Homeostasis as Regulated by Activated Macrophage. IV. Analgesic Effect of LPSw, a Lipopolysaccharide of Wheat Flour

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The effect of LPSw, a lipopolysaccharide from a water extract of wheat flour, on pain response was investigated using an acetic acid-induced writhing test in mice. LPSw inhibited writhing dose-dependently in the range of 10 ng—10 μg/mouse i.v. This effect reached its maximum 1.5—3 h after the LPSw inoculation and was detectable even after 8 h. The analgesic effect of LPSw was inhibited by i.v. injection of naloxone and also β-endorphin was detected in serum and brain tissue following injection of LPSw.

Preliminary clinical trials were done in which LPSw was administered percutaneously to relieve the pain of patients with herpes. The results showed that pain was relieved by this application. LPSw may be the best analgesic drug so far known, since it induces the endogenous mediator of analgesia, β-endorphin.

Keywords lipopolysaccharide; analgesic effect; β-endorphin; clinical trial; herpes

Introduction

Pain is considered to be an important warning signal for the human body to protect it from further damage. However, chronic pain appears to be one of the most serious clinical problems. Narcotic analgesic drugs have long been used for severe pain, however, these therapeutics are usually accompanied by some serious side effects such as narcotic addiction, or inhibition of breathing. Development of a new analgesic drug which is free from such side effects has therefore been urgently desired.

Endogenous analgesic substances such as opioid peptide and Kyotorphin were identified.[1-4] Recently, mediators of inflammation like tumor necrosis factor (TNF), interleukin 1 (IL-1) and interferon (IFN) have been reported to have analgesic effects.[5,6] Inducers of activated macrophages, *Escherichia coli* (*E. coli*) LPS and muramyldipeptide (MDP), have also been reported to have analgesic effect.[5,7] However, these substances cannot be used clinically due either to their severe side effects or to their inappropriate administration routes. Looking for a new drug which is clinically applicable, we recently discovered LPSw, a lipopolysaccharide which was purified from a water extract of wheat flour.[8]

The LPSw is a Limulus positive substance and activates macrophages to the primed stage for endogenous production of TNF. It differs from *E. coli* lipopolysaccharide (LPS) and LPS from other origins in several points, especially in its molecular weight and phosphorus content.[8] LPSw has lower molecular weight (5 kilodaltons (kDa)) than does *E. coli* LPS (30 kDa), and can be applied either orally or percutaneously.[8]

This paper deals with the analgesic effect of LPSw in mice, which was found to be inhibited by i.v. injection of naloxon. β-endorphin was detected when LPSw was i.v. injected. We also report preliminary accounts of percutaneous administration of LPSw to patients with herpes as an analgesic therapeutic.

Materials and Methods

Animal Eight week old male C3H/He mice were purchased from Shizuoka Experimental Animal Farm (Shizuoka, Japan).

Chemical Reagents Phenylbutazone and naloxone were purchased from Nacalai Tesque (Kyoto, Japan) and Sigma Chemical Co. (St. Louis, U.S.A)., respectively.

Purification of LPSw LPSw was purified from a water extract of wheat flour as described previously.[8] Purity of the sample was 91% as calculated by Limulus reaction.[8]

Analgesic Effect of LPSw Analgesic effect of LPSw was estimated in mice using a writhing syndrome method. Briefly, LPSw (1 ng—10 μg/mouse) or phenylbutazone (1 mg/mouse) was injected into C3H/He mice (6 mice/group) intravenously. Zero to eighteen hours after the administration, 0.5 ml of a 1% acetic acid solution was injected into the mice intraperitoneally and the number of stretching movements was counted for 30 min. The analgesic effect of LPSw was expressed as % inhibition as compared with that in the saline injected control group.

β-Endorphin Assay LPSw (10 μg/mouse) was injected into C3H/He mice intravenously. Zero to eight hours after the administration, each mouse was bled to obtain serum and brain tissue. The brain tissue was homogenized for 10 s in a PBS containing 10% fetal calf serum (FCS), by a polytron homogenizer (Kinematika, Switzerland). The homogenates were centrifuged at 7000 *g* for 10 min to obtain a supernatant. β-Endorphin in the brain tissue homogenate and serum was assayed by a β-endorphin radioimmunoassay (RIA) kit (Du Pont, Boston, U.S.A.).

Analgesic Effect of LPSw on Patient with Herpes Partially purified LPSw condensed by ultra-filtration (10^5 cut off) was dissolved in 50% (w/v) glycerol. LPSw content as measured by Limulus reaction was 1 μg/ml in the liniment. It was administered percutaneously on the herpes lesion every day, and the analgesic effect was evaluated in terms of disappearance of pain.

Statistical Analysis Statistical analysis was done by a Student *t*-test.

