Antinociceptive Effect of Vitamin K$_2$ (Menatetrenone) in Diabetic Mice

Kenji Onodera$^{1,*}$, Ko Zushida$^2$ and Junzo Kamei$^2$

$^1$Department of Dental Pharmacology, Okayama University Dental School, Sikata-cho, Okayama 700-8525, Japan
$^2$Department of Pathophysiology & Therapeutics, Faculty of Pharmaceutical Sciences, Hoshi University, Shinagawa-ku, Tokyo 142-8501, Japan

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ABSTRACT—The antinociceptive effect of vitamin K$_2$ (menatetrenone) in diabetic mice was examined using a tail-pressure test. Intraperitoneal injection of menatetrenone (10 – 100 mg/kg) produced a dose-dependent increase in the nociceptive threshold in diabetic mice. There was no significant difference between non-diabetic and diabetic mice in the menatetrenone-induced changes in the nociceptive threshold. The results suggest the therapeutical usefulness of menatetrenone for treating painful diabetic neuropathy and osteoporosis.

Keywords: Vitamin K$_2$ (menatetrenone), Antinociception, Painful diabetic neuropathy

Vitamin K$_2$ (menatetrenone) is clinically used for preventing postmenopausal osteoporosis and has been effective in the treatment of patients with osteoporosis (1, 2). The therapeutic utility of menatetrenone has recently focused on the analgesic action in patients with osteoporosis. Female osteoporosis patients whose pain had not been relieved by vitamin D$_3$, elcatonin, non-steroid anti-inflammatory drugs and/or physical therapy showed significant decrease in pain scores after treatment with menatetrenone for 2 – 4 weeks (2).

Diabetic neuropathy accompanied by anomalies in pain perception is one of the most frequent complications in insulin-dependent diabetes (3). Many clinical and experimental studies have suggested that diabetes or hyperglycemia alters pain sensitivity (4). Diabetic neuropathy can be associated with a sensation of burning or tactile hypersensitivity (4). Since pain related to diabetic neuropathy is difficult to control by nonsteroidal anti-inflammatory drugs, such pain is treated with lidocaine and mexiletine (5).

Under these conditions, we investigated the antinociceptive effect of menatetrenone in diabetic mice using a tail-pressure test, to test the therapeutic utility of menatetrenone for pain related to diabetic neuropathy.

Male ICR mice (Tokyo Laboratory Animals Science Co., Ltd., Tokyo), 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 24 ± 1°C with a 12-h light-dark cycle. Animals were rendered diabetic by an injection of streptozotocin (STZ; 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. The experiments were conducted 2 weeks after the injection of streptozotocin or vehicle. Mice with serum glucose levels above 400 mg/dl were considered diabetic. This study was carried out in accordance with the guidelines for the care and use of laboratory animals of The Japanese Pharmacological Society. Changes in nociceptive threshold were determined by the tail-pressure test using an analgesimeter (Natsume, Tokyo). In brief, the tail of the mouse was pressed 1.5 cm from the root using a plastic plate (2-mm-thick and 10-mm-wide) at a loading rate 250 g/s. The weight (g) when the mouse withdrew its tail or struggled was considered the nociceptive threshold. Menatetrenone (Kaytwo N Injection) and its vehicle were generously supplied by Eisai Co., Ltd., Tokyo. Lidocaine HCl was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The vehicle for menatetrenone, a mixture of glycerine, D-sorbitol and purified soybean lecithin, was the same composition as that of Kaytwo N Injection. Lidocaine HCl was dissolved in 0.9 % saline and injected intraperitoneally (i.p.) 30 min before the tail pressure test. Menatetrenone and its vehicle were i.p. administered. The data are expressed as the mean ± S.E.M. The statistical significance of differences was assessed with the Bonferroni/Dunn test.

