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Preventive effect of L-carnosine on changes in the thermal nociceptive threshold in streptozotocin-induced diabetic mice

Junzo Kamei [a,*], Masahiro Ohsawa [a], Shigeo Miyata [a], Shun-ichi Tanaka [b]

[a] Department of Pathophysiology and Therapeutics, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-Chome, Shinagawa-Ku, Tokyo 142-8501 Japan
[b] Kanazawa Medical Clinic, 6-3, Minatomirai 3-chome, Nishi-ku, Yokohama, Kanagawa 220-0012, Japan

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ABSTRACT

Sensory abnormality is one of the serious complications in diabetes. Since the effective therapeutic regimen to ameliorate the diabetic sensory abnormality is very few, the present study was then designed to investigate the effect of zinc L-carnosine on the changes of nociceptive threshold in diabetic mice. Zinc L-carnosine (75–300 mg/kg, p.o.) was administered once daily from 1 day after streptozotocin treatment. Diabetic mice showed shorter tail-flick latency at 1–4 weeks after streptozotocin treatment and longer tail-flick latency at 6–9 weeks after its treatment. The shortened tail-flick latency in early stage of diabetic mice was ameliorated by treatment with zinc L-carnosine. Moreover, zinc L-carnosine also slowed the onset of hypoalgesia in diabetic mice. Tail-flick latency in non-diabetic mice was not affected by the zinc L-carnosine treatment, indicating that zinc L-carnosine did not affect normal nociceptive transmission. Moreover, L-carnosine, but not zinc sulfate, ameliorated the abnormal sensory perception in diabetic mice. Interestingly, the ameliorative effect of zinc L-carnosine on the abnormal sensory perception in diabetic mice is much stronger than that of L-carnosine. These results provide the evidence of the ameliorative potential of zinc L-carnosine on the progressive diabetic neuropathy. Moreover, L-carnosine combined with zinc shows more potent amelioration of abnormal sensory perception in diabetic mice than by itself.

1. Introduction

Diabetic patients frequently exhibit devastating complications: almost half of diabetic patients have some degree of diabetic neuropathy, and the progression of diabetic neuropathy lowers their quality of life (Vinik et al., 2000). Clinical indications of peripheral diabetic neuropathy include increased vibration and decreased thermal perceptive thresholds followed by progressive sensory loss. This loss of sensory perception occurs in conjunction with the degeneration of all types of peripheral nerve fibers (Calcutt, 2002).

Several factors are known to underlie diabetic neuropathy. Multiple aetiologies, including increased aldose reductase activity (Nakamura et al., 1999; Yagihashi et al., 2001; Obrosova et al., 2002; Song et al., 2003), nonenzymatic glycation/glycoxidation (Dickinson et al., 2002; Thornalley, 2002), activation of protein kinase C (Nakamura et al., 1998; Cameron et al., 1999; Ohsawa and Kamei, 1999), reduction of nerve growth factor (Kakinoki et al., 2006), and oxidative stress (Vincent et al., 2004) have been investigated. Agents that affect these factors have been shown to ameliorate the symptoms of diabetic neuropathy in animal studies, but have failed to deliver convincing results in clinical trials. Therefore, new targets for treating the symptoms of diabetic neuropathy are needed.

Zinc L-carnosine, a chelate compound consisting of zinc ion and L-carnosine, is an anti-ulcer drug that is commonly used in the treatment of gastric ulcer in Japan (Ueki et al., 1989). The mechanisms of the anti-ulcer effect of zinc L-carnosine have been proposed to involve its membrane-stabilizing and antioxidative effects (Yoshikawa et al., 1991). In addition, dipeptide carnosine can suppress many of the biochemical changes (e.g., protein oxidation, glycation, AGE formation, and cross-linking) that accompany aging and associated pathologies, such as diabetes (see review, Hipkiss, 2006). The effects of L-carnosine on these biological changes led us to speculate that treatment with L-carnosine may ameliorate the abnormal sensory perception in patients with diabetes. The present study was undertaken to examine if zinc L-carnosine could ameliorate the abnormal nociceptive threshold in diabetic mice.

2. Materials and methods

This study was carried out in accordance with the Declaration of Helsinki and/or with the guide for the committee on the care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture.

2.1. Animal

Male ICR 4-week-old mice (Tokyo Animal Laboratories Inc., 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

* Corresponding author. Tel./fax: +81 3 5498 5030.
E-mail address: kamei@hoshi.ac.jp (J. Kamei).

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was maintained at 24±1 °C with a 12-h light–dark cycle (light on at 08:00, light off at 20:00). 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.

2.2. Antinociceptive assessment

The nociceptive response was evaluated by recording the latency to withdrawal of the tail in response to noxious skin heating. Briefly, the tails of mice were exposed to a focused beam of light from a 50 W projection bulb. The heat intensity was set by adjusting the source voltage of the bulb to 50 V (Ohsawa and Kamei, 1999). The heat intensities at 50 V produced surface skin heating rates of 0.9 °C/s. When a withdrawal response occurred, the stimulus was terminated and the response latency was measured electronically. In the absence of a response up to a predetermined maximum latency (30 s), the trial was terminated to prevent tissue damage. The tail-flick latency was measured before and once a week for 8 consecutive weeks after the injection of streptozotocin or vehicle. Mice were moved to the testing room at 08:30 to habituate to the experimental environment, and the experiment was begun at 13:00. Mice were given a thermal stimulus only once in each assessment of the nociceptive response and used only once at each drug administration. The experiments were blinded with respect to the drug treatment.

