Research report

Attenuation of morphine withdrawal signs by low level laser therapy in rats

Iraj Mirzaii-Dizgah, Reza Ojagh, Hamid Reza Sadeghipour-Roodsari*, Seyed Morteza Karimian, Hamid Sohanaki

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In the present study, the effects of low-intensity laser therapy (LILT) on naloxone-induced withdrawal signs of morphine-dependent rats were examined. Low-intensity lasers with a power density of 12.5 J/cm² have been used by a Ga–Al–As laser. One-way ANOVA showed that the LILT which applied immediately or 15 min prior to naloxone injection significantly decreased total withdrawal score (TWS). These results suggest that LILT prior to naloxone injection attenuates the expression of withdrawal signs in morphine-dependent rats. Further studies may elucidate the likely role of LILT in clinical management of opioid withdrawal syndrome.

1. Introduction

The potent pharmacological effects of opiates have been recognized by human since ancient times. Today, opiates are still used in medical settings for their analgesic virtue and abused as ‘street’ drugs for their recreational properties. Chronic exposure to opiates leads to dramatic behavioral and neural changes known as tolerance and physical dependence [1]. Cessation of drug administration then leads to a withdrawal syndrome that is characterized by severe physiological disturbances and a plethora of withdrawal signs [2]. Morphine withdrawal was found to be associated with neurochemical and behavioral changes [3,4].

Low-intensity laser therapy (LILT) is widely used in clinical medicine as a therapeutic tool and has been found effective in the treatment of a variety of diseases and conditions [5,6]. It is supposed to be a non-invasive, painless and athermal therapy with minimal side effects. The therapeutical properties attributed to LILT are reduction in pain and promotion of wound healing, properties that are often referred to as biostimulation [7]. Regarding laser analgesia, previous studies indicated that LILT would selectively block the depolarization of slowly conducting nociceptive afferents, particularly unmyelinated C-fibers [8,9]. It has also been hypothesized that laser irradiation would instead act on large myelinated Aβ-fibers, and thus re-establish the gate-control system for pain control [10]. Other pain relieving factors may be related to cellular and vascular mechanisms, such as inhibited production of chemical mediators [11] and improved microcirculation [12].

It was previously shown that the LILT enhances ATP production in human neuronal cells in culture [13]. It was also indicated that the LILT up-regulated TGF-β1 in the ischemic brain, which is considered neuroprotective [14] and reduced neurological deficits in mice [15] and rats when applied post-stroke [16].

As pain is a common manifestation of opioid withdrawal and LILT demonstrates efficacy in treating patients with various pain syndromes and also in a number of pain models [17], to understand whether LILT might attenuate opioid withdrawal symptoms, we examined the effects of LILT on naloxone-induced withdrawal in the morphine-dependent rats.

2. Materials and methods

2.1. Animals

Forty adult male Wistar rats, weighing 220–280 g, were randomly assigned and housed collectively (four per cage) in an animal room on a 12-h light/dark cycle, with free access to food and water. The temperature was kept at 22–25 °C. The animals were equally divided into 5 groups each with eight rats. One group was non-dependent and the remaining 4 groups were dependent with incremental doses of morphine sulfate. Saline treated group and one of morphine treated group (as control) did not receive LILT but three morphine treated groups received LILT before naloxone injection as follows: Laser 0 group received LILT immediately before naloxone injection. Laser 15 and Laser 30 groups received LILT 15 min and 30 min before naloxone injection...
naloxone injection. The dependent rats received daily subcutaneous injection of morphine sulfate (2 ml/kg) for 7 days starting with 6, then 16, 26, 36, 46, 56, and 66 mg/kg by a single dose injection [18]. Non-dependent animals received saline (2 ml/kg) for the same period. Morphine injections were done at the same time every day during the whole period of study. Opioid withdrawal was precipitated by naloxone hydrochloride (3 mg/kg, s.c.) 1 day post-last morphine dose.

The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals provided and propagated by Tehran University of Medical Science ethical committee.

2.2. Laser irradiation

A continuous GaAlAs laser wave length 830 nm (ADVANCE, AMN50, Australia) was used in the study. The laser was applied to the shaved skin of the animal skull at the cross-point between interaural line and midline of head. The duration of laser irradiation was 55 s, the laser beam was 0.22 cm² and its power was 50 mW (0.05 W). This translates to a power density of 12.5 J/cm² [(0.05 J/s ÷ 30 min) × (0.05 J/s ÷ 30 s)] = 12.5 J/cm².

