

Suppression of Hyperalgesia in Streptozotocin-Induced Diabetic Mice by a Lipopolysaccharide from *Pantoea agglomerans*

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The ability of a lipopolysaccharide from *Pantoea agglomerans* (LPSp) to relieve hyperalgesia was examined by observing its inhibition of the decrease in the threshold for nociceptive perception, as determined by the tail-pinch test, in streptozotocin-induced diabetic mice. Subcutaneous injection of LPSp suppressed hyperalgesia in streptozotocin-induced diabetic mice and also exerted a therapeutic effect on hyperalgesia in these animals. The present data suggest that LPSp may be effective in relieving the pain associated with diabetic neuropathy.

Keywords diabetes; hyperalgesia; painful diabetic neuropathy; lipopolysaccharide; macrophage

The pain associated with diabetic neuropathy is a serious clinical problem. In this regard, we previously reported that the threshold for pain perception in response to noxious mechanical stimuli was reduced in diabetic animals.^{1,2} However, we did not observe any significant changes in the nociceptive threshold when noxious thermal stimuli were applied.² We also demonstrated that a lipopolysaccharide from *Pantoea agglomerans* (LPSp) produced antinociception by induction of β -endorphin through macrophage activation.³ LPSp has been administered percutaneously in clinical trials for the relief of pain in herpetic neuralgia and an analgesic effect was observed even in patients who had been suffering pain for a long period.³ We recently reported that LPSp produces a marked antinociceptive effect in diabetic mice when the nociceptive threshold was determined by applying noxious thermal stimuli, such as in the tail-flick method.⁴ Thus, it is possible that LPSp may produce an antinociceptive effect in diabetic mice when the nociceptive threshold is determined by application of noxious mechanical stimuli. Furthermore, we demonstrated that LPSp suppresses the incidence of type 1 diabetes in non-obese diabetic mice.⁵ It is also possible that LPSp may suppresses the occurrence of hyperalgesia in diabetic mice. However, experimental support for this has yet to be provided.

Therefore, the present study had two basic purposes: 1) to investigate the therapeutic effects of LPSp on the hyperalgesia in diabetic animals, and 2) to investigate the effect of LPSp on the occurrence of hyperalgesia in diabetic animals. Thus, in the present study, we examined the effect of LPSp on the threshold of nociceptive perception, as determined by the tail-pinch test, in both diabetic and non-diabetic mice.

MATERIALS AND METHODS

Animals Male ICR mice (Tokyo Laboratory Animals Science Co., Ltd., Tokyo, Japan), weighing about 20 g at the beginning of the experiments, were used. They had free access to food and water in an animal room that was maintained at $22 \pm 1^\circ\text{C}$ with a 12-h light-dark cycle. Animals were rendered diabetic by an injection of

streptozotocin (200 mg/kg, i.v.) prepared in 0.1 N citrate buffer at pH 4.5. Age-matched non-diabetic mice were injected with vehicle alone. Mice with serum glucose levels above 400 mg/dl were considered diabetic.

Assessment of the Threshold of Nociceptive Perception The threshold of nociceptive perception was determined by recording the latency in the tail-pinch test according to the modified Haffner method.⁶

Experimental Schedules The present studies were conducted over a 3-week period. LPSp (0.1 mg/kg) was injected s.c. once a week. The threshold of nociceptive perception was determined immediately prior to the administration of LPSp and then once every 7 d. To examine the suppressive effect on the occurrence of hyperalgesia, treatment with LPSp was started on the same day as the administration of streptozotocin. Furthermore, to examine the therapeutic effect of LPSp on hyperalgesia, treatment with LPSp was started 2 weeks after administration of streptozotocin.

Drugs The drugs used in this study were streptozotocin (Sigma Chemical Co., St. Louis, MO, U.S.A.) and a LPSp. LPSp was purified by the Westphal method in our laboratory (purity >95%).⁷

Statistical Analysis Data are expressed as means \pm S.E. Duncan's multiple range test was used for statistical analyses.

