (1, 2, 4, and 7 d after injection of indomethacin). The amount of radioactivity in each tissue was calculated as the percentage injected dose per gram of tissue (%ID/g).

Macroautoradiography

Macroautoradiography was performed using a modified method described in our previous study, as reported by Matsumura et al. (*25*). Briefly, ^{18}F-FDG (55.5 MBq/kg) in 0.3 mL of physiologic saline was administered to rats via the tail vein. At 45 min after administration, the rats were deeply anesthetized with diethyl ether and then euthanized, and the blood was totally withdrawn by perfusing saline through the left ventricle. The small intestine was then rapidly removed, washed with saline, and placed on an imaging plate (BAS-IV MS 2040; Fuji Photo Film) for 20 min. The plate was scanned with a bioimaging analyzer (BAS-5000; Fuji Photo Film), and the images were displayed by Image Gauge software (version 4.2.1; Fuji Photo Film).

Microautoradiography Combined with Immunohistochemistry

Microautoradiography was performed using a modified method reported by Kubota et al. (*26*). Briefly, healthy rats and those with ulcers were injected intravenously with ^{18}F-FDG (74 MBq). They were euthanized under deep ether anesthesia at 45 min after the administration. Blood was totally withdrawn by perfusing saline through the left ventricle. The small intestine was quickly dissected and frozen with dry ice. Under a safety light, 5-μm-thick sections were made and mounted on slides coated with NTB2 nuclear emulsion (Kodak) diluted 13:7 with distilled water. The slides were immediately frozen on a dry ice block and kept in exposure boxes cooled with dry ice. After a 6-h exposure, sections were transferred to ethanol and acetic acid (ratio, 19:1) at −70°C and 25°C for 1 min each. After washing with water twice for 3 min, the sections were developed in D-19 developer (Kodak) for 5 min, fixed in Fuji Fix (Fuji Photo Film) for 15 min at room temperature, and then washed in water for 10 min.

In the combined microautoradiography and immunohistochemistry study, sections were fixed by 4% paraformaldehyde at 4°C for 3 min after development and fixation for microautoradiography. We inactivated the endogenous peroxidase by immersing the sections in 0.3% H_2O_2 in isotonic phosphate-buffered saline with 0.3% Triton X-100 (PBS-T) (Sigma-Aldrich) at room temperature for at least 2 h and then washing the sections with PBS-T 3 times for 5 min each. After immersing the sections in a blocking solution (3% normal serum in PBS-T) at room temperature for 1 h, we incubated the sections with each antibody at 4°C for 18 h as follows: monoclonal antibodies against rat CD31 (an endothelial cell marker; BD Biosciences Pharmingen) (1:200 dilution), rat ED2 (a macrophage marker; Serotec Ltd.) (1:100 dilution), and human α-smooth muscle actin (α-SMA, a myofibroblast marker; R&D Systems) (1:200 dilution), and a polyclonal antibody against Ki67 (a proliferation marker; Novocastra Laboratories) (1:1,000 dilution). After we washed the sections in PBS-T 3 times, we incubated them with biotinylated antimouse or antirabbit IgG (Vector Laboratories; 1:400 dilution) at room temperature for 4 h, washed them with PBS-T 3 times, and then incubated them with peroxidase-labeled streptavidin (Vectastain; Vector Laboratories) at room temperature for 1 h. These immunoreactions were visualized by the reaction with 3,3′-diaminobenzidine (Wako) and 0.03% H_2O_2. Counterstaining was performed with Mayer's hematoxylin.

Statistical Analysis

Parametric data are presented as mean ± SD. Statistical significance was determined using a 2-tailed Student *t* test. A *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

Induction of Indomethacin-Induced Intestinal Ulcer and Myeloperoxidase Activity Assay

Administration of indomethacin (10 mg/kg) caused small-intestine ulceration on the side of the mesenteric attachment 24 h after administration, mainly in the ileum. Intestinal ulcers were most severe at day 1 (24 h after indomethacin administration), after which gradual healing was observed (Fig. 1A). Macroscopically, ulcers were almost healed in 7 d (Fig. 1A). Histologic observation revealed denuded epithelium and severe edema in the submucosa on day 1. Granulation tissue was clearly observed on the ulcer bed on day 4, and neoepithelial cells covered the damaged areas on day 7 (Fig. 1B). These macro- and microscopic observations are consistent with a report by Hatazawa et al. (*27*).

To evaluate the severity of ulceration by quantifying infiltration of neutrophils, myeloperoxidase activity was assayed in the small-intestine mucosa. In the control group (Fig. 1C), little myeloperoxidase activity was detected in the intestine (<0.01 μmol H_2O_2/mg protein). On day 1, myeloperoxidase activity was markedly elevated (0.38 ± 0.04 μmol H_2O_2/mg protein), a value significantly higher than that of the control group ($P < 0.01$). Myeloperoxidase activity began to decline on day 2 (0.21 ± 0.10 μmol H_2O_2/mg protein [$P < 0.05$] on day 2 and 0.04 ± 0.01 μmol H_2O_2/mg [$P < 0.05$] on day 4), and activity was negligible on day 7 (<0.01 μmol H_2O_2/mg protein).

γ-Counting Study

^{18}F-FDG accumulation in the small intestine on day 1 after indomethacin administration was evaluated by γ-counting using small intestines taken at 5, 15, 30, 45, and 90 min after ^{18}F-FDG injection (Supplemental Fig. 1; supplemental materials are available online only at http://jnm.snmjournals.org). Accumulation in the intestines with ulcers reached a maximum at 45 min after ^{18}F-FDG injection, before decreasing. In contrast, ^{18}F-FDG uptake in control animals injected with vehicle alone peaked 5 min after ^{18}F-FDG injection, before decreasing.

In the course of small-intestine ulceration and healing for the 7 d after indomethacin administration, γ-counting was performed 45 min after the injection on days 1, 2, 4, and 7 in indomethacin-treated and control (vehicle-injected) rats (Fig. 2). In indomethacin-treated rats, the highest value was observed on day 1 (1.05 ± 0.37 %ID/g, $n = 6$ animals),

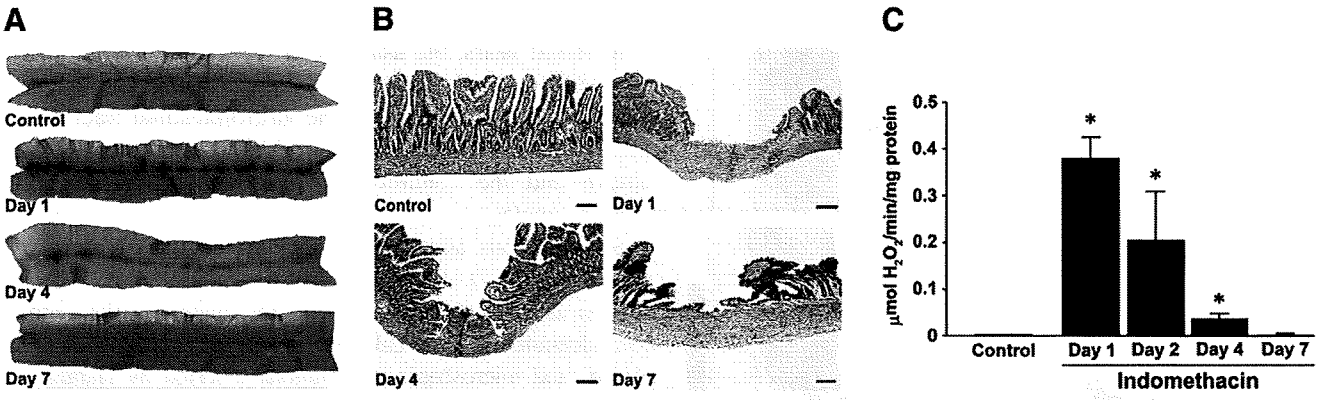

FIGURE 1. Macroscopic (A) and microscopic (B) observations of indomethacin-induced small-intestine ulceration during healing in rats. (A) Continuous circular lesions were seen on side of mesenteric attachment on day 1 or 4. (B) Histologically, denuded epithelium and severe edema in submucosa (day 1), granulation tissue on ulcer bed (day 4), and neoepithelial cells (day 7) were observed (hematoxylin and eosin staining; scale bar, 200 µm). (C) Change in myeloperoxidase activity as marker of neutrophil infiltration during indomethacin-induced intestinal ulceration and healing. Myeloperoxidase activity was evaluated in animals after administration of indomethacin (days 1, 2, 4, and 7) and in vehicle-injected animals (control). Data are shown as mean ± SD; $n = 4$–5 animals. *$P < 0.05$.

after which the values gradually decreased (0.78 ± 0.17 %ID/g, $n = 6$ animals on day 2 and 0.55 ± 0.15 %ID/g, $n = 6$ animals on day 4), returning to the value of 0.21 ± 0.12 %ID/g on day 7 (the same level as in the control rats [0.25 ± 0.07 %ID/g, $n = 4$ animals]).

