Antinociceptive effect of amitriptyline in mice of acute pain models

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Tricyclic antidepressant drugs induce antinociceptive effect and suggest that their analgesic action could be related to the monoaminergic activity of the drugs. The analgesic activity of amitriptyline was observed in mouse models of acute pain. Mice were divided into different groups and were given amitriptyline in different doses alone and in combination with morphine. Reaction time in Hot-Plate and Tail-Flick tests was observed. Results showed that amitriptyline had antinociceptive effect in acute pain state in experimental models. Amitriptyline in combination with morphine had better analgesic effect than the morphine alone in Hot-Plate test.

Keywords: Acute pain, Amitriptyline, Antinociception, Mice

Acute pain is a protective response to injury and most often it is nociceptive (i.e. resulting from injury or inflammation of somatic or visceral tissue)\(^1\). Studies indicate that specialized neural pathways are involved in transmission of pain and these pathways are sensitive to changes in stimulus features, such as intensity, quality and duration which are modulated by opioid peptides, serotonin and norepinephrine\(^2\). Transmission of painful stimulus through spinal column and CNS is modulated by excitatory and inhibitory neurotransmitters, as well as action at sodium and potassium channels; norepinephrine and serotonin may be excitatory or inhibitory but they are inhibitory on pain transmission\(^3\).

Nociceptive pain is usually treated with anti-inflammatory or analgesic agents\(^4\). Non-steroidal anti-inflammatory drugs suppress noxious signals by reducing the sensitivity of peripheral nociceptors and opioid drugs are administered to provide long-lasting pain relief\(^5\). Continued and prolonged use of narcotics in patients with pain is not recommended because of serious behavioral consequences, the development of tolerance, and dependence liability. Long-term use of analgesics usually produces behavioral complications that are more difficult to manage than the pain it was desired to eliminate\(^6\).

The recent literature on pain study shows that pain threshold is relatively constant for an individual, but pain tolerance is influenced by psychological state. For example, the patients with acute pain show normal personality profile, but the degree of pain experienced is related to the degree of anxiety present\(^4\). Psychotropic medication, particularly the tricyclic antidepressants, sometimes in combination with phenothiazines and anti-histamines, are effective in many instances of central pain and help increase the tolerance and decrease the need for the narcotics in other pain states\(^4\).

Metanalyses\(^5\),\(^6\) have confirmed the efficacy of tricyclic antidepressants in the treatment of neuropathic pain\(^1\). Tricyclic antidepressant drugs induce antinociceptive effect and it is suggested that their analgesic action could be related to the monoaminergic activity of the drugs\(^7\). However studies on antinociceptive effect of these agents in acute pain management are lacking to support their use in such a pain state, though the amitriptyline has shown to have some analgesic effect in patients with acute pain\(^8\). Therefore the present study involves the antinociceptive effect of amitriptyline in acute pain models of mice through Hot-Plate and Tail-Flick tests.

Materials and Methods

**Animals**— Experiments were performed on adult albino mice (n = 50) weighing 20-30 g. The mice were maintained under controlled room temperature (25°±2°C) and light and dark (12:12 hr) conditions and were given food pellets and water *ad libitum*. Before conducting the experiment, ethical clearance...
was obtained from the Local Ethical Committee on Animal Research and ethical guidelines for investigations of experimental pain in conscious animals were followed in accordance with IASP\(^9\) (International Association for the Study of Pain). The mice were randomly divided into following five groups of 10 each: group A (control – saline), group B (standard control – morphine, 10mg/kg), group C (low dose amitriptyline, 10mg/kg), group D (high dose amitriptyline, 50mg/kg), group E (morphine, 5mg/kg + amitriptyline, 25mg/kg). Drugs were injected intraperitoneally 30 min prior to the experiment. Pain was induced by placing the mice in the Hot-Plate meter and Tail-flick instrument.

**Hot-Plate test** — The thermal noxious stimulus was produced in the mice by placing them on the hot-plate (Hot-plate, UGO BASILE, Italy) maintained at 55°C and the reaction time was recorded. Reaction time was taken as the period between placing the mice on the hot-plate and time when they licked their paws. A cut-off time of 30 sec was used to prevent any thermal injury to mice\(^{10,11}\).

