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Mice with neuropathic pain exhibit morphine tolerance due to a decrease in the morphine concentration in the brain

Wataru Ochiai [a,1], Mitsumasa Kaneta [a,1], Marina Nagae [a], Ami yuzuhara [a], Xin Li [a], Haruka Suzuki [a], Mika Hanagata [a], Satoshi Kitaoka [a], Wataru Suto [a], Yoshiki Kusunoki [a], Risako Kon [a], Kazuhiko Miyashita [b], Daiki Masukawa [b], Nobutomo Ikarashi [a], Minoru Narita [c], Tsutomu Suzuki [b], Kiyoshi Sugiyama [a,*]

[a] Department of Clinical Pharmacokinetics, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
[b] Department of Toxicology, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
[c] Department of Pharmacology, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

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ABSTRACT

The chronic administration of morphine to patients with neuropathic pain results in the development of a gradual tolerance to morphine. Although the detailed mechanism of this effect has not yet been elucidated, one of the known causes is a decrease in μ-opioid receptor function with regard to the active metabolite of morphine, M-6-G(morphine-6-glucuronide), in the ventrotegmental area of the midbrain. In this study, the relationship between the concentration of morphine in the brain and its analgesic effect was examined after the administration of morphine in the presence of neuropathic pain. Morphine was orally administered to mice with neuropathic pain, and the relationship between morphine's analgesic effect and its concentration in the brain was analysed. In addition, the expression levels of the conjugation enzyme, UGT2B (uridine diphosphate glucuronosyltransferase), which has morphine as its substrate, and P-gp, which is a transporter involved in morphine excretion, were examined. In mice with neuropathic pain, the concentration of morphine in the brain was significantly decreased, and a correlation was found between this decrease and the decrease in the analgesic effect. It was considered possible that this decrease in the brain morphine concentration may be due to an increase in the expression level of P-gp in the small intestine and to an increase in the expression level and binding activity of UGT2B in the liver. The results of this study suggest the possibility that a sufficient analgesic effect may not be obtained when morphine is administered in the presence of neuropathic pain due to a decrease in the total amount of morphine and M-6-G that reach the brain.

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1. Introduction

Morphine is a potent analgesic agent that is widely used for the control of both acute and chronic pain. The administration of morphine is unavoidable in the treatment of cancer patients with pain. The analgesic effect of morphine is exerted via binding to μ-opioid receptors in the CNS. However, for patients with neuropathic pain, a gradual decrease in the analgesic effect that occurs with chronic morphine administration poses a problem (Narita et al., 2008; Sounvoravong et al., 2004). One of the causes of this problem is known to involve a decrease in the function of the μ-opioid receptor, to which M-6-G in the ventrotegmental area of the midbrain, one of the active metabolites of morphine, binds (Narita et al., 2008). It has been reported that morphine tolerance is not observed when glycogen synthetase kinase 3β (GSK3 β) inhibited (Dobashi et al., 2010a,b). Recently, it has also been reported that signalling via PDGF (platelet-derived growth factor) receptor-β plays an important role in the development of morphine tolerance. (Wang et al., 2012). However, it is believed that the development of a tolerance to morphine may not be explained by these mechanisms alone.

The majority of orally administered morphine is absorbed in the small intestine. One of the molecules that controls this absorption is the drug transporter, P-gp (Kharasch et al., 2003). It has been reported that when morphine is administered to P-gp knockout mice, its analgesic effect is enhanced due to an increase in the morphine concentration in the brain (Hamabe et al., 2006).

After absorption in the small intestine, morphine is metabolized by glucuronic acid conjugation enzyme (UGT2B7) to M-6-G, which possesses analgesic activity, and to M-3-G, which does not (Coffman et al., 1997; Ohno et al., 2008; Osborne et al., 1988; Takeda et al., 2005). Morphine, M-3-G, and M-6-G that reach the circulation are transported into the brain through the blood–brain barrier. The amount of morphine, but

* Corresponding author. Tel.: +81 3 5498 5772.
E-mail address: sugiyama@hoshi.ac.jp (K. Sugiyama).
[1] These authors contributed equally to this work as first.

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not M-6-G (Bourasset et al., 2003), transported into the brain is regulated by P-gp, which is located in the blood–brain barrier (Hamabe et al., 2007). The level of morphine transport into the brain is low; however, the levels of transport of the glucuronic acid conjugates of morphine (M-6-G and M-3-G) are even lower than that of morphine (Bickel et al., 1996; Laizure, 1994; Lotsch et al., 1998; Wu et al., 1997).

In this study, the decrease in the concentration of morphine in the brain was examined as a potential cause of the lowered analgesic effect of morphine in the presence of neuropathic pain. The concentration of morphine in the brains of mice with neuropathic pain was measured after the oral administration of morphine. In addition, the expression level and activity of P-gp and UGT2B were analysed to elucidate the cause of the observed decrease in brain morphine concentration.

2. Material and methods

2.1. Animal handling

Male ICR mice (20–25 g) were purchased from Japan SLC, Inc. (Tokyo Laboratory Animals Science Co. Ltd, Tokyo, Japan). The mice were kept at room temperature (24 ± 1 °C) and 55 ± 5% humidity with 12 h of light (artificial illumination; 8:00–20:00). Food and water were available ad libitum. Each animal was used only once. The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, as adopted by the Committee on Animal Research at Hoshi University.

