Short Communication

Beneficial Action of 2,4,4-Trimethyl-3-(15-hydroxypentadecyl)-2-cyclohexen-1-one, a Novel Long-Chain Fatty Alcohol, on Diabetic Hypoalgesia and Neuropathic Hyperalgesia

Yutaka Tamura¹, Mayuko Monden¹, Hiroto Suzuki², Masashi Yamada², Keizo Koyama², and Hirohito Shiomi¹,*

¹Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Fukuyama, Hiroshima 729-0292, Japan
²Pharmaceuticals Department, Developmental Section, Meiji Dairies Corporation, 1-2-1 Shinzuna, Koto-ku, Tokyo 136-8908, Japan

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Abstract. The effects of 2,4,4-trimethyl-3-(15-hydroxypentadecyl)-2-cyclohexen-1-one (tCFA15) on diabetic hypoalgesia and neuropathic hyperalgesia were examined. Treatments of streptozotocin (STZ)-pretreated mice with tCFA15 (8 – 40 mg/kg, i.p.) for 7 days significantly reversed the depressed inflammatory nociceptive licking response in the formalin test. In addition, similar drug treatments and dosing in 7-day postoperative neuropathic pain model rats (prepared by the method of Bennett and Xie) yielded a similarly favorable outcome by significantly reversing decreased nociceptive thresholds in the paw pressure test. These results suggest that tCFA15 may have the potential to normalize sensory nerve abnormalities induced in experimental diabetes and nerve injury.

Keywords: diabetic hypoalgesia, long-chain fatty alcohol, chronic constriction injury

Peripheral neuropathies, the most common and frequent complications found in diabetic mellitus (DM) and shingles, display predominant pathological features such as axonal degeneration (axonal or neuronal neuropathies) or paranodal or segmental demyelination. Diabetes-induced sensory problems include deep muscular aches, lancinating pains, and persistent burning pain or tingling sensation usually in the extremities. Furthermore, the inability to detect thermal stimuli, loss of vibration sensation, and loss of pain perception are also encountered (1). Diabetic neuropathic pain is usually treated with antidepressants, anticonvulsants, nonsteroidal anti-inflammatory drugs (NSAID), opioids, and aldose reductase inhibitors (2). Despite the many agents available for treatment, potent therapeutics against diabetic hypoalgesia have not been available as yet. Streptozotocin (STZ)-treated rats and mice are experimental models known to develop neuropathic complications that resemble clinical symptoms observed in human DM patients (3). Thus, the STZ-induced diabetic mouse has been widely used as an experimental model of insulin-dependent DM.

Conventional pharmacotherapy is often of little help for patients with neuropathic pain. Therefore, similar to diabetic neuropathy, shingle-associated neuropathic hyperalgesia is one of the most difficult pains to control. Chronic nerve constriction injury animals (CCI model), such as the Bennett rat and Chung rat models, are used as experimental models for the investigation of neuropathic hyperalgesia and/or allodynia. These model animals typically display a continuous decrease in the nociceptive threshold; viz., responding nociceptively to non-noxious stimuli, which would have been trivial to the same normal animals.

Long-chain fatty alcohols, including 2,4,4-trimethyl-3-(15-hydroxypentadecyl)-2-cyclohexen-1-one (tCFA15), synthesized by Girlanda-Junges et al. (4), have been reported to display various physiological functions such as neurotrophic activity, calcium mobilization, stimulation of neuropeptide secretion, and preventive effects
on ischemic-reperfusion injury of the bladder (5–9). In this study, we investigated if tCFA15 would normalize the sensory nerve abnormalities of STZ-treated diabetic mice and CCI model rats.

