Study of the Intraplantar Injection of Lidocaine and Morphine on Pain Perception and the Influence of Morphine Dependence and Withdrawal on Lidocaine-Induced Analgesia in Rats

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ABSTRACT

Background: Morphine and lidocaine are known to influence the perception of pain. The present study sought to determine the influence of local administration of morphine on lidocaine-induced analgesia in morphine non-dependent (MND), morphine dependent (MD) and morphine withdrawal (MW) animals. **Methods:** Adult male Wistar rats were divided into four groups: Control, MND, MD and MW rats. Lidocaine (0.5, 1 and 2%) and morphine (200, 400 and 800 µg) were injected in the plantar surface of the right paw. MD animals received chronic oral morphine (0.1, 0.2, 0.3 and 0.4 mg/ml in their drinking water) for 20 days. Twenty four hours before experiment, the animals in the MW group were deprived of morphine in their drinking water (physical dependence was observed by precipitating an abstinence syndrome with naloxone 2 mg/kg i.p.). Analgesia was assessed using hot-plate apparatus. **Results:** Morphine (400 µg) and lidocaine (2%) produce local analgesia in MND group. In MND rats, non-analgesic doses of each drug (200 µg morphine and 1% lidocaine) were used in combination and produced analgesia. In MD animals, all doses of lidocaine produced analgesia, while in MW animals, it failed to produce analgesia. In this situation, local administration of morphine could eventually influence the analgesic effect of lidocaine. **Conclusion:** Opioid withdrawal is one of the most common problems in clinic. This study determined the analgesic effect of lidocaine in MW animals in which lidocaine had no analgesic effect. In this regard, local administration of morphine with combination of lidocaine could probably produce an effective analgesia. *Iran. Biomed. J. 14 (4): 164-170, 2010*

Keywords: Morphine, Lidocaine, Local analgesia, Dependence, Withdrawal

INTRODUCTION

Local administration of analgesic drugs avoids many of the problematic side effects of systemic agents. By limiting the exposure of a drug to the periphery, central side effects can be markedly reduced [1]. For opioids, this might decrease some side effects such as sedation, respiratory depression and nausea. Further limiting the drug to the actual local site of action has even more advantages by avoiding peripherally mediated side effects such as constipation [2].

Recent studies suggest that peripheral administration of morphine may represent a valuable treatment in painful inflammatory conditions [3].

Local intra-articular administration of morphine in knee surgery has demonstrated potent and long-lasting postoperative analgesia and is believed to support the hypothesis of peripheral opioid receptor activation in inflammation [4]. Painful orofacial conditions also support the clinical use of peripheral opioid administration [5]. On the other hand, local application of morphine reduced pain in rat model of bone damage [6]. Opioid receptors have been identified at peripheral nerve terminals of primary afferent neurons [7, 8]. Under inflammatory conditions, the axonal transport of opioid receptors from the dorsal root ganglia to the peripheral nerve endings is enhanced, resulting in an increased number of opioid receptors in the inflamed tissue

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[9]. Opioid actions also can be modulated by non-opioid drugs such as local anesthetics [2, 10, 11]. Lidocaine, a local anesthetic, acts topically by blocking sodium channels, a mechanism distinct from the opioids [12]. Many studies have suggested that local anesthetics may be combined with other drugs such as opioids [2, 10, 13]. Clinical and basic studies have shown advantages to the combination of intrathecal lidocaine and opioids [14-16].