RESULTS

Effect of LPSp on the Tail-Pinch Latencies in Both Diabetic and Non-diabetic Mice Subcutaneous injection of LPSp (0.1 mg/kg) had no significant effect on the tail-pinch latencies in both diabetic (pre-drug latency, 0.9 ± 0.2 s, $n=6$; post-drug latency, 1.1 ± 0.2 s, $n=6$) and non-diabetic mice (pre-drug latency, 2.6 ± 0.5 s, $n=6$; post-drug latency, 2.1 ± 0.4 s, $n=6$), as determined 90 min after LPSp treatment.

Suppressive Effect of LPSp on the Occurrence of Hyperalgesia The mean tail-pinch latency in naive mice was 2.1 ± 0.2 s ($n=32$). As shown in Fig. 1, the tail-pinch latency decreased according to the duration of the treatment with streptozotocin. Indeed, the tail-pinch

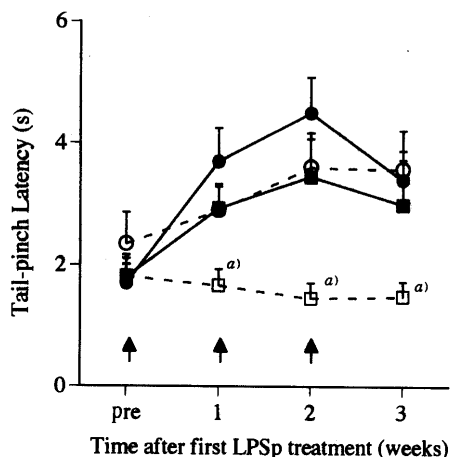


Fig. 1. Suppressive Effect of LPSp on the Occurrence of Hyperalgesia in Streptozotocin-Induced Diabetic Mice

Eight to ten mice were used in each group. The threshold of nociceptive perception, as determined by recording the latency in the tail-pinch test, was used as an index of hyperalgesia. LPSp (0.1 mg/kg) was injected s.c. once a week. The threshold of nociceptive perception was determined immediately prior to the administration of LPSp and then once every 7 d after LPSp administration. Treatment with LPSp was started on the same day as treatment with streptozotocin. LPSp was injected at the time indicated by the arrows. (○), saline-treated non-diabetic mice (vehicle + saline); (●), LPSp-treated non-diabetic mice (vehicle + LPSp); (□), saline-treated diabetic mice (streptozotocin + saline); (■), LPSp-treated diabetic mice (streptozotocin + LPSp). *a)* significantly different ($p < 0.05$) from saline-treated non-diabetic mice.

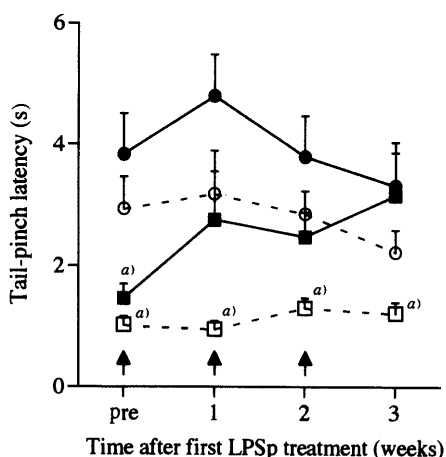


Fig. 2. Therapeutic Effect of LPSp on Hyperalgesia in Streptozotocin-Induced Diabetic Mice

Eight to ten mice were used in each group. The threshold of nociceptive perception, as determined by recording the latency in the tail-pinch test, was used as an index of hyperalgesia. LPSp (0.1 mg/kg) was injected s.c. once a week. The threshold of nociceptive perception was determined immediately prior to the administration of LPSp and then once every 7 d after LPSp administration. Treatment with LPSp was started 2 weeks after treatment with streptozotocin. LPSp was injected at the time indicated by the arrows. (○), saline-treated non-diabetic mice (vehicle + saline); (●), LPSp-treated non-diabetic mice (vehicle + LPSp); (□), saline-treated diabetic mice (streptozotocin + saline); (■), LPSp-treated diabetic mice (streptozotocin + LPSp). *a)* significantly different ($p < 0.05$) from saline-treated non-diabetic mice.

latency in streptozotocin-treated mice was significantly reduced even when the latency was determined just 1 week after streptozotocin. When LPSp was administered once a week, the changes in the tail-pinch latency in streptozotocin-induced diabetic mice were similar to those in saline-treated non-diabetic mice (Fig. 1). However, repeated treatment with LPSp had no significant effect on the tail-pinch latency in non-diabetic mice.

