Increased ^{18}F-FDG Uptake in a Model of Inflammation: Concanavalin A–Mediated Lymphocyte Activation

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The aim of this project was to study a mechanism that might explain the increased uptake of ^{18}F-labeled FDG seen in inflammation. The approach chosen was to examine the effect on ^{18}F-FDG uptake of acute activation of murine lymphocytes by concanavalin A (Con A). **Methods:** Immunocompetent BALB/c mice and nude mice received an intravenous injection of 10 mg/kg Con A. Twenty-four hours later, the mice received an intravenous injection of 0.74 MBq (20 μCi) ^{18}F-FDG. One hour later, biodistribution was determined. The distribution of the radiolabel in the liver was also evaluated by autoradiography. In vitro ^{18}F-FDG uptake to splenocytes from BALB/c mice with and without Con A pretreatment were determined 30, 60, and 120 min after the splenocytes were mixed with ^{18}F-FDG (0.74 MBq [20 μCi]/200 μL). **Results:** In immunocompetent BALB/c mice, pretreatment with Con A significantly increased ^{18}F-FDG uptake in the spleen and liver. Autoradiographs of the liver showed that pretreatment with Con A produced a specific localization of ^{18}F-FDG at periportal areas, where numerous sites of cellular infiltration were observed. In vitro binding of ^{18}F-FDG to the splenocytes was significantly higher for Con A–pretreated BALB/c mice than for control mice. **Conclusion:** This study showed that Con A increased ^{18}F-FDG uptake. Con A–activated lymphocytes actively took up ^{18}F-FDG both in vitro and in vivo, and ^{18}F-FDG specifically accumulated in Con A–mediated acute inflammatory tissues.

Key Words: ^{18}F-FDG PET; concanavalin A; lymphocyte; acute inflammation

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Application of PET with ^{18}F-labeled FDG has been widespread in clinical oncology (*1–3*). However, ^{18}F-FDG is not a cancer-specific agent and is known to accumulate in cases of acute inflammation, in granulomatous diseases, and in autoimmune diseases (*4–6*). Under such conditions, the suggestion is that ^{18}F-FDG is taken up by infiltrating cells such as macrophages, lymphocytes, and granulocytes. However, only a few studies have described ^{18}F-FDG uptake under these conditions using animal models (*7*).

Tiegs et al. (*8*) described a model of experimental liver injury induced by concanavalin A (Con A) in mice. This plant lectin derived from the jack bean is known to mitogenically activate T lymphocytes in vitro to produce lymphokines and monokines such as tumor necrosis factor (*9*). Two other studies have revealed that Con A induced T cell–dependent acute liver injury in mice (*10,11*). The purpose of this study was to investigate the effect of acute activation of murine lymphocytes by Con A on ^{18}F-FDG uptake.

MATERIALS AND METHODS

^{18}F-FDG Preparation

^{18}F was produced by a ^{20}Ne (d, α) ^{18}F nuclear reaction, and ^{18}F-FDG was synthesized by the acetyl hypofluorite method (*12*).

Animal Model and Biodistribution Study

Five-week-old female BALB/c mice and BALB/c nu/nu mice were obtained from Japan SLC Co. Ltd. (Hamamatsu, Japan). Group of mice (n = 5 for each group) received an injection of 10 mg/kg Con A (Sigma, St. Louis, MO) through their tail veins. Twenty-four hours after injection of the Con A, the mice received an intravenous injection of 0.74 MBq (20 μCi) ^{18}F-FDG. The mice were killed 60 min later by ether inhalation, and blood and various organs were removed, weighed, and measured for radioactivity using a γ-counter. The percentage injected dose per gram of tissue was determined and was normalized to a 20-g mouse. All animal experiments were performed in accordance with the regulations of Kyoto University Animal Facility regarding animal care and handling.

Autoradiography and Hematoxylin–Eosin Staining

Distribution of the radiolabel in the liver was evaluated using autoradiography. One hour after 37 MBq (1 mCi) ^{18}F-FDG had been injected into Con A–treated and untreated BALB/c mice, the

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livers were removed and quickly frozen in an optimal-cutting-temperature compound (Sakura Finetrek U.S.A. Inc., Torrance, CA), from which 15-μm frozen sections were made. Dried sections of the liver were placed in a lighttight cassette directly contacting X-OMAT XAR film (Eastman Kodak, Rochester, NY). After 4 h of exposure at room temperature, the films were processed through an automatic developer. The same sections were then stained with hematoxylin and eosin (H & E) for histologic comparison.

