Intrathecal cyclooxygenase inhibitor administration attenuates morphine antinociceptive tolerance in rats

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Several lines of evidence suggest that the N-methyl-D-aspartate receptor (NMDA) and nitric oxide (NO) systems are involved in morphine tolerance. Cyclooxygenase (COX) inhibitors may also play a role in morphine tolerance by interacting with both systems. In the present study, we examined the effects of the COX inhibitors N-(2-cyclohexyloxy-4-nitrophenyl) methanesulphonamide (NS-398, selective COX₂ inhibitor) and indomethacin (non-selective COX inhibitor) on the development of antinociceptive tolerance of morphine in a rat spinal model. The antinociceptive effect was determined by the tail-flick test. Tolerance was induced by injection of morphine 50 μg intrathecally (i.t.) twice daily for 5 days. The effects of NS-398 and indomethacin on morphine antinociceptive tolerance were examined after administering these drugs i.t. 10 min before each morphine injection. Neither NS-398 nor indomethacin alone produced an antinociception effect at doses up to 40 μg. NS-398 and indomethacin did not enhance the antinociceptive effect of morphine in naïve and morphine-tolerant rats. However, they shifted the morphine antinociceptive dose–response curve to the left when co-administered with morphine during tolerance induction, and reduced the increase in the ED₅₀ of morphine (dose producing 50% of the maximum response) three- to four-fold. Collectively, these findings and previous studies suggest that COX may be involved in the development of morphine tolerance without directly enhancing its antinociceptive effect.

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Non-steroidal anti-inflammatory drugs (NSAIDs) produce their therapeutic effect by inhibiting COX activity, thus reducing the synthesis of prostaglandins (PGs).¹ Combinations of NSAIDs with morphine have been used in clinical pain management, particularly in terminal cancer patients.² The potential advantages of this combination are that analgesia can be maximized while minimizing the adverse effects of morphine. Animal and clinical studies have shown additive or, possibly, synergistic interaction between opioids and NSAIDs.³ ⁴ Interactions between NSAIDs and morphine have also been reported in the visceral nociception and neuropathic pain models.⁵ ⁶

There is a large body of evidence indicating the involvement of the NMDA receptor and NO systems in opioid tolerance. NMDA antagonists and NO inhibitors have been shown to attenuate or prevent morphine tolerance.⁷–¹⁴ Recently, we further demonstrated that NMDA receptor antagonists inhibit morphine tolerance not only by modulating the binding characteristics of µ-opioid receptors¹¹ but also by partially preventing the constitutive neuronal expression of NO synthase (NOS).¹⁴

Mao et al. proposed that opioid tolerance and neuropathic pain syndromes share a common intracellular mechanism; both are expressed as a loss of analgesic effect of opioids.¹⁵ In addition, Malmberg and Yaksh reported that NSAIDs could attenuate the hyperalgesia mediated by glutamate receptors.¹⁶ Interaction between NMDA- and PG receptor-mediated events during inflammatory nociception has also been reported.¹⁷ PGE₂ was shown to stimulate the release of NO from rat spinal cord by NMDA receptor activation through EP1 receptors.¹⁸ Moreover, cross-communication between the NOS and COX systems has also been
demonstrated.\textsuperscript{19,20} NO interacts directly with COX to enhance its enzymatic activity.\textsuperscript{19} Inducible NOS activation may increase NO release and subsequently increase PG release, via COX activation.\textsuperscript{20} These findings imply complicated interactions among NMDA receptors and the NO and COX systems. In the light of these findings, we propose that COX inhibitors modulate antinociceptive tolerance of morphine via interaction with the NMDA–NO system. The present study was designed to examine the effects of the COX-selective inhibitors NS-398 (a selective COX\textsubscript{2} inhibitor) and the non-selective COX inhibitor indomethacin on the development of morphine antinociceptive tolerance in a rat spinal model.

Materials and methods

The experimental protocol was approved by the Animal Care and Use Committee of the National Defense Medical Center. Male Sprague–Dawley rats (weighing 300–350 g) were anaesthetized with intraperitoneal chloral hydrate (400 mg kg\textsuperscript{-1}), and intrathecal (i.t.) catheters were implanted for drug administration. The rats were then housed in the animal facility at the National Defense Medical Center for a 3-day recovery period. The antinociceptive effects of various drugs were measured by the tail-flick test, as in our previous studies.\textsuperscript{11–14} A microsyringe (Hamilton, 25 μl) was attached to the i.t. catheter (PE\textsubscript{10}) for drug administration. Drugs were administered in 5 μl of solution, and drug administration was followed by flushing with 10 μl of saline. Morphine sulphate was purchased from the Narcotics Control Bureau of the Health Department of the Republic of China (Taiwan), and was dissolved in saline. The antinociceptive effects of NS-398 and indomethacin were examined, and the effects of i.t. administration of NS-398 and indomethacin on morphine antinociceptive tolerance were examined by injecting these drugs 10 min before morphine administration. Antinociceptive responses were examined 30 min after morphine injection. The thermal intensity used in the tail-flick test was determined from the mean tail-flick latency (3.0±0.1 s) of 30 rats injected with saline before the study.