Therapeutic Effect of LPSp on Hyperalgesia Two weeks after the induction of diabetes, these mice had lower

nociceptive threshold values than the non-diabetic controls, as shown by a significant ($p < 0.05$) decrease in tail-pinch latency. When LPSp was injected once a week, the tail-pinch latency in diabetic mice was gradually prolonged. There were no significant differences in tail-pinch latencies between saline-treated non-diabetic mice and LPSp-treated diabetic mice (Fig. 2). Furthermore, LPSp had no significant effect on the tail-pinch latencies in non-diabetic mice. Repeated treatment with saline had no significant effect on the tail-pinch latencies in either non-diabetic or diabetic mice.

DISCUSSION

The present results clearly indicate that subcutaneous LPSp suppresses the incidence of hyperalgesia in streptozotocin-induced diabetic mice, as shown by the suppression of the decrease the tail-pinch latency. LPSp also has a therapeutic effect on hyperalgesia. As previously reported, although the antinociceptive effects of LPSp in diabetic mice do not last for 24 h, LPSp at this dose produces a marked antinociceptive effect in diabetic mice, compared with non-diabetic controls, when the nociceptive threshold is determined by application of noxious thermal stimuli, such as in the tail-flick method.⁴⁾ The present study shows, however, that LPSp (0.1 mg/kg, s.c.) has no significant effect on the nociceptive threshold as determined by the application of noxious mechanical stimuli, when the threshold of nociceptive perception was measured 90 min after LPSp treatment. Thus, it seems likely that the suppressive and therapeutic effects of LPSp on hyperalgesia are not due to the antinociceptive effects of LPSp itself.

We previously reported that LPSp can activate macrophages to prime the endogenous production of tumor necrosis factor (TNF)^{8,9)} resulting in "ontogenic inflammation".¹⁰⁾ Production of ontogenic inflammation in adults, especially in patients with intractable diseases, may restore their homeostasis to normal.⁷⁾ We also previously demonstrated that significantly more binding sites for substance P were found in the spinal cord of diabetic rats than in non-diabetic rats.¹⁾ Furthermore, we have also reported that the rate of K^+ -evoked release of substance P from the spinal cord of diabetic rats was greater than that in non-diabetic rats.¹¹⁾ These results suggest to us that diabetes selectively enhances the neurotransmission that involves substance P in the spinal cord. It has been reported that application of noxious mechanical stimuli specifically increase the release of substance P from the dorsal horn of the spinal cord.¹²⁾ Furthermore, the threshold for nociceptive perception in response to such mechanical stimuli is selectively reduced in diabetic animals.^{1,2)}

From these results, we conclude that the abnormal neurotransmission with respect to substance P may be correlated with the hyperalgesia in diabetic animals.^{1,2,11)} Therefore, it is reasonable to speculate that "ontogenic inflammation", which regulates the homeostasis of ontogenesis by endogenous production of TNF by LPSp, may restore the homeostasis of neurotransmission that involves substance P. This possibility is one of the very

plausible explanations for the mechanism of action of LPSp in suppressing hyperalgesia in streptozotocin-induced diabetic mice. However, at present, the exact mechanism of the suppressive action of LPSp on hyperalgesia remains unclear. Further studies are needed to clarify this. We also suggest that the primed stage for endogenous production of macrophages is essential for the suppression of the incidence of type 1 diabetes in non-obese diabetic mice by LPSp.⁵⁾ In the present study, however, other diabetic symptoms exhibited by the streptozotocin-induced diabetic mice, such as high serum glucose and body weight loss, were not suppressed by LPSp (data not shown). Although the detailed mechanism is unclear, it is possible that the differences in the effects of LPSp on the other diabetic symptoms may be related to the origin of the diabetes (*i.e.*, genetic or experimental).

In conclusion, the present results suggest that LPSp may be useful for the treatment of the pain associated with diabetic neuropathy.

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