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Translational Research

TRANSLATIONAL RESEARCH

Leukemia inhibitory factor (LIF) potentiates antinociception activity and inhibits tolerance induction of opioids

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Abstract

Background: The efficacy of opioids typically decreases after long-term use owing to the development of tolerance. Glial activation and the upregulation of proinflammatory cytokines are related to the induction of tolerance. We investigated the effect of leukemia inhibitory factor (LIF) on morphine analgesia and tolerance.

Methods: LIF concentrations in rat spinal cords were measured by polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) after morphine administration. LIF distribution was examined using confocal microscopy. To evaluate the effects of LIF on morphine analgesia and tolerance, LIF was intrathecally administered 30 min before morphine injection. The analgesic effect of morphine was evaluated by measuring tail-flick latency. Human LIF concentrations from the cerebrospinal fluid (CSF) of opioid tolerant patients were also determined by specific ELISA.

Results: Chronic morphine administration upregulated LIF concentrations in rat spinal cords. Intrathecal injection of LIF potentiated the analgesic action of morphine. Patch clamp recording of spinal cord slices showed that LIF enhanced DAMGO ([D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin)-induced outward potassium current. The development of tolerance was markedly suppressed by exogenous LIF, whereas neutralizing the endogenously released LIF with anti-LIF antibodies accelerated the tolerance induction. Moreover, LIF concentrations in the CSF of opioid-tolerant patients were higher than those in the opioid-naive controls.

Conclusions: Intrathecal administration of LIF potentiated morphine antinociceptive activity and attenuated the development of morphine tolerance. Upregulation of endogenously released LIF by long-term use of opioids might counterbalance the tolerance induction effects of other proinflammatory cytokines. LIF might be a novel drug candidate for inhibiting opioid tolerance induction.

Key words: glia; leukemia inhibitory factor; opioid

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Editor's key points

- Opioid tolerance is a significant clinical problem, with novel strategies needed to prevent it.
- The cytokine, Leukemia Inhibitory Factor (LIF), is upregulated in rat spinal cord after chronic morphine.
- Intrathecal LIF reduced morphine tolerance in rats. Opioid tolerant patients had higher intrathecal LIF concentrations.
- Further study of the potential role of LIF in reducing opioid tolerance is needed.

Opioid analgesics, such as morphine, provide effective pain relief and are considered the therapy of choice for managing moderate-to-severe pain, particularly cancer pain.[1] However, clinical use of opioid analgesics is usually restricted by their side-effects, especially the development of tolerance, which arises because of chronic use. As tolerance develops, a higher opioid dose is required to achieve the same analgesic level, which might lead to serious side-effects and physical dependence. Several hypotheses have been proposed for explaining the tolerance mechanism: receptor desensitization,[2] upregulation of NMDA receptors,[3] and downregulation of glutamate receptors.[4] Glial cells have also been reported to mediate morphine tolerance.[5–8] Chronic morphine treatment stimulates glial cell activation, leading to the release of proinflammatory cytokines.[9 10] These findings suggest that cytokines may play a pivotal role in tolerance development.

Leukemia inhibitory factor (LIF), a gp130 member of IL-6 family, is involved in inflammation,[11] pain,[12] and neurogenesis[13] and its receptors are widely expressed in various regions of the central nervous system.[14] Recent studies also indicate that LIF is a key mediator in sensory neurones.[15] Here we examined the potential effect of LIF on morphine-induced analgesic activity and tolerance.

Methods

Animals

Adult male Sprague–Dawley rats (250–275 g) were purchased from BioLASCO Taiwan Co., Ltd. All procedures were performed in accordance with the International Association for the Study of Pain (IASP) and the NIH guidelines on laboratory animal welfare. Rats were anaesthetized with isoflurane. The occipital muscles were bluntly separated using microscopic scissors. A sterile polyethylene catheter-10 was inserted through an incision in the cisterna magna and advanced caudally to the lumbar enlargement of the spinal cord. The end of the tube, polished to avoid physical damage to the spinal cord, was placed between the L4 and L5 vertebrae. The lower back was sutured, and the exterior end was subcutaneously tunnelled to exit through a small incision at the back of the neck.[16] After surgery, the rats were kept in the same cages for a week for recovery.

