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Improvement Effect of Dewaxed Brown Rice on Constipation in Antibiotic-treated Mice

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Abstract. *Background/Aim: A decrease in gastrointestinal motility causing weakened lipopolysaccharide (LPS) – toll-like receptor (TLR)4 signaling along with a decline in the number of enteric bacteria is known to be a cause of constipation due to the administration of antibiotics. A new type of brown rice with its wax layer removed, resulting in quick-cooking and tasty product, contains 100-times more LPS than polished white rice. In this study, the improvement effect on constipation due to intake of dewaxed brown rice was examined. Materials and Methods: Dewaxed brown rice was prepared at Toyo Rice from brown rice. Mice were given powdered feed to which powdered rice containing 0-50% of dewaxed brown rice was added. Antibiotics were administered for 10 or 27 days in drinking water containing vancomycin, metronidazole and neomycin. LPS, used as a control, was freely provided in drinking water. The defecation frequency, stool weight per hour and body weight were determined on the last day. Results: Although the 10-day administration of antibiotics reduced the stool weight per hour to half, the dewaxed brown rice and LPS groups showed a trend towards improvement at a level comparable to the group receiving no antibiotics. The body weight significantly decreased after the 27-day administration of antibiotics but was improved in the 50% dewaxed brown rice group at a level comparable to the group receiving no antibiotics. Though the defecation frequency and wet and dry stool weights per hour were reduced by as much as 50% in the group receiving antibiotics, a significant improvement in constipation was observed in the 50% dewaxed brown rice group. Conclusion: As the improvement effect of dewaxed brown rice on body weight loss and constipation caused by the long-term administration of antibiotics has been confirmed in animal experimentation, the introduction of dewaxed brown rice as a staple food to patients under long-term antibiotic treatment may improve constipation.*

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In recent years, enteric floras have been thought to play a critical role in the maintenance of homeostasis of a living organism through crosstalk (1). Enteric floras have been shown to be associated with the development of diseases, including infections, diabetes, allergies and cancers (1). Cancer treatment often involves the long-term administration of various kinds of antibiotics to prevent or reduce infection after chemotherapy or surgery. Constipation, one of the adverse reactions of the long-term administration of antibiotics, is known to produce a significant reduction in the number of Gram-negative bacteria in enteric floras (2). Accordingly, reduced toll-like receptor (TLR)4 signalling results in a decline in bone morphogenic protein 2 expression through enteric muscularis macrophages, reducing gastrointestinal motility. Meanwhile, constipation due to the administration of antibiotics can be avoided by the oral administration of lipopolysacchride (LPS) in animal experimentation (3). Moreover, a low dose of LPS, which is a ligand of TLR4, reportedly contributes to the survival of enteric neuronal cells (4). It has also been shown that the stool output of TLR4-deficient mice is half that of normal mice (4).

LPS, found in the outer membrane of Gram-negative bacteria, is known to control innate immunity by activating macrophages and enteric epithelial cells through TLR4 (5). Oral administration of LPS reportedly results in various improvements (6-8). Our screening of various dietary plants has demonstrated that some plants contain a high proportion of LPS derived from symbiotic Gram-negative bacteria (9).

We have found that brown rice is particularly rich in LPS and reported that it provides activation of innate immunity (10).

Brown rice, with bran layer and germ, is nutrient-dense and richer than polished white rice in vitamins E and B1, dietary fibres and malto-oligosaccharides (11). However, the bran layer of brown rice is hard and the outmost wax layer repels water. Brown rice has a low water absorption rate and its starch is often insufficiently gelatinised during cooking. This degrades the texture and interferes with digestion. Therefore, brown rice is not common in Japan, in spite of being recognised as a functional food (12). In contrast, brown rice whose outer wax layer has been removed (dewaxed brown rice) does not repel water. Water easily flows inwards, resulting in easy digestion and good eating quality under the same cooking conditions used for cooking normal, polished white rice. Due to these qualities, it is offered to inpatients at some Hospitals in Japan.

We have previously reported that dewaxed brown rice is rich in LPS and results in innate immunity activation mainly mediated by TLR4 (10). In this research paper, we conducted a study on antibiotic-treated mice to examine whether the intake of dewaxed brown rice, rich in LPS, can reduce antibiotic-induced constipation.