Splenocyte Preparation and In Vitro Binding of ^{18}F-FDG

Groups of BALB/c mice ($n = 4$ for each group) with and without Con A pretreatment were killed by cervical dislocation, the spleens were excised, and single-cell suspensions of splenocytes were prepared. Splenocytes (2×10^6 cells per 200 μL) were then mixed with ^{18}F-FDG (0.74 MBq [20 μCi]/200 μL). After 30, 60, and 120 min of incubation at room temperature, the cells were spun and were washed twice with phosphate-buffered saline. The radioactivity of the cell pellets was then measured using a γ-counter.

Statistical Analysis

Data were analyzed using the Mann–Whitney *U* test, and a probability value of <0.05 was considered significant.

RESULTS

Biodistribution of ^{18}F-FDG in Mice That Received Con A

The biodistributions of ^{18}F-FDG in immunocompetent and immunodeficient mice with and without Con A pretreatment are summarized in Figure 1. In immunocompetent BALB/c mice, pretreatment with Con A markedly increased ^{18}F-FDG uptake in the spleen (19.107% ± 5.900% with Con A vs. 2.645% ± 0.695% without Con A, $P = 0.009$). ^{18}F-FDG uptake in the liver was also 3.3-fold higher in the Con A–treated group than in the control group ($P = 0.009$). In addition, ^{18}F-FDG accumulation in the kidney, intestine, lung, and bone increased after Con A treatment.

In contrast, Con A pretreatment of immunodeficient mice did not significantly change the ^{18}F-FDG uptake, except in bone and blood.

Autoradiography and Histology of Liver

Autoradiographs of the liver of Con A–treated and untreated BALB/c mice are shown in Figures 2 and 3, along with the same section stained with H & E. Without Con A pretreatment, the livers appeared histologically normal, with