Animals were accommodated in a room maintained at 23 ± 0.5°C and 70 ± 5% relative humidity and illuminated with an alternating 12-h light-dark cycle (light: 08:00 – 20:00 h). Animals were handled according to the Fukuyma University Guidelines for Animal Experiments. Sixty male ddY mice (Japan SLC, Ishikawa), initially weighing 20 – 25 g, were used for induction of diabetic hyperalgesia. The mice were rendered diabetic by i.v. injection through the tail vein with a single bolus administration of 200 mg/kg STZ (Sigma, St. Louis, MO, USA), previously dissolved in 0.1 N citrate buffer at pH 4.5. Induction of diabetes was confirmed by measurements of blood glucose levels of the tail vein with Ascensia BREEZE (Bayer, German). Mice with glucose levels >400 mg/dl were considered diabetic and used in experiments. Diabetic mice were treated with tCFA15 from day 0. After the final administration of tCFA15 (day 7), we collected and measured the glucose contents of blood samples accordingly. Twenty-four male Sprague-Dawley rats (Charles River Japan, Yokohama), initially weighing 110 – 130 g, were used for induction of neuropathic hyperalgesia. Four groups of 6 animals each were housed in separate cages at 6 rats/cage, and the animals were given food and water ad libitum throughout the experiments. CCI-induced hyperalgesia model rats were prepared by using the method described by Bennett and Xie (10). Briefly, 4-week-old rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.; Dainippon Pharmaceutical Co., Ltd., Osaka). The left common sciatic nerve, exposed at the middle portion of the thigh by blunt dissection through the biceps femoris, was looped with four loose ligatures (5-0 MONOCRYL Y303H; Ethicon, Inc., Somerville, NJ, USA) at 1-mm axial intervals along the nerve. The CCI model rats exhibited decreases of >30% in nociceptive thresholds of the injured paw compared with pre-operative values with the paw pressure test, thus, a higher sensitivity to the same nociceptive stimulus was induced.

In the formalin test with groups of 10 mice each, the nociceptive paw-licking time was performed after 7-day STZ treatment. Since adaptation to the test environment decreases variables in formalin-evoked behavior, animals were handled daily (for 4 – 5 h) in the test room for a week. Although accommodated in another room nearby, STZ- and vehicle-treated mice were brought to the test room 2 h before testing. Injection of 1% formalin (20 µL) into the hindpaw elicited a characteristic biphasic nociceptive licking response in the treated paw. The first (early) phase began immediately after formalin injection and lasted for 5 – 6 min, followed by the second (late) phase 10 – 15 min after formalin injection. The second phase lasted for 20 – 25 min. Although the nociceptive licking response during the first phase was not affected, significant decreases in the second-phase licking time were noted in STZ-induced diabetic mice compared with controls. Injections with tCFA15 in STZ-treated mice for 7 days significantly reversed the depressed second-phase licking response at doses of 24 and 40 mg/kg. The licking times in non-diabetic, diabetic (vehicle), diabetic (tCFA 8 mg/kg), diabetic (tCFA 24 mg/kg), and diabetic (tCFA 40 mg/kg) mice were 127.2 ± 19.8, 40.5 ± 21.9, 28.7 ± 5.7, 110.2 ± 16.7, and 124.1 ± 27.8 s, respectively (Fig. 1: A and B). However, tCFA15 did not affect licking times of either the first or second phase in non-diabetic mice even at the higher dose (40 mg/kg) (data not shown). Since tCFA15 reversed STZ-induced hyperalgesia, we examined the effects of tCFA15 on blood glucose levels and body weights in similarly induced diabetic mice. On examination of blood samples collected from the tail vein of STZ-induced diabetic mice 3 h after the final of the 7-day tCFA15 administration, the STZ-induced high blood glucose levels were not affected (Table 1). Moreover, STZ-induced body-weight loss was not reversed with tCFA15 (40 mg/kg, i.p.) in STZ-treated mice.

The antinociceptive effect of tCFA15 on neuropathic hyperalgesia was determined by measuring the foot-withdrawal threshold elicited by mechanopressure on the hindpaw using the analgesy meter (model: 37215; Ugo Basile, Italy). The nociceptive paw pressure test was performed on day 14 after CCI operation. The nociceptive threshold of the tested (left) paw was significantly decreased compared with the contralateral sham-operated (right) paw of the animal. Furthermore, tCFA15 administration (8 – 40 mg/kg, i.p.) for 7 days from CCI postoperative day 7 significantly elevated the nociceptive threshold of injured paws in a dose-dependent manner without affecting that of contralateral sham-operated paws (Fig. 2).