In a preliminary study, the effect of 30 μg of NS-398 or indomethacin, alone or with morphine, was not significantly different from that of 20 μg in the tail-flick test. Therefore, a dose of 20 μg (i.t.) was used for NS-398 and indomethacin in the present study. Tolerance to the antinociceptive effect of morphine was induced by injection of morphine (50 μg, i.t.) twice daily for 5 days. To investigate the effects of COX inhibitors (NS-398 and indomethacin, 20 μg) on morphine tolerance, we calculated the ED\textsubscript{50} for morphine antinociception after morphine tolerance had developed. The COX inhibitors were administered 10 min before each morphine injection on each of the 5 days of tolerance induction. The effects of COX inhibitors on the morphine antinociceptive dose–response curve were examined on the first and fifth days of tolerance induction. The tail-flick test was performed daily.

The tail-flick response was converted from a defined latency to the maximum per cent effect (MPE) as follows:

\[
MPE\% = \frac{\text{maximum latency} - \text{baseline latency}}{\text{cut-off latency}(10\text{s}) - \text{baseline latency}} \times 100
\]

NS-398 and indomethacin (Cayman, MI, USA) were dissolved in dimethyl sulphoxide (DMSO) and saline (1:1). DMSO had no significant effects on antinociception using the tail-flick test.\textsuperscript{21}

The morphine antinociceptive dose–response latency was analysed by computer-assisted linear regression (Cricket Graph 1.32; Islandia, NY, USA). The ED\textsubscript{50} was defined as the morphine dose that induced a 50% MPE measured by the tail-flick test and was calculated using the linear regression equations. All data are presented as mean and SEM. The data were subjected to analysis of variance and the Dunnett test, and \(P\) values <0.05 were considered significant.

Results

Intrathecal administration of NS-398 or indomethacin alone failed to produce any antinociceptive effect (Fig. 1). The maximum antinociceptive effect of morphine was observed on day 1 during the induction of morphine tolerance (Fig. 2). The tail-flick latency for NS-398 or indomethacin co-administered with morphine was higher than that of morphine alone, but the difference was not statistically significant. Morphine antinociceptive tolerance developed by day 3. Morphine maintained an antinociceptive effect when co-administered with NS-398 or indomethacin during tolerance induction; i.e. both NS-398 and indomethacin attenuated morphine antinociceptive tolerance (\(P < 0.01\)) (Fig. 2). On day 5, maximum tolerance was attained in the rats treated with morphine alone. Neither indomethacin nor NS-398 treatment alone produced any antinociceptive effect.

![Image](image_url)

Fig 1 The thermal antinociceptive effects of intrathecally administered NS-398 and indomethacin in rats, determined by the tail-flick test. Neither drug had any antinociceptive effect. All data points are mean and SEM for at least six rats.
during the 5-day test (Fig. 2). As in our previous study\(^{14}\), normal saline injections did not influence tail-flick latency in the rats used as controls (data not shown).

The effects of COX inhibitors on the morphine antinociception dose–response curve in morphine-tolerant rats are shown in Fig. 3. On day 1, morphine administration (0.1–5 μg, i.t.) produced a dose-dependent antinociceptive effect, with an ED\(_{50}\) of 0.51 μg (Table 1). Co-administration of a COX inhibitor (NS-398 or indomethacin) did not change the morphine antinociception dose–response curves either before (day 1) or after (day 5) morphine tolerance had developed (Fig. 3). However, when the COX inhibitors were co-administered with each morphine injection during tolerance induction, the dose–response curves of morphine antinociception shifted to the left (Fig. 3). On day 5, the ED\(_{50}\) values were 85.12, 17.38 and 22.19 μg for morphine alone, morphine plus NS-398, and morphine plus indomethacin respectively (Table 1). Furthermore, the mean baseline tail-flick latency of morphine-tolerant rats was lower (0.2–1 s) than that of the saline-injected control rats (data not shown).

