Homeostasis as Regulated by Activated Macrophage. III. Protective Effect of LPSw (Lipopolysaccharide (LPS) of Wheat Flour) on Gastric Ulcer in Mice as Compared with Those of Other LPS from Various Sources

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Protective effect of lipopolysaccharide (LPS) from various sources on gastric ulcer has been examined in mice using parenteral as well as oral route. Ulcer is induced by indomethacin, stress or alcohol. LPS was prepared from 6 species of bacteria (*Escherichia coli*, *Pantoea agglomerans*, *Serratia ficaria*, *Enterobacter cloacae*, *Bordetella pertussis*, *Alcaligenes faecalis*) and from wheat flour. When administered intravenously, LPS of *Pantoea agglomerans* was the most effective among other LPS examined. Lipopolysaccharide of wheat flour (LPSw) showed a significantly protective effect by the oral route, especially when given *ad libitum* in drinking water to mice.

Keywords gastric ulcer; lipopolysaccharide; macrophage activation; homeostasis; *Pantoea agglomerans*

Introduction

We earlier proposed that tumor necrosis factor (TNF)-driven inflammation in embryogenesis, which we called "ontogenic inflammation"[1] could regulate the homeostasis of our body, so long as it is appropriately reproduced in adults. In previous reports of this series[2] we described that lipopolysaccharide (LPS) prepared from wheat flour (LPSw) can also reproduce ontogenic inflammation. Many kinds of LPS have been known most of them from gram-negative bacteria. In this report we demonstrate the protective effect of LPSw on gastric ulcer in mice and compare the effect with various other LPS.

Ulcer is provoked in mice by indomethacin, stress or ethanol by some devices on the conventional rat system.[3] Anti-ulcer effect of each LPS was examined using parenteral and oral route. The results show that all LPS examined have some degree of anti-ulcer effect when given intravenously. LPSw demonstrates its strong effect when uptaken orally *ad libitum* in drinking water.

Materials and Methods

Mice Male C3H/He and BALB/c mice 7 to 12 weeks of age were obtained from Shizuoka Experimental Farm (Shizuoka, Japan). All mice were given a standard laboratory diet and water *ad libitum*. The mice were deprived of food but not water for 24 h prior to the experiment.

LPS LPS from *Escherichia coli* (*E. coli*) 0127:B8 was purchased from Difco Lab. (Detroit, U.S.A.). LPS from *Pantoea agglomerans* (*P. aggl.*), *Serratia ficaria* (*S. fic.*), *Enterobacter cloacae* (*E. cloac.*), *Alcaligenes faecalis* (*A. faec.*), *Agrobacterium radiobacter* (*A. rad.*) and *Bordetella pertussis* (*B. pert.*) were purified by the conventional method of Westphal *et al.*[4] These LPSs were purified nearly homogeneity to more than 95% by conventional methods.[5] The method of LPS preparation from wheat flour was described previously.[2] Briefly, low molecular fractions (<5 kilodaltons (kDa)) were excluded from the water extracts of wheat flour by ultra filtration. This preparation, called LPSw-H, contained 0.01—0.1% of LPSw. LPSw was purified from LPSw-H by TCA-extraction, gel-filtration, enzyme digestion and ion-exchange chromatography.

Gastric Ulcer Protection Experimental gastric ulcers in mice were induced by indomethacin (IM), water-stress or ethanol according to the methods of the rat model.[3]

IM-Induced Gastric Ulcer: IM was administered subcutaneously at a dose of 60 mg/kg and mice were killed 6 h later for assessment of ulcer damage. One hour prior to administration of IM, the mice were given LPS samples *via* an intravenous route. After the resection the stomach was filled with 1 ml of 5% formalin solution, fixed overnight, and then opened and turned inside out. Hemorrhagic scars were photographed and the images were enlarged 1.7 times. The hemorrhagic damage was measured to give an "ulcer index" which is the sum of the lesion length (mm). The ratio of ulcer index of a test sample to that of control (A) gave the "inhibition percentage" $(1-A)\times 100\%$.

Water-Stress Induced Ulcer: Mice were fixed in a tube (i.d. 27 mm) and immersed in water. 1) One hour prior to stress, the mice were administered LPSw-H *per os* by sonde, or 2) 4 to 6 d prior to stress, the mice were given LPSw-H solution *ad libitum* in drinking water. After 7 h of stressing, the mice were killed. Gastric ulcer index was scored by the same method used for the IM-induced ulcer above.

