Short communication

Protective role of Delphinium denudatum (Jadwar) against morphine induced tolerance and dependence in mice

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Abstract

Chronic treatment with Delphinium denudatum (Dd) (Jadwar) (family: Ranunculaceae, 200–1600 mg/kg) suppressed morphine withdrawal jumps in a dose-dependent manner, a sign of the development of dependence to opiate as assessed by naloxone (2 mg/kg) precipitation withdrawal on day 10 of testing in mice. Repeated administration of Dd (200–1600 mg/kg) for 9 days attenuated the development of tolerance to the analgesic effect of morphine (10 mg/kg), also produces significant change in tail-flick latency from the saline pretreated group in a dose-dependent manner. © 2001 Published by Elsevier Science Ireland Ltd.

Keywords: Delphinium denudatum; Morphine; Tolerance; Dependence; Naloxone

1. Introduction

The development of physical dependence and tolerance with repeated use is a characteristic feature of all the opioid drugs and offers major limitations in their clinical use. Tolerance and dependence are thought to result from neural adaptations produced by repeated exposure.

The daily administration of morphine or other opioids to rodents in one or more injections per day has been used for many years to produce a tolerant state which develops at a rate dependent on the specific drug, the dosage schedule, the interval between doses and the sensitivity of pharmacological assay. Tolerance is measured by the classical method of assessing the antinociceptive response whereas dependence is measured in tolerant animals by the evocation of abstinence signs by abrupt drug withdrawal or the administration of a narcotic antagonist or both. Blasig et al. (1973) suggested that jumping was the most suitable sign of measuring abstinence quantitatively because jumps are easily counted and the jumping rate increased when dependence increased or dose of antagonist increased.

Delphinium denudatum, Wall (Jadwar, Family: Ranunculaceae), a plant which possesses anticonvulsant properties in rats (Khan, 1981, 1982). Investigations on Dd revealed its beneficial effects in cardioprotection (Khan, 1984, 1989), hepatoprotection (Khan and Taiyab, 1981) and immunomodulation (Siddiqui et al., 1990). Recently, it has been reported that Jawahar Mohra (JM), which is a compound formulation of Unani medicine containing Dd, has shown antistress activity against diverse stressors (Ahmad et al., 1998).

The root of this plant is also reputed to be in Unani medicine for its beneficial effects in nervous disorders and opium addiction (Husain, 1875, 1897) but the claim of its efficacy for the same has not been scientifically explored. Therefore, the present experiments were undertaken to study the protective effect of Dd root extract on the development of tolerance and dependence to morphine in mice.
2. Materials and methods

2.1. Drugs

Roots of Delphinium denudatum were procured from the market. The authenticity and identity of the drug was confirmed at the Department of Botany, Faculty of Science, Hamdard University, New Delhi. The aqueous extract of the drug was obtained by the help of soxhlet’s apparatus. The yield of the extract was 30% w/w in terms of dried starting material. Morphine sulphate (Narcotic Division, Ghazipur, India) and naloxone hydrochloride (David Bul Laboratories, Australia) were obtained from their respective sources.

2.2. Animals

Swiss albino mice (weighing 20–30 g) of either sex were bred in the Central Animal House Facility of Hamdard University. The animals were housed under standard laboratory conditions with food and water provided ad libitum. The experiments were performed between 09:00 and 17:00 h.

2.3. Treatment schedule

In acute studies, the animals received saline or Dd root extract (200–1600 mg/kg, p.o.) using distilled water as the dose vehicle, followed 30 min later by saline or morphine (10 mg/kg, s.c.). The antinociceptive response to morphine was assessed by the tail-flick test 30 min after the second injection.

