Antinociceptive Effect of Intrathecal Nefopam and Interaction with Morphine in Formalin-Induced Pain of Rats

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Background:
Nefopam, a non-opiate analgesic, has been regarded as a substance that reduces the requirement for morphine, but conflicting results have also been reported. The inhibition of monoamine reuptake is a mechanism of action for the analgesia of nefopam. The spinal cord is an important site for the action of monoamines however, the antinociceptive effect of intrathecal nefopam was not clear. This study was performed to examine the antinociceptive effect of intrathecal (i.t.) nefopam and the pattern of pharmacologic interaction with i.t. morphine in the formalin test.

Methods:
Male Sprague-Dawley rats were implanted with an i.t. catheter, and were randomly treated with a vehicle, nefopam, or morphine. Formalin was injected into the hind-paw 10 min. after an i.t. injection of the above experiment drugs. After obtaining antinociceptive ED$_{50}$ of nefopam and morphine, the mixture of nefopam and morphine was tested for the antinociceptive effect in the formalin test at a dose of 1/8, 1/4, 1/2 of ED$_{50}$, or ED$_{50}$ of each drug followed by an isobolographic analysis.

Results:
Intrathecal nefopam significantly reduced the flinching responses in both phases of the formalin test in a dose-dependent manner. Its effect, however, peaked at a dose of 30 $\mu$g in phase 1 (39.8% of control) and 10 $\mu$g during phase 2 (37.6% of control). The isobolographic analysis indicated an additive interaction of nefopam and morphine during phase 2, and a synergy effect in antinociception during phase 1.

Conclusions:
This study demonstrated that i.t. nefopam produces an antinociceptive effect in formalin induced pain behavior during both phases of the formalin test, while interacting differently with i.t. morphine, synergistically during phase 1, and additively during phase 2. (Korean J Pain 2013; 26: 14-20)

Key Words:
antinociception, formalin, interaction, morphine, nefopam.
INTRODUCTION

Nefopam has been known to be a non-opioid analgesic, and is structurally unrelated to other analogs like NSAIDs [1]. Although supportive data is still lacking, the inhibition of monoamine reuptake has been suggested as a mechanism for the antinociceptive effect of nefopam [2,3]. It has been widely used, mainly in Europe, for controlling acute postoperative pain and other chronic pain conditions due to its relatively favorable side effects, while also providing beneficial properties like the morphine sparing effect [3–5]. However, different results have been reported on the interactions of morphine and nefopam [8,6–12]. In addition, most of the studies on its antinociceptive effect and the interactions with other analgesics were performed with the systemic administration of nefopam. Spinal nefopam was also shown to have an antinociceptive effect [13,14]. The spinal cord is also an important site of action for monoamines, which mediates descending pain modulation, the direction of which could be either inhibitory or facilitatory, depending on the pain stimuli [15]. In view of the mechanism of nefopam involving the inhibition of monoamine uptake, the nature of the pharmacologic interaction with morphine at the spinal level could be different according to the pain stimuli applied. This study examined the interaction of the intrathecal administration of morphine and nefopam using an isobolographic analysis in the formalin test in which two distinct phases of pain behavior are derived from different mechanisms.

MATERIALS AND METHODS

1. Animals and intrathecal catheter implantation

Male Sprague–Dawley rats weighing 225–250 g were used, with all animals being housed in a room with a constant temperature of 22–23°C and an alternating 12 h light/dark cycle. Water and food were allowed with no limitations. All experiments were performed according to the IASP guidelines for the Use of Animals in Research. The protocol was approved by the Institutional Animal Care and Use Committee, Research Institute of Medical Science, Chonnam National University Medical School.

Animals were implanted with a polyethylene–5 (PE–5) catheter into the i.t space for the administration of the experiment drug according to the previous study [16]. Under general anesthesia using sevoflurane, a PE–5 catheter was introduced through the atlanto–occipital membrane and was advanced caudally, 8.5 cm, to the level of the lumbar enlargement. The other end was externalized through the skin of the top of the head and plugged with a stainless steel wire for drug administration. Rats with a neurological deficit after the catheter implantation were sacrificed immediately with an overdose of inhalational anesthetics. Rats were housed in individual cages after surgery.

