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Original Article

The antinociceptive effects of folic acid using formalin and acetic acid tests in male mice

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Abstract

Background and aims: Antidepressant agents such as imipramine are clinically used to control and treat different types of pain, especially neuropathic pain. Studies have shown the antidepressant-like activity of folic acid (FA). This study aimed to investigate the potential antinociceptive effects of FA using formalin and acetic acid tests in male mice.

Methods: Sixty male albino mice (20-30 g) were randomly divided into 10 groups (n=6 in each group) of negative control, positive control (morphine or indomethacin), and FA (10, 15, and 30 mg/kg) groups. In the formalin test, duration of paw licking and biting the right hind paw during acute (0-5 minutes) and chronic (15-60 minutes) pain after intraplantar injection of formalin 2.5% (25 µL) was recorded. In the writhing test, the abdominal constrictions were recorded after the intraperitoneal injection of acetic acid 1%.

Results: Only a high dose (30 mg/kg) of FA significantly reduced acute pain ($P=0.001$) compared with the control group. But all doses of FA significantly decreased chronic pain ($P=0.001$). In addition, morphine significantly reduced both phases of pain ($P=0.020$ and $P=0.001$, respectively). Moreover, indomethacin and all doses of FA decreased the number of abdominal constrictions induced by acetic acid ($P=0.001$).

Conclusion: Compared with acute (neurogenic) pain, FA more potently decreases chronic (inflammatory) pain. Furthermore, FA decreases the parietal pain that could potentially represent antinociceptive effect. However, further studies are needed to elucidate the exact mechanism of FA's analgesic activity.

Keywords: Folic acid, Antinociceptive, Antidepressants, Formalin test, Writhing test, Mice

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Introduction

Pain is a common and uncomfortable complication of many disorders such as tumors, surgical procedures, physical trauma, and dangerous chemical stimulations (1). Pain can be acute or chronic. Acute (neurological or first phase) pain is the result of a sudden and rapid injury to a member and disappears as the causative agent is removed. However, chronic (inflammatory or second phase) pain is long-lasting and is the result of injuries caused throughout long periods (2). Studies have identified that various neurotransmitters such as opioids, glutamate (GLU), substance P (SP), serotonin (5-HT), noradrenaline (NA), histamine (His), nerve growth factor (NGF), adenosine and adenosine phosphate, and nitric oxide (NO), as well as capsaicin and vanilloid receptors are involved in pain pathophysiology (3-8). Currently, different drugs are used to alleviate pain including opioid analgesics (such as morphine) and non-steroidal anti-inflammatory agents (NSAIDs) such as indomethacin. But conventional analgesics cause various side effects such as drowsiness, nausea, respiratory weakness, bleeding, and gastrointestinal ulcers that limit their clinical use. Therefore, it is currently essential to seek out new analgesic agents with fewer side effects (9).

Apart from conventional analgesics, some studies have emphasized the antinociceptive effects of antidepressants, such as imipramine. In addition, antidepressants are clinically used to control and treat different types of pain, especially neuropathic pain (10-14).

Folic acid (FA) or folate is a water-soluble vitamin. The human body is unable to synthesize FA and therefore must receive it from food. A small amount of FA passes through the brain and its effects on the brain depend on its passage rate from the blood-brain barrier. FA plays a critical role in the function of the central nervous system (CNS). Moreover, recent studies have demonstrated the antidepressant-like effect of FA in animal models of depression. Studies have also shown the role of

serotonergic, noradrenergic, and opioidergic systems in the antidepressant-like effects of FA (15,16). Taking into account the possible increase in the levels of some neurotransmitters (e.g. 5HT and NA) after FA treatment and the antinociceptive potential of antidepressants and since there has not yet been any report on the antinociceptive effects of FA, the present study aimed to investigate the antinociceptive effects of FA using formalin and acetic acid tests in male mice.

Materials and Methods

Animals

This experimental study was carried out in West Azerbaijan (Urmia, Iran) in 2015. For this purpose, male albino mice (20-30 g) were purchased form animal house of Urmia University (Urmia, Iran). Animals were maintained in standard conditions (23± 2°C, 12 hours light/dark cycles and 60%-65% humidity) and cages were kept separately. During this period, the animals had access to standard food and tap water. All experiments were performed during the illumination period, i.e., from 8 AM to 14 PM.

Drugs

Morphine sulfate (Temad, Iran), FA (Sigma, USA), indomethacin (Arya Pharmaceutical Co), and formalin (Merck, Germany) were used in this study. The drugs were dissolved in normal saline (NS) 0.9% and administered through intraperitoneal (i.p.) and intraplantar (i.pl.) routes at a constant dose of 10 mL/kg and 25 µL per hint paw (right), respectively. We used 1 N NaOH for adjusting the pH.

