Anti-inflammatory effect of fermented brown rice and rice bran with Aspergillus oryzae on mice

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ABSTRACT
Aim: In this study, we examined the anti-inflammatory effect of fermented brown rice and rice bran with Aspergillus oryzae (FBRA) on acute and chronic inflammation mouse models.

Methods: As an acute inflammation model, we used a 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema model. As a chronic inflammation model, we used an imiquimod (IMQ)-induced psoriasis model and ragweed pollen-induced allergic rhinitis model. We also investigated the effect of FBRA on the expression of inflammatory mediators in skin biopsies from the imiquimod-induced psoriasis model.

Results: The mice fed the 10% FBRA-containing diet showed lower-level inflammation among the three different experimental models. In the IMQ-induced psoriasis model, mRNA expressions of IL-17A, IL-1β and COX-2 were significantly inhibited in the skin tissue of mice fed 10% FBRA diet.

Conclusion: Our data suggest the potential benefit of FBRA as a functional food to prevent excessive inflammation.

KEY WORDS: fermented brown rice and rice bran, inflammation, psoriasis, rhinitis

INTRODUCTION
Inflammation is a type of immune response against tissue damage or infection to repair damaged tissue or to defend against pathogens such as viruses or bacteria. Additionally, mounting evidence suggests that inflammation is also associated with the development and maintenance of many types of complicated diseases including allergy, dermatitis, and cancer. While different types of anti-inflammatory drugs have been developed such as corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs), traditional medicines and functional foods are also widely used to ameliorate symptoms of inflammation.

Fermented brown rice and rice bran with Aspergillus oryzae (FBRA) is a functional food manufactured by fermenting a mixture of brown rice (Oryzae Fructus) and rice bran with Aspergillus oryzae to improve its digestibility. Aspergillus oryzae is known to contain several types of enzymes that metabolize carbohydrates and proteins to produce a variety of functional substances during fermentation. In addition, brown rice has also been used as one of the constituents of traditional Kampo medicine known as ‘Kobei’. Its medicinal activities are known to be nourishing effects, the fortifying of qi and the spleen, and anti-inflammatory effects. The potential active components of Kobei are vitamins (E, B1, B2), starch, dextrin, and γ-oryzanol [1]. Previous studies reported that FBRA has chemopreventive effects in several different chemical carcinogenesis models [2–7,8,9] and an inflammation-related carcinogenesis model [10], as well as hepatitis [11] and colitis [12] models.

In this study, we examined the anti-inflammatory effect of FBRA in both acute and chronic inflammation mouse models. As an acute inflammation model, we used a 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema model. As a chronic inflammation model, we used an imiquimod (IMQ)-induced psoriasis model and a ragweed pollen-induced allergic rhinitis model. We also investigated the effect of FBRA on the expression of inflammatory mediators in skin biopsies from the imiquimod-induced psoriasis model.

METHODS
Mice
C57BL/6 (B6) and BALB/c mice were purchased from Nippon SLC (Hamamatsu, Japan). Mice were fed a basal diet...
(MF, Oriental Yeast Co., Ltd., Tokyo, Japan), or MF supplemented with a processed food prepared by fermenting brown rice and its bran with Aspergillus oryzae (FBRA, 10% per weight). FBRA was provided by Genmai Koso Co., Ltd. (Sapporo, Japan) and its composition is shown in Table 1. All experiments were approved and performed according to the guidelines of the Animal Care and Use Committee of the Graduate School of Pharmaceutical Sciences of the University of Tokyo, and of the Care and Use of Laboratory Animals of the University of Toyama.

**TPA-induced ear edema model**
The right ear of B6 mice was challenged with TPA (Sigma, St. Louis, MO, USA, 2 μg/ear). Ear thickness was measured by a thickness gauge 24, 48, and 72 h after the TPA challenge. Mice were fed a control diet or 10% FBRA diet for 10 days before the TPA challenge.

