Research report

Antinociceptive effect of Er:YAG laser irradiation in the orofacial formalin test

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Abstract

Low-power, soft, or low-level laser irradiation has been successfully used to provide analgesia in injured or diseased tissues. In this study, we tested the possible antinociceptive effect of laser irradiation when applied to a normal tissue before the onset of a painful stimulus. Male Wistar rats (350–380 g) were used. A 1.5% formalin solution (50 μL s.c., diluted in saline) was injected into the right upper lip of the test animals (n=9) immediately after 10 min of low-power Er:YAG laser irradiation (wavelength: 2.94 μm; energy: 0.1 J/cm²/pulse at 10 Hz). Control animals (n=9) were restrained for 10 min without laser application. The nociceptive response, i.e., the amount of time the rats spent rubbing the formalin injected area, was measured by an investigator blind to whether the animals had been laser irradiated or not. On laser irradiated rats, significantly less nociceptive behavior was observed only during the late phase (12–39 min) of the test. This result is similar to that reported for nonsteroid antiinflammatory drugs (NSAIDs) and other peripherally acting antiinflammatory agents. We conclude that low-power laser irradiation have a tonic antinociceptive effect on inflammatory pain even when applied before tissue injury.

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1. Introduction

Low-level laser irradiation (LLLI) has been found effective in the treatment of a variety of diseases and conditions [21,29]. The therapeutical properties attributed to LLLI are reduction in pain and promotion of wound healing, properties that are often referred to as biostimulation [17]. Several mechanisms have been proposed to explain LLLI biostimulation, among which, mechanisms related the pain reduction effect include antiinflammatory, analgesic, and antiedematous properties of LLLI [21,26,31].

Most laser systems used in LLLI studies are therapeutical lasers that operate in the red to near-infrared spectrum of light and have low-power outputs. However, surgical lasers operating in the mid- to near-infrared spectrum, such as the CO₂ [8], the Nd:YAG [23], and the Er:YAG [32] lasers, may also be used as therapeutical lasers, provided that the beam is defocused and the power output level can be regulated to avoid thermal damage to the irradiated surface. As a surgical laser, the Er:YAG laser system is one of the only laser systems capable of cutting hard and soft tissues alike without causing the thermal damage that most other laser systems cause [14]. Therefore, it is of particular interest to evaluate the applicability of Er:YAG laser irradiation for therapeutical purposes as well.

The effects of LLLI have been studied mainly on diseased or injured tissues [21]. In this study, we aimed at clarifying whether LLLI could produce analgesia if applied to a normal tissue before the onset of a painful stimulus.
2. Materials and methods

A total of 36 male Wistar rats weighing 350–380 g were used in this study. Every effort was taken to minimize animal suffering and the number of animals used. The methods described here follow the ethical guidelines proposed by the International Association for the Study of Pain and received approval by the Animal Welfare Committee of Nagasaki University.

2.1. Laser irradiation

Laser irradiation was applied to the right upper lip of the test animals immediately before (n = 9) and 10 and 20 min (n = 6 each) before the orofacial formalin test. During laser irradiation, the animals were restrained in an acrylic cylinder (Fig. 1A). The restraining cylinder had an opening at the snout to allow for the laser application and the formalin injection. Control animals (n = 9) were kept in the restraining cylinder for 10 min immediately before the orofacial formalin test without laser irradiation. To assess the effect of prolonged animal restraint, the orofacial formalin test was also applied to a group of nonrestraint animals (n = 6).

We used a pulsed Er:YAG laser system (Dentlite™, Hoya Photonics, Tokyo, Japan; wavelength 2.94 μm). The laser tip was fixed at a distance of about 2 cm from the target tissue, producing a 6-mm-wide irradiation surface. The energy density was 0.106 J/cm²/pulse, the pulse rate was 10 Hz, and the irradiation time was 10 min.

2.2. Orofacial formalin test

The experiments were performed in a quiet and uniformly illuminated room. Ambient temperature was kept stable at 23 ± 1 °C. To minimize the animals’ stress, we allowed an acclimatization period of at least 4 h before each experiment.

We injected 50 μL of a 1.5% formalin solution into the right upper lip of the test animals. After the injection, each animal was placed in an acrylic box (30 × 30 × 30 cm) with reflecting surfaces on three sides. The rat’s behavior was recorded on videotape for later analysis. The magnitude of nociceptive response was quantified by how long the animals spent rubbing the formalin-injected area with the ipsilateral fore- or hind paw (Fig. 1B). Measurements were performed always by the same investigator, who was blind to the animals’ group assignment.