2. Nociceptive test and behavioral study

Animals were injected subcutaneously with 50 μl of 5% formalin into the center of the hind–paw of the rat using a 30 gauge needle. The formalin test was conducted with the rats restrained in a cylinder. Intraplantar formalin injection produced a typical flinching response, which has two distinct phases. An initial acute phase (phase I) was a relatively short quiets period, followed by a prolonged tonic response (phase 2 beginning about 10 min after the formalin injection). Phase 1 represents acute nociception, while phase 2 is thought to involve the central sensitization of the dorsal horn neurons, as well as the sensitization of the peripheral neurons [17]. Flinching responses were quantified by counting the number of responses at 1 and 5 min (phase 1, 0–9 min), and every 5 min up to 60 min thereafter, after the injection of formalin (phase 2, 10–60 min). Each count was done for 1 min. The person who carried out the behavioral testing was blind to the treatment.

3. Antinociceptive effect of intrathecal nefopam and morphine

The antinociceptive effect of spinal nefopam hydrochloride (Pharmbio, Korea) and morphine sulfate (Sigma Aldrich, USA) were tested in the formalin test. Animals were placed in a restraint cylinder and allowed to adapt for 20 min before being allocated to receive one of the experimental drugs. They were randomly given nefopam (1, 3, 10, 30 μg), morphine (1, 3, 10, 30 μg), or saline through the i.t. catheter 10 min prior to the formalin injection into the hindpaw. Drugs were injected using a hand gear–driven Hamilton syringe with a volume of 20 μl followed by a flushing with a 10 μl vehicle.

4. Isobolographic analysis of interaction of nefopam with morphine

On completion of the antinociceptive study, dose–response was analyzed and ED50 (a dose that produced
Fig. 1. Formalin induced flinching responses are attenuated dose-dependently by intrathecal injection of morphine in a dose-dependent manner. Time course after formalin injection (top panel), and dose response curve (middle and bottom) are shown. Each line represents the mean ± SEM of 6–8 rats/group. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.
malin significantly reduced the flinching responses at both phases in a dose-dependent manner (Fig. 1). Intrathecal nefopam also significantly attenuated the flinching responses in both phases of the formalin test when compared to the control group (Fig. 2). The antinociceptive effect of intrathecal nefopam was dose-dependent, but the maximum effect was achieved at a dose of 30 μg in phase 1 (39.8% of control), 10 μg in phase 2 (37.6% of control), while higher doses produced no difference in the antinociceptive effect. This finding is consistent with the ceiling effect, which was shown in previous studies.

The ED50 value (95% confidence intervals) of i.t. nefopam was 13.7 (7.9–23.5) μg during phase 1, which is about 1.7-fold larger than the ED50 of i.t. morphine, 8.0 (5.5–11.5) μg. During phase 2, the ED50 of i.t. nefopam was 10.5 (5.0–21.8) μg, which is about 2.5-fold larger than the ED50 of i.t. morphine, 4.0 (3.0–5.2) μg.

2. Antinociceptive interaction of nefopam with morphine

The isobolographic analysis showed that the experimental ED50 (4.5 (3.5–5.8) μg) was significantly lower than the calculated theoretical ED50 (10.8 (7.0–14.6) μg) during phase 1, which indicated a synergistic interaction between nefopam and morphine (Fig. 3). In contrast to phase 1, co-administration of morphine and nefopam produced an additive antinociceptive interaction by showing no significant differences between the theoretical (7.2 (3.5–11.0) μg) and the experimental ED50 (4.6 (3.4–6.1) μg) of phase 2.

