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Short Communication

Can repeated exposure to morphine change the spinal analgesic effects of lidocaine in rats?*

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Abstract

BACKGROUND: Chronic opium exposure leads to altered response to opioid compounds. The aim of this study was to assess the behavioral effects of opium tolerance on the analgesic effects of intrathecal lidocaine in rats.

METHODS: Twenty-four adult male Sprague Dawley rats with intrathecal (IT) catheters were divided into 3 groups of 8. The first group was morphine tolerant and received IT lidocaine (ML). Rats in the second group were not morphine tolerant and received IT lidocaine (L), while the third group consisted of not morphine tolerant rats that received IT placebo. Tail flick test was done and maximal possible antinociceptive effects (MPAE) were compared using analysis of variance (ANOVA).

RESULTS: While percent of MPAE significantly increased in the L group, it had a significant reduction in the ML group ($P < 0.001$).

CONCLUSIONS: After intrathecal lidocaine administration, a hyperalgesic response was seen in morphine tolerant rats and an analgesic response was seen in the lidocaine group.

KEYWORDS: Morphine, Intrathecal, Lidocaine, Local Anesthetic.

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Chronic opium exposure leads to a number of physiologic alterations[1] especially regarding both clinical and receptor-level response to opioid compounds including neuroplastic changes in the spinal cord and response to anesthetic drugs.[2, 3] In addition, chronic use of opioids causes abnormal pain states[1-4] and enhanced abnormal sensitivity to painful stimuli.[5] Besides, decreased effect of intrathecal local anesthetics has been detected in chronic opium abusers undergoing spinal anesthesia with local anesthetic drugs.[6, 7] Some studies have noted the effect of analgesics in increasing spinal acetylcholine.[8] Others have demonstrated decreased efficacy of spinal morphine possibly due to the reduced influence through brainstem-spinal pathways.[9, 10] However, since no previous study has focused on the cross tolerance between them, it is not still clear how the effect of lidocaine in spinal anesthesia is decreased among opium abusers. The aim of this study was to assess the effects of opium tolerance on the behavioral analgesic response after intrathecal lidocaine administration in rats.

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Methods

This study was the result of a university research proposal (project number: 87-01-141-5601) financially supported by the Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

After ethical approval of the research by the institutional review board (IRB), 24 adult male Sprague Dawley rats weighing 250-280 grams were divided into three groups of eight. Rats were bred using standard animal housing (individual cages, having available food pellets, free water, and a 12-hour dark and light cycle). They were 10 to 14 weeks old and matched by age. All the animals out of this classification or with failed catheter implantation were excluded.

The first group included opium tolerant rats which were implanted with intrathecal catheters for intrathecal administration of lidocaine (the ML group). The rats of the second group were not opium tolerant but had intrathecal catheters implanted for intrathecal lidocaine administration (the L group). Finally, the rats of the third group were not opium tolerant while they had intrathecal catheters to receive intrathecal normal saline as placebo (the control group).

In order to insert intrathecal catheter, intraperitoneal ketamine was administered for analgesia and the rats were cannulated with chronic indwelling intrathecal catheter (Yaksh and Rudy method).[11] After a midline incision on the skull, from a line between the ears to a point 2 cm caudal, the fascia was retracted from the skull, about 0.5 cm on either side of the midline.[11] Using a stereotaxis device, implantation of intrathecal polyethylene (PE)-10 catheters was done rostrally for 7 to 8 cm, passing the lumbar enlargement of the spinal cord. Finally, a 5-day recovery period was allowed.

The first group (ML) became tolerant to morphine sulphate compound (10 mg vials, Daroupakhsh, Iran) using two subcutaneous injections of 10 μg/g of body weight per day for 5 days (Javan et al.).[12] Tail flick test was performed (Mao et al. and Liu et al.)[2, 3] every morning, before and after morphine injection and the results were recorded. On the last day, intrathecal lidocaine was administered and tail flick test was done before and after intrathecal lidocaine administration.

The second group (L) did not receive subcutaneous morphine injections. However, intrathecal lidocaine was administered and tail flick test was done like the first group. Although the tail flick test was performed on the third group (control group) with exactly the same procedure followed for the other 2 groups, they did not receive morphine or intrathecal lidocaine. Instead of lidocaine, the same volume of normal saline (considered as placebo) was administered through the intrathecal catheter.

In order to make rats accustomed to restrainer devices and to prevent their agitation on the day of the test, every rat had a restrainer device in his cage for 3 days before tail flick test. Finally, they were anesthetized using ketamine and put under a CO2 hood until they died.