Materials and Methods

Preparation of rice samples. Brown rice of Koshihikari (from Nagano prefecture, produced in 2014) was dewaxed and polished rice was prepared using the Saika-style rice polishing process in Toyo Rice Corp. Both varieties were powdered with blender.

For LPS measurements, one gram of each sample was added up to 10 ml distilled water and heat-treated (90˚C for 20 min). The samples were subsequently sonicated for 15 min and centrifuged for 30 min at 3,500 rpm. The collected supernatants were used for LPS measurement (hereinafter 100 mg/ml rice extract of each sample). The LPS amount in samples was assayed by the kinetic turbidimetric method with a Toxinometer ET-201 (Wako Pure Chemical Industries Ltd. Tokyo, Japan) as described previously (10). LPS content in dewaxed brown rice and polished rice was 2.64±0.35 μg/g and 0.038 μg/g±0.004 μg/g, respectively. LPS content in standard diet American Institute of Nutrition (AIN)93 (CLEA, Japan, Tokyo) was 3.6 ng/g.

Antibiotic-induced constipation assessment. AIN93 was used as powdered feed. In the brown rice intake study, mice were given AIN93 to which powdered rice containing 0-50% dewaxed brown rice was added. Antibiotics were administered to 10-week-old male C57BL/6 mice (CLEA) for 10 or 27 days through a drinking water cocktail containing 250 μg/ml vancomycin (Sigma, Japan, Tokyo), 250 μg/ml metronidazole (Sigma) and 125 μg/ml neomycin (Sigma). LPS (highly purified LPS derived from *Pantoea agglomerans*; Macrophi Inc., Kagawa, Japan) 10 μg/ml was freely provided in drinking water. The defecation frequency, stool weight per hour and body weight were determined on the last day of the study.

Statistical analysis. Statistical analysis was performed using Ekuseru-Toukei 2008 ver. 1.07 (SSRI, Tokyo, Japan). The Dunnett's multiple comparison test was used.

Results

Improvement effect of the 10-day intake of dewaxed brown rice on antibiotic-induced constipation. Constipation was induced in mice given normal AIN93 diet during the 10-day intake of the antibiotic cocktail. As shown in Figure 1, this resulted in a decrease in the defecation frequency (Figure 1A) and wet stool weight (Figure 1B) to 63% and 52%, respectively.

The defecation frequency and wet stool weight in the group receiving *Pantoea agglomerans* LPS and antibiotics through drinking water were 1.9-times higher than those in the antibiotic-only group and 120% and 97% of the levels in the control group (without antibiotics administration and AIN93 standard diet) respectively. This result supported that LPS improved defecation. Meanwhile, the defecation frequency and wet stool weight in the 50% dewaxed brown rice group were 1.9-times higher than those in the groups receiving antibiotics and 117% and 97% of the levels in the control group, respectively. This suggests that the intake of 50% dewaxed brown rice improves antibiotic-induced constipation to an extent as large as that seen in the LPS group. There was no difference in body weight among the groups (data not shown).

Improvement effect of the 27-day intake of dewaxed brown rice on antibiotic-induced constipation. Based on the results of Figure 1, the effect of a longer intake of brown rice was assessed. Powdered dewaxed brown rice was mixed at ratios of 0%, 50% and 100% with polished white rice powder. The prepared powdered rice mixtures were further mixed with normal AIN93 at a 1:1 ratio so that final feed powders contained 0%, 25% and 50% dewaxed brown rice. The feed was given to C57BL/6 mice that received the antibiotic cocktail for 27 days to induce constipation.

Figure 2 shows the weekly measured body weights of mice. The groups receiving antibiotics experienced significant weight loss throughout the study period compared to the no-antibiotic control group (Figure 2A). Bars indicate the body weight on day 27 of the administration of antibiotics (Figure 2B). A weight loss of 7.9% was observed in the group receiving antibiotics compared with the no-antibiotic control group. The mice in the 0%, 25% and 50% dewaxed brown rice groups lost 10.0%, 6.9% and 1.7% of their body weight, respectively. Significant suppression of weight loss was seen only in the 50% dewaxed brown rice group compared to the group receiving only antibiotics.