Small-Animal PET Study

In the ^{18}F-FDG PET study, specific ^{18}F-FDG accumulation was visualized and quantified in coronal images of the abdominal areas of 4 rats in the course of small-intestine ulceration and healing for 7 d after indomethacin administration (Fig. 3). Over the course of the experiment, accumulations were commonly observed in the kidney and urinary bladder. On day 1, robust tubelike signals were seen in the abdominal area (Fig. 3A, arrowhead). Similar but weak signals were seen on day 2. These specific signals finally disappeared on day 7 (Fig. 3A).

Figure 3B indicates the mean SUV in the abdominal areas of the animals on each experimental day of the ^{18}F-FDG PET study. SUV was highest on day 1 (2.16 ± 0.28, $P < 0.01$), began to decrease on day 2 (1.91 ± 0.87, $P < 0.05$), and returned to the pretreatment level (0.70 ± 0.08) on day 7 (0.91 ± 0.17), consistent with the findings of the γ-counting study.

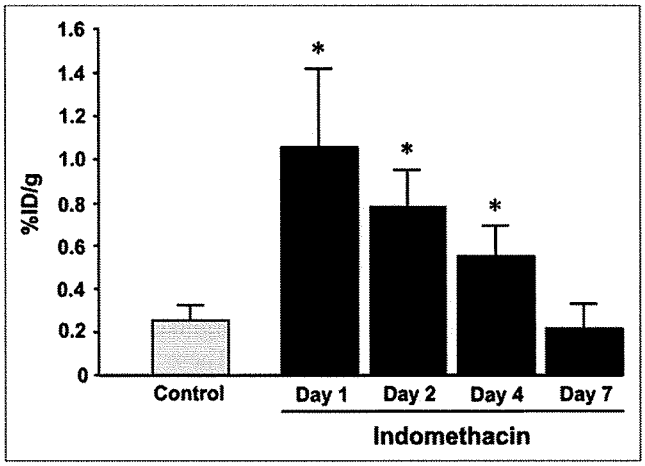

FIGURE 2. γ-counting study of ^{18}F-FDG uptake in ulcerated intestine, showing ^{18}F-FDG accumulation in small intestine at time points after indomethacin administration (days 1, 2, 4, and 7) and after vehicle injection (control). Data are shown as mean ± SD; $n = 6$–8 animals for each time point.

Macroautoradiography

The autoradiography findings were analyzed for ex vivo confirmation of the region of ^{18}F-FDG accumulation in the small intestine (Fig. 4). In the control group (injected with vehicle alone), low and uniform distribution of ^{18}F-FDG was observed throughout the intestine. On day 1 after indomethacin administration, discontinuous dotlike accumulation was clearly identified, mainly in the ileum. In the specimen opened along the longitudinal axis, intensive accumulation of ^{18}F-FDG was observed at the mesenteric side of the intestinal mucosa in which ulceration was observed (Fig. 4, arrowhead). On day 7, the ^{18}F-FDG imaging profile was similar to that of the control.

Microautoradiography Combined with Immunohistochemistry

Microautoradiography of the intestine was performed to identify ^{18}F-FDG accumulation at the cellular level (Figs. 5 and 6). In animals injected with indomethacin, labeling with silver grains of a high density was observed in ulcerated areas, especially in the submucosal and smooth muscle layers on day 1 (Figs. 5A–5C). Combined with peroxidase staining, silver grains were seen in the ulcerated

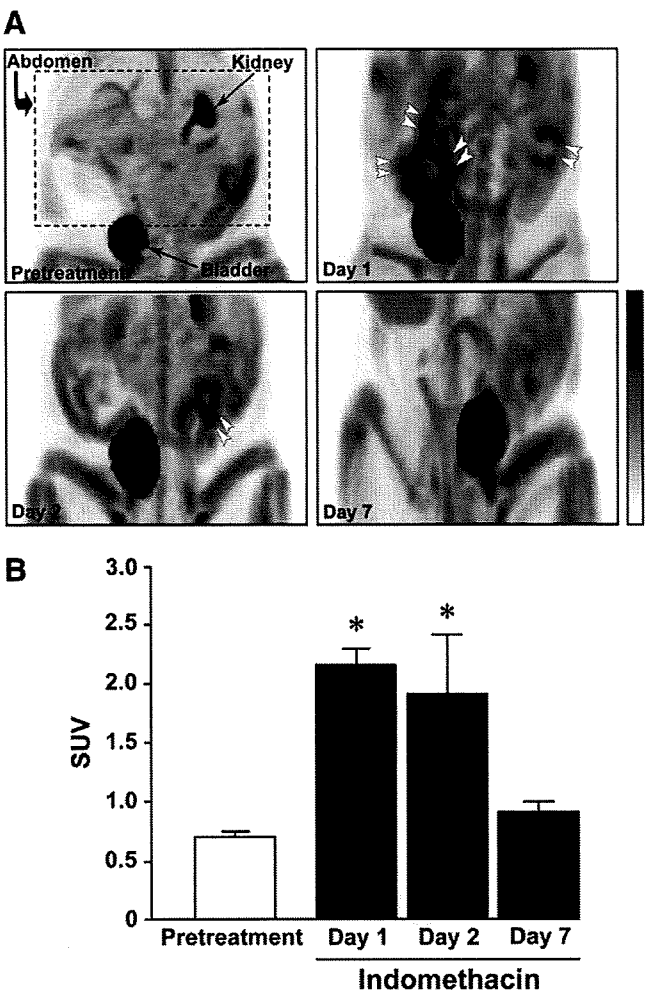

FIGURE 3. ^{18}F-FDG PET study of indomethacin-induced intestinal ulceration. (A) Abdominal PET images (coronal images) in same rat at different times after indomethacin administration. Arrowheads indicate characteristic accumulation of ^{18}F-FDG. (B) Quantification of ^{18}F-FDG uptake in PET study. Mean SUVs are shown as mean ± SD; $n = 4$ animals. *$P < 0.05$.

area that included the majority of the peroxidase-positive cells (Figs. 6A and 6B).

On day 4, dense labeling with silver grains was seen on cells forming granulation tissue (Figs. 5D and 5E). In granulation tissue, combined microautoradiography and immunohistochemistry revealed silver grains localized on the cytoplasm of α-SMA–positive myofibroblasts, a few ED2-positive macrophages, and CD31-positive endothelial cells (Figs. 6C–6E).

^{18}F-FDG accumulation was observed not only in the intestinal crypts and around ulcerated areas but also in the intact region of the intestine. The microautoradiographic study combined with immunohistochemical detection of Ki67, a cell cycle marker, revealed that such an accumulation of ^{18}F-FDG was involved in many proliferating cells in the crypts (Fig. 6F).

DISCUSSION

In recent years, the usability of ^{18}F-FDG PET has been reported for the detection of malignant and inflammatory diseases, including those of the gastrointestinal tract (*18–21,28–30*); however, the relationship between ^{18}F-FDG uptake and the condition of gastrointestinal ulceration remains unclear.

