Proposal of a New Modified Injection Method for Development of Morphine Dependency in Male Rats

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Abstract: The rapid induction of dependency is the priority of injection methods, but high mortality rate due to medullary depression is the most important limitation of injection methods in laboratory animals. This study was carried out on 5 groups of adult male wistar rats weighing 250-350 g which were maintained in standard light and temperature with free access to food and water. Four methods in which morphine dependency was achieved during a 5 days period were chosen and the mortality rate and the time of mortality were compared with the new group which received morphine (10 mg kg\(^{-1}\) on the first day and 20 mg kg\(^{-1}\) from 2nd to 5th day). Precipitation of Morphine withdrawal signs was performed on the 5th days, 4 h after the last injection of morphine and withdrawal signs were recorded in 5 min intervals for 20 min. The results of this study showed that the new modified method is an effective method for induction of morphine dependency. The least mortality rate (19%) was observed in the new modified morphine dependency method. Mortality rate in other procedures were ranged from 33-60%. The results of this study showed that first dose of morphine as well as the pattern of increasing dosage regimen are important factors which determine mortality rate. So we propose that this new method would be suitable for rapid induction of morphine dependency.

Key words: Morphine dependency, injection method

INTRODUCTION

Opioid dependency is a reversible chronic disorder in which there is an increasing tendency toward opioid use, despite their negative effects (Cami and Farre, 2003; Gerdeman et al., 2003). No opioid analgesic without dependency moiety has been discovered, yet (Cami and Farre, 2003; Gerdeman et al., 2003). So development of opioid dependency in laboratory animals is needed to evaluate the mechanisms of opioid dependency, opioid withdrawal syndrome and opioid adverse effects. Different methods are used for development of morphine dependency. The choice of each method is based on its advantages and disadvantages. Development of morphine dependency in a short period and low mortality rates are the major advantages of the dependency methods in some of the experimental procedures. Drug dependency could be induced by oral (Badawy et al., 1982; Gellert and Holtzman, 1978), subcutaneous pellets (Aricoglu et al., 2004; Bhargava, 1995; Cicero et al., 2002) and injection methods (Cami and Farre, 2003; Trang et al., 2003). The rapid induction of dependency is the priority of injection methods, but high mortality rate due to medullary depression is the most important limitation of injection methods in laboratory animals (Trang et al., 2003; Schumacher et al., 2004). Different injection methods are used for morphine dependency by different authors, which show differences in initial and final dose, injection period and number of injections day\(^{-1}\) (Aricoglu et al., 2004; Bhargava, 1995; Cicero et al., 2002). In most of the reported protocols, morphine is injected either by Subcutaneous (SC) or intraperitoneal (i.p.) route (Trang et al., 2003; Fiserova et al., 1999; Maeda et al., 2002).

The mortality rate of laboratory animals is the major limitation for morphine dependency, especially in those experiments which needs surgical procedures such as cannula implantation in to CNS nuclei in which the experiments usually start about one week after surgery. Since the mortality rates of different experimentally used methods for morphine dependency is not reported yet, so this study was performed to evaluate the mortality rate in some of the experimentally approved methods and

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development of a new injection method for morphine dependency.

**MATERIALS AND METHODS**

This study was carried out on 5 groups of adult male Wistar rats weighing 250-350 g (provided by the Kerman Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran). The experiments were approved by Animal Care and Use Committee and Neuroscience Research Council of Kerman University of Medical Sciences, Kerman, Iran. Rats were housed in plastic cages (four rats per cage) with soft bedding and free access to food and water. Animal room temperature was maintained at 23±1°C and light controlled (12:12 h dark: light cycle with light on at 08:00 h). Test sessions took place during the light phase between 10:00 a.m. and 12:00 a.m. in a quiet room maintained at 23-24°C. Each rat were used only once and was sacrificed at the end of the experiment by the administration of lethal dose of thiopental.

Among different injection methods for induction of morphine dependency, 4 methods in which drug dependency was achieved during a 5 days period were chosen and the mortality rate and time of mortality were recorded in each group. Morphine sulfate (Temad Co. Iran) was injected according to the following protocol for induction of dependency:

**Protocol No. 1:** (15-105 mg/kg/i.p. twice a day for 5 days) (Afify et al., 2001) 15 mg/kg on day 1, 20 mg/kg/ 2nd day at 8:00 a.m.; 30 mg/kg 2nd day at 4:00 p.m., 45 mg/kg 3rd day at 8:00 a.m.; 60 mg/kg/ 3rd day at 4:00 p.m.; 75 mg/kg/ 4th day at 8:00 a.m.; 90 mg/kg/ 4th day at 4:00 p.m.; 105 mg/kg/ 5th day at 8:00 a.m.

**Protocol No. 2:** (10-50 mg/kg/i.p twice a day for 5 days) (Chou et al., 2002) 10 mg/kg/i.p on the first day at 8:00 a.m. and increasing to 50 mg/kg/i.p on the 5th days.

**Protocol No. 3:** (10-40 mg/kg/i.p once a day for 5 days) (Fiserova et al., 1999) 10 mg/kg/i.p on the first day at 8:00 a.m. and increasing to 40 mg/kg/i.p on the 4th and 5th days.