Previous studies indicate that similar advantages might be seen when these drugs are used topically [2, 10, 11]. Moreover, potentiation of the analgesic effect of morphine by dypirone and the possible participation of a peripheral mechanism in such synergy were studied with the formalin test in rats [17]. In the inflamed tissue, morphine administered into intraligamentary space of a chronically inflamed hyperalgesic tooth has produced analgesia [18, 19]. This analgesic effect is mediated by a local mechanism in the inflamed tissue because subcutaneous administration of morphine failed to elicit an analgesic response [18]. However, the results have shown that morphine added to a local anesthetic for dental surgery results in a significant improvement of postoperative analgesia [19]. On this background and regarding to some unpublished clinical reports indicating that opioid abusers claim that in their cases, local anesthetics fail to produce analgesia in dental procedures and based on the above mentioned reports concerning the local analgesic effect of morphine in inflamed tissues, we designed to study the local analgesic effect of morphine in non-inflamed tissues, the influence of morphine dependence and deprivation on the local analgesic effect of lidocaine, and the effect of combination therapy in this situation in rats.

MATERIALS AND METHODS

Animals. Adult male Wistar rats (n = 7-9 in each group), weighing 180-250 g, were used in this study. They were maintained at the constant temperature of 22 ± 2ºC on a 12 h light/dark cycle (light on 7:30 am to 7:30 pm). Animals had free access to food and water except during the experimental sessions. All experiments were carried out according to the International Association for the Study of Pain Guide on the Care and Use of Animals for research [20]. Animals were used only once.

Drugs. The following drugs were used: morphine sulfate (MacFarlan Smith Ltd., England), lidocaine hydrochloride (Sigma, USA) and naloxone hydrochloride (Sigma, USA). Drugs were dissolved in 0.9% saline.

Measurement of analgesia. Analgesia was measured before and 15 minutes after drug injection into the right hind paw of animals using the hot-plate test according to the method of Vitale *et al.* [21]. This test involves placement of a rat on a metal surface (19 cm diameter) heated to a temperature of 55 ± 0.5ºC with a cylindrical plexiglass (20 cm height and 19 cm diameter). The apparatus was equipped with a timer and a thermocouple to maintain a constant temperature (CENCO instrument, the Netherlands). The time reaction required for paw licking or lifting from the surface was taken as the response. To avoid tissue damage, a cut-off time for the latency of response was taken as 45 seconds [21]. After determination of baseline latencies, rat received intraplantar injection of drugs and the reaction time was determined 15 min after injection. The paw latencies were converted to the percentage of analgesia using the following formula:

%Analgesia= (latency of test – basal latency time)/ (cut off time – basal latency time) ×100.

In all experiments the right paw was compared in different groups of animals.

Animal groups and treatment. Animals were divided randomly into several experimental groups: 1) control (0.9% saline), 2) morphine non-dependent (MND), animals who did not receive morphine in their drinking water; 3) morphine dependent (MD) and 4) morphine withdrawal (MW) animals. Morphine (200, 400 and 800 µg) and lidocaine (0.5, 1 and 2%) were injected into the plantar surface of the right hind paw. Morphine was dissolved in 50 µl of 0.9% saline (in all experiments two successive injections of 50 µl of drugs were made as: saline + saline (control animals), saline + morphine (MND, MD and MW animals), saline + lidocaine (MND, MD and MW animals) and morphine + lidocaine (MND, MW animals). Doses of morphine and lidocaine that produce the same response and failed to produce analgesia (200 µg morphine + 1% lidocaine and 200 µg morphine + 2% lidocaine) were applied in MND and MW rats, respectively. Naloxone (2 mg/kg i.p.) was used to assess the degree of morphine dependence.