Subcutaneous administration of indomethacin, a representative NSAID (10 mg/kg), caused small-intestine ulceration on the mesenteric attachment side, mainly in the ileum, with ulceration almost healed in 1 wk, as previously shown in macro- and microscopic observations reported by Hatazawa et al. (*27*). In the present PET study, we used propofol 4 times (before treatment and on days 1, 2, and 7) to anesthetize each rat. Propofol is known to modulate gamma-aminobutyric acid (GABA)-ergic transmission and exerts its pharmacologic effects by enhancing the function of the GABA-activated chloride channels (*31*). Therefore, we cannot rule out the possibility that feeding behavior and locomotor activity were affected during the experiment. The dose used in this study, however, did not affect the severity or healing of the intestinal ulcers (data not shown).

In the present study, characteristic abdominal ^{18}F-FDG uptake was observed in indomethacin-induced ulceration, with maximum uptake observed 1 d after indomethacin administration. We used SUV to quantify ^{18}F-FDG uptake in the PET study. The mean SUV was approximately 3 times greater in the indomethacin treatment group than in the pretreatment group on day 1 and returned to the pretreatment level on 7 d. This time course of ulceration was also confirmed by γ-counting. Our data showed the ulcerated areas to have an ^{18}F-FDG SUV of 2.16 ± 0.28, compared with 0.70 ± 0.08 for the normal areas ($P < 0.01$). Therefore, a threshold SUV of 1.60 (mean − 2 SDs) could be applied for PET evaluation of tissue suspected of being ulcer-positive. These findings indicated that ^{18}F-FDG PET could evaluate not only the severity but also the process of healing on indomethacin-induced intestinal ulceration. Thus, ^{18}F-FDG PET could be useful for noninvasive detection of gastrointestinal ulcers and in follow-up, even in the clinical cases.

The microautoradiographic study provided an understanding of tissue ^{18}F-FDG accumulation at the cellular level. One day after administration of indomethacin (when the mucosal lesions were most severe), marked ^{18}F-FDG accumulations were observed at the marginal, submucosal, and muscular regions of ulceration. These regions contained many peroxidase-positive cells, suggesting that ^{18}F-FDG accumulated in inflammatory cells such as neutrophils and macrophages. On day 4, the number of peroxidase-positive inflammatory cells decreased, and granulation tissue was formed on the ulcer bed. Accumulation of ^{18}F-FDG was found in the granulation tissue. The microautoradiographic study, combined with immunohistochemistry, revealed that ^{18}F-FDG accumulation was observed in α-SMA–positive

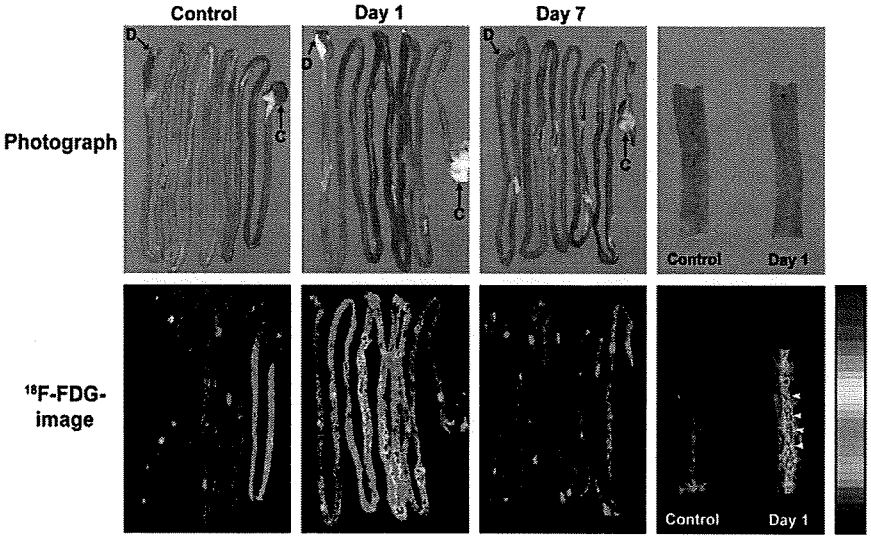

FIGURE 4. ^{18}F-FDG macroautoradiography of intestinal ulceration. Rats were intravenously injected with ^{18}F-FDG and euthanized 45 min later. Discontinuous dotlike ^{18}F-FDG accumulation was clearly identified mainly in ileum on day 1. ^{18}F-FDG imaging profile was similar to that of control on day 7. Images at far right show plain photograph (upper) and ^{18}F-FDG image (lower) of intestines opened along longitudinal axis for control and at day 1. D = duodenum; C = cecum.

myofibroblasts, CD31-positive endothelial cells, and a few ED2-positive macrophages.

Myofibroblasts are thought to play an important role in the repair of injured gut (*32,33*); contraction of intestinal myofibroblasts rapidly reduces mucosal defects and decreases the area requiring reepithelialization (*33*). CD31-immunopositive endothelial cells are also well known to play an important role in ulcer healing by angiogenesis (*34,35*). Kubota et al. (*36*) reported that ^{18}F-FDG accumulation in macrophages and neutrophils likely occurred via the use of glucose as the energy source for their chemotaxis and phagocytosis and in fibroblasts for proliferation in and around tumors, although those ^{18}F-FDG–accumulated cells were not identified immunohistochemically. These observations support the findings of the present study obtained by microautoradiography combined with immunohistochemical identification of cells.

In addition to cells with accumulated ^{18}F-FDG, we found that cells located in deeper regions of the intestinal crypts, and in normal tissue, were heavily radiolabeled with ^{18}F-FDG. The combination study of ^{18}F-FDG microautoradiography with immunohistochemistry revealed that the cells were immunopositive for Ki67. Such regions of the intestinal crypts are known to contain many intestinal stem cells expressing Ki67 (*37*). The stem cells actively proliferate and differentiate mainly into absorptive cells, the major constituent of the epithelial cells of the villus, migrating upward along the crypt-villus axis (*38,39*). These findings indicate that proliferating cells in the intestinal epithelium actively take up ^{18}F-FDG. Ki67 is known to be included in the granular components of the nucleolus during late G1, S, G2, and M phases (*40*); therefore, additional studies using cell cycle markers are required to understand the relationship between each cell cycle phase and glucose utility. To the best of our knowledge, the present study is the first to show ^{18}F-FDG uptake at the single-cell level by a combination of microautoradiography and immunohistochemistry. Our results indicated that ^{18}F-FDG accumulates in such inflammatory cells during the acute phase of mucosal ulceration and also in the cells forming granulation during the healing phase.

NSAIDs, some of the most commonly prescribed drugs worldwide for antipyresis and analgesia, act by inhibition of endogenous prostaglandin synthesis. Prostaglandins are well known to maintain the mucosal integrity of the gastrointes-

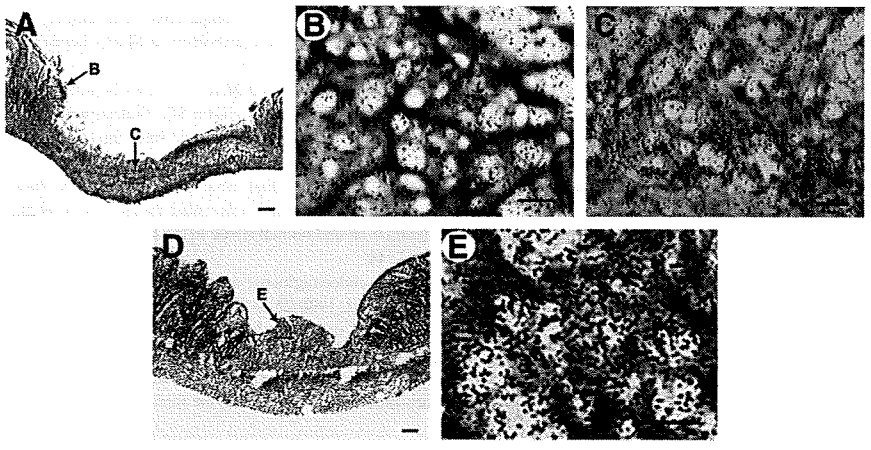

FIGURE 5. ^{18}F-FDG microautoradiography of indomethacin-induced intestinal lesions combined with hematoxylin and eosin staining. (A–C) At 1 d after administration of indomethacin, silver grains of high density were observed in ulcerated area, especially at ulcer margin (B) and submucosal and smooth muscle layer (C). B and C are magnified views of areas indicated by arrows B and C in A. (D and E) On day 4, grains were accumulated on cells forming granulation tissue. (E) Magnified view of the region indicated by arrow E in D. Scale bars: 100 μm (A and D) and 20 μm (B, C, and E).