**Protocol No. 4:** (20 mg/kg/SC once a day for 5 days) (Maeda et al., 2002). Since the mortality rate was observed in all experimental groups, so a new modified method for morphine dependency was examined by clinical trial and error and morphine was administered according to the following protocol: 10 mg/kg/SC at 8:00 a.m. on the first day, followed by 20 mg/kg/SC at 8:00 a.m. from 2nd to 5th days.

Precipitation of Morphine withdrawal signs was performed on the 5th days, 4 h after the last injection of morphine (Afify et al., 2001). Each animal was weighted before observation period, thereafter, rats were challenged for withdrawal signs by the administration of naloxone (4 mg/kg/i.p) (Chou et al., 2002; Fukuraga et al., 1998; Gomaa et al., 2003). Finally after observation period, animals were weighted again. The following signs including jumping, rearing, weight loss and wet dog shakes were scored on a quantitative basis. Other withdrawal signs observed include diarrhea, ptosis, teeth chattering which were evaluated by Gellert and Holtzman (1978) scale (Gellert and Holtzman, 1978). Weight loss was calculated as difference of weight before and one hour after naloxone injection and each 1% of weight loss was quantified as one. Morphine withdrawal signs were recorded in 5 min intervals for 20 min.

**RESULTS AND DISCUSSION**

All groups of treated rats showed morphine withdrawal signs (jumping, rearing, weight loss, ptosis, teeth chattering, wet dog shakes) following naloxone administration.

Comparison of the mortality rate showed that the least mortality rate (19%) was observed in the new modified morphine dependency method. Mortality rate in other procedures were ranged from 33-60 percent and most deaths were occurred in the first 3 days (Table 1).

The results of this study showed that the new modified method, as well as other experimental procedures, is an effective method for induction of morphine dependency and the first dose of morphine is an important factor which determine mortality rate. Starting dose of 10 mg kg⁻¹ of morphine had the least mortality rate (19%) and 20 mg kg⁻¹ morphine showed the highest mortality rate (60%) and all deaths were occurred in the first day.

Also mortality rate depends on the pattern of increasing dosage regimen, i.e., that rat could tolerate the increasing dosage from 10 to 20 mg kg⁻¹, but increasing morphine dosage from 20 to 30 mg kg⁻¹, 30 to 40 mg kg⁻¹ and 40 to 50 mg kg⁻¹ causes an increase in mortality rate. The causes of the most of the mortalities are respiratory depression due to morphine over dosages (Bhargava, 1995; Schumacher et al., 2004).

Rapid induction of morphine dependency is the most important priority of injection methods, however, respiratory depression and mortality rate are the major limitations of injection methods (Schumacher et al., 2004). This side effect become very important when the preparation of animals needs certain procedures which may lasts at least 1-2 weeks. Since some of the animals will
Table 1: Mortality rate during injection protocols used for induction of morphine dependency in male rats

<table>
<thead>
<tr>
<th>Morphine dependency protocol</th>
<th>No. of animals</th>
<th>Mortality frequency</th>
<th>Mortality (%)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol No. 1 15-105 mg/kg/ip/bid</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td>1 death in 2nd day and 4 death in 3rd day</td>
</tr>
<tr>
<td>Protocol No. 2 10-50 mg/kg/SC/bid'</td>
<td>12</td>
<td>4</td>
<td>33</td>
<td>3 death in 3rd day and 1 death in 5th day</td>
</tr>
<tr>
<td>Protocol No. 3 10-40 mg/kg/d</td>
<td>23</td>
<td>11</td>
<td>47.8</td>
<td>Most mortalities in 3rd to 5th day</td>
</tr>
<tr>
<td>Protocol No. 4 20 mg/kg/SC/d</td>
<td>10</td>
<td>6</td>
<td>60</td>
<td>All mortalities in the 1st day</td>
</tr>
<tr>
<td>New protocol 16-20 mg/kg/SC/d</td>
<td>23</td>
<td>4</td>
<td>19</td>
<td>Most mortalities in the 1st day</td>
</tr>
</tbody>
</table>

Mortality dependency was induced by either Subcutaneous (SC) or intraperitoneal (i.p.) injection of morphine sulfate during a 5 days period.

die during or after surgical operations (Chou et al., 2002; Fukunaga et al., 1998; Wang et al., 2004), so the use of a safe method with a low mortality rate is highly valuable in such experiments (Fiserova et al., 1999; Chou et al., 2002; Fukunaga et al., 1998; Wang et al., 2004).

Since the mortality rate in the new method of morphine dependency was lower than other experimental procedures, so we propose that this new method would be suitable for rapid induction of morphine dependency.

As we know, the mortality rate of injection methods for morphine dependency is not reported, so this study is a preliminary work which report the mortality rate of some of the examined protocols (Fiserova et al., 1999; Chou et al., 2002; Fukunaga et al., 1998; Wang et al., 2004).

Beside the medullary depression following morphine administration, other factors such as species variations and gender related differences may be involved in the mortality rate (Schumacher et al., 2004; Chou et al., 2002), so it is proposed that the effects of different doses of morphine on various laboratory animals, especially rats and mice, should be investigated in future.

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REFERENCES