Induction of morphine dependence. Oral administration of morphine was used to induce
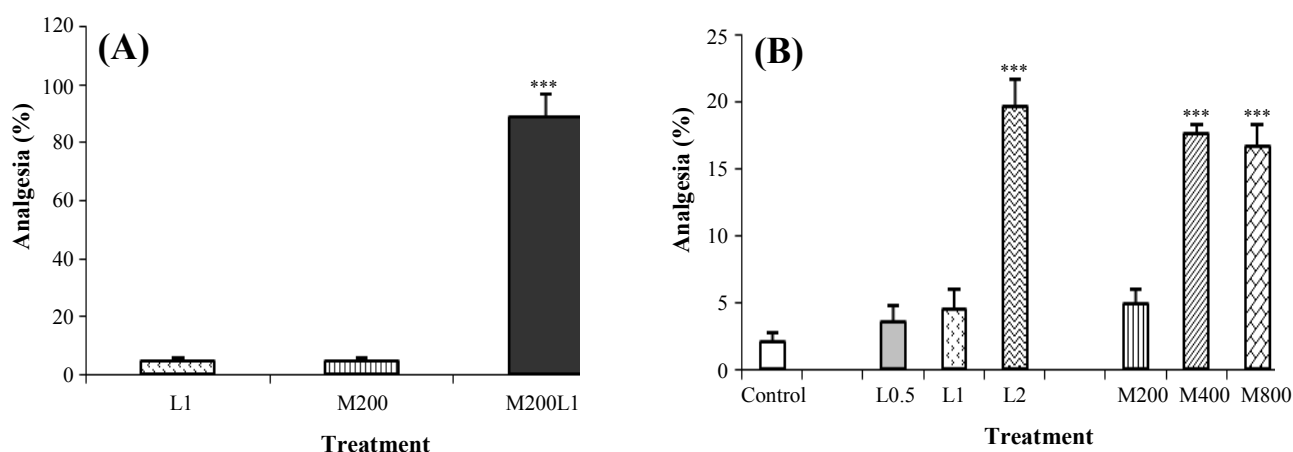
Fig. 1. Effect of intraplantar injection **(A)** and co-administration of morphine and lidocaine **(B)** on morphine non-dependent rats. (A) In animals treated with 2% lidocaine and 400 and 800 μg morphine, analgesia was significantly different (***$P<0.001$) from the control animals. (B) A volume of 200 μg morphine + 1% lidocaine injected intraplantarly produced analgesia significantly different (***$P<0.001$) from animals treated with 200 μg morphine and 1% lidocaine. L, lidocaine; M, morphine.

morphine dependence according to the method of Leung *et al*. [22]. We used glucose 5% w/v in ordinary tap water to mask the bitter taste of morphine sulfate which was chronically administered in the drinking fluid. Morphine was given in increasing concentrations (48 h apart) of 0.1, 0.2, 0.3 and finally 0.4 mg/ml. The last concentration was used until the end of the 20th day of the experimental period.

Morphine withdrawal. To confirm the morphine dependency, in MD and MW animals, the degree of morphine dependence was assessed by observing a number of characteristic signs of naloxone-precipitated withdrawal according to the method of Collier *et al*. [23]. Withdrawal signs such as jumping, diarrhea, writhing, restlessness, climbing, rearing and grooming were observed for 60 minutes after i.p. injection of naloxone randomly to one rat in each group (data not shown). Animals that were received naloxone were omitted from experiments. In the MW group, we discontinued morphine from their drinking water 24 hours before the experiment and we assessed withdrawal signs as mentioned previously.

Data analysis. Data are shown as the Mean ± S.E.M of percentage of analgesia. The difference in percentage of analgesia between groups was determined using analysis of variance (ANOVA) followed by the Newman-Keuls test. A value of $P<0.05$ was considered as significant.

RESULTS

The effect of lidocaine and morphine in MND rats. Lidocaine (0.5, 1 and 2%) and morphine (200, 400 and 800 μg) were injected intraplantarly. Lidocaine at a concentration of 2% ($P<0.001$) produced analgesia significantly different when compared to control animals. Analgesic response was seen after intraplantar injection of 400 and 800 μg morphine ($P<0.001$) compared to control groups (Fig. 1A).

The effect of co-administration of lidocaine and morphine in MND rats. Here, we studied the effect of drug combination. A volume of 200 μg morphine + 1% lidocaine were injected intraplantarly. In MND rats, significant analgesia was induced with 200μg morphine + 1% lidocaine ($P<0.001$) compared to 200μg morphine and1% lidocaine in MND rats (Fig 1B).

