A Study Report on the Protective Activity of Modified Arabinoxylan from Rice Bran (BioBran/MGN-3)

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Introduction

Modified Alabinoxylan from Rice Bran (BioBran/MGN-3) developed by Daiwa Pharmaceutical Co., Ltd. has been shown to activate rat NK cells and is predicted to have an anticancer effect. To determine whether BioBran/MGN-3 prevents bacterial infection, in this study, mice were inoculated with either virulent or attenuated strains of *Salmonella*, or enterohaemorrhagic *Escherichia coli* O157, and the effect of BioBran/MGN-3 in preventing infection was observed.

Experimental method

Female BALB/cAJc16 mice aged 7 weeks (12 mice per group) were given BioBran/MGN-3 or a placebo (1 mg/mL) orally *ad libitum* (1-2 mL/day/mouse). In the experiment, BioBran/MGN-3 or the placebo (1 mg/mouse: 50 mg/kg) was administered to the mice intraperitoneally either 5 days or 1 day before infection and the mice were then given the same substance (1 mg/mL) orally *ad libitum*

This is a report of collaborative study result conducted with International Medical Center of Japan.

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Table 1 Effect of BioBran/MGN-3 on *Salmonella enteritidis* No.11 infection

	No. of survivals at 15days after inoculation			LD_{50}
	3.0×10^1	3.0×10^2	3.0×10^3	
MGN-3	0	0	0	$<3.0\times10^1$
Placebo	0	0	0	$<3.0\times10^1$

No difference was shown between BioBran/MGN-3 and placebo groups

Table 2 Effect of BioBran/MGN-3 on *Escherichia coli* O157: H7 (EHEC-SMR) infection

	MGN-3	Placebo
One day after inoculation	①$1.0\times10^2$ CFU/spleen ②$3.0\times10^2$ CFU/spleen	①$1.0\times10^2$ CFU/spleen ②$2.0\times10^2$ CFU/spleen
	①<100 CFU/liver ②<100 CFU/liver	①<100 CFU/liver ②<100 CFU/liver
	①<100 CFU/ kidney ②<100 CFU/kidney	①<100 CFU/ kidney ②<100 CFU/kidney
Two days after inoculation	①<100 CFU/spleen ②<100 CFU/ spleen	①<100 CFU/spleen ②<100 CFU/ spleen
	①<100 CFU/liver ②<100 CFU/liver	①<100 CFU/liver ②<100 CFU/liver
	①<100 CFU/ kidney ②<100 CFU/kidney	①<100 CFU/ kidney ②<100 CFU/kidney

(1-2mL/day/mouse). One of *Salmonella enteritidis* No. 11, *Salmonella typhimurium* LT2, or streptomycin-resistant enterohaemorrhagic *Escherichia coli* O157:H7 (EHEC-SMR) was inoculated into the abdominal cavity. The mortality rate of the mice infected with *Salmonella enteritidis* No. 11 and *Salmonella typhimurium* was observed. In the case of *Escherichia coli* o157:H7 (EHEC-SMR), the number of bacterial cells present in organs was counted 24 and 48 hours after inoculation.

Results

Salmonella enteritidis No. 11

Mice were given BioBran/MGN-3 or a placebo (1mg/mL) orally *ad libitum* for 5 days and intraperitoneally administered the virulent strain *Salmonella enteritidis* No. 11 in quantities of 3.0×10, 3.0×10^2, and 3.0×10^3 CFU/ mouse. As shown in **Table 1**, all mice in both the placebo and BioBran/MGN-3

Table 3 Effect of oral administration of BioBran/MGN-3 on *Salmonella typhimmurium* LT2 infection mice

Salmonella typhimmurium	Mortality	
	MGN-3	Placebo
1.0×10^{1} CFU/mouse	0	0
1.0×10^{2} CFU/mouse	0	0
1.0×10^{3} CFU/mouse	8	12
1.0×10^{4} CFU/mouse	12	12
1.0×10^{5} CFU/mouse	12	12
1.0×10^{6} CFU/mouse	12	12
LD50	7.0×10^{2} CFU/mouse	4.5×10^{2} CFU/mouse

Table 4 Effect of intraperitoneal administration of BioBran/MGN-3 on *Salmonella typhimmurium* LT2 infection mice

Salmonella typhimmurium	Mortality	
	MGN-3	Placebo
2.0×10^{2} CFU/mouse	0	0
2.0×10^{3} CFU/mouse	0	5
2.0×10^{4} CFU/mouse	12	12
2.0×10^{5} CFU/mouse	12	12
LD50	6.4×10^{3} CFU/mouse	3.0×10^{3} CFU/mouse

groups died. The death rate was 100% after inoculation with only 30 viable cells of this strain of bacteria. BioBran/MGN-3 did not prevent death from infection and there was no significant difference between the survival curves for the two groups.

Escherichia coli O157:H7 (EHEC-SMR)

Mice given BioBran/MGN-3 or a placebo (1 mg/mL) orally *ad libitum* for 5 days were inoculated with *Escherichia coli* O157:H7 (EHEC-SMR) at 1.0×10^{5} and the number of bacterial cells present in organs was measured 24 and 48 hours after inoculation. It is known that the mice were not affected in this experimental system (1). The number of bacterial cells was measured to observe the clearance of the bacteria. **Table 2** shows no difference between the number of bacterial cells for the BioBran/MGN-3 and placebo groups.

Salmonella typhimurium LT2

Mice given BioBran/MGN-3 or a placebo (1 mg/mL) orally *ad libitum* for 5 days were intraperitoneally administered with the attenuated strain *Salmonella typhimurium* LT2 in amounts of between 1.0×10 and 1.0×10^{6} CFU/ mouse. As shown in **Table 3**, all mice in the placebo group inoculated with 1.0×10^{3} CFU/mouse died and the LD_{50} was 4.5×10^{2} CFU/mouse. In the BioBran/MGN-3 group, all mice died after inoculation with 1.0×10^{4} CFU/mouse, but 33% survived 1.0×10^{3} CFU/mouse. The LD_{50} was 7.0×10^{2} CFU/mouse.

The same dose-dependent effect was observed in mice given an increased dose of BioBran/MGN-3 and

then inoculated with 2.0×10^3 CFU/mouse of *Salmonella typhimurium* LT2 (**Table 4**).

Discussion

The preventive effect of BioBran/MGN-3, a derivative of rice bran arabinoxylan developed by Daiwa Pharmaceutical Co., Ltd. against infections, was studied using virulent and attenuated strains of *Salmonella* and enterohaemorrhagic *Escherichia coli* O157. The results showed that BioBran/MGN-3 acted as a biological defense against *Salmonella* injection. *Salmonella* is an intracellular bacterium, suggesting that BioBran/MGN-3 may have the same effect on intracellular *Mycobacterium* tuberculosis and *Listeria*.