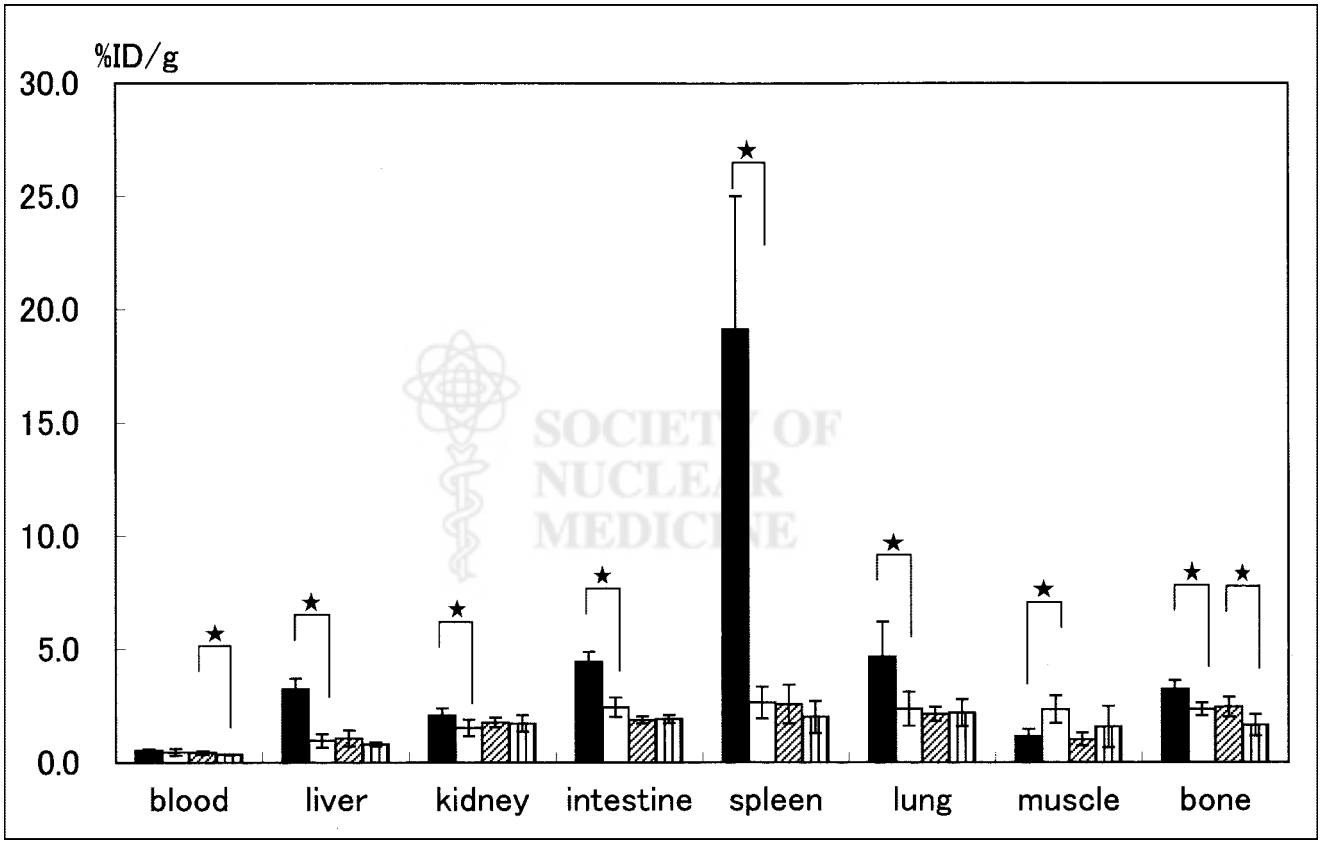

FIGURE 1. Biodistribution of ^{18}F-FDG at 1 h after injection in immunocompetent BALB/c mice with Con A pretreatment (filled bar) or without Con A pretreatment (open bar) and in nude mice with Con A pretreatment (hatched bar) or without Con A pretreatment (striped bar). Values are expressed as mean and SD for percentage injected dose (%ID) per gram of tissue ($n = 5$ for each group). Stars indicate significant differences ($P < 0.05$).

