Role of fosaprepitant, a neurokinin Type 1 receptor antagonist, in morphine-induced antinociception in rats

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Abstract:
Objectives: Opioids such as morphine form the cornerstone in the treatment of moderate to severe pain. However, opioids also produce serious side effects such as tolerance. Fosaprepitant is a substance P (SP) receptor antagonist, which is used for treating chemotherapy-induced nausea and vomiting. SP is an important neuropeptide mediating transmission of pain at the spinal level. Thus, it was hypothesized that combining morphine with fosaprepitant would increase the antinociceptive effect of morphine. The objectives were to evaluate the effect of fosaprepitant on morphine-induced antinociception in rats and to investigate its mechanism of action.

Methods: Sprague-Dawley rats were injected with morphine (10 mg/kg twice daily) and/or fosaprepitant (30 mg/kg once daily) for 7 days. Pain threshold was assessed by the hot plate test. Expression of SP and calcitonin gene-related peptide (CGRP) in the spinal cords of these rats was evaluated by immunohistochemistry.

Results: Morphine administration resulted in an antinociceptive effect compared to the control group (day 1 and to a lesser extent on day 4). The decreased antinociception despite continued morphine treatment indicated development of tolerance. Co-administration of fosaprepitant attenuated tolerance to morphine (days 1 and 3) and increased the antinociceptive effect compared to control group (days 1–4). Expression of SP was increased in the morphine + fosaprepitant group.

Conclusions: The results show that fosaprepitant attenuates the development of tolerance to morphine and thereby, increases the antinociceptive effect. This is likely linked to decreased release of SP from presynaptic terminals.

Key words: Breakthrough pain, morphine tolerance, nociception, rodent, thermal escape behavior, withdrawal threshold
neurons, leading to lowering of nociceptive threshold known as central sensitization.\cite{55} Correspondingly, ablation of the NK1r expressing neurons by SP-Saporin complex impairs central sensitization without affecting basal pain sensitivity.\cite{56} Thus, it was hypothesized that combining morphine with an NK1r antagonist such as fosaprepitant might attenuate the development of tolerance to morphine. The NK1r antagonist aprepitant (Emend\textsuperscript{9}) is often used for treating chemotherapy-induced nausea and vomiting.\cite{57} Fosaprepitant (dimeglumine), a water-soluble prodrug of aprepitant, was later introduced in the market. Fosaprepitant administration gives rise to high NK1r occupancy in the CNS for a prolonged period of time (41–75% receptor occupancy at 120 h).\cite{58} The expression of SP in the spinal cord was determined at the end of the behavioral study. Apart from SP, calcitonin gene-related peptide (CGRP) is also present in primary nociceptive afferents and takes part in the process of central sensitization.\cite{59} Its expression was also evaluated.

### Methods

Male Sprague-Dawley rats (n = 24) weighing approximately 250 g were used for the study. Permission for the experimental work was obtained from the Institutional Animal Ethics Committee (767/IAEC/13 dated 1-1-2014). Rats were divided into 4 equal groups and injected physiological saline (twice daily subcutaneously in the morning and evening; Group I), morphine sulfate (10 mg/kg twice daily subcutaneously; Group II), fosaprepitant (30 mg/kg once by intraperitoneal route; Group III), and finally, the last group was co-administered both morphine and fosaprepitant (Group IV) in the same doses as in Group II and III, respectively, for a week. In the last group, fosaprepitant was injected 30 min before the morphine injection. The dose of fosaprepitant (30 mg/kg) was higher than that used in an earlier study (25 mg/kg).\cite{60} The approximate LD\textsubscript{50} is >200 mg/kg in rats. The rationale for selecting a higher dose was the rapid conversion of fosaprepitant to aprepitant (half-life ~30 min) in the rat compared to dogs and humans. Hot plate latency or the thermal escape behaviour was determined in the morning, 40 min after saline (Group I), fosaprepitant (Group III), or morphine injection (Group II and IV). This time point was previously standardized as corresponding with maximum antinociceptive effect of morphine. Testing for latency was done at 24 h intervals. The hot plate test is commonly employed for screening the putative antinociceptive property of drugs. The advantages of this test are the brief nature of the noxious stimulus, which does not produce any tissue damage and that multiple testing can be done in the same animal.\cite{61,62} The predictability of this test to clinical situations is high for morphine and related opioid substances.\cite{63}