**Imiquimod-induced psoriasis model**
An experimental psoriasis model was established by following the methods of van der Fits et al. [13] with some modifications. Balb/c mice received a daily topical dose of 20 μg of commercially available imiquimod (IMQ) cream (5%) (Aldara; Mochida Pharmaceuticals, Tokyo, Japan) on the right ear for five consecutive days, equivalent to a daily dose of 1 μg of the active compound. Ear thickness was measured by a thickness gauge 24 h after each IMQ challenge. Mice were fed a control diet or 10% FBRA diet for 10 days before the first IMQ challenge.

**Experimental allergic rhinitis models**
Experimental allergic rhinitis models were established by following the methods of Haenuki et al. (immunization model) [14] or the methods of Kato et al. (nasal sensitization model) [15] with some modifications.

For the immunization model, Balb/c mice were immunized (i.p.) with a mixture of ragweed pollen (100 μg in 200 μl) and aluminum hydroxide hydrate gel (1 mg in 200 μl; Sigma-Aldrich, St Louis, MO, USA) on day 0, and subsequently boosted (i.p.) with ragweed pollen in phosphate-buffered saline (PBS; 100 mg/200 μl) on day 7. A week after the boost, mice were intra-nasally challenged with ragweed pollen (1 mg in 20 μl of PBS) or PBS (20 μl) for two consecutive days. Immediately after each nasal challenge, the frequency of sneezing was counted for 10 min. Mice were fed a control diet or 10% FBRA diet starting seven days before the immunization, and continued to be fed with the same diet throughout the experiment. A group of mice was treated with ketotifen (p.o., Wako Pure Chemicals, 0.2 mg/mouse) 1 h before the nasal challenge.

For the nasal sensitization model, Balb/c mice were nasally administered ragweed pollen (1 mg in 20 μl PBS) every 3–4 days for three weeks. The frequency of sneezing in the 10 min immediately after nasal challenge was determined on days 7, 14, 21, and 28. Mice were fed a control diet or 10% FBRA diet starting a day before the first challenge, and continued to be fed with the same diet throughout the experiment.

**Real-time PCR**
Total RNAs were prepared using the RNeasy Plus Mini kit (Qiagen, Hilden, Germany) and subjected to real-time polymerase chain reaction (RT-PCR) on an ABI Prism 7300 sequence detection system (Life Technologies Corporation, Carlsbad, CA, USA). Expression levels of IL-17A, IL-1β, and COX-2 mRNAs were normalized to β-actin mRNA. The primers used were: 5'-CAC CTC ACA CGA GGC ACA AG-3' (sense) and 5'-GCA GCA ACA GCA TCA GAG ACA-3' (anti-sense) for IL-17A mRNA, 5'-TCC AGG ATG ACG ACA TGA GCA C-3' (sense) and 5'-GAA CGT CAC ACA CCA GCA GGT TA-3' (anti-sense) for IL-1β mRNA, 5'-GTG TGC GAC ATA CTC AAG CAG GA-3' (sense) and 5'-TGA AGT GGT AAC CGC TCA GTT G-3' (anti-sense) for COX-2 mRNA, and 5'-GCA CAG AGC CTC GCC TT-3' (sense) and 5'-GGT GTC GAC GAC GAG CG-3' (anti-sense) for β-actin mRNA.

**Statistical analysis**
All data were obtained from a group of 3–6 mice and are representative of at least two independent experiments. Data were analyzed for significance using Student’s t-test. P-values less than 0.05 were considered significant.