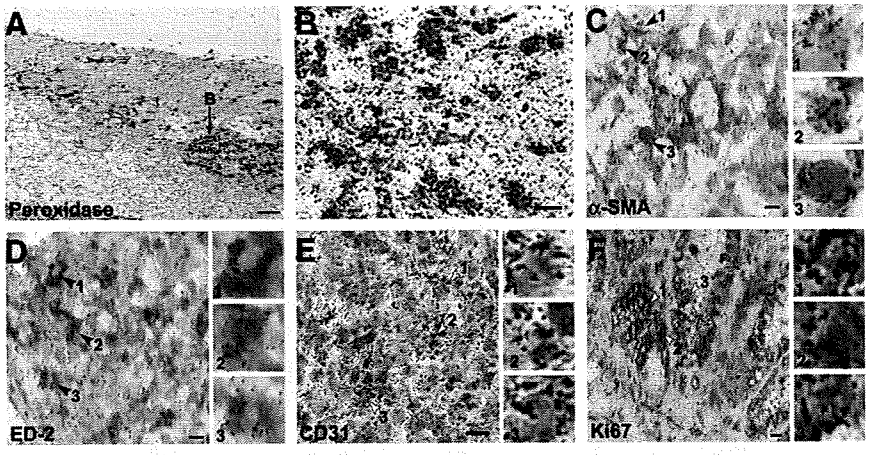

FIGURE 6. Microautoradiography combined with immunohistochemistry. In submucosal area at day 1 after indomethacin administration, ^{18}F-FDG uptake was observed mainly in peroxidase-positive cells (A and B). On granulation tissue at day 4, myofibroblasts (C; α-SMA–positive), macrophages (D; ED2-positive), and endothelial cells (E; CD31-positive) were visualized. In normal tissue, silver grains of high density reflecting ^{18}F-FDG radioactivity were observed in Ki67-positive crypt cells (F). Insets show magnified view of region indicated by arrowheads in each panel. Scale bars: 100 μm (A), 10 μm (B), and 5 μm (C–F).

tinal tract; therefore, NSAIDs commonly cause gastrointestinal damage as an adverse effect. The recently developed technique of capsule enteroscopy has enabled examination of damage to the small bowel and is now considered the gold standard for diagnosis of Crohn disease of the intestine (*4,5*). However, in this technique, the images are magnified to differing extents depending on the amount of surrounding fluid, making it difficult to evaluate lesion size (*7*). Furthermore, capsule enteroscopy carries a risk of capsule retention if there is obstruction of the digestive tract, which may occur in intestinal disorders. In the present study, we demonstrated the usability of ^{18}F-FDG PET and also the meaning of physiologic and pathologic glucose uptake for indomethacin-induced intestinal ulceration. Because ^{18}F-FDG PET is used clinically for diagnosis of cancer or tumors and has low adverse effects, the safety of the method for patients is well established. Clinical studies are needed to evaluate whether ^{18}F-FDG PET exhibits sufficient sensitivity and specificity for the detection of indomethacin-induced small-intestine ulcers in humans. ^{18}F-FDG PET will enable detection of intestinal lesions, staging of disease, and assessment of clinical treatment and reveal recurrence of disease.

CONCLUSION

Our experimental data suggest that ^{18}F-FDG PET may be useful for evaluating indomethacin-induced small-intestine ulcers. ^{18}F-FDG accumulation was observed mainly in inflammatory cells in the acute phase and in myofibroblasts and endothelial cells forming granulation tissue in the healing phase. PET enables detection of accumulation during the course of ulceration.

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^{11}C-PK11195 PET for the In Vivo Evaluation of Neuroinflammation in the Rat Brain After Cortical Spreading Depression

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Neurogenic inflammation triggered by extravasation of plasma protein has been hypothesized as a key factor in the generation of the pain sensation associated with migraine. The principal immune cell that responds to this inflammation is the parenchymal microglia of the central nervous system. **Methods:** Using a PET technique with ^{11}C-(*R*)-[1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide] (^{11}C-PK11195), a PET ligand for peripheral type–benzodiazepine receptor, we evaluated the microglial activation in the rat brain after generation of unilateral cortical spreading depression, a stimulation used to bring up an experimental animal model of migraine. **Results:** We found a significant increase in the brain uptake of ^{11}C-PK11195, which was completely displaceable by the excess amounts of unlabeled ligands, in the ipsilateral hemisphere of the spreading depression–generated rats. Moreover, the binding potential of ^{11}C-PK11195 in the spreading depression–generated rats was significantly higher than that in the sham-operated control rats. **Conclusion:** These results suggest that as an inflammatory reaction, microglial cells are activated in response to the nociceptive stimuli induced by cortical spreading depression in the rat brain. Therefore, the ^{11}C-PK11195 PET technique could have a potential for diagnostic and therapeutic monitoring of neurologic disorders related to neuroinflammation such as migraine.

Key Words: binding potential; microglia; peripheral benzodiazepine receptor; migraine

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Neuroinflammation is a process whereby glial cells are activated in response to infection, disease, or injuries involved in the central nervous system. Such inflammatory reactions are implicated in several neurologic disorders such as migraine, Alzheimer disease, stroke, Parkinson disease, brain trauma, spinal cord injury, and multiple sclerosis (*1,2*). Neurogenic inflammation has been implicated as a key factor in the generation of the pain sensation associated with migraine headaches (*3–5*). It is hypothesized that the proinflammatory peptides, such as substance P and calcitonin gene–related peptide, released from trigeminocervical nerve terminals in response to meningeal nociceptive stimuli, induce vasodilation and plasma protein extravasation. Such neurogenic inflammatory reactions were thought to trigger headache via a stimulation of trigeminal afferents (*4,5*). Consistent with this theory, vasogenic leakage (*6,7*) and an increase in calcitonin gene–related peptide in the jugular vein (*8*) have been reported in migraine patients during migraine attack. However, recent clinical trials have shown that several drugs that selectively inhibit plasma protein extravasation in rodents have failed to reduce pain severity in patients with migraine (*9*). These observations indicate that a noninvasive evaluation method for neuroinflammation is necessary to investigate whether and how the neuroinflammation is involved in migraine etiology and verify the extrapolated data from an animal study to the human condition.

As the principal immune cells in the central nervous system, the microglial cells are activated in response to such neurogenic inflammation. The process of microglial activation is thought to be related to an increase in the number of microglial cells and the expression of numerous proteins such as peripheral benzodiazepine receptor (PBR) (*10*). The PBR, which is a mitochondrial outer membrane protein and is expressed at low levels in the normal brain on resting microglial cells and astrocytes, is known to be upregulated in the activated microglial cells (*11–13*). ^{11}C-labeled PK11195, a ligand that specifically binds to PBR, has been used extensively for imaging of activated microglial cells by PET in several neurologic disorders, such as stroke (*14*), multiple sclerosis (*15*), Alzheimer disease (*16*), Parkinson disease (*17*), and Huntington disease (*18*). However, no literature has described the

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microglial activation in the brain of migraine patients using PET with ^{11}C-PK11195.