The hourly frequency of defecation on day 27 of the administration of antibiotics decreased by 52% (Figure 3). No significant difference (p=0.082) was seen; however, the 50% dewaxed brown rice group showed a trend towards improvement (80% of the frequency observed in the no-antibiotic group). Compared to the no-antibiotic control group, the wet (Figure 4A)

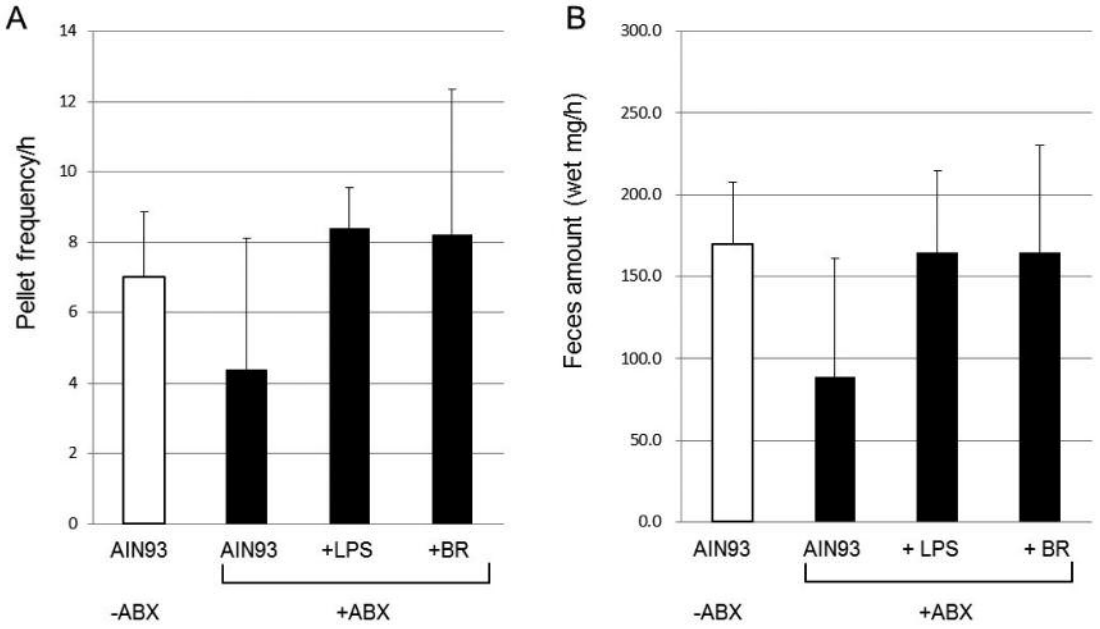

Figure 1. *Improvement effect of constipation induced by a 10-day administration of antibiotics due to the intake of lipopolysaccharide (LPS) dissolved in drinking water and brown rice/dewaxed brown rice powder. A: Hourly frequency of defecation on day 10 of the administration of antibiotics, B: Total wet stool weight during one hour on day 10. American Institute of Nutrition (AIN)93, standard diet; ABX, the mixed antibiotics administration in drinking water; LPS, LPS provided in drinking water (10 µg/ml) with ABX; +brown rice powder (+BR), AIN93 containing 50% dewaxed brown rice powder. Columns and bars indicate means and standard deviations (n=6), respectively.*

