

Effect of curcumin on morphine-induced antinociception in acute corneal pain in rats

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Abstract

In the present study, the effects of the acute and chronic oral administrations (po) of curcumin in the absence and presence of morphine and naloxone was investigated on the sensation of acute corneal pain in rats. Acute corneal pain was induced by the local application of hypertonic saline (5 M NaCl) on the corneal surface, and the number of eye wipes was then counted for 30 s. Subcutaneous (sc) injections of morphine (1, 2 and 4 mg/kg) significantly suppressed corneal pain ($p < 0.05$). Naloxone (2 mg/kg, sc) did not change the intensity of pain when used alone, but pretreatment of the rats with naloxone (2 mg/kg, sc) significantly prevented the antinociceptive effect induced by morphine (2 mg/kg, sc; $p < 0.05$). The short-term and long-term oral administration of curcumin (either a single dose of 200 mg/kg or dosages of 6.25, 12.5 of 25 mg/kg for 15 days each, respectively) both significantly decreased the number of eye wipes ($p < 0.05$). The antinociceptive effect induced by morphine was significantly ($p < 0.05$) enhanced by both the acute (200 mg/kg, once, po) and chronic (25 mg/kg, 15 days, po) curcumin treatments. Naloxone (2 mg/kg, sc) did not change the antinociception that was induced by acute (200 mg/kg, once, po) and chronic (25 mg/kg, 15 days, po) treatments of curcumin. The present findings suggest that morphine produced an analgesic effect through a naloxone-sensitive mechanism in acute corneal pain. Both high-dose acute and lower-dose chronic oral administrations of curcumin suppressed corneal pain. Moreover, curcumin enhanced morphine-induced antinociception.

Introduction

Spices possess several medicinal properties and have been used effectively by indigenous populations in their systems of medicine (Srinivasan, 2005). Curcumin is the major yellow-orange pigment extracted from turmeric, a commonly used spice, which is derived from the rhizome of the herb, *Curcuma longa*. It has a wide range of biological and pharmacological effects including anti-carcinogenic, anti-mutagenic, anti-diabetic, antibacterial, antiviral, anti-inflammatory and antioxidative effects (Maheshwari *et al.*, 2006).

Several lines of evidence also suggest that *Curcuma longa* and its active substance, curcumin, have antinociceptive properties. Intraperitoneal (ip) injection of *Curcuma longa* extracts suppress nociception in both tail flick and acetic acid-induced writhing tests (John *et al.*, 2009). Chronic treatment with curcumin suppresses both thermal hyperalgesia

and hot-plate latencies in a diabetic mouse model of neuropathic pain (Sharma *et al.*, 2006). In addition, acute oral treatments with curcumin attenuated the late phase of the pain response that was induced by an intraplantar injection of formalin in rats (Tajik *et al.*, 2007). A single ip injection of curcumin reduced face grooming in both the acute and tonic phases of formalin-induced orofacial pain in rats (Mittal *et al.*, 2009). In acetic acid-induced visceral nociception, the chronic oral administration of curcumin reduced the number of abdominal wall contractions (Tamaddonfard *et al.*, 2008). Chronic treatment with curcumin decreased lipopolysaccharide- and *Brucella abortus*-induced hyperalgesia in mice (Gupta *et al.*, 2009).

The cornea is a densely innervated organ (Muller *et al.*, 2003), and it is used for study of nociception in the trigeminal system because corneal nociceptive receptors have a large representation in the trigeminal ganglion through the ophthalmic branch of trigeminal

nerve (Felipe *et al.*, 1999). Moreover, nociceptive neurons in the trigeminal nucleus respond vigorously when capsaicin, nicotine and 5 M NaCl applied on corneal surface (Carstens *et al.*, 1998). Recently, the eye wiping induced by local application of 5 M NaCl solution on corneal surface, have been introduced as a sensitive animal model for study of acute trigeminal pain mechanisms (Farazifard *et al.*, 2005; Tamaddonfard *et al.*, 2008).

Curcumin has beneficial effects on pathologies of the eyes. In patients with chronic anterior uveitis, chronic treatment with curcumin induces subjective and objective improvement in vision along with relief of pain, redness and lacrimation (Lal *et al.*, 1999). Curcumin was shown to inhibit corneal neovascularization in the corneal alkaline burn model in rats (Bian *et al.*, 2008). The barrier function of the corneal epithelium contributes to corneal homeostasis and can be impaired by inflammation. Curcumin inhibits interleukin-1 β -induced barrier disruption and dysfunction of the corneal epithelium by blocking the subcellular localization of the ZO-1 and occluding proteins that are important for cellular adherence and the formation of tight junctions of the corneal epithelium (Kimura *et al.*, 2009).