Ethanol-Induced Ulcer: Gastric damage was induced by oral administration of 200 μl of 50% ethanol. The mice were pretreated with LPS (i.v. or *p.o.*) or the vehicle (saline or distilled water) 1 h prior to ethanol administration. One hour after administration, the mice were killed. Gastric ulcer index was scored by the same method used for the IM-induced ulcer above. Cimetidine was used as a positive control for ulcer protection in some cases.

Acute Toxicity of LPS Inbred male BALB/c mice were used. Various doses of each LPS were administered intravenously. After 48 h, dead and surviving mice were counted, and LD_{50} values were calculated by the method of Behren Kärber.

SDS-PAGE of LPS Polyacrylamide gel electrophoresis (PAGE) in sodium dodecyl sulfate (SDS) was carried out in 1.0 mm-thich 20% acrylamide concentration slab gel by the method of Schägger and Jagow.[6] Details were described in the preceding paper.[2]

Reagents IM, ethanol and formalin were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). Cimetidine were obtained from Fujisawa Pharm. Ind. (Osaka, Japan).

Statistical Analysis Statistical evaluation of differences between the groups was made by Student's t-test.

Results

Protective Effect of Various LPS on Gastric Ulcer in Mice by Intravenous Route The anti-ulcer index was examined using IM (Fig. 1). One μg per mouse of LPSw could prevent the incidence of ulcer significantly ($p<0.01$) when given intravenously. Other LPSs showed a similar effect (Table I), in the order: LPSw, LPS of *B. pert.*, *P. aggl.*, *S. fic.* which were better than those of *E. coli* and *E. cloac.*

Figure 2 shows the test to determine the most appropriate timing of LPSw administration. It is effective when given 1 h prior to the administration of IM, whereas there is no effect if it follows the IM-administration. Crude sample of LPSw (LPSw-H) which will be used for the oral route is also effective for the intravenous route (Table II).

Protective Effect of Various LPS on Gastric Ulcer in Mice by Oral Route Because of the site of the disease, gastric ulcer might be favorably protected by the oral route. Efficacy of LPSw and 4 kinds of LPSs from various sources

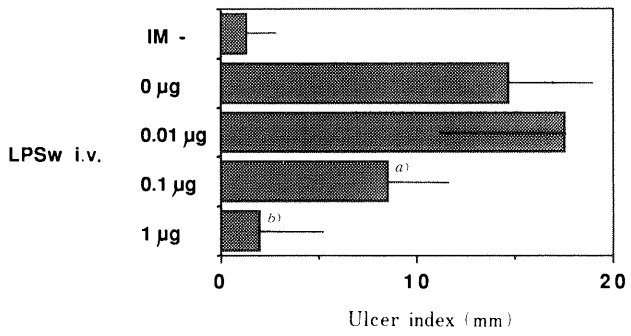

Fig. 1. Dose Response of LPSw for Protective Effect of IM-Induced Ulcer *via* Intravenous Administration

LPSw (0.01—1 μg/mouse) was injected i.v. 1 h before IM administration. Column shows the average ulcer index of five mice and bar their standard deviation. Significant differences (*a*) $p<0.05$, *b*) $p<0.01$) from the value for the control not tested with LPSw by Student's *t*-test.

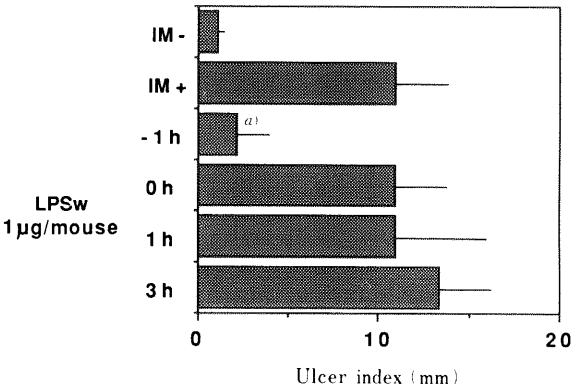

Fig. 2. Effect of the Timing of LPSw Injection before or after IM Administration

LPSw (1 μg/mouse) was administered i.v. 1 h before, at the same time or 3 h after indomethacin injection. Column shows the average ulcer index of five mice and bars their standard deviation. Significant differences (*a*) $p<0.01$) from the value for the control not tested with LPSw by Student's *t*-test.