**Discussion**

The present study shows that both COX\(_{2}\)-selective and non-selective COX inhibitors attenuated morphine antinocicep-
We found that a selective and a non-selective inhibitor of COX attenuated morphine antinociceptive tolerance. This is consistent with a recent study showing that the non-selective COX inhibitors ketorolac and ibuprofen inhibited the development of morphine tolerance.\textsuperscript{22} The morphine-tolerant rats had lower mean baseline tail-flick latency than the control group, suggesting that thermal hyperalgesia may develop in association with the development of morphine tolerance.\textsuperscript{10,15} It is interesting that COX\textsubscript{2} is not only inducibly expressed after the inflammation process but is also constitutively expressed in the spinal cord of normal rats.\textsuperscript{23–25} Furthermore, COX\textsubscript{2} is distributed in the superficial layer of the dorsal horn and is related to spinal nociceptive processing in the normal condition.\textsuperscript{25} However, it is not clear which COX isoforms are involved in morphine tolerance in the rat spinal cord. The effects of NS-398, a COX\textsubscript{2}-selective inhibitor, on morphine tolerance and morphine ED\textsubscript{50} values were slightly greater than those of indomethacin, implying that the inhibition of COX\textsubscript{2} may play a role in the development of morphine tolerance. Moreover, the present results agree with those of previous reports showing that COX inhibitors did not produce any thermal antinociceptive effects in the tail-flick test.\textsuperscript{21,26}

Although the non-selective COX inhibitor ketorolac has been shown to potentiate the analgesic effects of opioids by modulating the function of the opioid receptor in visceral nociception\textsuperscript{6}, the present results demonstrate that NS-398 and indomethacin did not potentiate morphine antinociception either before or after the development of morphine tolerance. However, these data also confirm that neither COX\textsubscript{1} nor COX\textsubscript{2} was directly involved in phasic thermal nociceptive transmission in the rat spinal cord.\textsuperscript{26}

Our previous studies have demonstrated that several drugs attenuate morphine tolerance and maintain the antinociceptive efficacy of morphine in a rat spinal model.\textsuperscript{11,14} Although the non-selective COX inhibitor ketorolac has been shown to potentiate the analgesic effect of opioids by modulating opioid receptor function in visceral nociception,\textsuperscript{5} in the present study neither NS-398 nor indomethacin potentiated morphine antinociception, either before or after the development of morphine tolerance. We found previously that the NMDA receptor antagonist MK-801 attenuated morphine tolerance by preventing the reduction of high-affinity \( \mu \)-opioid receptor sites.\textsuperscript{11,14} In the present study, COX inhibitors were found to attenuate morphine tolerance but did not enhance the antinociceptive effect of morphine. Because NMDA receptor antagonists and COX inhibitors shift the morphine dose–response curve in different directions, our present results suggest that COX inhibitors inhibit PG synthesis and related neurotransmission rather than having a direct inhibitory effect on conformational change of the \( \mu \)-opioid receptor.

In summary, the present results show that COX inhibitors can attenuate the development of morphine tolerance. However, both the non-selective COX inhibitor indomethacin and COX\textsubscript{2} inhibitors failed to produce any analgesic effects or potentiation of morphine antinociception either before or after the development of morphine tolerance. Which isoforms of COX are expressed and what interactions occur among the NMDA, NO and COX systems during morphine tolerance in the spinal cord are worthy of further investigation.

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References
5 Maves TJ, Pechman PS, Meller ST, Gebhart GF. Keterolac potentiates morphine antinociception during visceral nociception in the rat. Anesthesiology 1994; 80: 1094–101
7 Kolesnikov YA, Piek CG, Pasternak GW. NG-nitro-L-arginine prevents morphine tolerance. Eur J Pharmacol 1992; 221: 399–400
8 Trujillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 1991; 251: 85–7
11 Wong CS, Cherng CH, Luk HN, Ho ST, Tung CS. Effects of NMDA receptor antagonists on inhibition of morphine tolerance in rats: binding at \( \mu \)-opioid receptors. Eur J Pharmacol 1996; 297: 27–33
13 Elliott K, Minami N, Kolesnikov YA, Pasternak GW, Inturrisi CE. The NMDA receptor antagonists, LY274614 and MK-801, and the nitric oxide synthase inhibitor, NG-nitro-L-arginine, attenuate analgesic tolerance to the \( \mu \)-opioid morphine but not to \( \kappa \) opioids. Pain 1994; 56: 69–75
14 Wong CS, Hsu MM, Chou YY, Tao PL, Tung CS. Morphine tolerance increases \( [3H] \) MK-801 binding affinity and constitutive


16 Malmberg AB, Yaksh TL. Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. Science 1992; 257: 1276–9


24 Hay CH, Trevechick MA, Wheeldon A, Bowers JS, de Belleruche JS. The potential role of spinal cord cyclooxygenase-2 in the development of Freund’s complete adjuvant-induced changes in hyperalgesia and allodynia. Neuroscience 1997; 78: 843–50