Animals were initially acclimatized to the testing chamber for 15 min at room temperature. Testing was done in a quiet room with the ambient temperature between 22°C and 25°C. On the day of the experiment, the testing platform of the hot plate apparatus (Stoelting, USA) was set at a constant temperature of 52.5°C. The rats were placed on the hot plate, and the behavioral end points were either licking of the hindpaw or jumping.\cite{64} The test was repeated thrice at 5–7 min intervals and the average of these values was the latency period (sec). A 40 s, cut-off was fixed to prevent damage to the completely analgesic paw after morphine injection. Transformation of these values was done by calculating the percent maximum possible effect (%MPE) as follows:

\[
\text{(%MPE) = } \left( \frac{\text{Drug induced latency − basal response time}}{\text{40 s − basal response time}} \right) \times 100
\]

On day 8 (morning), rats were anesthetized by pentobarbitral injection (100 mg/kg intraperitoneal). This was followed by intracardiac perfusion with 4% paraformaldehyde solution in 0.1 M phosphate buffer saline. The cervical enlargement (upper part) was isolated after laminectomy. Transverse sections of the spinal cord (20 µm thick) were obtained in a cryostat and processed for immunohistochemical localization of SP and CGRP using specific antibodies (anti-SP antibody, Abcam, UK; anti-CGRP antibody, Calbiochem, USA; 1:500 titer) using the Avidin-Biotin complex method (Vector Labs, USA).\cite{65} Representative sections (3/rat; systematic random sampling) were later viewed under a microscope and the images captured. The expression of SP and CGRP in the superficial laminae (Rexed’s laminae I-II) of the gray matter was quantitated by Image J software (NIH, USA). Specific expression was obtained after deducting background staining in the white matter (lateral funiculus) from the total value obtained from the superficial laminae. Some of the cryostat sections were stained with 0.5% Cresyl violet for localization of neurons in the dorsal horn.

Statistical evaluation of data was done by GraphPad Prism version 5 (GraphPad software, La Jolla, San Diego, USA). Values of latency period of the different groups of animals were independently analyzed at each time point by one-way analysis of variance followed by Bonferroni multiple comparison test. P < 0.05 was considered statistically significant. Values are represented as mean ± standard error of mean. Each experimental group had 6 animals.

### Results

The various groups of rats had different baseline values, which affected the subsequent comparison of the antinociceptive effect of the drugs [Figure 1a]. Hence, normalization of the data was done by calculating the %MPE [Figure 1b]. The %MPE values of control and the fosaprepitant-treated groups did not show any statistically significant change during the experiment. Morphine injection produced significantly higher antinociceptive effect on day 1 (P < 0.001) and to a lesser extent day 4 (P < 0.05) compared to the control group. The sharp decrease in the antinociceptive effect from day 2 in the morphine treated group indicated the development of tolerance. Morphine + fosaprepitant combination delayed the onset of tolerance in comparison to morphine treated group on days 1 (P < 0.01) and 3 (P < 0.001). Compared to control, antinociception was higher between days 1 and 4 (P < 0.001) for this group.

Cresyl violet staining showed the dorsal horn neurons arranged in various laminae [Figure 2]. Immunohistochemical study revealed the expression of SP and CGRP over the superficial laminae (laminae I-II) of the dorsal horn [Figure 2]. Quantitative image analysis revealed increased SP expression in the morphine + fosaprepitant treated group compared to others [Figure 3]. Statistically significant difference was present between morphine and morphine + fosaprepitant co-treatment.
Discussion