TABLE I. Anti-ulcer Effect of Various LPS *via* Intravenous Route against IM-Ulcer

IM	Percent inhibition of gastric ulcer[a]					
	E. cloac.	*E. coli*	*B. pert.*	*S. fic.*	LPSw	*P. aggl.*
0	36[b]	44[c]	76[d]	84[d]	91[e]	100[e]

a) One μg/mouse of LPS was administered 1 h prior to IM injection. Ulcer index is compared for inhibition with control. This result is the average of two independent experiments. Three to five mice were used in one group. *b*) Significant difference is $p<0.2$ by Student's *t*-test. *c*) $p<0.05$. *d*) $p<0.01$. *e*) $p<0.001$.

administered orally was compared. LPSw was tested using its crude sample LPSw-H. Table III shows a summary of results expressed as percentage of incidence from 6 independent experiments using LPSw-H. Gastric ulcers employed were IM-, water-stress- and ethanol-induced. About 50% inhibition of the incidence was observed in 6 cases. Cimetidine, known as H2-brocker[7] and used as the control showed a similar effect. Figure 3 shows the test to determine the most appropriate timing of LPSw-H administration. It is effective when given 1 h prior to the IM administration. Table IV shows the anti-ulcer activity demonstrated by 5 kinds of LPSs (*B. pert.*, *P. aggl.*, *A. faec.*, LPSw, *E. coli*) *via* an oral route. Molecular size of LPS as observed in SDS–PAGE is proportional to the inhibition.

TABLE II. Protective Effect of Intravenously Injected LPSw and LPSw-H on IM- or Stress-Induced Ulcer

	Percent inhibition of gastric ulcer[a]			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Ulcer inducer	IM	IM	IM	Stress
Distilled water	0	0	0	0
LPSw-H[b]	55[c]	60[c]	53[c]	42
LPSw[b]	<100[c]	85[c]	ND	62[c]

a) Five to eight mice were used in one group. *b*) One μg of LPSw or 4 mg of LPSw-H (LPSw, 1 μg) was administered i.v. 1 h prior to ulcer induction. *c*) Significant differences ($p<0.05$) from the value for the control by Student's *t*-test. ND, not done.

TABLE III. Anti-ulcer Effect of *p.o.* Administration of LPSw-H by Sonde

	Percent inhibition of gastric ulcer[a]					
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6
Ulcer inducer	IM	IM	IM	Stress	EtOH	EtOH
Distilled water	0	0	0	0	0	0
LPSw-H[b]	55[c]	60[c]	ND	45	65[c]	53[c]
Cimetidine[b]	ND	ND	59[c]	61[c]	35	ND

a) Five to eight mice were used in one group. *b*) LPSw-H containing 50 μg of LPSw or 4 mg of cimetidine was administered *p.o.* 1 h prior to ulcer induction. *c*) Significant differences ($p<0.05$) from the value for the control by Student's *t*-test. ND, not done.

TABLE IV. Anti-ulcer Effect *via per os* Route and SDS–PAGE Pattern of Various LPSs

LPS[a]	Inhibition %[b]	SDS–PAGE pattern (kDa)	
		Main band	Minor band
DW	0	—	
A. faec.	0	20	4
E. coli	15	5—50[e]	
P. aggl.	27	5	30—60
LPSw-H[c]	50[d]	4—6	
B. pert.	60[d]	3.5—5	

a) LPS (200 μg/mouse) was orally administered by sonde. *b*) Results are the average of 4 independent experiments. *c*) LPSw-H (50 mg/mouse) containing 0.1% of LPSw was administered by sonde. *d*) Significant differences ($p<0.05$) from the values for the DW control by Student's *t*-test. *e*) The gel pattern of *E. coli* LPS was multi-ladder in 5—50 kDa.

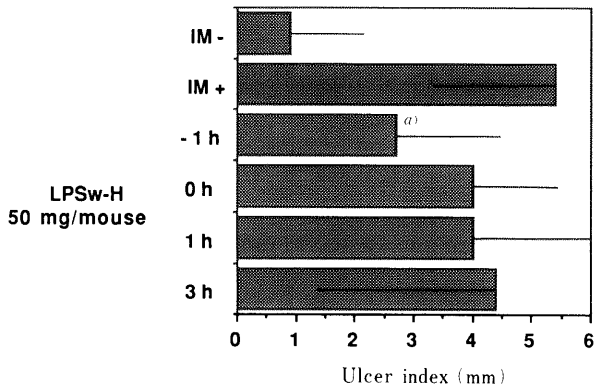

Fig. 3. Timing of LPSw-H Administration *per os* before or after IM-Injection

A solution of LPSw-H containing 50 μg of LPSw was administered *per os* by sonde 1 h prior, at the same time, and 1 and 3 h after IM injection. Column shows the average ulcer index of five to seven mice and bars their standard deviation. Significant differences (*a*) $p<0.05$) from the control not tested with LPSw-H by Student's *t*-test.