For induction of tolerance to morphine, animals received the morphine (10 mg/kg, s.c.) injection twice daily (09:00 and 16:00 h) for 9 days. On day 1, 3 and 9, antinociceptive response was assessed by the tail-flick test 30 min after morphine injection. Various treatment groups (pretreatment:treatment) included: (i) saline:saline; (ii) saline:morphine; (iii) Dd (200 mg/kg):saline; (iv) Dd (400 mg/kg):saline; (v) Dd (800 mg/kg):saline; (vi) Dd (1600 mg/kg):saline; (vii) Dd (200 mg/kg):morphine; (viii) Dd (400 mg/kg):morphine; (ix) Dd (800 mg/kg):morphine and (x) Dd (1600 mg/kg):morphine. On the 10th day, the treatment were reversed so that the animals that had been treated with Dd followed by saline for 9 days were challenged with Dd followed by morphine on day 10. In addition the animals that had received Dd followed by morphine for 9 were challenged with saline followed by morphine (Trujillo and Akil, 1991).

2.4. Technique

Antinociceptive response was assessed by measuring tail-flick latency to radiant heat as described by D’Armoour and Smith (1941) and as modified by Kulkarni (1980). A cut-off time of 10 s was observed to prevent any injury to the tail. A minimum of three trials was recorded for each animal.

To assess the morphine withdrawal, mice were injected with naloxone (2 mg/kg i.p.) immediately after the tail-flick test on day 10. The withdrawal syndrome was assessed by placing each mouse in a clear plexiglass box (base area: 22 x 17 cm height: 47 cm), open at the top for observation. The incidence of escape jumps was recorded for 15 min.

2.5. Statistical analysis

The data expressed as mean ± S.E.M. were analyzed by one way analysis of variance (ANOVA) followed by Student’s t-test. A value of P < 0.05 was considered statistically significant.

3. Results

3.1. Effect of acute and chronic administration of morphine on antinociceptive response

Animals receiving acute treatment with morphine (10 mg/kg) displayed maximal analgesia on day 1 and 3. But the chronic treatment in the same animals with morphine (10 mg/kg) for 10 days exhibited tolerance to morphine on the 9th and 10th day of testing as reaction time was reduced (Table 1).

3.2. Effect of acute and chronic concomitant treatment with Dd and morphine on development of tolerance to morphine

The acute treatment with Dd (200–1600 mg/kg) and morphine (10 mg/kg) showed maximal analgesia (10 s) on day 1 and 3. Dd (200–1600 mg/kg) and morphine (10 mg/kg) prevented the development of tolerance to the antinociceptive response of morphine on day 9 of testing when the same animals received the chronic treatment for 9 days. But these animals that had been treated with Dd and morphine on days 1–9, and then given morphine alone on day 10, also displayed considerable analgesia at 200 mg/kg and maximal analgesia (10 s) at 400–1600 mg/kg, respectively, (Table 1).

3.3. Effect of acute and chronic treatment with Dd on antinociceptive response

Acute and chronic administration of Dd (200–1600 mg/kg) followed by saline displayed a more significant analgesic response than saline pretreated animals on days 1–9. But on day 10 when these animals were challenged with Dd followed by morphine (10 mg/kg), a significant antinociceptive response comparable to
3.4. Effect of chronic treatment with Dd and morphine on naloxone precipitated withdrawal jumps

Animals that had received repeated administration of saline followed by morphine (10 mg/kg) displayed numerous escape jumps in response to an injection of naloxone (2 mg/kg) on day 10. In contrast, the animals treated with Dd (200–1600 mg/kg) and morphine (10 mg/kg) on day 1–9, and then with saline and morphine on day 10, displayed significantly fewer jumps after naloxone (2 mg/kg) administration (Table 2).

4. Discussion and conclusion

In the present study, Dd inhibited the development of tolerance to analgesic response of morphine and its physical dependence. Acute and chronic treatment with Dd also produced analgesia, demonstrating the effect of Dd on pain responsiveness and did not block morphine-induced analgesia as well. The mechanism of this observed inhibition of the abstinence syndrome in morphine tolerant and dependent animals by Dd root extract remains unclear. Earlier, an anti-addictive profile of a compound Unani formulation, a safe analgesic polyherbal preparation containing Dd as one of the components has been reported (Shahana et al., 1994; Zafar et al., 1991). The present study show that anti-addictive profile of a compound Unani formulation could be mainly due to the presence of Dd in it, as Dd per se has been shown to protect against development of tolerance and dependence to morphine. Dd has a wide spectrum of psychotropic and antistress activities, and being analgesic per se, it may be used safely in de-addiction profile.