The effect of lidocaine and morphine in MD rats. In this part of study, we investigated the effect of chronic administration of morphine. Intraplantar injection of lidocaine (0.5, 1 and 2%) produced a significant analgesic effect ($P<0.001$) compared to the control group. Morphine (200, 400 and 800 μg) did not produce analgesia when compared to the control group (Fig. 2).

The effect of lidocaine and morphine in MW rats. Lidocaine (0.5, 1 and 2%) and morphine (200, 400 and 800 μg) were injected intraplantarly. Lidocaine
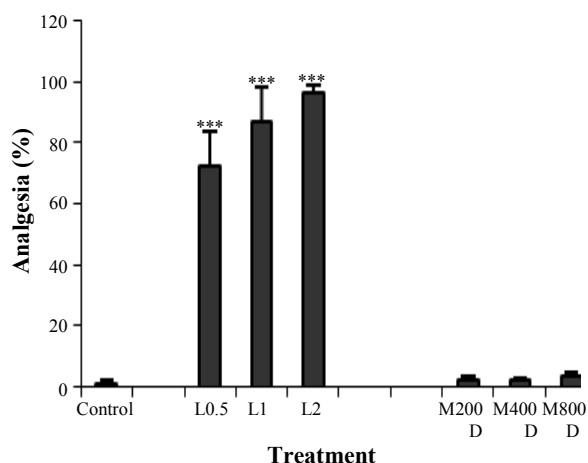
Fig. 2. Effect of intraplantar injection of different doses of lidocaine (0.5, 1 and 2%) and morphine (200, 400 and 800 μg) in morphine-dependent rats. Lidocaine treatment resulted in analgesia that was significantly different (***P<0.001) from untreated control animals. Analgesia was not seen in animals treated with morphine. L, lidocaine; M, morphine; D, morphine dependent.

did not produce analgesia but morphine 400 and 800 μg (*P*<0.01) showed a significant analgesic effect compared to control animals (Fig. 3A).

The effect of co-administration of morphine and lidocaine in MW rats. In MW animals, co-administration of 200 μg morphine + 2% lidocaine showed that analgesia is significantly different (*P*<0.001) from 200 μg morphine and 2% lidocaine (Fig. 3B).
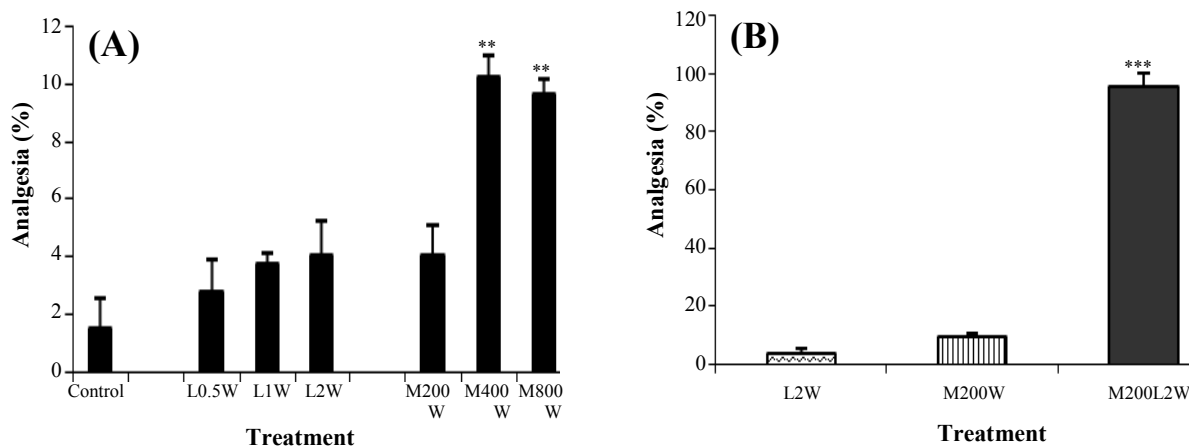
Comparison of local analgesic effect of lidocaine and morphine in MND, MD and MW rats. In MD animals, intraplantar injection of lidocaine (0.5, 1 and 2%) produced analgesic effect significantly different, *P*<0.001) compare with MND and MW groups (Fig. 4).