The Aδ and C groups of nerve fibers carry pain from the periphery to the spinal cord. Majority of these fibers end in laminae I and II of the dorsal horn. The peptidergic subgroup of these nerve fibers contains neuropeptides such as SP and CGRP, which are released into the synaptic cleft following noxious stimulation. These neuropeptides diffuse to the postsynaptic terminal, where they bind to the corresponding receptors. Morphine-induced antinociception is linked to decreased release of glutamate, SP, CGRP, etc., from presynaptic terminals and greater postsynaptic hyperpolarization due to the activation of inwardly rectifying potassium channels.[23] Both these effect decrease onward neurotransmission of pain signals. Co-administration of fosaprepitant possibly interfered with the binding of SP to the NK1r expressed by postsynaptic neurons in the spinal cord. The results of this study show that combining morphine with fosaprepitant (Group IV) delayed the development of morphine tolerance and concurrently increased the antinociceptive effect compared to the control group. As mentioned earlier, this could be due to decreased binding of SP to the NK1r. Evidence from electrophysiological experiments indicates that noxious stimuli produce slow and prolonged excitatory potentials in postsynaptic dorsal horn neurons, which are inhibited by NK1r antagonist.[18] Since fosaprepitant treatment (Group III) alone did not result in an antinociceptive effect, the interaction between morphine and fosaprepitant is likely synergistic in nature. Previously, injection of a bifunctional peptide having both μ-opioid receptor agonist and NK1r antagonist properties relieved pain in neuropathic rats without producing tolerance.[19] In a different study, Tumati et al. reported that intrathecal administration of both morphine and a NK1r antagonist reduced subsequent opioid withdrawal-induced hyperalgesia.[20] Paradoxically, a SP-opioid receptor agonist chimera inhibited development of opioid tolerance.[21] Pharmacokinetic interaction between fosaprepitant/aprepitant and morphine could also contribute to the enhanced antinociceptive effect. Aprepitant is mainly metabolized by CYP3A4 enzyme.[22] N-Demethylation of morphine by CYP3A4 is an important biotransformation pathway in rodents. Hence, morphine concentration in the nervous system could be increased by concurrent administration of these drugs.

Significant alterations in the expression of SP and CGRP were not observed except in the group treated with morphine + fosaprepitant combination (Group IV). This was unexpected because prevailing evidence suggests that NK1r does not regulate the release of SP in the spinal cord. However, contrary to this, NK1r has been recently reported to be crucial for release of SP from dorsal root ganglion neurons.[23] Thus, a possibility exists that fosaprepitant decreased release of SP from presynaptic terminals. Moreover, it has been speculated that there could be SP autoreceptors on presynaptic terminals, which can modulate SP release.[24] But, fosaprepitant treatment
alone did not increase SP immunoreactivity in the present work. As noted earlier, morphine inhibits the release of SP under acute conditions but rats chronically treated with morphine in our study did not show a statistically significant increase in SP expression. Presumably, both NK1 autoreceptors as well as morphine-induced inhibition of SP release might have contributed to the increased SP expression in Group IV [Figure 4]. A limitation of this study was that the expression of SP and CGRP was evaluated at the end of the observation period and not between days 1 and 4, when the antinociceptive effect was maximum. Another limitation was the use of a fixed-dose combination of morphine and fosaprepitant.

The antinociceptive effect of morphine decreased rapidly following daily administration, indicating development of tolerance. Morphine tolerance has been reported to be counter-adapting processes, which maintains the status quo in the spinal cord. Similarly, the potentiation of the antinociceptive effect of morphine by fosaprepitant is also lost on continued administration (day 5 onward). Identical result was reported with a chimera possessing both opioid agonist and NK1r antagonist properties. Morphine tolerance is a complex phenomenon with several factors contributing to it. Moreover, the factors can differ depending upon the specific µ-opioid receptor agonist used for producing the tolerance.

**Conclusion**

The results indicate that addition of fosaprepitant to morphine can delay morphine tolerance for a limited period of time. This information could be used to treat breakthrough pain in cancer patients. To the best of our knowledge, this is the first report on the novel antinociceptive effect of morphine + fosaprepitant combination. Further studies are required to further elucidate this novel antinociceptive effect of morphine + fosaprepitant combination.

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**Conflicts of Interest**

There are no conflicts of interest.

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