2.3. Tissue temperature recording

To test thermal effects on the laser irradiation, a thermocouple probe (IT-23, Physitemp Instr., Clifton, NJ) was inserted subcutaneously below the laser-irradiated area with a 25G needle in one lightly anesthetized rat (80 mg/kg i.p., Isozol®, Yoshitomi Pharmaceutical, Osaka, Japan). The probe was connected to a thermometer (BAT-12, Physitemp Instr.) for temperature reading. Then the animal was laser irradiated for 10 min as in the actual experiment, using the same irradiation parameters as those described above.

2.4. Data analysis

The time the rats spent rubbing the formalin-injected site was measured with a stopwatch and grouped in bins of 3 min. The total observation period was 45 min. The reaction to formalin injection was also analyzed in terms of early and late phases, the early phase being the first 3 min after formalin injection, and the late phase is the period between 12 and 39 min after formalin injection. Variations in local tissue temperature were measured for 20 min, including 10 min of laser irradiation and 5 min before and after laser irradiation. Data values are expressed as mean ± S.E.M. The Kruskal–Wallis test was used to analyze differences between the experimental groups. A P < 0.05 was considered significant.

3. Results

3.1. Orofacial formalin test

The time course of nociceptive responses (face rubbing) is shown in Fig. 2. The classic two-phase response after formalin injection was observed; the first 3 min marked by vigorous face rubbing, followed by a relatively quiet period of about 9 min, then another burst of face rubbing activity. The late phase was not always as intense as the early phase but lasted much longer (about 27 min).

In rats irradiated immediately before the formalin injection, the overall magnitude of nociceptive response was lower than in control rats but reached statistical significance only between 21 and 30 min of the test. Rats irradiated 10 or 20 min before the formalin injection showed depressed nociceptive behavior only between 24 and 27 min of the test, which was also the period of peak face rubbing activity in the control group. When the face rubbing activity
was compared between laser and control groups during early and late phases, statistically significant difference was found only in the late phase of rats irradiated immediately before the formalin injection (Fig. 3).

3.2. Effects of animal restraint

To evaluate the effect of stress from prolonged restraint on behavioral nociceptive responses, the orofacial formalin test was applied to a small sample of nonrestraint controls. Comparison between control and nonrestraint control groups showed significantly higher face rubbing activity in nonrestrained animals during the late phase of the orofacial formalin test. Stress from prolonged restraint did not affect the early phase of the formalin test. The expression of nociceptive behavior in nonrestrained animals was markedly higher in the first half of the late phase of the test. Significant differences from the control group are indicated as *$P < 0.05$ and **$P < 0.01$ in the Kruskal–Wallis test.

3.3. Tissue temperature

A minor raise in tissue temperature of about 5°C could be recorded from a thermocouple inserted into the laser-irradiated tissue (Fig. 4). The tissue temperature was stable during the 10-min laser irradiation period, the maximum tissue temperature raise was about 5°C. Temperature values returned to normal limits within 2 min after laser irradiation.

Fig. 2. Time course of nociceptive behavior. Laser irradiation was applied immediately, 10 or 20 min before the orofacial formalin test. Control rats were restrained but not laser irradiated, and nonstress control rats were not restrained before the test. Although the overall face rubbing activity was lower in laser-irradiated rats, statistical significance was observed only between 21 and 30 min after formalin injection in rats irradiated immediately before the test and between 24 and 27 min in other laser-irradiated groups. Nonstress control group showed higher nociceptive behavior in the first half of the late phase of the test. Significant differences from the control group are indicated as *$P < 0.05$ and **$P < 0.01$ in the Kruskal–Wallis test.

Fig. 3. Comparison of nociceptive behavior between laser and control groups during early and late phases of the orofacial formalin test. Statistically significant differences were not found between any of the experimental groups in the early phase of the formalin test. In the late phase, nociceptive behavior was significantly lower in rats laser irradiated immediately before the formalin test and significantly higher in nonrestrained rats. *$P < 0.05$ against control in the Kruskal–Wallis test.

Fig. 4. Variations in local tissue temperature. A thermocouple was inserted subcutaneously below the laser-irradiated area in a lightly anesthetized rat. During the 10-min laser irradiation period, the maximum tissue temperature raise was about 5°C. Temperature values returned to normal limits within 2 min after laser irradiation.
acting opioids [18]. The early phase of the formalin test can be blocked by local anesthetics [1,6] or centrally acting analgesic agents, such as morphine, codeine, nefopam, orphenadrine, and remifentanil [1,13]. Therefore, the early phase of the formalin test is sensitive to analgesic treatments that affect conduction or modulation and perception of pain. These treatments also act on the second phase of the test provided they have a sustained effect and/or are applied continuously.