Cortical spreading depression, described first by Leao (*19*) in 1944, is implicated in the pathogenesis of migraine. Cortical spreading depression is characterized by the spreading of neuronal or glial membrane depolarization accompanied by temporal elevation of the cerebral blood flow (CBF) throughout the cerebral cortical hemisphere at a rate of 2–5 mm/min (*20,21*). The rate of spread correlates with the observed spread of the aura of classic migraine (*22*), which is characterized by either a paracentral scotoma or a small scintillating area of bright light. A spreading oligemia has been observed at a similar velocity during migraine attacks in experimental cortical spreading depression models (*23,24*). Hadjikhani et al. (*25*) also reported that a neurovascular event closely resembling cortical spreading depression has been shown with functional MRI during the migraine visual aura. On the basis of these observations and other experimental data, the cortical spreading depression has been hypothesized as an endogenous event involved in migraine etiology (*26,27*). In this study, we used ^{11}C-PK11195 PET to investigate the neurogenic inflammation in the cerebral cortex induced by unilateral cortical spreading depression.

MATERIALS AND METHODS

All experimental protocols were approved by the Ethics Committee on Animal Care and Use of the Institute of Physical and Chemical Research and were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (*28*).

Chemicals

(*R*)-[1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methyl-propyl)-3-isoquinoline-carboxamide] ((*R*)-*N*-desmethyl-PK11195) was obtained from Advanced Biochemical Compounds (ABX). ^{11}C-labeled PK11195 was synthesized according to the procedures described by Shah et al. (*29*), with slight modifications. Briefly, 1 mg of (*R*)-*N*-desmethyl-PK11195 was dissolved in 200 μL of anhydrous dimethyl sulfoxide, containing 1 mg of KOH. After trapping of the ^{11}C-CH_3I, the vial was heated at 90°C for 4 min. Purification was performed by high-performance liquid chromatography on a COSMOSIL C_{18}-AR-II column (10 × 250 mm, 5-μm particle size) (Nakalai) using acetonitrile:water (70:30) as the mobile phase. The purified fraction was evaporated to dryness and reconstituted with 4 mL of a saline solution including 0.3 mL of propylene glycol and 0.05 mL of polysorbate 80. The specific activity ranged from 30 to 70 GBq/μmol. Radiochemical purity was higher than 99%.

Animal Preparation for Generation of Spreading Depression

Male Sprague–Dawley rats (SLC), weighing approximately 300 g, were used. To prepare the spreading depression rat model, the head of each rat was fixed in a stereotactic apparatus (type 1430, David Kopf) under 1.5% isoflurane anesthesia. A thermocouple probe was connected with a thermocontroller and inserted into the rectum to maintain the body temperature at 37°C. A small burr hole was drilled in the skull at the frontal cortex (2.0–3.0 mm anterior and 2.0–3.0 mm lateral to the bregma). A glass micropipette (internal diameter of the tip, 50 μm) was inserted 1 mm below the cortical surface through the burr hole for subsequent microinjection. Two hours after the insertion, a microinjection of 1 M KCl was performed (at a rate of 0.2 μL/min for 1 min) every 10 min for a period of 2 h (12 injections; 2.4 μL of total volume). Sham controls (n = 4 animals) were injected with 1 M NaCl at an analogous rate, duration, and frequency.

A laser Doppler flowmetry (LDF) (type FLO-N1; ω-wave) probe was stereotactically placed in the parietal cortex over the skull for recording changes in CBF. The absolute value from LDF does not mean actual perfusion units; therefore, relative change in CBF is displayed (the data are normalized to prelevel).

PET Studies

Rats (n = 39) were anesthetized and maintained with a mixture of 1.5% isoflurane and nitrous oxide and oxygen (7:3) and positioned in the gantry of a PET scanner (microPET Focus 220; Siemens Co., Ltd.). After a bolus injection of ^{11}C-PK11195 (~100 MBq per animal) via a tail vein, a 60-min emission scan was performed with 400–650 keV as the energy window and 6 ns as the coincidence time window. Unlabeled ligands (n = 4, 1 mg/kg) were injected intravenously 20 min after the injection of radiotracers for the displacement experiment. Emission data were acquired in the list mode. The acquired data were sorted into single sinogram (for static image) and dynamic sinograms (6 × 10 s, 6 × 30 s, 11 × 60 s, and 15 × 180 s, for a total of 38 frames). The data were reconstructed by standard 2-dimensional filtered backprojection (FBP) with ramp filter and cutoff frequency at 0.5 cycles per pixel or by a statistical maximum a posteriori probability (MAP) algorithm (12 iterations with point spread function effect). Compared with FBP, MAP-reconstructed images have been shown to result in improved spatial resolution and noise properties on small-animal PET images, an advantage for image coregistration. Meanwhile, FBP-reconstructed images were used for quantification. The radioactivity concentrations were normalized with cylinder phantom data and were expressed as standardized uptake values.

Image Analysis

A 3-dimensional T2-weighted MRI template, which was aligned in space with the rat brain atlas of Paxinos (*30*), was used for defining regions of interest (ROIs). PET images were manually coregistered with the MRI template using an image-processing small-animal PET software package (ASIPro 6.05; Siemens Co., Ltd.). ROIs were defined for each rat on the region of microinjection (core, as delineated by the hypersignal seen in the MAP-reconstructed image), ipsilateral surrounding areas (ipsilateral), and corresponding contralateral areas (contralateral, as shown in Fig. 1). Each region was primarily drawn on coronal slices and then confirmed on sagittal and horizontal slices. All related ROIs were stacked into the volume of interest (VOI), and the mean value in each VOI was used to generate regional time–activity curves.

Radioactivity and Metabolite Analysis in Plasma

Arterial blood was collected from the femoral artery at 10, 20, 30, 40, 50, and 60 s and 2, 5, 10, 25, and 40 min after administration. The radioactivity of blood and plasma was measured by a well-type γ-counter (Wallac1470; PerkinElmer) and corrected for decay. For the metabolite analysis, plasma

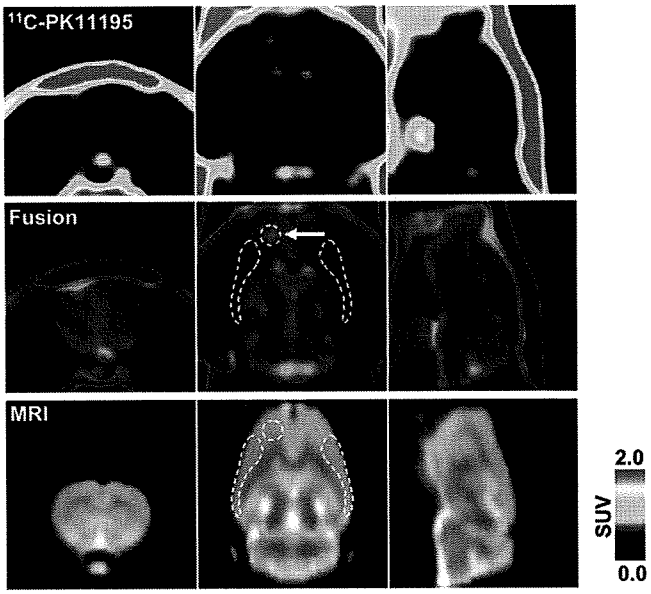

FIGURE 1. Representative ^{11}C-PK11195 PET image coregistered with MRI template 8 d after generation of unilateral (left hemisphere) cortical spreading depression. PET image was reconstructed with MAP algorithm and summated from 5 to 60 min after radioligand injection. Arrow in middle panel indicates KCl-microinjected area (core). White broken lines in middle and bottom panels indicate ROIs (core, ipsilateral, and contralateral, respectively) defined in this study.

samples (1, 2, 5, 10, 25, and 40 min after administration) were extracted by acetonitrile, and the extracts were applied to an RP-18 thin-layer chromatography (TLC) plate (Merck). The plate was developed with acetonitrile:water (70:30) as the mobile phase. After migration, the TLC plates were dried and exposed on imaging plates (BAS TR2040; Fuji Photo Film) for 40 min. The signal of radioactivity on the imaging plates was measured with a digital PSL autoradiography analyzer (FLA-7000; Fuji Photo Film), and the data were estimated using imaging-analysis software (MultiGauge; Fuji Photo Film).