Table V. LD_{50} of Purified LPS from 4 Sources

LPS	LD_{50} mg/kg	Anti-ulcer effect[a]	
		i.v.[b]	*p.o.*[c]
Vehicle		−	−
E. coli	2.9	+	±
P. aggl.	2.2	+++	±
B. pert.	9.4	+++	++
LPSw (LPSw-H)	2.9	+++	+

a) Anti-ulcer activity was calculated as the result of percent ulcer-inhibition in Tables I and IV and expressed as follows, −, <10%; ±, 10—25%; +, 26—50%; ++, 51—75%; +++, >76%. *b*) One μg of LPS per mouse was administered intravenously. *c*) Bacterial LPS (200 μg/mouse) or LPSw-H (50 mg/mouse) was administered orally.

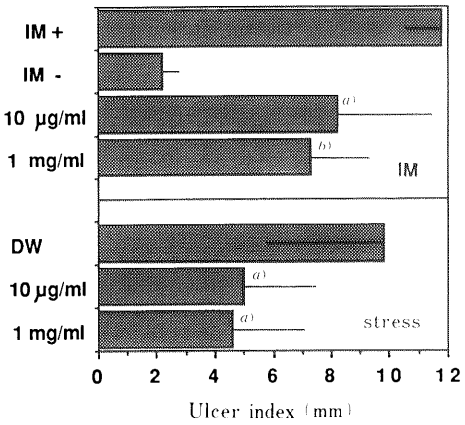

Fig. 4. Protective Effect of LPSw-H with *ad Libitum* Administration in Drinking Water on IM- or Water-Stress Induced Ulcer

One mg/ml, 10 μg/ml of LPSw-H containing 0.1% of LPSw in drinking water was supplied for 4—5 d. Column shows the average ulcer index of 6—8 mice and bars their standard deviation. Significant differences (*a*) $p<0.05$, *b*) $p<0.01$) from the control not tested with LPSw-H by Student's *t*-test.

LD_{50} of Various LPS as Compared with Anti-ulcer Activity LD_{50} of 4 LPS was examined. Table V shows summarized data as compared with their anti-ulcer activities. LD_{50} of LPSw is comparable with LPS of *E. coli* and *P. aggl.* In the case of oral administration, LPSw-H may be the best material because wheat flour is not harmful.

Protective Effect of LPSw-H on Gastric Ulcer in Mice Uptaken *ad Libitum* LPSw-H was tested for its protective effect on gastric ulcer of mouse, when uptaken *ad libitum*. Figure 4 shows the results and its significant effect for protection against both IM-induced and stress-induced ulcer incidence.

Discussion

LPSw, a new lipopolysaccharide derived from wheat flour[2] has been shown to be protective against incidence of ulcer even when used orally, irrespective of whether the ulcer inducer is IM, water-stress or ethanol (Tables II, III).

LPSw-H showed remarkably anti-ulcer activity equal to cimetidine (Table III), which is one of most effective of the commercially available anti-ulcer drugs.[7] These facts suggest that LPSw-H is applicable for clinical usage in the treatment of ulcers.

As shown in Table I, the most effective anti-ulcer LPS *via* intravenous administration is that of *Pantoea agglomerans* which is known to be a concomitant gram-negative bacteria in wheat, cotton and other plants.[8] We found that *P. aggl.* LPS was also most effective in inducing the priming state for endogenous TNF production (data not shown). Therefore, *P. aggl.* LPS may be the most hopeful LPS for use parenterally.

The most effective LPS by *per os* route is *B. pert.* LPS as shown in Table IV. On the other hand, LPSw-H, which is an extract of wheat flour, can be taken every day because wheat flour is not harmful. This makes this wheat extract a better and safer material for ulcer prevention than *B. pert.* LPS.

Various LPSs derived from different species of gram-negative bacteria were compared for their protective effect on incidence of gastric ulcer induced by stress, IM, water-stress and ethanol. An assay method was newly developed by modifying the original assay system using rat.[3] Our system can simultaneously compare 6 different LPSs for their protective effect (Table I).

The precise mechanism of the effect of LPSw remains unclear. However, macrophage activated to the primed stage for endogenous production of TNF may be the effector cell. LPS, in general, can activate macrophage up to this stage even at low doses as shown in this work (1 μg/mouse).[2] We have shown also that the macrophage primed for endogenous production of TNF produces precursor TNF (26 kDa) which may be fixed around the surface of the activated macrophage.[9] Prostaglandin E_2, which is produced as a result of activation of macrophages, may act as one of effector molecule to protect the onset of gastric ulcer.[10]

The present report has shown that two LPSs are worthy of selection for further testing of their therapeutic effect on intractable diseases. One is LPSw used orally, and the other is LPS from *Pantoea agglomerans* used percutaneously or by other parenteral means.

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