It is well known that injection of formalin into the hindpaw elicits a biphasic nociceptive licking response. The first phase of the paw-licking response was believed to be the result of direct activation of nociceptors (fast pain), whereas the second phase was mediated by a combination of ongoing activity of primary afferents and increased sensitivity of spinal cord neurons (inflammatory response, chronic pain). In this study, tCFA15 significantly suppressed licking responses during the second phase in diabetic (compared with non-diabetic) mice without affecting STZ-induced hyperglycemia.
This result suggests that tCFA15 favorably influenced diabetes-induced hypoalgesia without the involvement of glucose metabolism.

Treatment with tCFA15 for 7 days reversed chemogenic hypoalgesia at doses of 24 and 40 mg/kg. Nerve growth factor (NGF) is essential for the development and functional maintenance of sensory neurons, and endogenous NGF is known to decrease in diabetic patients (11). Therefore, changes in endogenous NGF levels could be of relevance to the pathogenesis of diabetic neuropathies. Christianson et al. have demonstrated cutaneous axon loss in STZ-induced diabetic mice (12), improvement of diabetes-induced innervation deficits by intrathecal treatment of neurotrophic factors such as glial cell-line derived neurotrophic factor (GDNF) and neurturin (NTN), and reversal of diabetes-

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**Table 1.** Changes in the body weight and blood glucose level of vehicle-treated, tCFA15-treated diabetic mice and non-diabetic (control) mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight (g)</th>
<th>Blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>23.4 ± 0.34</td>
<td>27.1 ± 0.52**</td>
</tr>
<tr>
<td>Diabetic (vehicle)</td>
<td>25.3 ± 0.15</td>
<td>26.1 ± 1.04</td>
</tr>
<tr>
<td>Diabetic (tCFA15)</td>
<td>27.3 ± 0.30</td>
<td>25.2 ± 0.80*</td>
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Each value represents the mean ± S.E.M. Statistical significance of differences between any two groups was assessed by the Wilcoxon pair-matched signed-rank test. Differences where \(P<0.05\) (*) or \(P<0.01\) (**) were considered significant when compared with the pre-test value. Ten animals were designated for each experimental group.
Normalizes the Sensory Function

induced depression of chemogenic nociceptive response with GDNF in the formalin test (13). In addition, Apfel et al. have also reported that NGF administrations protect against diabetic sensory neuropathies (14). Compound tCFA15 has previously been documented to have neurotrophic activity (5), which could have contributed favorably to improving diabetic hypoalgesia in STZ-treated mice.

Furthermore, tCFA15 treatment for 7 days reversed decreased the nociceptive threshold of the injured paws without affecting those of the contralateral (control) paws. In this experiment, tCFA15 was administered for 7 days from CCI postoperative day 7, where decreases in the nociceptive threshold had already occurred and thereafter remained persistently (see controls). Therefore, this antinociceptive effect of tCFA15 may be interpreted as a therapeutic action. Ren et al. have shown that the CCI-induced decrease in the nociceptive mechanical threshold can also be abolished with NGF treatment (15). Thus, the neurotrophic effect of tCFA15 probably contributed to the therapeutic action by repairing nerve damage and/or promoting neuronal regrowth to improve the decreased nociceptive threshold induced by sciatic nerve injury.

In summary, the novel long-chain fatty alcohol tCFA15 may have the potential to normalize the pathologically/chemogenically inflicted abnormalities of the sensory nerve system in patients with type II diabetes and nerve injuries. It is noteworthy that tCFA15 improved not only neuropathic hyperalgesia but also reversed diabetic hypoalgesia. Therefore, tCFA15 may be developed as a useful therapeutic drug against diabetes-induced and/or nerve injury-derived neuropathies. As the therapeutic action of tCFA15 remains unclear, further investigations are warranted to better understand the underlying mechanism(s) of action in diabetes-related and nerve injury-associated neuropathies.

References


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