Experimental design

Formalin test groups

In this test, 30 mice were divided into five groups (n=6 in each) as follows:

Group 1: Negative control group received i.p. NS at 10mL/kg, and 15 minutes later formalin (25 µL) was injected into their paws.

Group 2: Positive control or morphine group received i.p. morphine at 5 mg/kg, and 15 minutes later formalin (25 µL) was injected into their paws.

Groups 3-5: These groups received i.p. different doses of FA at 10, 15, and 30 mg/kg 15 minutes before the beginning of the test, and 15 minutes later formalin (25 µL) was injected into their paws.

Writhing test groups

In this test, 30 mice were divided into five groups (n=6 in each) as follows:

Group 1: Negative control group received i.p. NS at 10 mL/kg, and 45 minutes later acetic acid 1% (10 mL/kg) was injected by i.p. route.

Group 2: Positive control received i.p. indomethacin at 10 mg/kg, and 45 minutes later acetic acid 1% (10 mL/kg) was injected by i.p. route.

Groups 3-5: These groups received i.p. different doses of FA at 10, 15, and 30 mg/kg 15 minutes before the beginning of the test, and 45 minutes later acetic acid 1% (10 mL/kg) was injected by i.p. route. In this study, drug doses and administration schedules were selected based on previous studies (15-18).

Formalin paw test

In this test, a glass box (30 × 30 × 30 cm^3) with mirrors (inserted at a 45-degree angle) was used to enable a total panorama of pain-related behaviors. To induce pain, the animals were injected under the plantar surface skin of the right hind paw with 25 µL formalin (2.5%) using a 30-gauge needle. In the formalin test, licking or biting of the injected foot was considered as a response to painful stimuli and measured in seconds using a chronometer (Casio, Japan). This test consists of two phases, the first interval of 0-15 minutes was considered as the acute phase and the second 15-60 min interval following injection as the chronic phase (19). In this test, mice were placed in a glass container for 30 min three times a day before the experiments.

Acetic acid-induced writhing test

To induce parietal pain, the animals were injected i.p. with 1% acetic acid (10 mL/kg). The results regarding writhings were recorded 10 minutes after acetic acid injection and counted for 10 minutes using a counter (Haley counters, China). Antinociceptive activity was distinguished by a decrease in the average number of writhings in the treatment groups compared with the control group (20).

Statistical analysis

The data were expressed as mean ± SEM (standard error of mean) and analyzed by one-way ANOVA followed by Tukey's post hoc test. Since our data were normally distributed according to the Kolmogorov-Smirnov normality test, parametric statistics was used. $P<0.05$ was considered as significant level. All statistical analyses were performed using SPSS software version 22.

Results

The effect of different doses of FA on formalin-induced acute pain in male mice

As illustrated in Figure 1, only a high dose (30 mg/kg) of FA significantly ($P=0.001$) reduced acute pain (first phase) compared to the control group ($F(4, 25)=16.789$, $P=0.001$).

The results also showed that a high dose (30 mg/kg) of FA significantly decreased the acute pain more potently than morphine (5 mg/kg) ($P=0.002$), but there was no significant difference between morphine and other doses of FA ($P=0.235$ and $P=0.885$, respectively).

The effect of different doses of FA on formalin-induced chronic pain in male mice

As illustrated in Figure 2, all doses of FA significantly ($P=0.001$) reduced chronic pain (second phase) compared

to the control group (F (4, 25) =27.827, $P=0.001$). The results also showed that all doses of FA reduced chronic pain even more potently than morphine ($P=0.041$, $P=0.013$, and $P=0.001$, respectively).

The effect of different doses of FA on acetic acid-induced writhing

As illustrated in Figure 3, all doses of of FA and indomethacin significantly ($P=0.001$) reduced the number of writhings induced by acetic acid compared to the control group (F (4, 25) =56.062, $P=0.001$). Furthermore, indomethacin decreased the number of writhings more potently than 10 mg/kg of FA ($P=0.001$).

Discussion

The results of our study showed that only a high dose (30 mg/kg) of FA reduced acute pain but chronic pain was significantly reduced by all doses of FA (10-30 mg/kg). Furthermore, a high dose and all doses of FA reduced acute and chronic pain more potently than morphine (5 mg/kg), respectively. Following formalin injection in mice, two phases of pain are noted: first-phase or neurogenic