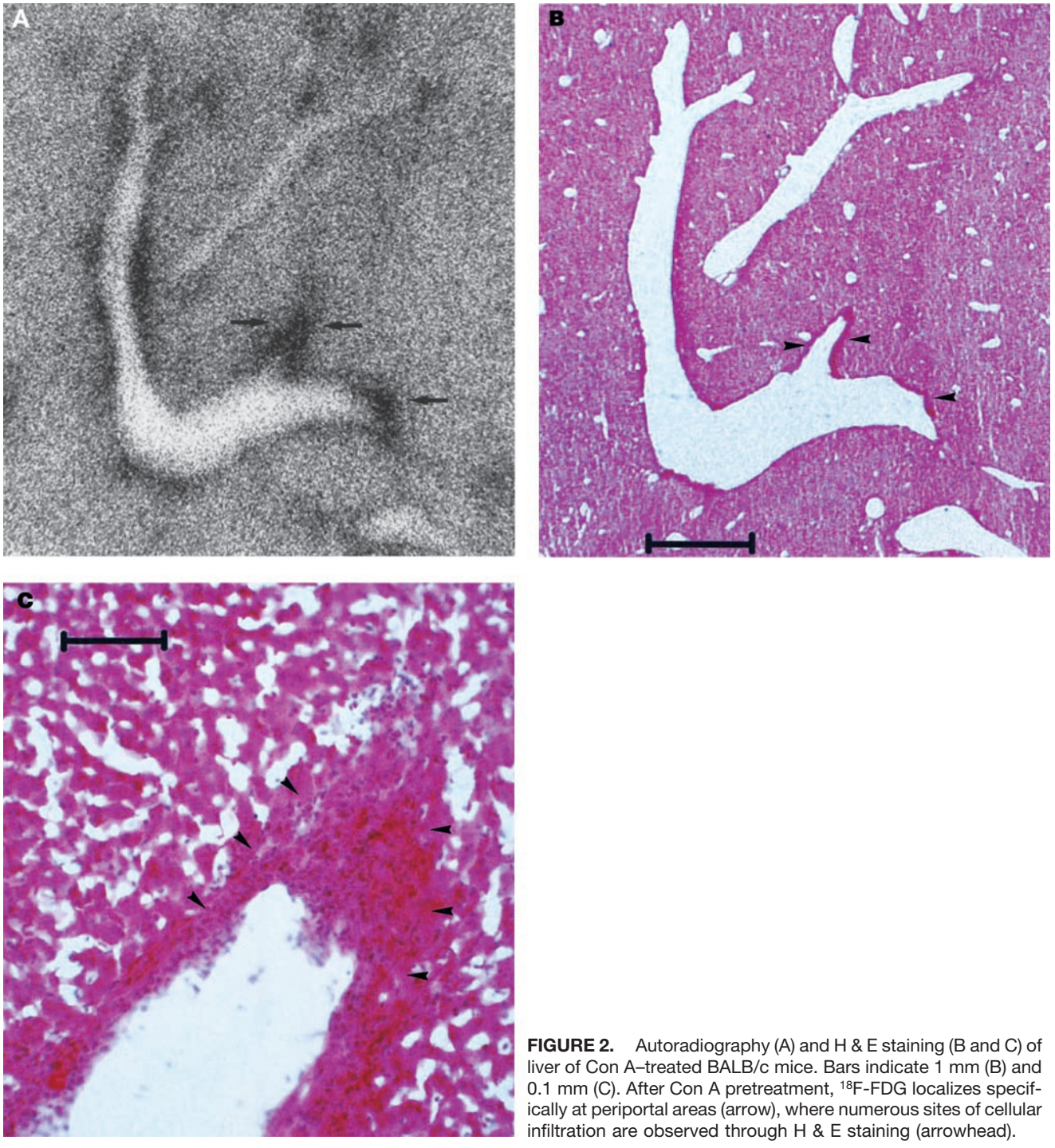

FIGURE 2. Autoradiography (A) and H & E staining (B and C) of liver of Con A–treated BALB/c mice. Bars indicate 1 mm (B) and 0.1 mm (C). After Con A pretreatment, ^{18}F-FDG localizes specifically at periportal areas (arrow), where numerous sites of cellular infiltration are observed through H & E staining (arrowhead).

no specific localization of ^{18}F-FDG detected by autoradiography (Figs. 2A and 3A). In contrast, 24 h after injection of Con A, a specific localization of ^{18}F-FDG was seen at periportal areas, where numerous sites of cellular infiltration were observed through H & E staining, corresponding to Con A–induced acute liver injury (Figs. 2A–2C). The infiltrated cells were mainly lymphocytes.

In Vitro ^{18}F-FDG Uptake to In Vivo Activated Splenocytes

In vitro binding of ^{18}F-FDG to the splenocytes from BALB/c mice was significantly higher when the mice were pretreated with Con A (Fig. 4). The higher uptake was first observed after 30 min of incubation and increased thereafter (from 8.06% ± 2.38% at 30 min to 13.54% ± 6.06% at 2 h).