All of the results taken together show that Dd did not antagonise morphine analgesia but inhibited the development of analgesic tolerance and physical dependence. Thus we presume that Dd can be developed for the treatment of opiate addiction.

Acknowledgements

The supply of morphine sulfate (Narcotic division, Ghazipur, India) by Department of Medical Elementology and Toxicology, Faculty of science, Hamdard University is acknowledged.

Table 1
Effect of morphine (10 mg/kg), Dd (200–1600 mg/kg) and combination of Dd (200–1600 mg/kg) and morphine (10 mg/kg) on analgesic response on day 1, 3, 9 and 10

<table>
<thead>
<tr>
<th>Gp. number</th>
<th>Treatment (mg/kg)</th>
<th>n</th>
<th>Tail-flick latency (Sec. ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>(i)</td>
<td>Saline:saline</td>
<td>6</td>
<td>3.01 ± 0.10</td>
</tr>
<tr>
<td>(ii)</td>
<td>Saline:mor (10)</td>
<td>6</td>
<td>10 ± 0</td>
</tr>
<tr>
<td>(iii)</td>
<td>Dd (200):saline</td>
<td>6</td>
<td>3.5 ± 0.13*</td>
</tr>
<tr>
<td>(iv)</td>
<td>Dd (400):saline</td>
<td>6</td>
<td>4.16 ± 0.17***</td>
</tr>
<tr>
<td>(v)</td>
<td>Dd (800):saline</td>
<td>6</td>
<td>4.75 ± 0.24***</td>
</tr>
<tr>
<td>(vi)</td>
<td>Dd (1600):saline</td>
<td>6</td>
<td>6.1 ± 0.18***</td>
</tr>
<tr>
<td>(vii)</td>
<td>Dd (200):mor (10)</td>
<td>6</td>
<td>10 ± 0NS</td>
</tr>
<tr>
<td>(viii)</td>
<td>Dd (400):mor (10)</td>
<td>6</td>
<td>10 ± 0NS</td>
</tr>
<tr>
<td>(ix)</td>
<td>Dd (800):mor (10)</td>
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<td>10 ± 0NS</td>
</tr>
<tr>
<td>(x)</td>
<td>Dd (1600):mor (10)</td>
<td>6</td>
<td>10 ± 0NS</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01 and ***P < 0.001 as compared with saline:morphine on day 9 and 10 with saline:morphine on day 1 and 3; Dd: morphine with saline: morphine; and Dd: saline with the saline: saline treated group (statistically significant). NSP > 0.05 (non significant).

Table 2
Effect of Dd on naloxone-precipitated morphine withdrawal jumps in mice

<table>
<thead>
<tr>
<th>Gp. Number</th>
<th>Treatment (mg/kg)*</th>
<th>n</th>
<th>Number of jumps (Mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i)</td>
<td>Saline:saline</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>(ii)</td>
<td>Saline:morphine (10)</td>
<td>6</td>
<td>24.83 ± 3.97</td>
</tr>
<tr>
<td>(iii)</td>
<td>Dd (200):saline</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>(iv)</td>
<td>Dd (400):saline</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>(v)</td>
<td>Dd (800):saline</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>(vi)</td>
<td>Dd (1600):saline</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>(vii)</td>
<td>Dd (200):morphine</td>
<td>6</td>
<td>15.66 ± 6.68 NS</td>
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<tr>
<td>(viii)</td>
<td>Dd (400):morphine</td>
<td>6</td>
<td>9.33 ± 1.76*</td>
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<tr>
<td>(ix)</td>
<td>Dd (800):morphine</td>
<td>6</td>
<td>6.66 ± 1.20***</td>
</tr>
<tr>
<td>(x)</td>
<td>Dd (1600):morphine</td>
<td>6</td>
<td>2.33 ± 0.95***</td>
</tr>
</tbody>
</table>

Naloxone (2 mg/kg i.p.) was given immediately after the tail-flick test on day 10. NSP > 0.05 (non significant), *P < 0.05 and **P < 0.01 as compared with the saline:morphine-treated control (statistically significant).

* See Section 2.
References