DISCUSSION

Chronic opioid-induced drug dependence and withdrawal syndrome after opioid cessation remains a severe obstacle in clinical treatment of chronic pain and opioid drug addiction. Opioids have been used for treating moderate to severe pain, but chronic treatment with these drugs lead to the development of tolerance and dependence.

Previous studies have reported that local application of morphine reduced pain in different conditions [1, 3, 4, 18]. In the present study, intraplantar injection of morphine produced local analgesia; this effect was consistent with the previous reports. Lidocaine is a widely used local anesthetic [24].

Lidocaine acts through the blockade of sodium channels, a mechanism distinct from the opioids [12]. We also found that intraplantar injection of all doses of lidocaine produced analgesia in MD animals, while in MND rats, analgesia was seen only with a high dose of lidocaine; however, in MW animals, lidocaine was not effective. One of the key symptoms during opioid withdrawal is a state of sensitized pain. The most significant molecular

Fig 3. Effect of intraplantar injection **(A)** and co-administration of morphine and lidocaine **(B)** on morphine withdrawal rats. (A) Analgesia was not seen in animals treated with lidocaine (0.5, 1 and 2%). Animals treated with morphine 400 and 800 μg showed that analgesia is significantly different (**P<0.01) from control animals. (B) Intraplantar injection of 200 μg morphine + 2% lidocaine produced analgesia significantly different (***P<0.001) from animals treated with 200 μg morphine and 2% lidocaine. L, lidocaine; M, morphine; W, morphine withdrawal.
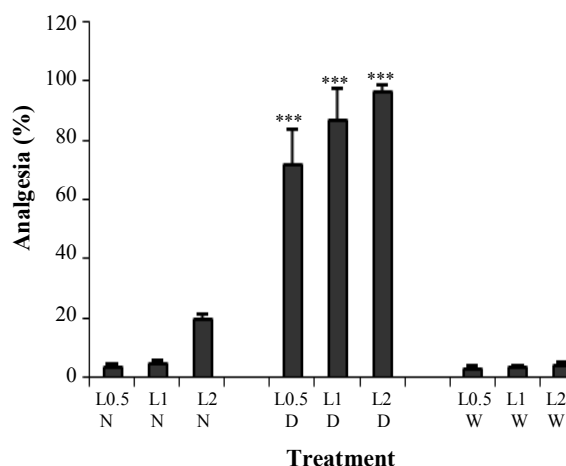
Fig. 4. Comparison of intraplantar injection of different doses of lidocaine (0.5, 1 and 2%) in morphine non-dependent, morphine dependent and morphine withdrawal rats. All doses of lidocaine produced analgesia in morphine dependent rats when compared to morphine non-dependent and morphine withdrawal groups (***P<0.001). L, lidocaine; N, morphine non-dependent; D, morphine dependent, W, morphine withdrawal.

adaptation induced by chronic opioids in the brain is up-regulation of the cAMP-signaling pathway [25, 26].

Opioids acutely inhibit adenylyl cyclase (AC) and cAMP-dependent protein phosphorylation in the locus coeruleus [27, 28]; the μ-opioid receptor is coupled to a G protein that activates potassium channels and inhibits AC, resulting in membrane hyperpolarization and in decreased production of cAMP. Because cAMP activates protein kinase A, which in turn regulates the threshold of the voltage-gated sodium channel, the decreased cAMP levels indirectly decrease sodium channel conductance.