First, we examined the time course of the antinociception produced by menatetrenone (10 – 100 mg/kg, i.p.), as shown in Fig. 1, A and B. The basal nociceptive threshold (g) in diabetic mice (1133.9 ± 92.0 g) was significantly (P<0.01) lower than that in non-diabetic mice (1647.8 ± 135.8 g).
Menatetrenone increased the nociceptive threshold in diabetic and non-diabetic mice. The effect reached its peak 30 min after injection, then gradually declined, and finally the nociceptive threshold returned to the preinjection level at 120 min after menatetrenone injection. Figure 1C shows the effect of menatetrenone on the nociceptive threshold (Δg) 30 min after each injection in mice. Menatetrenone (10 – 100 mg/kg, i.p.) produced a dose-dependent increase in the nociceptive threshold in both non-diabetic mice and diabetic mice. There was no significant difference between non-diabetic mice and diabetic mice in the amount of increases in the nociceptive threshold produced by each dose of menatetrenone tested. As a reference, we examined the effect of lidocaine on the nociceptive threshold (g) 30 min after each injection in mice. As shown in Fig. 2, lidocaine (50 mg/kg, i.p.) produced a significant increase in the nociceptive threshold in both non-diabetic mice and diabetic mice, but there was no significant difference between non-diabetic and diabetic mice 30 min after each injection.

The present study indicated that i.p. administration of menatetrenone has an antinociceptive effect on diabetic mice. Furthermore, menatetrenone produced an equivalent antinociceptive effect in diabetic mice and in non-diabetic mice. In addition, in diabetic mice, menatetrenone completely reversed the nociceptive threshold to the basal nociceptive threshold in non-diabetic mice. These results support previous data on the antinociceptive activity of menatetrenone in mice and humans (2, 6). We reported previously that menatetrenone significantly reduces the duration of intrathecally administered bradykinin-, but not substance P-, prostaglandin E₂- or NMDA-induced nociceptive responses in mice (6). We suggested that the inhibi-

Fig. 1. Antinociceptive effect of vitamin K₃ (menatetrenone) in diabetic mice. Time course of the antinociceptive effect of menatetrenone on the nociceptive threshold in non-diabetic (A) and diabetic mice (B). The effects of menatetrenone (10 mg/kg, closed diamonds; 50 mg/kg, open circles; 100 mg/kg, closed circles) and vehicle (open triangles) were measured 30, 60, 90 and 120 min after i.p. injection. Changes in nociceptive threshold were determined by the tail-pressure test. Each point represents the mean with S.E.M. for at least 10 mice in each group. *P<0.05 vs respective vehicle-treated group. C) Effect of menatetrenone (10, 50 and 100 mg/kg, i.p.) on the nociceptive threshold in non-diabetic (open columns) and diabetic mice (hatched columns). The effects of menatetrenone (10, 50 and 100 mg/kg) and vehicle were measured 30 min after i.p. injection. Δg = post-drug value – pre-drug value. Each column represents the mean with S.E.M. for at least 10 mice in each group. *P<0.05 vs respective vehicle-treated group.

Fig. 2. Effect of lidocaine on the nociceptive threshold in non-diabetic (open columns) and diabetic mice (hatched columns). The effects of lidocaine (30 and 50 mg/kg) and vehicle (saline) were measured 30 min after i.p. injection. Changes in nociceptive threshold were determined by the tail-pressure test. Δg = post-drug value – pre-drug value. Each column represents the mean with S.E.M. for at least 10 mice in each group. *P<0.05 vs respective saline-treated group.
tion of bradykinin-, but not of prostaglandin E\textsubscript{2}, substance P- and NMDA-dependent nociceptive transmission in the spinal cord may be at least partially responsible for the menatetrenone-induced antinociception (6). However, aspirin and diclofenac, nonsteroidal anti-inflammatory drugs, showed a lack of antinociceptive effects in streptozotocin-induced diabetic rats (5). Kamei and Zushida previously suggested that the antinociceptive effect of mexiletine, a lidocaine-like antiarrhythmic drug, may involve the inhibition of substance-P- and somatostatin-mediated nociceptive transmission in the spinal cord in mice (7). Therefore, the inhibition of bradikinin-dependent nociceptive transmission alone can not explain the potent antinociceptive effect of menatetrenone in diabetic mice as assessed by the tail pressure test.

Lidocaine and mexiletine are known to be useful drugs whose antinociceptive activities are increased in diabetes (5). Pain related to diabetic neuropathy is difficult to control by nonsteroidal anti-inflammatory drugs (7). Although further studies are necessary to clarify how menatetrenone produces its antinociception, it is notable that menatetrenone shows antinociception resemblance to lidocaine in diabetic mice.

Menatetrenone, vitamin K\textsubscript{2} with 4 isoprene units, has not only high \(\gamma\)-carboxylation activity in hypoprothrombinaemic rats (8), but also has prevented bone-loss induced by ovariectomy (9) or by predonisolone administration in rats (10). In addition, the present results suggest therapeutic usefulness of menatetrenone for treating pain related to osteoporosis and diabetic neuropathy.

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