**Tail-Flick test** — For the tail flick method pain was induced by giving infrared light on the tail of the mice (Tail-Flick Unit, UGO BASILE, Italy) 5 cm away from the tip of the tail. Reaction time was noted by observing the interval between placing the tail on the infrared light and the withdrawal of the tail. A cut off time of 30 sec was used\(^{11}\).

**Statistical analysis** — The non-parametric Wilcoxon unpaired signed ranks test was used for the comparison of control and test groups. This test was selected as the data did not have equal variance and a normal distribution. \(P\) value < 0.05 was considered significant.

### Results and Discussion

Amitriptyline had antinociceptive activity both in low and high doses in acute thermal nociception which was statistically significant and in combination with morphine it produced more superior results than did morphine alone in Hot-Plate test (Table 1). In Tail-Flick test, high dose amitriptyline had antinociceptive activity which was significant when compared to saline but it differed significantly from morphine. Study showed that combination of amitriptyline with morphine was more effective than morphine alone in Hot-Plate test. This better result of the combined drugs (antidepressant and morphine) can be explained by the fact that combined re-uptake inhibition of serotonin and noradrenaline, and the modulation of pain transmission by opioids appears to confer a greater degree of antinocepcion in animal models of experimental pain than the single agent\(^{12}\).

Morphine remains the reference compound among centrally acting analgesics both for acute and chronic pain. Its mechanism of action and the role of opiate receptors are well known\(^{13}\). It has also been established that there are three major families of opioid peptides in the brain; the enkephalins, the dynorphins, and the endorphins\(^7\) associated with the neurobiology of pain and its modulation. Besides these opioid receptors, serotonin and norepinephrine play a role in the modulation of signals related to tissue damage\(^7\).

Antinociceptive effect of high dose amitriptyline did not differ significantly from morphine \((P>0.05)\) in Hot-Plate test but it differed significantly \((P<0.01)\) from morphine though it had antinociceptive effect \((P<0.05)\) when compared to saline in Tail Flick test. Ardid et al.\(^{14}\) explained that antinociceptive potency of re-uptake inhibitors varies according to their monoamine specificity and the nature of stimuli. Serotonergic inhibitors were more effective in Hot-Plate test. However, analgesic responses and the mechanisms implicated were dependent in analgesiometer test used, and antinociceptive responses were more potent in formaline test than in Tail-Flick test\(^{15}\).

Valverde et al.\(^{15}\) have shown that tricyclic antidepressants produce antinociception partly via the participation of endogenous opioid system and partly

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction time (sec) in Hot-Plate test</td>
<td>6.4±1.78</td>
<td>18.6±7.04(^b)</td>
<td>12.5±2.28(^a)</td>
<td>17.10±4.2(^b)</td>
<td>26.6±3.75(^b,d)</td>
</tr>
<tr>
<td>Reaction time (sec) in Tail-Flick test</td>
<td>1.6±0.84</td>
<td>11.2±7.15(^b)</td>
<td>1.8±0.42(^d)</td>
<td>2.6±0.97(^c,d)</td>
<td>5.6±3.03(^a,c)</td>
</tr>
</tbody>
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\(P\) values: \(^a\) <0.05, \(^b\) < 0.01 compared to control
\(^c\) < 0.05, \(^d\) < 0.01 compared to standard control
by further activating nor-adrenergic and serotonergic pathways. This study showed that amitriptyline can be combined with morphine and can be used in acute pain states to reduce the dose of morphine. Further research activities are essential to prove the effectiveness of such a combined therapy in patients. Analgesic activity of amitriptyline observed in the present study is in agreement with earlier findings\(^8,16-18\).

In conclusion, the present study showed that amitriptyline has analgesic activity in acute pain state in the experimental models. Amitriptyline in combination with morphine has better antinociceptive effect than the morphine alone in Hot-Plate test. Further studies and clinical trials are required to use amitriptyline alone or in combination with morphine in acute pain states.

References