2.3. Drugs

L-Carnosine and zinc sulfate were purchased from Wako Pure Industries, Ltd. (Osaka, Japan). Zinc L-carnosine was freshly prepared by mixing zinc sulfate and L-carnosine (1:1 at molecular weight) in distilled water. Treatment with zinc L-carnosine, L-carnosine or zinc sulfate was started from 1 day after the injection of streptozotocin. All chemicals were resolved in distilled water and all treatments were continued once a day for 8 weeks after streptozotocin administration. All injections were completed 23 h before measurement of the thermal nociceptive threshold.

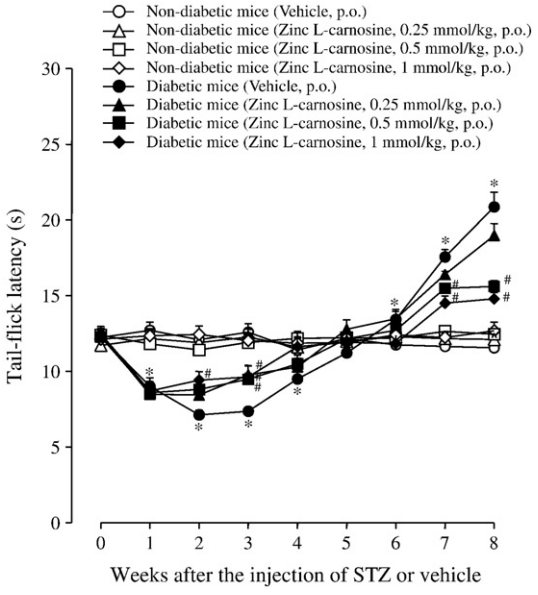

Fig. 1. Effect of zinc L-carnosine on the tail-flick latency in non-diabetic and diabetic mice. Daily treatment with zinc L-carnosine was started 1 day after the injection of streptozotocin or vehicle for 8 weeks. Each point represents the mean with S.E.M. of non-diabetic (n=10–14; open symbols) and diabetic mice (n=10–13; closed symbols). *P<0.05 vs. vehicle-treated non-diabetic mice (Bonferroni test). #P<0.05 vs. respective vehicle-treated group (Bonferroni test).

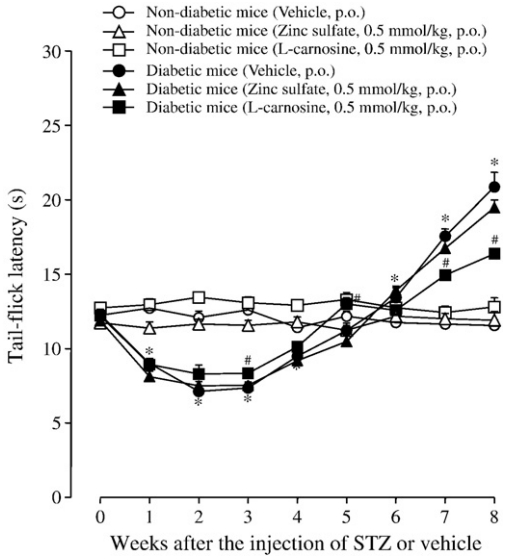

Fig. 2. Effects of L-carnosine and zinc sulfate on the tail-flick latency in non-diabetic and diabetic mice. Daily treatment with L-carnosine and zinc sulfate was started 1 day after the injection of streptozotocin or vehicle for 8 weeks. Each point represents the mean with S.E.M. of non-diabetic (n=10–14; open symbols) and diabetic mice (n=10–16; closed symbols). *P<0.05 vs. vehicle-treated non-diabetic mice (Bonferroni test). #P<0.05 vs. respective vehicle-treated group (Bonferroni test).

2.4. Statistical analysis

The data are expressed as mean±S.E.M. The statistical significance of factorial differences (Diabetes, Drug treatment, Duration after treatment with streptozotocin) was assessed by a three-way analysis of variance (ANOVA) with repeated measures. The statistical significance of differences between groups was assessed with ANOVA followed by the Bonferroni test. A level of probability of 0.05 or less was considered significant.

3. Results

Three-way ANOVA with repeated measures revealed that zinc L-carnosine (0.25–1 mmol/kg) significantly affected the tail-flick latencies in streptozotocin-treated and vehicle-treated mice, depending on the duration after treatment with streptozotocin [Interaction of Diabetes×Drug treatment×Duration after treatment with streptozotocin; $F(24, 656)=5.144$; $P<0.001$] (Fig. 1). Tail-flick latencies in vehicle-treated diabetic mice were significantly shortened at 1–3 weeks after streptozotocin treatment and significantly prolonged at 7–8 weeks after streptozotocin treatment compared with those in vehicle-treated non-diabetic mice (Fig. 1). These results are consistent with our previous report (Ohsawa et al., 2008). Oral treatment with zinc L-carnosine (0.25–1 mmol/kg) was started 1 day after streptozotocin injection and treatment was continued once a day for 8 consecutive weeks. Chronic treatment with zinc L-carnosine (0.25–1 mmol/kg, p.o.) dose-dependently and significantly improved both the decrease in tail-flick latencies observed at weeks 2–3 in diabetic mice, and the prolongation of tail-flick latencies observed at weeks 7–8. In contrast to the results in diabetic mice, chronic treatment with zinc L-carnosine (0.25–1 mmol/kg, p.o.) had no significant effect on the tail-flick latency in non-diabetic mice. Single treatment with zinc L-carnosine (1 mmol/kg) did not affect tail-flick latency 2 h after its treatment (data not shown), indicating the long-term treatment with zinc L-carnosine is needed to improve both the decrease in tail-flick latencies observed at weeks 2–3 in diabetic mice, and the prolongation of tail-flick latencies observed at weeks 7–8. At the end of the experiment, the body weight of vehicle-treated diabetic mice (36.8±0.6 g) was significantly lower than that of vehicle-treated non-diabetic mice (42.1±1.1 g) (P<0.01, Bonferroni test).