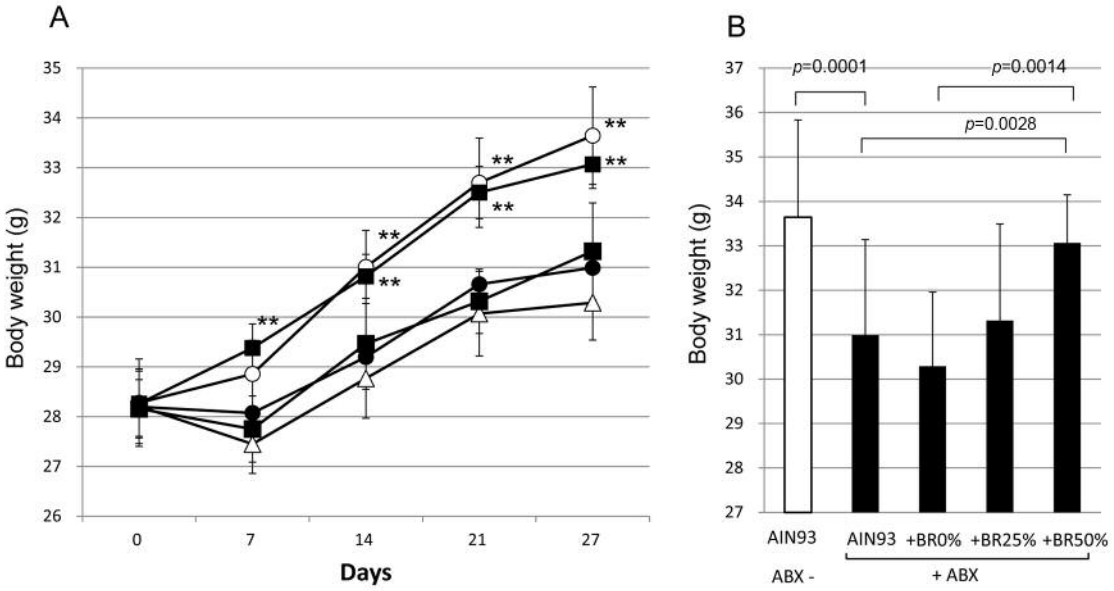

Figure 2. *Improvement in body weight loss in mice treated with antibiotics for 27 days with dewaxed brown rice. A: Weekly measured body weights of mice during 27 days of the mixed antibiotics administration (ABX). Open circle, drinking water without ABX and American Institute of Nutrition (AIN)93 standard diet (control); closed circle, drinking water with ABX and with AIN93 (antibiotics control); open square, drinking water with ABX and AIN93 containing 50% polished white rice powder (WR) without dewaxed brown rice powder (BR); closed squared; drinking water with ABX and AIN93 with 25% WR and 25% BR powder; open triangle, drinking water with ABX and AIN93 containing 50% BR without WR; **p<0.01 vs. antibiotics control. B: body weight on day 27 of the antibiotics administration. American Institute of Nutrition (AIN)93, standard diet; ABX, the mixed antibiotics administration in drinking water; LPS, lipopolysaccharide provided in drinking water (10 µg/ml) with ABX; +brown rice powder (+BR), AIN93 (containing 50% dewaxed brown rice powder). Columns and bars indicate means and standard deviations (n=6), respectively.*

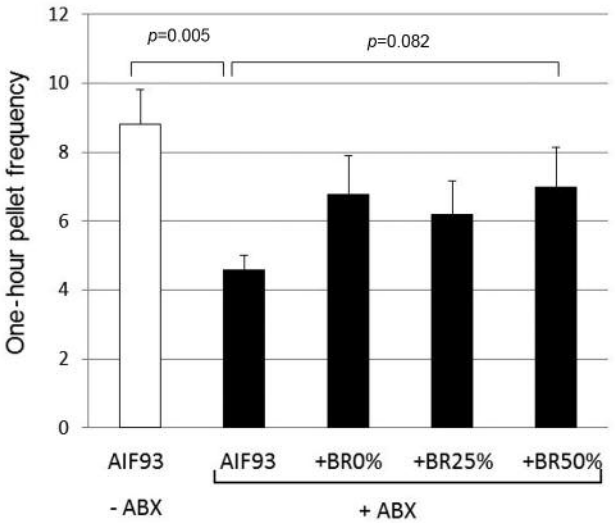

Figure 3. *Improvement in hourly frequency of defecation in mice treated with antibiotics for 27 days with dewaxed brown rice. American Institute of Nutrition (AIN)93, standard diet; ABX, the mixed antibiotics administration in drinking water; brown rice powder (BR)0%, AIN93 containing 50% polished white rice powder (WR) without dewaxed brown rice powder (BR); BR25%, AIN93 containing 25% WR and 25% BR powder; BR50%, 50% BR without WR. Columns and bars indicate means and standard deviations (n=6), respectively.*