Decreased cAMP also influences the activation of the transcription factor cAMP response element-binding protein (CREB), which regulates the level of adenylyl cyclase expression [29]. This inhibition is reversed with chronic opiate administration (tolerance) and increases far above normal upon administration of an opioid receptor antagonist showing dependence and withdrawal [27]. The mechanism underlying tolerance to morphine after chronic administration of drug and thereby reducing its analgesic effects involves up-regulation of CREB, which stimulates the transcription of AC, which in turn restores cAMP production toward normal levels. The increased cAMP stimulates protein kinase A, which phosphorylates (and thereby activates) both CREB and the voltage-gated sodium channel [29]. The up-regulated cAMP pathway increases the neuronal electrical excitability by

activating the Na$^+$ channels, thereby serving as a mechanism for tolerance and dependence [29, 30]. Moreover, up-regulation of dihydropyridine-sensitive Ca^{2+} channels is one of the mechanisms underlying the development of morphine tolerance [31-33]. In this part of our study, we tested the chronic effect of morphine on the analgesic effect of lidocaine. Our results showed that lidocaine produced analgesia in MD animals. It has been reported that a resting nerve is much less sensitive to a local anesthetic than one that is repetitively stimulated; a higher frequency of stimulation and more positive membrane potential causes a greater degree of anesthetic block [12].

Based on the previous reports on the mechanism responsible for opioid-induced tolerance/ dependence, the increased electrical excitability of neurons due to activation of the Na$^+$ current may eventually account for the analgesic effect of lidocaine in MD animals. In contrast to this effect, lidocaine failed to produce analgesia in MW rats. However, some reports have indicated that the nucleus raphe magnus is a brainstem site which is critically involved in opioid modulation of pain. MW also enhances the hyperpolarization-activated current in these neurons by increasing the intracellular cAMP [26] and chronic opiate results in an increase in GABA tone in these neurons [28].

Moreover, data have shown that the probability of GABA release was increased during withdrawal from chronic morphine treatment and that this effect resulted from an up-regulation of the cAMP-dependent cascade [34]. Since our study showed that lidocaine was not able to produce analgesia in MW animals, we suggest that hyperpolarization-activated current due to intracellular increase of cAMP and the release of GABA in the neurons during withdrawal may be the reason why lidocaine failed to produce analgesia. On the other hand, we also observed that in MW animals, local administration of morphine produced analgesic effects. We suggest that this result may be due to the inhibition of the GABAergic inhibitory interneurons [35].

We also determined the effect of concomitant administration of morphine and lidocaine. Several lines of evidence indicated that the opioids were used in combination with other classes of analgesic drugs [2, 5, 11, 13]. In this regard local anesthetics are widely used in combination with opioid drugs [2, 10, 13]. We noted that in MND animals, non-analgesic doses of morphine and lidocaine, here in combination showed an analgesic effect. A similar result was obtained in MW rats. These doses of

drugs failed to produce analgesia as mentioned before. The present data are in agreement with the previous studies which pointed to the combination of opioids with other classes of analgesic drugs [2, 5, 11, 13].

Therefore, we concluded that the analgesic effect produced by intraplantar injection of morphine or lidocaine was not a systemic effect because animals tolerated heat stimulus on their right paw while they showed licking or lifting behavior of their left paw. In our study design, we also noted that the local analgesic effect of morphine was produced in non-inflamed tissues, consistent with the fact that opioid receptors have been identified on peripheral nerve terminals of primary afferent neurons [7, 8], while previous studies pointed to the local analgesic effect of morphine, especially in inflamed tissues [18, 19].

In summary, the results of this study suggest that local administration of morphine can influence the local analgesic effect of lidocaine. Chronic use of morphine influences the analgesic effect of lidocaine. Lidocaine failed to produce analgesia in MW animals. Our findings are consistent with unpublished reports indicating that opioid abusers do not respond to the analgesic effect of local anesthetics in dental procedures. In this regard, complementary clinical research is needed to compare the effects of lidocaine in abuser patients who do not receive morphine several hours before dental manipulation with those patients who receive their drug just before the procedure. In addition, more studies remain to be done to clarify the mechanisms by which lidocaine fails to produce analgesia in MW animals.

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