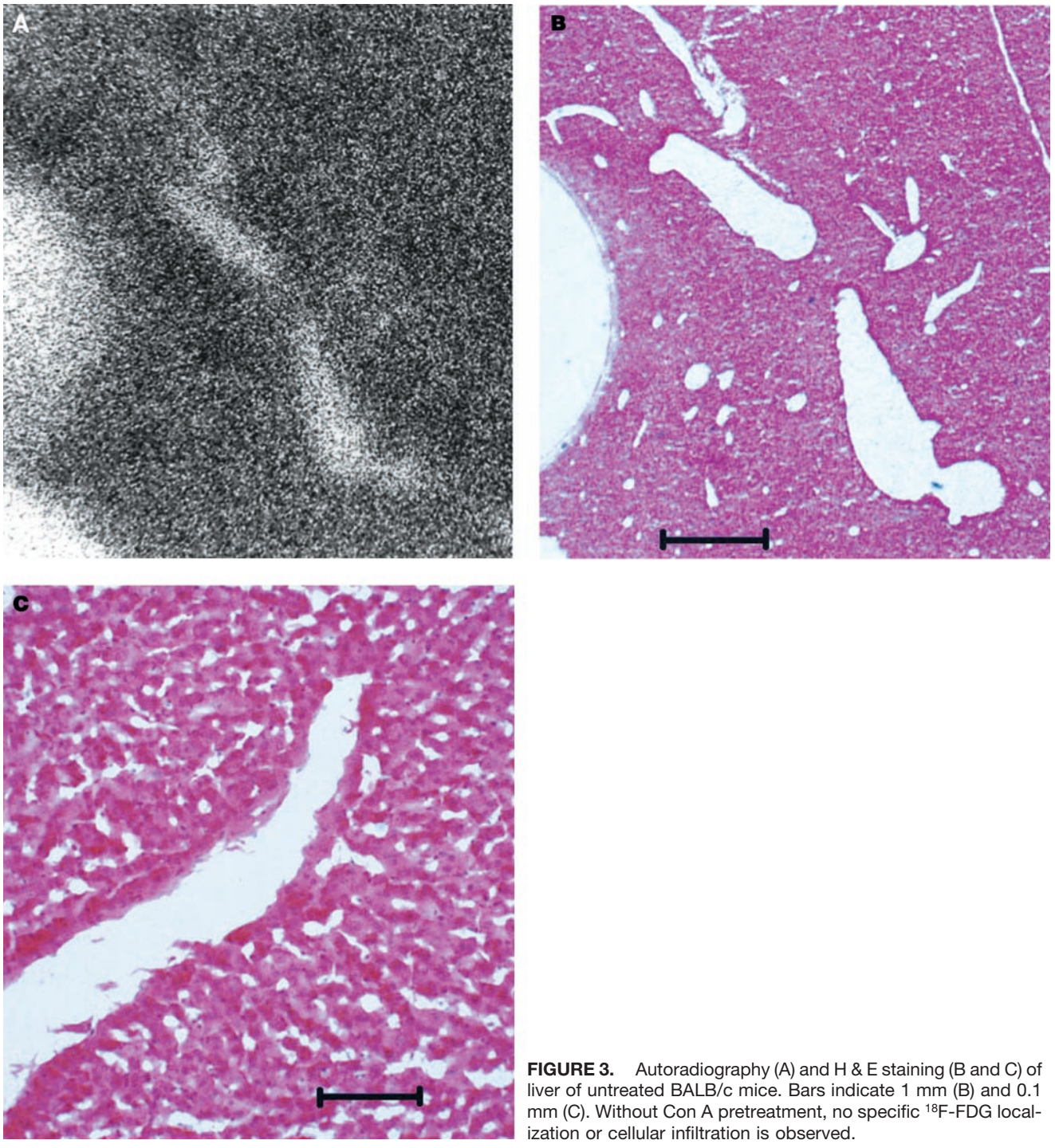

FIGURE 3. Autoradiography (A) and H & E staining (B and C) of liver of untreated BALB/c mice. Bars indicate 1 mm (B) and 0.1 mm (C). Without Con A pretreatment, no specific ^{18}F-FDG localization or cellular infiltration is observed.

DISCUSSION

^{18}F-FDG is widely used for the evaluation of malignant tumors in clinical oncology. At first, ^{18}F-FDG PET was expected to play an important role in the differentiation of malignant from benign diseases (*1–3*). However, it gave numerous false-positive results (*13–19*). In addition to accumulating in malignant tissues, ^{18}F-FDG has been shown to accumulate in nontumorous tissues, such as tissues with acute inflammation, granulomatous tissues, and tissues containing autoimmune lesions (*4–6,20*).

A full understanding of the mechanism of this nontumorous ^{18}F-FDG uptake requires basic investigations of ^{18}F-FDG uptake into the cells responsive to inflammation, granulomatous disease, and autoimmunity. However, only a few studies have investigated ^{18}F-FDG uptake and distribution in inflammation that was induced experimentally in animal