The routine tail flick test had baseline latencies of 4–6 seconds and a cutoff time of 10 seconds for antinociceptive effects of morphine.[2, 12-14]

The percentage of maximal possible antinociceptive effect (%MPAE) was calculated according to the following formula:

$$\%MPAE = [(TL - BL)/ (cutoff - BL)] * 100$$

in which BL is test latency before the test and TL is latency after drug injection.[2, 10, 12-14]

Data entry and analyses were performed by SPSS 11.5. Analysis of variance (ANOVA) was used to compare the three groups. A $p < 0.05$ was considered significant.

Results

The results of the tail flick test were calculated as %MPAE and are presented in Table 1. A significant difference between the 3 groups was observed regarding % MPAE, i.e. the highest scores for MPAE were seen in the pure lidocaine group (26.54 ± 27.62), while the morphine tolerant group (20.25 ± 8.44) and the control group (1.18 ± 4.42) stood next (ANOVA test; $F = 15.4$; $DF = 23$; $p < 0.001$). Therefore, a

significant increase in MPAE was observed in the lidocaine group (that were not made opium tolerant) compared with the placebo group. Moreover, there was a significant increase in MPAE in the lidocaine group (that were made opium tolerant) compared with the placebo group. The differences were found after ANOVA and its post hoc analysis which resulted in a statistically significant difference between the three groups.

Table 1. The results of the percentage of maximal possible antinociceptive effect in the 3 rat groups (8 rats in each group).

Group	Mean	Standard Deviation
ML	20.25	8.44
L	26.54	27.62
Control	1.18	4.42

Discussion

The results of this study demonstrated a statistically significant increase in %MPAE among rats that were not tolerant to morphine but received intrathecal lidocaine compared with the control group. A significant increase in %MPAE was also detected among rats that were morphine tolerant and received intrathecal lidocaine.

These results suggested that morphine tolerant rats had a hyperalgesic response to the tail flick test after administration of intrathecal lidocaine, while administering the same dose of intrathecal lidocaine with the same method to the other group (not tolerant to morphine) in similar conditions resulted in analgesic properties expressed as an increase in %MPAE. This hyperalgesic response to the tail flick test after intrathecal administration of lidocaine in morphine tolerant rats has not been reported before in similar studies of rats.

On the other hand, a state has been clinically seen in opium abusing patients when receiving intrathecal lidocaine[6] or bupivacaine[7] in which the duration of local anesthetics was shortened.

Therefore, as previously shown,[14-16] chronic morphine administration caused desensitization of the spinal cord receptors to morphine in rats in our study. In addition, Mao et al. found chronic morphine use to cause down regulation of spinal glutamate transporters and abnormal pain sensitivity.[2] In this study, morphine tolerant rats expressed an unpredicted response to the tail flick test after intrathecal administration of lidocaine.

This study has an aspect of novelty in explaining the human model, i.e. the clinical model applied in this study was not ever reported before[6, 7] in clinical or animal models resembling the opium abuser patients undergoing spinal anesthesia for surgery.[8-10, 12, 16]
A similar study did not create a model of opium tolerant rats and concluded that intrathecal infusion of lidocaine in combination with intrathecal infusion of morphine could not develop cross-tolerance.[10] Likewise, another study on rats demonstrated functional synaptic connections mediating tonic descending inhibition in the neonatal rats but did not indicate lack of morphine analgesia or barbiturate analgesic characteristics.[16]

Furthermore, this study found that when spinal cord receptors of rats encountered morphine, a change in their response happened and the antinociceptive response was changed to an unexpected hyperalgesic response expressed in this study as decreased %MPAE and hyperalgesia.

Spinal cord mediators could change the pain toleration process including glutamate[8-10, 12, 17] and adenylyl cyclase.[18-20] Opioid drugs affect through the process of activating inhibitory guanine nucleotide-binding regulatory protein-linked mu, delta, and kappa opioid receptors[21, 22] in which adenylyl cyclase type 5 receptor is an important component.[23, 24]

There were a number of limitations in our study. First, the study mandates a complementary assessment of mediators like adenylyl cyclase and glutamate. Second, the study could be completed after assessment of cerebrospinal fluid of the opium abuser patients after spinal anesthesia. Third, a complementary study to assess possible neuroplastic changes in the receptors of the spinal cord neurons in morphine tolerant rats would be necessary.

Conflict of Interests
Authors have no conflict of interests.

Authors' Contributions
AD planned and finalized the study, performed statistical analyses and prepared the first and final versions of the manuscript. HSM planned and finalized the study and took part in manuscript preparation. SFM provided assistance in laboratory phase and manuscript preparation. ZM took part in planning and laboratory phase and reviewed the manuscript. SR took part in design, statistical analyses, and laboratory phase of the study and reviewed the manuscript, too. All authors read and approved the final manuscript.

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