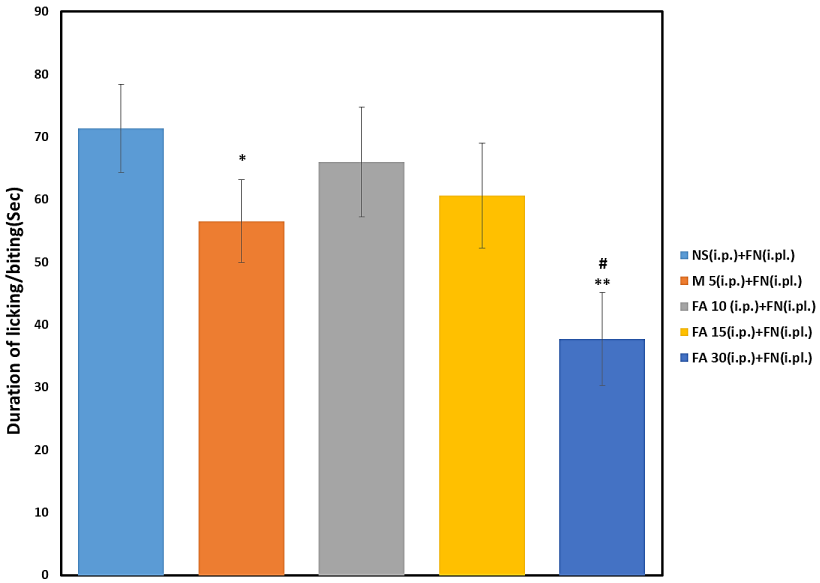

Figure 1. Effects of i.p. injection of morphine (5 mg/kg) and different doses of folic acid (10, 15 and 30 mg/kg) on 1st phase (0-5 min) response to formalin-induced pain in mice.
* $P<0.05$ and ** $P<0.01$ vs control group (NS+FN); # $P<0.05$ *vs* morphine group (M+FN).
NS: normal saline; FN: formalin; FA: folic acid; M: morphine; i.p.: intraperitoneal; i.pl: intraplantar.

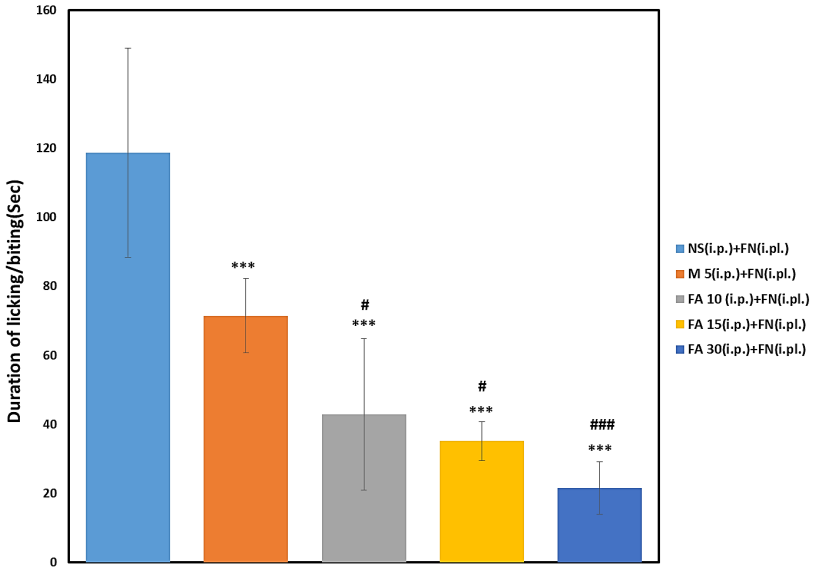

Figure 2. Effects of i.p. injection of morphine (5 mg/kg) and different doses of folic acid (10, 15 and 30 mg/kg) on 2nd phase (20-40 min) response to formalin-induced pain in mice.
*** $P<0.001$ *vs* control group (NS+FN); # $P<0.05$ and ### $P<0.001$ vs morphine group (M+FN).
NS: normal saline; FN: formalin; FA: folic acid; M: morphine; i.p.: intraperitoneal; i.pl: intraplantar.

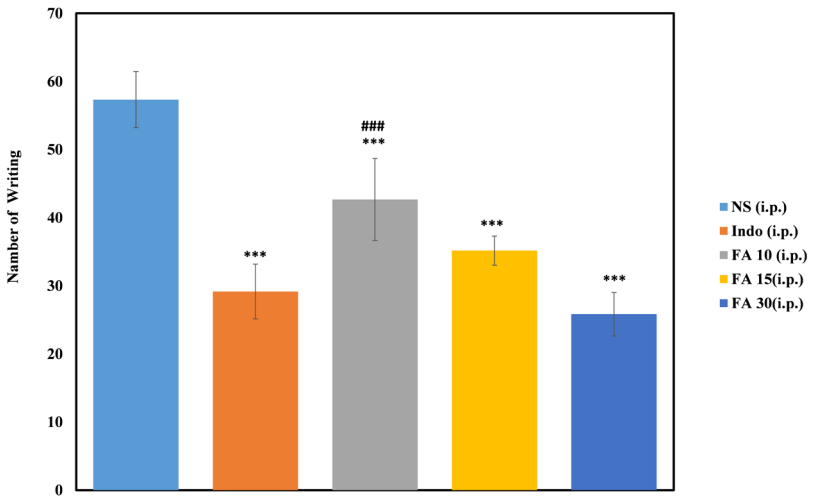

Figure 3 . Effects of i.p. injection of indomethacin (10 mg/kg) and different doses of folic acid (10, 15 and 30 mg/kg) on acid acetic-induced writhing in mice. *** $P<0.001$ *vs* control group (NS); ### $P<0.001$ vs indomethacin group (Indo). NS: normal saline; Indo: indomethacin; FA; folic acid; i.p.: intraperitoneal.