To our knowledge, there is a lack of studies that examine the effect of curcumin on the perception of corneal pain. Therefore, the present study was designed to investigate the effects of acute and chronic oral administrations of curcumin on acute corneal pain that was induced by hypertonic saline applied locally on the corneal surface in rats. In addition, to identify the mechanism that possibly mediates the effect of curcumin on pain perception, the contribution of the endogenous analgesic opioid system was assessed using subcutaneous injections of morphine (an opioid receptor agonist) and naloxone (an opioid receptor antagonist) in the absence and presence of curcumin.

Materials and Methods

Healthy adult male Wistar rats that weighed between 220 and 250 g were used in this study. Rats were maintained in polyethylene cages with food and water available *ad libitum*. They lived in a laboratory with a controlled ambient temperature ($22 \pm 0.5^\circ\text{C}$) with a 12 h light-dark cycle (lights on at 07:00 h). Eight rats were used in each treatment group. Experiments were performed between 11:00 h to 14:00 h. The experimental protocol was approved by the Laboratory Animal Care and Use Center of the Faculty of Veterinary Medicine of Urmia University, Iran.

The drugs that were used in the present study included curcumin (Sigma-Aldrich Inc., St Louis, MO, USA), morphine sulfate (Temad, Tehran, Iran) and naloxone hydrochloride (Sigma-Aldrich Inc., St Louis, MO, USA). Morphine and naloxone were

dissolved in normal saline. Normal saline (control), morphine (0.5, 1, 2 and 4 mg/kg) and naloxone (2 mg/kg) in a constant volume of 0.2 ml per rat were injected subcutaneously (sc) in the back of neck using a 25-gauge needle. The curcumin was dissolved in rice bran oil (Kulkarni *et al.*, 2008). In acute treatments, rice bran oil (control) and curcumin at doses of 25, 50, 100 and 200 mg/kg were administered orally (po) on a single occasion; in chronic treatments, pcurcumin was given at doses of 3.125, 6.25, 12.5 and 25 mg/kg once daily over a period of 15 days. Oral administrations of rice bran oil and curcumin were performed in a constant volume of 0.2 ml per rat over a period of between 1-2 min using a needle-free 1 ml syringe. The selected doses of curcumin and the time period schedule used in this study were similar to other studies that have been performed in rats and mice (Sharma *et al.*, 2006; Tajik *et al.*, 2007; Tajik *et al.*, 2008; Tamaddonfard *et al.*, 2008; Kulkarni *et al.*, 2008; Tamaddonfard *et al.*, 2009). Corneal pain was induced 1 h after the last oral administration of curcumin. Morphine and naloxone were administered sc 30 and 40 min before induction of corneal pain, respectively. In combined treatments, naloxone and morphine were injected sc at 20 and 30 min after the po administration of curcumin.

For the induction of corneal pain, each rat was placed on a 50 \times 50 cm wooden table. After a 15 min adaptation period, one 40 μl drop of 5 M NaCl solution was applied locally on the corneal surface using a fine dropper, and then the number of eye wipes performed with the ipsilateral forepaw was counted for a period of 30 sec (Farazifard *et al.*, 2005; Tamaddonfard *et al.*, 2008). In control group, one drop of 0.15 M NaCl (normal saline) solution was applied.

To evaluate significance differences among treated groups, a one-way analysis of variance (ANOVA) and Duncan's test were applied. All of the data were expressed as the mean \pm standard error of the mean (SEM). A value of $p < 0.05$ was considered to be statistically significant.

Results

None of the tested animals reacted to locally applied 0.15 M NaCl solution (0.0 ± 0.0), and therefore the results that were obtained from these animals were not shown in the figures. The number of eye wipes induced by the local corneal application of 5 M NaCl solution after the sc injection of normal saline was 16.2 ± 1.5 . The sc injection of morphine at a dose of 0.5 mg/kg did not give a significant effect, whereas at doses of 1, 2 and 4 mg/kg, morphine significantly decreased the number of eye wipes induced by topical application of hypertonic saline ($p < 0.05$). The sc injection of naloxone (2 mg/kg) did not change the intensity of corneal pain, whereas pretreatment with naloxone (2

mg/kg) prior to the administration of 2 mg/kg morphine significantly inhibited morphine-induced antinociception ($p < 0.05$; Fig. 1).