DISCUSSION

Multimodal analgesia is widely accepted as a good strategy to obtain optimal levels of analgesia, while reducing opioid-induced side effects [19]. The underlying mechanism of the multimodal analgesia is that the non-opioid drug has a different mode of analgesic action from the opioid, thus allowing the dose of opioid to be reduced, which results in the lowering of side effect incidences. The basic requirement for the employment of this strategy is the synergistic, or at least, an additive interaction between an opioid and another non-opioid drug.

The analgesic effects of systemic nefopam have been demonstrated in animal and clinical studies. However, the mechanism of action remains to be elucidated. Inhibition of monoamine reuptake, including serotonin and catechol-
Fig. 3. Isobologram for the combined intrathecal administration of nefopam and morphine during phase 1 (top panel) and phase 2 (bottom). ED50 with SEM of nefopam and morphine are plotted on x and y axes. The line connecting ED50 of nefopam and morphine on x and y axes is the theoretical additive line. The point (A) is the theoretical ED50 with SEM of drug combination, and point (B) represents the experimental ED50 with SEM. ***P < 0.001 vs. theoretical ED50.

amine, has been suggested as a mechanism for nefopam-induced antinociception, despite the inconsistent results [2,20]. Moreover, most of the documented studies were performed on an in vitro basis, or on an in vivo pharmacological study using systemically or peripherally injected nefopam [21,22]. There have been a few reports on the analgesic effects of i.t. nefopam with equivocal results [3,13,14]. However, the spinal cord was also shown to be a site for analgesic action for nefopam [23], which is supported by the findings of this current study in which nefopam has a dose-dependent antinociceptive effect when administered intrathecally.

The effect of the combined administration of nefopam and morphine has been investigated in several animal pain models, as well as in postoperative pain or opioid-induced hyperalgesia [6-9,24,25]. While several studies have reported a morphine sparing effect of nefopam in a postoperative analgesia setting using the combination of a subanalgesic dose of nefopam or morphine, less or no significant effects of nefopam were observed [8,10-12]. Furthermore, a study using the isobolographic analysis revealed an infra-additive interaction in postoperative pain between intravenous nefopam and morphine [10]. The difference in pain modality originated from the different kinds of surgeries, which could at least partially explain the different results, since animal studies have shown that the descending modulations mediated by serotonin or noradrenaline, the main targets of nefopam, could be inhibitory or facilitatory depending on the pain stimuli [15].

As for animal studies, nefopam has shown to spare other analgesics including paracetamol, COX-2 inhibitor, or NSAIDs, or enhance the efficacy of these analgesics [25-28]. In contrast, the interaction between nefopam and morphine has not been examined in only a few studies, in which nefopam enhanced the analgesic effect of morphine in the pain elicited by chronic constriction injury and carrageenan injection [24,25]. These studies, however, used only a combination of the sub-analgesic dose for both drugs to examine the interaction. To the best of our knowledge, this study is the first to address the interactions of morphine and nefopam using the isobolographic analysis at the spinal level.

The different patterns of interaction in phase 1 and phase 2 are likely to reflect the different mechanisms of the nociceptive behavior [17,29]. The acute pain of phase 1 is derived from the direct stimulation of the peripheral nociceptor, and the facilitated pain of phase 2 involves the increased excitability of the central neurons and the ongoing primary afferent input. Accordingly, the combination of nefopam and morphine could be regarded as being more useful for acute pain than chronic inflammatory pain. However, the additive interaction during phase 2 was different from the enhanced analgesic effect of morphine in the carrageenan-induced inflammatory pain. This discrepancy could be related to the previous findings, in which the descending serotonergic modulation is differentially in-
In conclusion, this study demonstrated that i.t. nefopam produces an antinociceptive effect in the behavior of formalin induced pain during both phase 1 and phase 2, while interacting with i.t. morphine synergistically during phase 1, and additively during phase 2. The results of this study support the usefulness of nefopam as a component of multimodal analgesia, and may facilitate further clinical studies for the optimal use of nefopam.

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