Results

Inhibitory Effect of LPSw on Acetic Acid Induced Writhing

The effect of LPSw on acetic acid induced writhing syndrome was first examined. Figure 1 shows the time course of this analgesic effect in mice. One μg of LPSw was intravenously injected into C3H/He mice. At 0, 0.5, 1.5, 3, 8 and 18 h after the administration, 0.5 ml of a 1% acetic acid solution was injected intraperitoneally and the number of stretching movements was counted for 30 min. Saline-injected mice were used as the control. Analgesic effect was expressed as per cent inhibition compared with that in the saline group. LPSw showed a marked inhibition on stretching movement. This effect reached its maximum at 1.5—3 h after the LPSw inoculation and then gradually decreased, but even after 8 h it was still detectable in an appreciative amount. The effect of LPSw was found to last longer than that of phenylbutazone, a positive control.

Dose dependency of LPSw on the analgesic effect is shown in Fig. 2. Ten ng of LPSw showed significant inhibi-

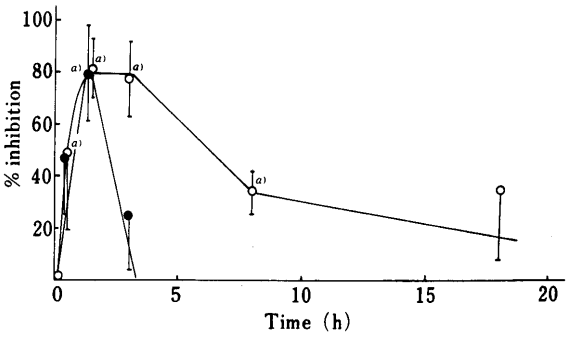

Fig. 1. Time Course of Analgesic Effect of LPSw

Analgesic effect was evaluated by an acetic acid induced writhing syndrome test. 1 μg of LPSw (○) or 1 mg of phenylbutazone (●) was injected into C3H/He mice intravenously. 0—18 h after the administration, 0.5 ml of a 1% acetic acid solution was injected intraperitoneally and the number of stretching movements was counted for 30 min. Analgesic effect was expressed by % inhibition as compared with that in the saline injected group as control. *a*) $p<0.01$.

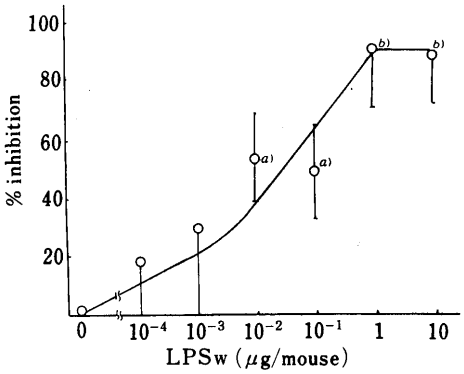

Fig. 2. Dose Dependency of Effect of LPSw on Its Analgesic Effect

0—10 μg of LPSw was injected into C3H/He mice intravenously. 3 h thereafter, 0.5 ml of a 1% acetic acid solution was injected intraperitoneally. For other details, see legend of Fig. 1. *a*) $p<0.01$, *b*) $p<0.001$.

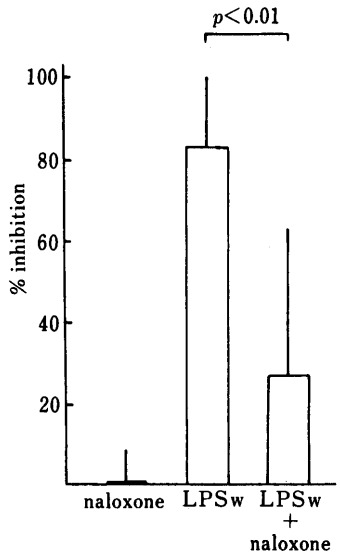

Fig. 3. Effect of Naloxone on Analgesic Effect of LPSw

100 μg of naloxone hydrochloride was intravenously injected to mice simultaneously with 1 μg of LPSw. 1.5 h after, 0.5 ml of a 1% acetic acid solution was injected intraperitoneally. For other details, see legend of Fig. 1.

tion on stretching movement and the inhibition reached its maximum at 1 μg per mouse.

Influence of Endogenous Opioid on Analgesic Effect of LPSw Examination of the effect of naloxone, an antagonist of opioid, was done by injection. 100 μg of naloxone hydrochloride and 1 μg of LPSw were injected intravenously into mice simultaneously. As shown in Fig. 3, about 67% of the analgesic effect of LPSw was inhibited by naloxone hydrochloride.

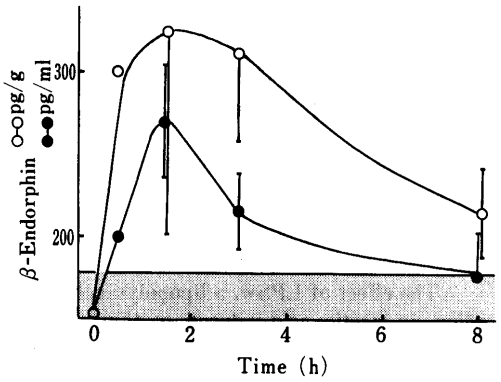

Fig. 4. Time Course of Production of β-Endorphin

10 μg of LPSw was injected into C3H/He mice intravenously. 0—8 h after, mice were bled to obtain serum and brain tissue. The brain tissue was homogenized and then centrifuged to obtain the supernatant. β-Endorphin in both brain homogenate (○) and serum (●) were assayed by RIA. Shadow area shows β-endorphin level of normal mice in the brain and serum.