pain induced by the direct activation of pain receptors such as nociceptors, that is comparably more sensitive to drugs acting through modulation of the opioid system; but second-phase or inflammatory pain (delayed phase) is caused by a combination of environmental factors and the spinal shock (21). In this regard, numerous reports have shown that drugs that primarily act on the CNS can inhibit both phases of pain. However, along with these drugs that act peripherally such NSAIDs, they mildly inhibit the initial phase of formalin-induced pain (2). Therefore, according to the results of the present study, FA may both peripherally and centrally decrease both phases of formalin-induced pain in mice. Consistent with our results, studies have shown that morphine reduces inflammatory pain by producing a peripheral effect, in addition to the central effect. Thus, both pain phases are inhibited by morphine (21).

The acetic acid-induced writhing is commonly described as a peripheral assessment of analgesic effect of different medicines (22). In this regard, all doses of FA reduced the number of writhings (parietal pain) induced by acetic acid, with the effect of the high dose of FA being similar to indomethacin. Our results are in accordance with the findings of Aoki et al that showed the antidepressants, such as imipramine and fluvoxamine enhance the antinociceptive effects of carbamazepine in the acetic acid-induced writhing test in mice (23). In addition, indomethacin decreases the number of writhings induced by acetic acid.

Opioid receptors are found in the peripheral ends of afferent nerve fibers so that the axonal transmission of these receptors increases in inflammation. Opioid-induced peripheral analgesia seems to be a physiological antinociceptive response because the number of endogenous opioids in the inflammatory tissue increases. In the ventromedial nucleus of the hypothalamus, the analgesic transmission is divided into two pathways, one is serotonin-mediated and the other is noradrenaline-mediated. Both pathways decrease via periaqueductal gray (24,25). Therefore, since NA and 5-HT are involved in the nociceptive (analgesic) pathway, antidepressants are also effective in controlling pain (26). In this regard, studies have shown that the serotonergic, noradrenergic, and opioidergic systems modulate the antidepressant-like effect of FA in animal models of depression (15,16).

A study has shown that the antidepressants, especially those that increase 5-HT and NA concentration, have numerous uses in the treatment of pain, especially chronic pain syndrome such as neuropathic pain, which is absolutely consistent with the results of the present study showing that FA was more effective in controlling or reducing chronic pain than acute pain (27). The selective serotonin reuptake inhibitors and selective noradrenaline reuptake inhibitors antidepressants act presynaptically on nociceptive transmission and increase potassium currents, thereby reducing the excitability of neurons involved in noxious stimuli (inhibiting the release of GLU and SP). In other words, NA and 5-HT, which act presynaptically, can increase the release of γ-aminobutyric acid and enkephalins. Some antidepressants may inhibit the excitability of neurons or antagonistic effects on GLU or His receptors (28,29). This analgesic effect of antidepressants on mixed receptors is stronger than that on single receptors. However, antidepressants that work on the noradrenergic system are also more potent than the serotonergic antidepressants (30, 31). In other words, the tricyclic antidepressants relieve pain more potently than selective serotonin reuptake inhibitors, but newer antidepressants cause fewer side effects than tricyclic antidepressants (32). Therefore, FA may also decrease both types of pain (acute and chronic pain) induced by formalin and parietal pain induced by acetic acid in mice through these mechanisms.

Conclusion

The results of the present study, for the first time, showed that FA decreased chronic (inflammatory) pain more potently than acute (neurogenic) pain. Moreover, FA decreases the parietal pain induced by acetic acid. Nevertheless, the main limitations of the present study are the lack of other animal models of pain, the lack of assessment of anti-inflammatory property, and evaluation of the exact action mechanism of FA. However, further studies are needed to determine the exact analgesic mechanism of FA.

Conflict of Interests

The authors of the article have no conflict of interests to disclose.

Ethical Approval

The study protocol was approved by the Research Ethics Committee of Urmia Branch, Islamic Azad University, Urmia, Iran (IR.IAU.REC.1394.1019).

Authors' Contribution

MH carried out tests and collected the data. SAM designed the study and analyzed the data.

SAM wrote and revised the manuscript. All authors approved the final version of the manuscript.

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