2.3. Measurement of behavioral signs during morphine withdrawal

For behavioral assessment of opioid withdrawal, animals were studied individually in a double chambers; an outer clear Plexiglas (50 cm × 25 cm × 15 cm) placed in an inner dark one to avoid environmental perturbations. A digital camera connected to a recording computer attached to the inner chamber for simultaneous rat behaviors recording. Each animal reactions were evaluated by an observer unaware of the treatment received by that animal. All animals’ behaviors were evaluated by the same observer. The records were replayed for meticulous analysis if needed. Total withdrawal score (TWS) was determined using Dizgah et al. modified method [18]. Briefly, 20 distinct withdrawal behaviors (16 scale): jumping, rearing, walk sniffing, sniffing, wet dog shakes, head shakes, body grooming, face wiping, penis licking, chewing, teeth chattering, swallowing, writhing, fore paw tremor, weight loss percentage and dysphoria time percentage; (2 ordinal): ptosis and diarrhea; and (2 checked) behaviors: irritability and eye twitch) were scored during a 30-min period following naloxone injection. Body weight was also measured before and 30 min after naloxone administration. Dysphoria time percentage was calculated using the following equation:

\[
\text{Dysphoria duration time (min) × 100}
\]

The score of each behavior was divided by a weighing factor (Table 1) and the results were added and to reach a total withdrawal score for each animal.

2.4. Drugs

All doses of morphine sulfate (Temad Tehran, Iran) were dissolved in a volume of 2 ml saline. Naloxone (Tocris, Bristol, UK) was also prepared in 3 mg/ml concentration.

2.5. Data analysis

Results were tested for statistical significance of the differences among groups by one-way analysis of variance, followed by Newman–Keuls multiple comparison as a post hoc test. Differences among means were considered statistically significant if P < 0.05. Data were expressed as mean ± S.E.M. for eight rats.

Table 1

<table>
<thead>
<tr>
<th>Behavior</th>
<th>WF</th>
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<tbody>
<tr>
<td>Jumping</td>
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<tr>
<td>Writhing</td>
<td>5</td>
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<tr>
<td>Wet-dog-shakes</td>
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<td>Head shakes</td>
<td>5</td>
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<td>Paw tremor</td>
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<td>Penis licking</td>
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<td>Body grooming</td>
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<td>Face wiping</td>
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<td>Teeth chattering</td>
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<td>Swallowing</td>
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<td>Dysphoria</td>
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<td>Rearing</td>
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<td>Chewing</td>
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Fig. 1. The expression of naloxone-induced total withdrawal score in morphine-dependent rats. Data are expressed as mean ± S.E.M. *Different from non-dependent group, P < 0.05.

Fig. 2. Effects of low-level laser irradiation at head on the expression of naloxone-induced withdrawal signs (TWS) in morphine-dependent rats. Data are expressed as mean ± S.E.M. *Different from control (morphine-dependent group). Laser 0, Laser 15 and Laser 30 groups were received head laser irradiation immediately, 15 min and 30 min before the naloxone injection, respectively.

3. Results

Administration of naloxone (3 mg/kg, subcutaneously) significantly increased total withdrawal score in morphine-dependent rats (25.43 ± 0.67) as compared with non-dependent rats (12.11 ± 0.66, P < 0.001) (Fig. 1). Total withdrawal score was considered as an index of abstinence throughout our study. Chronic administration of morphine sulfate caused weight loss (6–8%) and death (7%).

A one-way ANOVA indicated that the total withdrawal score was altered by low-level laser irradiation [F (3,28) = 4.2, P < 0.01] (Fig. 2). Post hoc analysis showed that the total withdrawal score was decreased in dependent rats received laser irradiation immediately (18.63 ± 0.98) and 15 min (19.63 ± 0.78) before naloxone injection (P < 0.05) as compared with dependent control animals (25.43 ± 0.67). But there were no significant differences between animals received laser irradiation 30 min prior to naloxone injection (22.75 ± 2.04) and control rats (Fig. 2).

4. Discussion

In the present study, effects of LILT on naloxone-induced withdrawal signs in morphine-dependent rats were investigated. Naloxone administration following chronic injections of morphine-induced behavioral signs of withdrawal in rats, which is in agreement with other studies [18,19]. We demonstrated that LILT exerts an alleviative effect on naloxone-precipitated withdrawal symptoms in morphine-dependent rats. Our results show that total withdrawal score was significantly decreased by LILT (Dose 25 J/cm²) applied immediately and 15 min before naloxone injection. On the other hand, LILT application 30 min before naloxone injection had no significant effect on withdrawal. So it seems that
this effect is time-dependent and will diminish with increasing time interval between LILT and naloxone injection. To our knowledge, there was no publication on attenuation of naloxone-induced withdrawal by LILT.

Although LILT has been widely used since its introduction in the 1960s [20], little is known about its mechanism of action. It affects many sub-cellular and cellular processes, although the mechanisms have not been well defined [21]. However, it is important to note that LILT does not produce significant tissue temperature changes, so any potential physiological effect appears to be non-thermal [22]. Even though therapies do not elevate tissue temperatures more than a few degrees, laboratory studies find that irradiation alters DNA synthesis and improves the function of damaged neurological tissue, increases the activation of cytoplasmic enzymes, oxygen consumption, ATP production and the synthesis of nucleic acids, proteins [13,23,24], serotonin and endogen opioid [25]. It is also assumed that the effect of low-power laser is based on the stabilization of nerve cell membrane, probably due to the more stable conformation of the lipid bilayers that is induced, and the associated integral proteins of the nerve cell membrane, which have been observed [26].

In conclusion, LILT prior to naloxone injection attenuates the expression of withdrawal signs in morphine-dependent rats. Further studies may elucidate the likely role of LILT in clinical management of opioid withdrawal syndrome.

References