Kinetic Analysis

Kinetic modeling was performed using the PMOD software package (version 2.85; PMOD Technologies). A metabolite-corrected plasma input function was obtained by fitting to an exponential function, as follows. The ^{11}C-PK11195 parent fraction in the plasma samples for each sampling point was multiplied by this exponential function to obtain the metabolite-corrected plasma input function. The metabolite-corrected plasma time–activity curves were fitted to 2 exponential functions to obtain the plasma pharmacokinetic parameter. The standard input function was obtained by averaging individual input functions from 3 satellite rats, and k_2' of the reference region (contralateral hemisphere), which represents the clearance from the reference region into the vascular compartment, was obtained using a 1-tissue-compartment model for each animal imaged. The averaged k_2' estimated in this study was 0.14/min. Binding potential (BP) was calculated for ipsilateral ROI kinetics using Logan noninvasive graphical analysis (*31*), which was applied to the target regions by the following equation:

$$\frac{\int_0^T C(t)dt}{C(T)} = DVR\left[\frac{\int_0^T C'(t)dt + C'(T)/k_2'}{C(T)}\right] + int',$$

where $C(T)$ is the radioactivity concentration in the tissue of interest, and $C'(T)$ is the radioactivity concentration in the reference tissue (contralateral ROI). DVR is the distribution volume ratio calculated by the regression slope, int′ is an intercept that becomes constant after an equilibration, and k_2' is the average tissue-to-plasma clearance, which has to be determined before this analysis. In this study, k_2' was determined directly using the standard input function as shown above. Finally, BP is evaluated as BP = DVR − 1.

Immunohistochemistry

The level of microglial activation was investigated by immunohistochemistry in the spreading depression–generated (n = 4) and sham control (n = 4) rats at 8 d after the operation. After the PET scan, the rats were anesthetized and perfused with 4% formaldehyde buffered with 0.1 M phosphate-buffered saline (pH 7.4). The brain was removed and further fixed in the same fixative at 4°C for 24 h. We prepared coronal brain sections (30-μm thickness) using a cryostat. To immunostain the microglial cells, mouse monoclonal antibody against rat OX-42 (1:100; Abcam) was used. The bound antibodies were visualized by the avidin–biotin complex method (Vectastain ABC kit; Vector) with 3, 3′-diaminobenzidine.

Data Analysis

All results were expressed as mean ± SD. The statistical differences in BP values between spreading depression–generated and sham-operated groups were assessed by 2-tailed unpaired t test. The statistical significance was set at P less than 0.05.

RESULTS

Spreading Depression in Rat Cerebral Cortex

Occurrence of cortical spreading depression in all rats used for the PET study was evaluated by transient CBF hyperperfusion recorded continuously from the ipsilateral hemisphere (7 mm posterior to the injection site). Transient CBF hyperperfusion always occurred after microinjection of 1 M KCl (at a rate of 0.2 μL/min for 1 min) in the ipsilateral hemisphere (Fig. 2), as is characteristic of spreading depression (*21*). Such transient CBF hyperperfusions are well known to be synchronously accompanied by the transient negative shifts of direct current (DC) potential, as described in our previous report (*21*) and in other literature (*32*). The mean number of transient hyperperfusions in the KCl-treated rats (n = 35) was 12.8 ± 3.3 over 2 h. However, microinjection of 1 M NaCl (n = 4) in an analogous fashion did not induce similar changes in CBF, indicating that cortical spreading depression was not induced in those rats.

PET Studies

To investigate the neurogenic inflammation after cortical spreading depression, we examined microglial activation using ^{11}C-PK11195 PET at 1, 3, 8, and 15 d after induction

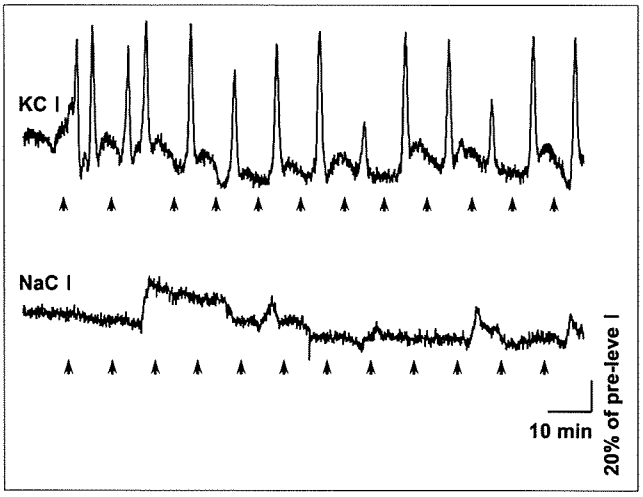

FIGURE 2. Cortical spreading depression–associated CBF changes in experimental and sham-operated rat. Dynamic changes in CBF were continuously recorded from parietal cortex over skull using LDF. Upper panel shows a representative CBF change recorded from parietal cortex in KCl-microinjected rat. Lower panel shows result from a rat who received NaCl microinjection using analogous paradigm, as sham operation. Arrows at bottom of each panel indicate time of microinjection. Note that spreading depression–associated CBF hyperperfusions were not observed in sham-operated rat.

of unilateral cortical spreading depression in the rats. The radioactivity of ^{11}C-PK11195 was barely observed within the brain under normal condition in the control rats, except for the cerebral ventricles, including the lateral ventricle, third ventricle, and fourth ventricle (data not shown). In the cortical spreading depression–generated rats, ^{11}C-PK11195 radioactivity was high in the ipsilateral hemisphere, as compared with that in the contralateral hemisphere. The ^{11}C-PK11195 radioactivity was detectable in the injection site 1 d after the KCl treatment, and that increased and spread extensively in the ipsilateral surrounding areas at 3, 8, and 15 d after the KCl treatment (Figs. 1 and 3). Figure 1 shows a representative ^{11}C-PK11195 PET image coregistered with the corresponding MR image at 8 d after the KCl treatment. The highest radioactivity was seen in the KCl-microinjected site (core), and moderate radioactivity was observed in the ipsilateral surrounding areas but not in the corresponding contralateral areas (Fig. 1). In the sham control rats, however, a slight increase in ^{11}C-PK11195 radioactivity was seen primarily in the NaCl-microinjected site at 8 d after the sham operation.

The temporal changes of ^{11}C-PK11195 uptake in the core, ipsilateral, and contralateral ROIs were similar (Fig. 3). The radioactivity of ^{11}C-PK11195 in the contralateral ROI reached a peak within the first minute after radiotracer injection and decreased rapidly thereafter. In the cortical spreading depression–generated rats, the decrement in the ipsilateral areas (core and ipsilateral ROIs) was slower than that in the contralateral areas (contralateral ROI). The obvious contrast of radioactivity between ipsi- and contralateral hemispheres appeared first 3 d after the operation and remained until the end of the study (Fig. 3). No obvious contrast between the 2 hemispheres was observed in the sham-operated control rats at 8 d after the NaCl treatment.

In vivo displacement was performed by injecting unlabeled PK11195 (n = 4, 1 mg/kg) at 20 min after the injection of ^{11}C-PK11195 (Fig. 4). Immediately after injection of unlabeled PK11195, the brain uptake of ^{11}C-PK11195 was transiently increased in both ipsi- and contralateral hemispheres because of a release of ^{11}C-PK11195 from peripheral organs into the blood circulation, as previously reported (*33,34*). The contrast of radioactivity between the 2 hemispheres completely disappeared after the unlabeled PK11195 injection, indicating ^{11}C-PK11195 was rapidly displaced by unlabeled PK11195 in the ipsilateral hemisphere.

Using Logan noninvasive graphical analysis, we estimated the BP value for ^{11}C-PK11195 in the core and ipsilateral ROIs. The BP values in the core and ipsilateral ROIs were increased with the time after the KCl treatment (Fig. 5). In the cortical spreading depression–generated rats, the mean value of BP in the core ROI reached 0.45 ± 0.10 (n = 5) at 3 d after the KCl treatment and maintained approximately the same level until 15 d (0.51 ± 0.36, n = 5). The mean values in the ipsilateral ROI were approximately half of those in the core ROI at each time of the examination, indicating that microglial activation was outstanding in the microinjected site. In contrast with the cortical spreading depression–generated rats, the BP values were lower in the sham-operated control (NaCl treatment) rats. Significant differences between spreading depression–generated and sham control rats were noted in both ROIs at 8 d after the operation (core, 0.48 ± 0.18 vs. 0.21 ± 0.05, and ipsilateral, 0.26 ± 0.07 vs. 0.14 ± 0.08, in the spreading depression–generated [n = 11] and sham control [n = 4] rats, respectively; $P < 0.05$, unpaired t test) (Fig. 6).