4. Discussion

The original formalin test, as described by Dubuisson and Dennis [7], was devised for use in rats and cats. It consisted of a subcutaneous (s.c.) injection of diluted formalin to the hind paw of the experimental animal and analysis of the subsequent nociceptive behavior. Formalin-induced pain is believed to resemble postinjury pain in man. It is of neurogenic nature and characterized by a diffuse and sustained noxious stimulus. The time course of nociceptive behavior consists of an early phase of about 3 to 5 min of intense response, followed by 9 to 15 min of relatively reduced response and a late phase of about 20 to 30 min of sustained nociceptive response. The original test was later adapted for use in mice, and more recently, a modification of the test was introduced for the assessment of pain and analgesia in the orofacial region of the rat [4]. In the orofacial formalin test, the formalin injection is directed to the upper lip, and the pain intensity is measured by the time the experimental animals spent rubbing the injected area. The pattern of response to the formalin injection, however, always shows the biphasic profile, regardless of the experimental animal used or the site of formalin injection [3]. Pain scores obtained by analysis of nociceptive behavior are consistent with electrophysiological recordings of convergent spinal dorsal horn and trigeminal brainstem neurons [6,19,20].

The early and late phases of the formalin response have differential properties that help in the assessment of the effectiveness and the mechanisms of action of potent, as well as mild, analgesic agents. The early phase of the formalin response is caused by a direct effect of the chemical irritant on peripheral nociceptors, and the late phase is caused by a peripheral inflammatory reaction [28]. Analgesic treatments can be directed to the periphery (pain transduction), along afferent nerve fibers (conduction), or at CNS sites (modulation and perception). In the formalin test, peripherally acting antiinflammatory agents produce analgesia in the late phase but have hardly any effect on the early phase of the test. Such agents include indomethacin and naproxen (nonsteroidal antiinflammatory drugs, NSAIDs), dexamethasone and hydrocortisone (corticosteroids) [11–13,22], bradykinin receptor antagonists [24], peripheral antisym pathetic treatments [5], and peripherally acting opioids [18]. The early phase of the formalin test can be blocked by local anesthetics [1,6] or centrally acting analgesic agents, such as morphine, codeine, nefopam, orphenadrine, and remifentanil [1,13]. Therefore, the early phase of the formalin test is sensitive to analgesic treatments that affect conduction or modulation and perception of pain. Analgesic mechanisms associated with LLLI have been attributed to inhibited release of inflammatory mediators [10,25], blocked depolarization of nociceptive afferents [15,30,32], and improved microcirculation in peripheral blood vessels [9,16]. We could speculate that the antinociceptive effect observed here (i.e., selective inhibition of the late phase of the formalin test) relied on the anti-inflammatory action of the laser irradiation, therefore acting on the transduction of pain from inflammatory mediators.

In this study, none of the laser-irradiated groups showed decreased nociceptive response in the early phase of the formalin test. In a previous study using the same irradiation parameters, we observed that the laser analgesia started 10 min after the irradiation and lasted for about 20 min [32]. Nevertheless, laser irradiation did not affect the early phase of the formalin test when applied either 10 or 20 min before the formalin injection. This result indicates that the LLLI effect in blocking the depolarization of nociceptive afferents [15,30,32] was not enough to suppress the nerve conduction in the early phase of the formalin response. Likewise, the hypothesis put forward by Tam [27], in which laser irradiation would act on large myelinated Aβ-fibers (non-nociceptive afferents) and produce analgesia by modulation at CNS sites, seems unlikely since such an effect would have affected the early phase of the formalin test as well.

As expected, animal restraint significantly reduced the nociceptive behavioral responses observed in this study, particularly in the first half of the late phase of the formalin test. Indeed, stressful stimulation is known to have an analgesic effect in rats (e.g., see Ref. [2]). Nevertheless, this effect was not strong enough as to mask antinociception obtained by laser irradiation. On the other hand, it could also be argued that the raise in local tissue temperature alone could have caused dilatation of peripheral blood vessels and thus improved the formalin inflammatory reaction. However, temperature changes were too mild and transient to produce such an effect. In addition, previous studies suggest that peripheral microcirculatory changes to LLLI are not merely a thermal phenomenon [16,21].

In conclusion, our results indicate that low-level laser irradiation may provide tonic analgesia even when applied to a normal tissue before the onset of a noxious stimulus. This effect probably relates to the antiinflammatory action of the laser irradiation.

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