Real-time PCR and cytokine measurement

After behavioural testing, rats were anaesthetized and perfused with saline at different time points. Total RNA was extracted from L4–L6 spinal cord segments using a TRIzol® kit (MDBio Inc., Taipei, Taiwan). Single-stranded cDNA was synthesized using a two-step MMLV reverse transcriptase system (Promega, Madison, WI, USA). Fifty nanograms of cDNA was mixed with 200 nM specific primers and SYBR® Green PCR Master Mix (Roche Molecular system, NJ, USA). Quantitative PCR assays were performed in triplicate on a StepOnePlus sequence detection system (Applied Biosystems, CA, USA). The change in gene expression relative to the control was calculated using the $2^{-\Delta\Delta CT}$ method.

The specific PCR primers used were as follows: rat GAPDH: sense, GGCAAGTTCAATGGCACAGT; antisense, TGGTGAAGACG CCAGTAGACTC. rat LIF: sense, AGTTGTGCCCCTGCTGTTGG; antisense, GTCACGTTGGGGCCACATAG.

To measure endogenous LIF expression, the L4–L6 spinal cord segments were isolated, and an extraction buffer (#9806, Cell Signaling Technology, MA, USA) was added to the tissue. Samples were homogenized and centrifuged at 4°C. The supernatant was transferred to a fresh tube. Rat LIF concentrations were determined using a specific ELISA kit (SEA085Ra, USCN Life Science Inc., Wuhan, Hubei, PRC).

Drug administration

Morphine hydrochloride (Factory for Controlled Drugs, Taiwan Food and Drug Administration) was dissolved in sterile endotoxin-free water. Recombinant rat LIF (rLIF) was purchased from EMD Millipore (LIF3010). To evaluate the effect of LIF on acute morphine analgesic action, exogenous LIF (10 ng in 5 μl) was slowly injected through the intrathecal catheter, followed by a flush of 10 μl sterile saline. The morphine (2 mg kg^{-1}) was injected subcutaneously 30 min later. Subsequently, tail-flick latency was assessed at 15 min intervals (Supplementary data, Fig. 1A).

For the tolerance studies, exogenous LIF was injected as mentioned above, and morphine (10 mg kg^{-1} day^{-1}, s.c.) was injected 30 min later. The antinociceptive activity of morphine was measured using a tail-flick test 30 min after morphine injection (Supplementary data, Fig. 1B).

To neutralize endogenously released LIF, a specific anti-LIF antibody (AB-449-NA, R&D) was used. Normal goat IgG (AB-108-C, R&D Systems) was used as a control. Both control goat IgG and anti-LIF (5 μg in 5 μl, followed by a 10 μl saline) were infused through an intrathecal catheter 30 min before morphine injection (10 mg kg^{-1} day^{-1}, s.c.). Tail-flick latency was measured 30 min after morphine administration.

Antinociception test

Thermal nociception was evaluated using a tail-flick apparatus (Columbus Instruments, Columbus, OH, USA). Rats were gently restrained with their tail positioned directly under a heat source (heat intensity setting at 15, approximately 60°C). The time it took for the rats to flick their tails were recorded, with a 15-s cut-off time to minimize the risk of tissue damage. The analgesic response of morphine was calculated as a percentage of the maximum possible effect (MPE) using the following equation:

$$\%\text{MPE} = \frac{\text{test latency - baseline latency}}{\text{cut - off time - baseline latency}} \times 100.$$

Electrophysiology in spinal cord slices

Adult male SD rats (150–200 g) were anaesthetized with isoflurane, and the spinal cord was rapidly removed from the vertebral canal through hydraulic extrusion, as reported in a previous study.[17] The fresh spinal cord containing the L4-L6 vertebrae, was quickly dissected and immersed in a chilled cutting solution as previously described.[18] Coronal spinal cord slices (300–350-μm-thick) were cut using a microslicer (DTK-1000, Dosaka, Kyoto, Japan) and subsequently transferred to a holding chamber