Immunohistochemical Studies

The activation of microglial cells was confirmed by immunohistochemical studies after the induction of spreading depression. As shown in Figure 7, a large number of immunosignals of OX-42 were observed in the ipsilateral hemisphere, compared with the corresponding area of the contralateral hemisphere, in the rats 8 d after unilateral spreading depression generation. Magnified photomicrographs taken from the corresponding parietal cortex showed that hypertrophied (enlarged, darkened soma with shorter, thicker processes) or ameboid (densely stained, enlarged soma with a few short processes) OX-42–positive microglial cells were often seen in the ipsilateral hemisphere (Fig. 7C). However, such a difference between the 2

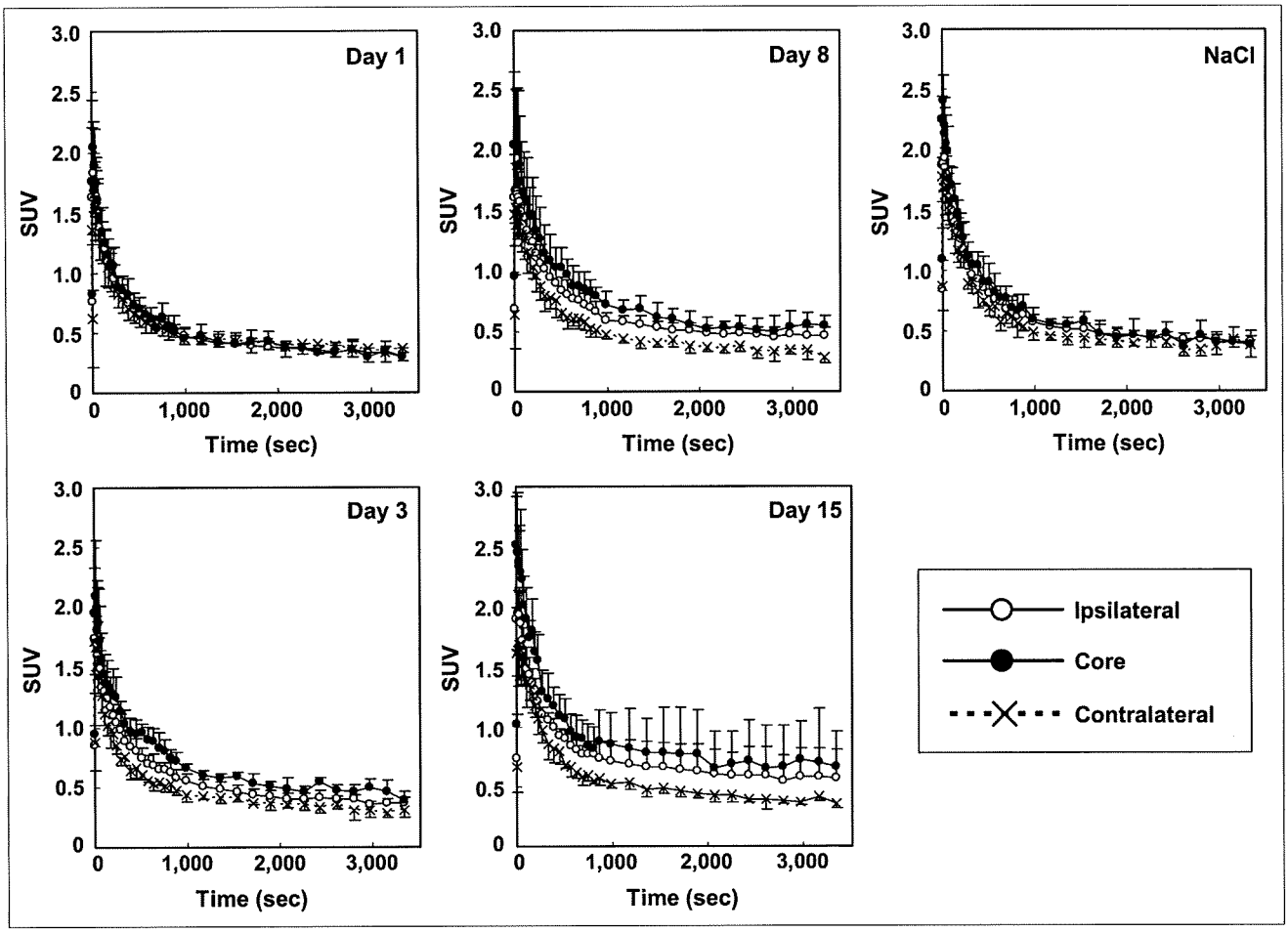

FIGURE 3. Time–activity curves for ^{11}C-PK11195 in brain regions of experimental (at 1, 3, 8, and 15 d after microinjection of KCl) and sham-operated (8 d after microinjection of NaCl) rats. Data were expressed as mean ± SD.

hemispheres was not observed in the rats treated with NaCl as a sham operation.

DISCUSSION

Neurogenic inflammation is thought to be a key factor in the generation of pain sensation in migraine headaches. In the present study, we evaluated neurogenic inflammation using an animal model of migraine and noninvasive PET. Our results show that in the unilateral spreading depression model rats, the uptake of ^{11}C-PK11195, a PET tracer for PBR, which is used extensively to image activated microglial cells in the central nervous system, was increased in the ipsilateral hemisphere and completely displaced by excess unlabeled ligands. In addition, quantitative analysis in spreading depression model rats, compared with that in the sham-operated control rats, showed that the BP values in the core and ipsilateral ROIs were significantly high. Finally, predominant microglial activation in the ipsilateral cerebral hemisphere of the spreading depression model rats was also confirmed by immunohistochemical study. These observations suggest that an inflammatory process may be involved in the pathologic state of migraine and that ^{11}C-PK11195 PET is a useful tool for evaluating the neurogenic inflammation in vivo.

On the basis of the expression pattern of the PBR in the pathophysiologic state, ^{11}C-labeled PK11195 has been developed as a specific PET ligand for the PBR to image activated microglial cells in the brain (*15,17,35,36*). The PBR is known to be highly expressed in activated microglial cells under neuropathologic conditions but barely expressed in healthy brain tissue (*12,13*). The upregulated level of PBR was known to be well correlated with the state of microglial activation (*15,37,38*). In the present study, we used unilateral cortical spreading depression as an animal model of migraine and demonstrated that the brain uptake of ^{11}C-PK11195 was specifically increased in the ipsilateral hemisphere. It is well known that cortical spreading depression–induced pathophysiologic changes, such as transient CBF hyperperfusion, negative DC potential shifts, and related biochemical events, are restricted to the ipsilateral hemisphere (*19,21,39*). Consistent with these characteristics of spreading depression, we demonstrated that micro-

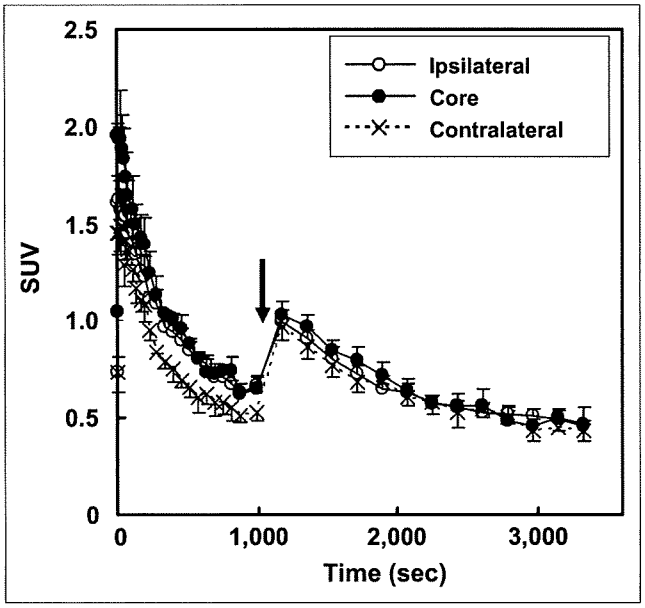

FIGURE 4. Time–activity curves for ^{11}C-PK11195 displaced by excess unlabeled PK11195 in brain regions 8 d after generation of unilateral cortical spreading depression. Unlabeled PK11195 (n = 4, 1 mg/kg) was administered 20 min after injection of radioligand. Arrow indicates time of injection of unlabeled ligand. Data were expressed as mean ± SD.