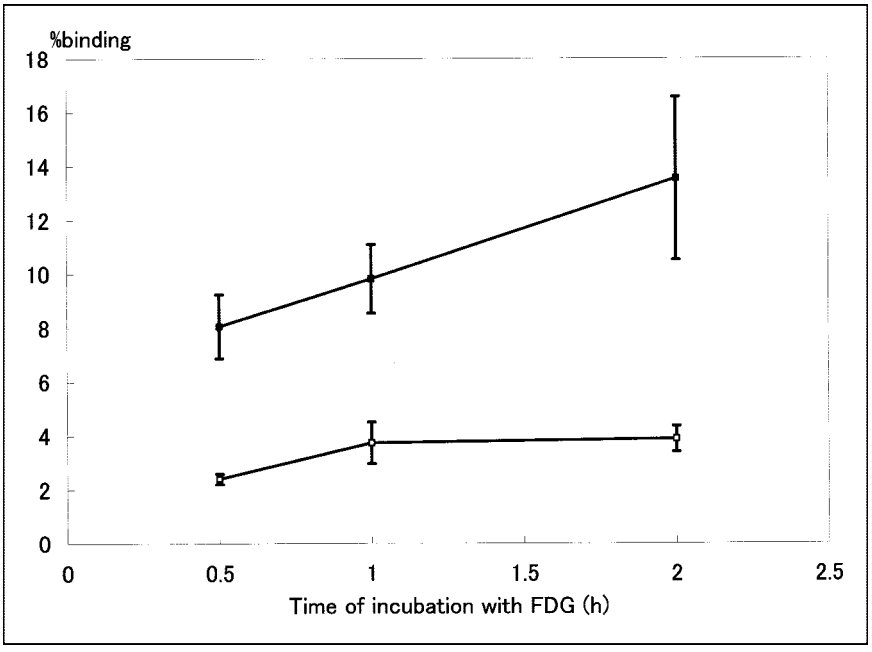

FIGURE 4. In vitro binding of ^{18}F-FDG to splenocytes from Con A–treated (■) and untreated (□) BALB/c mice. Values are expressed as mean and SD for percentage binding (n = 4 for each group).

models. Yamada et al. (*7*) reported that subcutaneous injection of turpentine oil could produce inflammation in rats, and high ^{18}F-FDG uptake in the inflammatory tissue was observed. Increased ^{18}F-FDG uptake in inflammation was also reported in animal models of bacterial infection (*21*,*22*), in which high ^{18}F-FDG uptake in the inflammatory cell infiltration was shown. In addition, Scharko et al. (*21*) studied ^{18}F-FDG distribution in activated lymphoid tissues after viral infection and showed high uptake in B cells. In this study, we selected Con A to activate murine lymphocytes and used this as a model to investigate ^{18}F-FDG uptake in inflammatory tissue and in activated lymphocytes. Con A is widely regarded as a model for investigating inflammation but has not yet been applied to ^{18}F-FDG–related experiments.

In this study, Con A pretreatment in immunocompetent mice caused a marked increase in ^{18}F-FDG uptake in the spleen, which is actually rich in lymphoid tissues. Increased ^{18}F-FDG uptake in the lung and intestine may also reflect the activation of lymphocytes in the lymphoid tissue of both organs. The effect of Con A was observed only with immunocompetent mice and not with T cell–deficient nude mice, indicating that Con A–mediated cellular activation takes place only in T lymphocytes and that activated T lymphocytes took up more ^{18}F-FDG than did nonactivated T lymphocytes. This finding was also confirmed by an in vitro binding study of ^{18}F-FDG to activated and nonactivated splenocytes—a study in which activated splenocytes showed more than 3-fold higher binding.

As reported by Tiegs et al. (*8*), Con A pretreatment induced acute liver injury that caused prominent cellular infiltration at periportal areas only in immunocompetent mice. As shown by autoradiography, ^{18}F-FDG specifically accumulated in these areas, confirming that these infiltrating cells were activated T lymphocytes that actively took up ^{18}F-FDG.

This situation caused by Con A treatment did not exactly correspond to the situation of natural inflammation, because inflammatory cells other than T cells are also involved and may contribute to the high ^{18}F-FDG uptake in inflammatory tissues. In experimental inflammatory tissue, young fibroblasts, endothelial cells of vessels, and phagocytes of neutrophils and macrophages were reported to show high ^{18}F-FDG uptake (*7*), and high ^{18}F-FDG uptake into B cells was also reported (*23*). However, in addition to acute inflammation, the processes of T cell activation are also involved in the formation of autoimmunity, in graft and tumor rejection, and in other such events, and this murine model of Con A activation can be used for evaluating the mechanism of various diseases and also for developing specific methods to reduce unfavorable ^{18}F-FDG uptake in nontumorous tissues.

CONCLUSION

This investigation showed that Con A activation of lymphocytes, a model of intense inflammation, markedly increased ^{18}F-FDG uptake by lymphocytes both in vitro and in vivo and that ^{18}F-FDG specifically accumulated in Con A–mediated acute inflammatory tissues. This experimental model would be applicable to further investigations to evaluate mechanisms of ^{18}F-FDG uptake in inflammatory cells.

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