TABLE I. Analgesic Effect of LPSw on Patients with Herpes

	Patient	Previous therapy[a]	Antigen[c]	Treatment	Disappearance of pain (d)
Acute herpes simplex of vulva	M.U.	ACV[b]	I, II	LPSw	3
	Y.T.	—	II	LPSw	3
	N.M.	—	II	LPSw	3
	H.I.	—	I	LPSw	3
	K.M.	—	?	LPSw	3
	S.N.	—	?	LPSw	2
Recurrent herpes of vulva	T.T.	ACV	II	LPSw	3

	Patient	Previous therapy[a]	Original disease	Treatment	Disappearance of pain (d)
Herpes zoster	A.S.	ACV[b]	—	LPSw	5
	T.H.	ACV	Cervical cancer	LPSw	4

Partially purified LPSw in a liniment (LPSw 1 μg/ml) was administered percutaneously to the herpes lesion. Analgesic effect was evaluated in term of disappearance of pain. *a*) Patient was pretreated with ACV for several days. *b*) ACV = acyclovir. *c*) I, herpes type I; II, herpes type II.

Detection of β-Endorphin Following Injection of LPSw We then examined the production of β-endorphin, an endogenous opioid, by LPSw; the time course of this production after intravenous injection of LPSw is shown in Fig. 4. The level in serum and brain tissue reached its maximum 0.5—3 h after the injection and then gradually decreased, consistent with the time course of the analgesic effect of LPSw.

Clinical Trial of LPSw on Herpes Patients Table I shows the analgesic effect of LPSw. As previously reported,[8] the substance can be applied percutaneously. Preliminarily, LPSw in a liniment (LPSw 1 μg/ml) was given to patients with herpes on their lesions and pain was disappeared 2—5 d after this administration on acute herpes, recurrent herpes and herpes zoster (Table I).

Discussion

An analgesic effect of LPSw was definitely observed, and the minimum effective dose was found to be small (10 ng/mouse) (Figs. 1, 2). This effect was obtained by the intravenous route, but we reported earlier that LPSw is also effective when applied percutaneously[8] and, as will be discussed later, efficacy has been proven by this route even in pain of patients with herpes.

The analgesic effect of LPSw was found to last longer than that of phenylbutazone, a positive control (Fig. 1). We reported that LPSw could induce both priming and triggering state depending on the dose, leading to the endogenous production of TNF through activation of macrophages. The priming state is that in which TNF precursor productions occur, while the triggering state is that in which mature TNF productions occur.[9,10] These features of time course and dose dependency of LPSw on analgesic effect resembled those in the priming state induced by LPSw,[8] suggesting that induction of the priming state by LPSw, that is, mild activation of macrophages where precursor TNF is produced may be essential for exertion of the analgesic effect of LPSw. This could well explain the fact that muramyl dipeptide (MDP), which has a priming effect but not a triggering effect, inhibits the writhing syndrome induced by acetic acid.[7,11]

Our results that the analgesic effect of LPSw was inhibited by naloxone and that β-endorphin was induced by LPSw injection suggest that the analgesic effect may be mediated by an opioid substance, particularly β-endorphin. Furthermore, from the fact that the time course of production of β-endorphin is correlated with that of the analgesic effect of LPSw, we are able to propose that the mechanism for this analgesic effect may be ascribable to the simultaneous production of β-endorphin with that of precursor TNF through macrophage activation by LPSw.

TNF, IL-1, IFN and other cytokines are also reported to have analgesic effect,[5] and we reported earlier that these could activate macrophage to produce precursor TNF endogenously.[9,12,13] They may therefore eventually exert an analgesic effect which is regulated by simultaneous production of endogenous opioid such as β-endorphin. Based on this concept, we can assume that LPSw may be more effective as a natural drug than exogenous opioids or conventional analgesic drugs, because it can last to induce an endogenous mediator of analgesia, β-endorphin.

A preliminary clinical trial was done to relieve pain in patients with herpes by administering LPSw percutaneously to the lesion. As shown in Table I the analgesic effect was marked even in patients with pain due to long-lasting sequela. We found that LPSw could be absorbed through mucous membrane and induced activation of macrophage. Therefore, it is suggested that percutaneously administered LPSw may be absorbed through the lesion of herpes resulting in exertion of analgesic effect.

IFN-α and MDP, both of which are priming agents for TNF production,[11,14] have been reported to effect morphine addiction.[15,16] This suggests that LPSw may also affect morphine addiction because it also induces the priming state for TNF production. LPS, in general, has similar activity regardless of its origin.[8] However, LPSw is even more useful since its small molecular size allows its percutaneous administration.

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