glial reactivity, indicated by immunosignal of OX-42, was observed predominantly in the ipsilateral hemisphere after cortical spreading depression. Indeed, Caggiano et al. (*40*) have also reported that reactive microglial cells appeared predominantly in the ipsilateral hemisphere after KCl-induced unilateral neocortical spreading depression by the immunohistochemical approach. Moreover, our displacement experiment demonstrated that such a superior brain

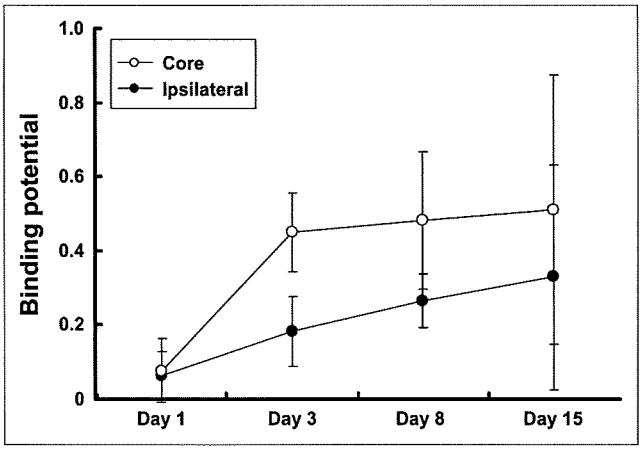

FIGURE 5. BP for ^{11}C-PK11195 in core and ipsilateral ROIs at 1, 3, 8, and 15 d after induction of unilateral cortical spreading depression by KCl microinjection. BP values were estimated in core and ipsilateral ROIs using Logan noninvasive graphical analysis. Corresponding contralateral ROI was used as reference. Data were expressed as mean ± SD.

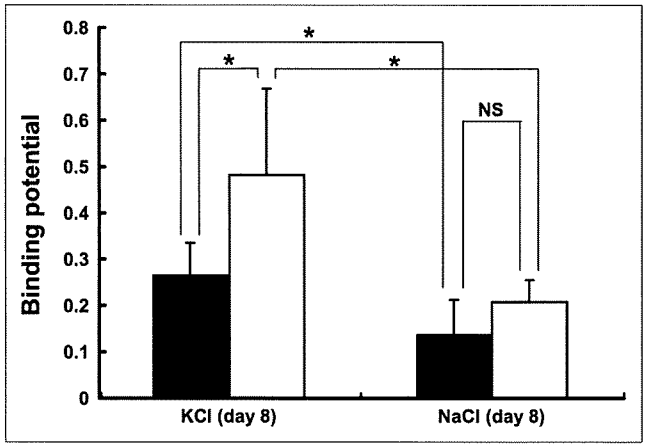

FIGURE 6. Comparison of BP for ^{11}C-PK11195 in ROIs (core and ipsilateral) between experimental and sham-operated rats. BP values were estimated from experimental (KCl microinjection, n = 11) and sham-operated (NaCl microinjection, n = 4) rats 8 d after operation. Open and closed bars indicate core and ipsilateral ROIs, respectively. Data were expressed as mean ± SD. NS = not significant. *$P < 0.05$, unpaired t test.

uptake of ^{11}C-PK11195 in the ipsilateral hemisphere was completely displaced by excess unlabeled ligands. Though astrocytes have also been reported to express PBR in vitro (*41*), the origin of ^{11}C-PK11195 radioactivity in vivo is thought to be primarily from activated microglial cells in the brain (*15,35,36*). Therefore, the increased radioactivity of ^{11}C-PK11195 in the ipsilateral hemisphere may originate from the activated microglial cells in response to the neurogenic inflammation after the unilateral spreading depression.

In the present study, BP value for ^{11}C-PK11195 was estimated using the Logan noninvasive graphical analysis (*31*). The mean BP value for ^{11}C-PK11195 was approximately 0.5 in the core ROI after spreading depression generation. That value was slightly lower than that reported in the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid–induced lesion area of the rat striatum (0.66 ± 0.15), in which the BP value was estimated by a simplified reference tissue model (*33*). Using the simplified reference tissue model, Cagnin et al. also have reported that mean BP value for ^{11}C-PK11195 in the cerebral cortex of healthy volunteers was 0.13 ± 0.04 and increased variably (≤0.79) in several cortical regions of patients with herpes simplex encephalitis (*36*). In the present study, the highest value of mean BP for ^{11}C-PK11195 was noted in the KCl-microinjected site (0.48 ± 0.18), and a moderate value was observed in the surrounding area of the injection site (0.26 ± 0.07) 8 d after the unilateral spreading depression generation. In contrast, the BP value in the ipsilateral hemisphere of sham-operated rats was 0.14 ± 0.08, which was almost equivalent to the value reported by Cagnin et al. for the cerebral cortex of healthy volunteers. (*36*). These

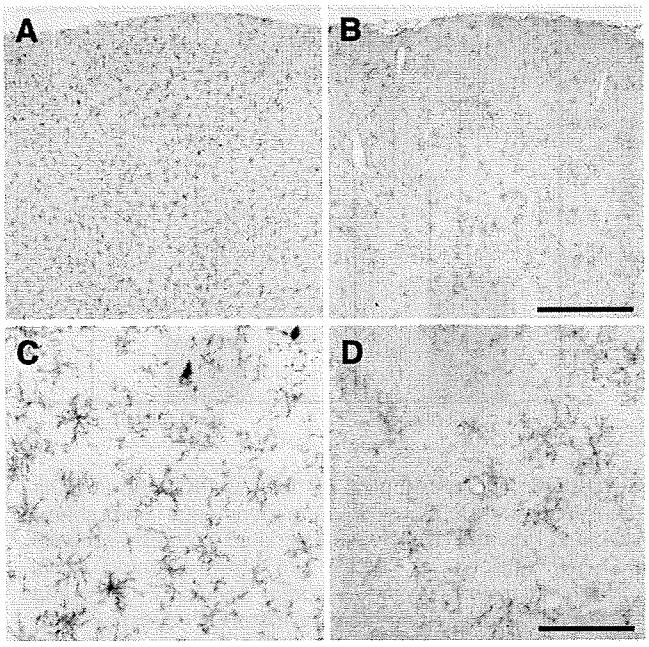

FIGURE 7. Photomicrographs of OX-42 immunoreactivity after cortical spreading depression. Images display OX-42 immunoreactivity 8 d after 2 h of recurrent spreading depression in left hemisphere (A and C), compared with contralateral hemisphere (B and D). (C and D) Magnified views of OX-42 immunoreactivity. Hypertrophied or ameboid OX-42–positive microglial cells were often seen in left hemisphere. Bars in B and D indicate 500 and 100 μm, respectively.

observations suggest that ^{11}C-PK11195 PET is potentially useful for the quantitative evaluation of temporal change in neurogenic inflammation in the rat model of migraine.

CONCLUSION

In the present study, we evaluated for the first time, to our knowledge, the neurogenic inflammation in the rat model of migraine using quantitative PET with ^{11}C-PK11195. The microglial activation quantified by ^{11}C-PK11195 PET was significantly increased in the cerebral hemisphere, which underwent unilateral cortical spreading depression. These results suggest that ^{11}C-PK11195 PET is useful for the evaluation of the neurogenic inflammatory process and may provide a new and powerful tool for both the diagnosis of migraine and the monitoring efficacy for migraine therapy.

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