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**Table 1 | Composition of FBRA†**

| Ingredient | Amount/100 g |
|------------|--------------|
| Energy     | 390 Kcal     |
| Protein    | 15.7 g       |
| Fat        | 22.8 g       |
| Carbohydrate | 17.1 g  |
| Fiber      | 26.7 g       |
| Ash        | 12.0 g       |
| Calcium    | 451 mg       |
| Sodium     | 15.3 mg      |
| Zinc       | 5.45 mg      |
| Copper     | 0.73 mg      |
| Phytic acid | 7.78 g     |
| Thiamin    | 1.76 mg      |
| Riboflavin | 0.82 mg      |
| Vitamin B6 | 3.40 mg      |
| Tocopherol | 9.5 mg       |
| SOD        | 1.5 × 10⁶ units |
| Amylase    | 1.6 × 10⁶ units |

† Data from Japan Food Research Laboratories (report number: 1608461001-0201) and provided by Genmai Koso Co., Ltd. (Sapporo, Japan), SOD, superoxide dismutase.
RESULTS

Inhibitory effect of FBRA on both acute and chronic skin inflammation mouse models

We first examined the effect of FBRA on acute inflammation induced by the topical application of TPA to induce skin edema. Mice were fed with either a control diet (Control) or 10% FBRA-containing diet (10% FBRA) for 10 days, and then challenged with TPA on the right ear. As shown in Figure 1, 10% FBRA mice showed significantly thinner ear skin compared with control mice at 24 h after the TPA challenge, and such inhibition of ear edema formation was seen up to 72 h after the initial TPA challenge.

Next, we examined the effect of FBRA on chronic inflammation induced by the topical application of imiquimod (IMQ), which is a toll-like receptor 4 (TLR4) ligand, to induce psoriasis-like skin inflammation. As shown in Figure 2, daily application of IMQ on the right ear of mice led to significant increases in ear thickness. Compared with the control group, the 10% FBRA diet (provided for 10 days prior to the initial IMQ application) significantly inhibited IMQ-induced skin inflammation (Fig. 2). Collectively, these results indicate the inhibitory effect of the FBRA-containing diet on both acute and chronic skin inflammation mouse models.

Inhibitory effect of FBRA on experimental allergic rhinitis models

In order to determine the effect of FBRA on allergic inflammation, we used two murine models of allergic rhinitis induced by ragweed pollen. For the immunization mode, BALB/c mice were immunized with ragweed pollen, and then intra-nasally challenged with ragweed pollen to induce sneezing behavior. For the nasal sensitization model, BALB/c mice were repeatedly intra-nasally challenged with ragweed pollen to induce sneezing behavior. As shown in Figure 3a, ragweed-challenged mice showed a significant increase in the frequency of sneezing compared with control PBS-challenged mice, suggesting that the ragweed pollen challenge induces allergic rhinitis-like symptoms. While the H1-antagonist ketotifen markedly inhibited the frequency of sneezing upon ragweed challenge, mice fed 10% FBRA showed a moderate but significant reduction in their sneezing behavior. Next, we investigated the effect of FBRA on a nasal sensitization model, in which repeated nasal sensitization with ragweed pollen induced an increase in sneezing over time (Fig. 3b). In mice fed 10% FBRA, the frequency of sneezing was significantly decreased on day 28. Collectively, these results indicate the inhibitory effect of a FBRA-containing diet on experimental allergic rhinitis models, possibly through the inhibition of both the induction and progression of allergic inflammation.

Inhibition of mRNA expression of inflammatory mediators by FBRA-containing diet

To further understand the mechanism of the anti-inflammatory effect of the FBRA-containing diet, we examined the mRNA expression of inflammatory mediators in skin biopsies from the IMQ-induced psoriasis model. As it is known that IL-17A is a key cytokine for IMQ-induced skin inflammation, we examined the mRNA expression of IL-17A, and the mRNA expression of other typical inflammatory mediators.

Figure 1 | Effect of FBRA-containing diet on TPA-induced ear edema model. B6 mice were challenged with TPA (2 μg) on the right ear. Ear thickness was measured by a thickness gauge 24, 48, and 72 h after the TPA challenge, and the increase of ear thickness from 0 h (before TPA challenge) was calculated. Mice were fed a control diet or 10% FBRA diet for 10 days before the TPA challenge. Data are shown as the mean ± SD, * P < 0.05 compared with control mice.