2.2. Neuropathic pain model

The mice were anesthetized with 3% isoflurane. We produced a partial sciatic nerve injury by tying a tight ligature with an 8-0 silk suture around approximately one-third to one-half the diameter of the sciatic nerve on the right side under a light microscope (SD30, Olympus, Tokyo) (Seltzer et al., 1990). In the sham-operated mice, the nerve was exposed without ligation.

2.3. Assessment of antinociception

Pain hypersensitivity to a heat stimulus was evaluated in mice with neuropathic pain using a paw flick test or tail flick test (Fruhstorfer et al., 2001; Kallina and Grau, 1995; Keizer et al., 2007, 2008; Mulder and Pritchett, 2004; Pitcher et al., 1999; Yasphal et al., 1982). Antinociception induced by morphine was determined by tail-flick test (Tail Flick Analgesia Meter Model MK330B, Muromachi Kikai Co. Ltd, Tokyo, Japan). The intensity of the heat stimulus was adjusted, so that the animal flicked its tail after 3–5 s. When the intensity of stimulation was enough to produce a basal movement within 3–5 s in mice, it was defined that pharmacological observation results from the spinal reflex and supraspinal modulations. The inhibition of this tail-flick response was expressed as a percentage of the maximum possible effect (% MPE), which was calculated as ((T1 − T0) × 100 / (T2 − T0)), where T0 and T1 were the tail-flick latencies before and after the administration of morphine and T2 was the cut-off time (set at 10 s) in the tests to avoid injury to the tail. In the present study, the antinociceptive assay was performed 28 days after partial sciatic nerve-ligation. Each group consists of 7–10 mice. Mechanical allodynia after sciatic nerve ligation was evaluated using the von Frey test (Fruhstorfer et al., 2001; Keizer et al., 2007; Pitcher et al., 1999).

2.4. Quantitation of morphine and morphine-3-glucuronide by LC/MS/MS

The mice were anesthetized using pentobarbital, and blood samples were collected from the inferior vena cava, followed by perfusion with phosphate-buffered saline (PBS) through the heart to flush residual blood from the tissues. The brain was homogenized with twice its volume of ultrapure water. The resulting serum and brain homogenates were treated with acetonitrile, and the concentration of morphine and M-3-G were determined using LC/MS/MS (Agilent 1100 Series) after the removal of proteins (Projean et al., 2003; Rook et al., 2005).

2.4.1. HPLC

The HPLC system consists of an Agilent 1100 series binary pump and autosampler (Agilent, Palo Alto, CA, USA). Auto-sampler temperature was remained at 4 °C. Ten microlitres of extract was injected to reverse phase YMC Pack Pro C8 columns (4.6 mm I.D. × 50 mm YMC, particle size 3 μm (YMC). Gradient elution was performed using a mixture of 0.1 5 vol.% formic acid in acetonitrile, as described in Table 1. Flow rate of the mobile phase was kept constant at 0.6 mL/min and total run time was 6.5 min. The column oven temperature was kept at 40 °C. The autosampler needle was washed with acetonitrile/isopropanol/H_2O (2:2:1, v/v) after injection.

2.4.2. MS/MS

2.4.2.1. MS/MS API4000 (AB SCIEX) triple quadrupole mass spectrometry system. Ions were created in the positive ion mode under atmosphere pressure at 5500 V and at a source temperature of 600 °C. Acetaminophen (0.1 μg/mL) was used as an internal standard material. Multiple reaction monitoring (MRM) was used for drug quantification. Selected ion masses of the protonated precursors and fragment ions are shown in Table 2.

2.5. Measurement of UGT activity

Along with 50 μL of reaction buffer, 10 μL of D-saccharic acid 1,4-lactone (a specific inhibitor of β-glucuronidase) aqueous solution, 10 μL of phosphatidylcholine suspension, and 10 μL of UDP-glucuronic acid aqueous solution were placed in a 1.5-mL microtube. The mixture was vortexed and pre-incubated for 10 min at 37 °C. Ten microlitres each of hepatic microsomal fraction and 4-MU (4-methylumbeliferone) solution were also pre-incubated for 10 min at 37 °C. All of these pre-incubated solutions were mixed and vortexed, followed by incubation for 30 min at 37 °C. The reaction was halted by the addition of 100 μL of ice-chilled methanol. After centrifugation at 10,000 ×*g* for 10 min at 4 °C, 150 μL of supernatant was collected.

2.6. Quantitation of 4-MUG

Quantitation of 4-MUG (4-methylumveliferyl-β-D-glucuronide hydrate) by HPLC was performed using the absolute calibration curve method. A total of 20 μL of sample solution was used for the HPLC analysis. The HPLC system consists of a 600S Controller, 616 Pump, and 717plus Autosampler, along with a Waters 2475 Multi λ Fluorescence Detector. The measurement data were recorded and analysed using the Empower analysis software. An Inertsil ODS-3 column was used. As a mobile phase, phosphate buffer/methanol (70/30, v/v) mixture was used at a flow rate of 1.1 mL/min after degassing by a sonicator and aspirator. The column temperature was set at 40 °C. The quantity of produced 4-MUG was determined using an excitation wavelength of 315 nm and an emission wavelength of 365 nm.

Table 1
HPLC eluent gradient, eluent A consists of 0.1% formic acid and eluent B of 100% acetonitrile.

Time (min)	Eluent A (%)	Eluent B (%)
0	95	5
0.5	95	5
3.5	10	90
4.5	10	90
4.51	95	5
6.5	95	5

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