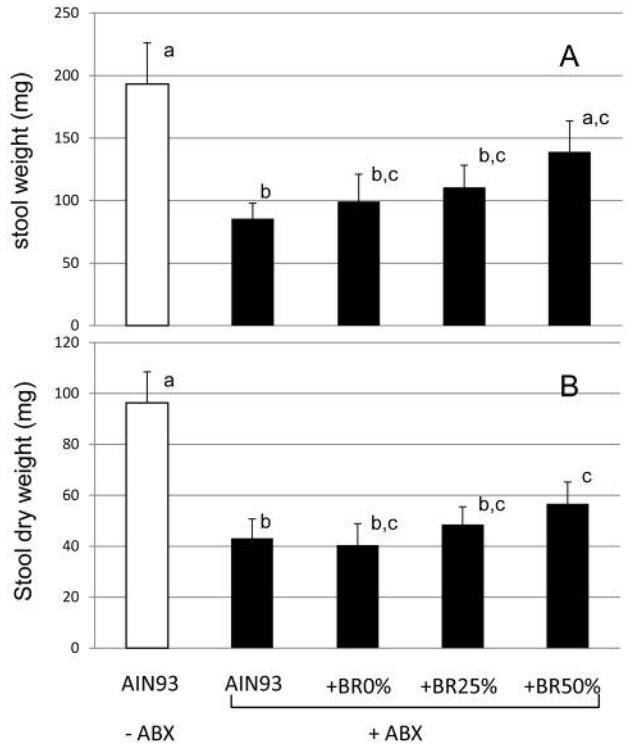

Figure 4. *Improvement in hourly frequency of defecation in mice treated with antibiotics for 27 days with dewaxed brown rice. A: Total wet stool weight during one hour on day 27. B: Total dry stool weight during one hour on day 10. American Institute of Nutrition (AIN)93, standard diet; ABX, the mixed antibiotics administration in drinking water; brown rice powder (BR)0%, AIN93 containing 50% polished white rice powder (WR) without dewaxed brown rice powder (BR); BR25%, AIN93 containing 25% WR and 25% BR powder; BR50%, 50% BR without WR. Columns and bars indicate means and standard deviations (n=6), respectively. Different letters indicate statistically significant differences between groups (p<0.05).*

and dry stool (Figure 4B) weights, that were determined from collected stool samples, were 44% and 45%, respectively, showing a significant drop in stool output due to administration of antibiotics. The wet and dry stool weights in the 0%, 25% and 50% brown rice groups were 51%, 57% and 72% and 42%, 50% and 59%, respectively, indicating significant defecation improvement in the 50% brown rice group.

Discussion

The high prevalence of constipation in patients with cancer has been reported (13). Antibiotics are commonly used to prevent increased susceptibility to infection due to a weakened immune system caused by the administration of anticancer drugs during treatment. However, the long-term administration of antibiotics is known to significantly change enteric floras (2). This research paper has confirmed that defecation and stool output are distinctly reduced in mice receiving long-term administration of antibiotics and that they can be improved by the intake of LPS added to drinking water (Figure 1). Our examination of the effect of brown rice powder using these constipation models demonstrated that the intake of powdered rice feed containing 50% brown rice significantly improved constipation (Figures 2-4).

Brown rice has various bioactive components in its bran layer. For instance, due to its low-density lipoprotein (LDL)-cholesterol-lowering effects, γ-Oryzanol has the potential to improve the hyperlipidaemia disorder (14, 15). In addition, dietary fibres reduce constipation by promoting the growth of enteric bacteria (16). Brown rice is a prospective functional food. However, its poor eating quality has reduced its popularity (12). Dewaxed brown rice has a water absorption rate as high as that of polished white rice, resulting in improved eating quality. This improvement helps solve the popularity problem of brown rice. The content of LPS amounts as a functional component in the dewaxed brown rice used in the present study was largely retained 50% of that in material brown rice (10).

We have previously found that the macrophage activation potency of dewaxed brown rice is about 100-times higher than that of white rice (10). Potency is mainly mediated by TLR4, but also by TLR2, and LPS is considered to be the primary macrophage-activated component. Though it is known that the systemic injection of LPS causes endotoxin shock, this is not

caused by oral administration and LPS is a safe substance (17). There are numerous studies suggesting the association of the oral administration of LPS with the maintenance of enteric physiological functions. For instance, oral administration of LPS is known to contribute to the stabilisation of enteric floras by the production of bactericidal peptides from Paneth cells in the intestines (18, 19). Accordingly, LPS is used to maintain good health through oral intake.

The stimulation of survival of enteric neuronal cells and gastrointestinal motility by TLR4 signalling in enteric bacteria are associated with the improvement effect of LPS on antibiotic-induced constipation (3, 4). However, the mechanism on how LPS in the gastrointestinal tract physiologically provides information on muscularis macrophages remains unknown (4). It is presumed that a different mechanism operates after oral administration compared to transmucosal administration because LPS is safe when transmucosally administered. The elucidation of the exact mechanism is a topic for future research.

Conflicts of Interest

The Authors have no financial conflicts of interest.

Acknowledgements

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