Figure 2 | Effect of FBRA-containing diet on IMQ-induced psoriasis model. Balb/c mice received a daily topical dose of imiquimod cream (5%, 20 μg) on the right ear for five consecutive days. Ear thickness was measured by a thickness gauge 24 h after the each IMQ challenge and the increase of ear thickness from day 0 (before IMQ challenge) was calculated. Mice were fed a control diet or 10% FBRA diet for 10 days before the first IMQ challenge. Data are shown as the mean ± SD, * P < 0.05 compared with control mice.
mediators, IL-1β and COX-2 in skin tissue samples from IMQ-treated mice. As shown in Figure 4, mRNA expressions of IL-17A, IL-1β, and COX-2 were significantly inhibited in mice fed a 10% FBRA diet, supporting the anti-inflammatory effect of FBRA in pathological tissues.

**DISCUSSION**

In this study, we found that oral administration of FBRA, i.e. brown rice and its bran fermented with *Aspergillus oryzae*, led to an anti-inflammatory effect in three different experimental models of inflammation. Considering that FBRA also showed preventive effects on chronic inflammation models such as hepatitis [11] and colitis [12] models as well as several different chemical carcinogenesis models in which inflammation plays a critical role in tumor initiation [2–6,8–11], FBRA is a promising functional food to resolve excessive inflammatory responses and inflammation-associated diseases.

Regarding the skin inflammation models used in this study, it is well known that different types of immune responses are involved. While topical application of TPA to the skin induces an acute inflammatory reaction as seen in erythema, edema, and polymorphonuclear leukocyte (PMN) infiltration [16], IMQ treatment is known to be closely related to human plaque-type psoriasis, as seen in skin symptoms and infiltration of inflammatory immune cells [13]. More specifically, the inflammatory cytokine IL-17A and its source Th17 and/or γδT17 cells are known to be critical to the development of both TPA-induced and IMQ-induced skin inflammation [13,16]. IL-17A is known to induce STAT3-dependent proliferative and anti-apoptotic gene expression along with epidermal cell proliferation and...
hyperplasia [17]. In addition, IL-17 is known to be associated with the induction of chemokine expression to attract inflammatory cells [18,19]. Considering that FBRA-treatment significantly inhibited the mRNA expression of IL-17 in IMQ-treated skin tissue, it is possible that FBRA and its active ingredients suppress those IL-17-producing immune cells to prevent inflammatory responses. In addition to those skin inflammation models, we also showed the beneficial effect of FBRA in the experimental allergic rhinitis models. Allergic rhinitis is one of the most common allergic inflammatory diseases, induced by nasal allergen exposure that results in IgE-mediated inflammation [14,15]. Seasonal allergic rhinitis is mostly regarded as pollinosis caused by exposure to allergenic pollens, such as ragweed or cedar pollen. Nasal symptoms such as sneezing and watery nasal discharge are the typical symptoms of allergic rhinitis. While the involvement of IL-17A in the pathogenesis of allergic rhinitis is unclear, the importance of IL-33 has been reported [14,20]. IL-33 is a nuclear cytokine of the IL-1 family constitutively expressed in epithelial barrier tissues and lymphoid organs, and plays important roles in type-2 innate immunity [21]. Together with the effect of FBRA on skin inflammation models, the present results suggest the involvement of innate immunity as a potential target for the anti-inflammatory activity of FBRA. Importantly, there was no observation of any clear toxicity of FBRA administration in animal experiments [10] or in a human subject [22], and FBRA has potential as a prebiotic to modify colonic microbiota [22,23]. Although the exact mechanism of how FBRA exerts its anti-inflammatory effect is unclear, the present results strongly suggest the beneficial effects of FBRA as a functional food to prevent excessive inflammation.

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CONFLICTS OF INTEREST

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