After the acute and chronic oral administrations of rice bran oil, the number of eye wipes obtained was 17.4 ± 1.5 and 16.5 ± 1.9 , respectively. The acute oral administration of curcumin at doses of 25, 50 and 100 mg/kg produced no significant effect on the corneal pain response, whereas at a dose of 200 mg/kg, curcumin significantly decreased the number of eye wipes ($p < 0.05$). The chronic oral administration of curcumin for 15 days at a dose of 3.125 mg/kg did not change the intensity of corneal pain, whereas at doses of 6.25, 12.5 and 25 mg/kg, curcumin significantly attenuated the corneal pain response ($p < 0.05$; Fig. 2).

Acute oral administration of curcumin at a dose of 200 mg/kg, but not at doses of 25, 50 and 100 mg/kg significantly increased the suppressive effect of morphine (2 mg/kg, sc) on corneal pain ($p < 0.05$). Naloxone (2 mg/kg, sc) did not reverse the antinociceptive effect of the acute oral administration of curcumin at a dose of 200 mg/kg (Fig. 3).

Chronic oral administration of curcumin for 15 days at doses of 3.125 mg/kg had no significant effect, but doses of 6.25, 12.5 and 25 mg/kg curcumin significantly enhanced the suppressive effect of 2 mg/kg sc morphine on corneal pain ($p < 0.05$). Naloxone (2 mg/kg, sc) did not reverse the effect of chronic oral administration of curcumin at a dose of 25 mg/kg (Fig. 4).

Discussion

In the present study, morphine attenuated the intensity of corneal pain, but naloxone did not change this. Pretreatment with naloxone prior to doses of morphine prevented the antinociceptive effect of morphine. These results confirmed that morphine

mediates its antinociceptive activity through a naloxone-sensitive mechanism in acute corneal pain.

Figure 2: The effect of the acute or chronic oral administration of curcumin on hypertonic saline-induced corneal pain in rats. Values are expressed as the mean \pm SEM, (n=8 rats in each treatment group). Rats within the control group had 5 M NaCl applied corneal surface after either the acute or chronic oral administration of rice bran oil. * $p < 0.05$ compared with control; po: oral administration.

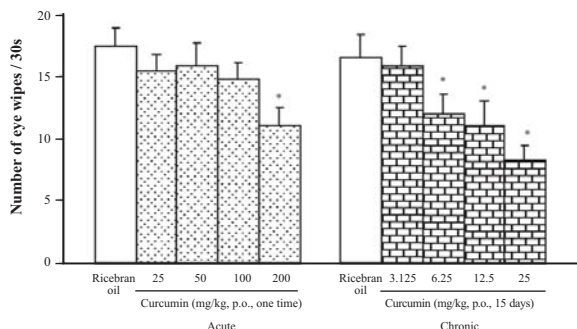


Figure 3: The effect of acute oral administration of curcumin on the morphine- and naloxone-induced changes in the perception of corneal pain in rats. Values are expressed as the mean \pm SEM. (n=8 rats in each treatment group). * $p < 0.05$ compared with morphine (2 mg/kg); † $p < 0.05$ compared with naloxone; po: oral administration; sc: subcutaneous.

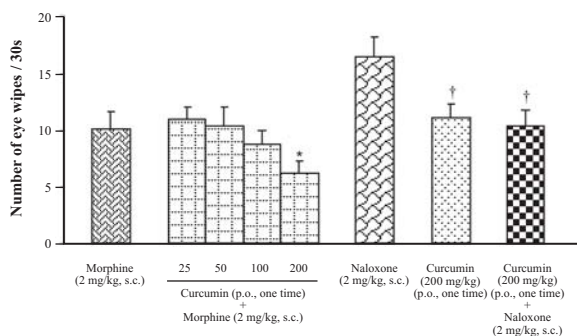


Figure 4: The effect of chronic oral administrations of curcumin on the morphine- and naloxone-induced changes in corneal pain in rats. Values are expressed as the mean \pm SEM. (n=8 rats for each treatment group). * $p < 0.05$ compared with morphine (2 mg/kg); † $p < 0.05$ compared with naloxone; po: oral administrations; sc: subcutaneous administration.

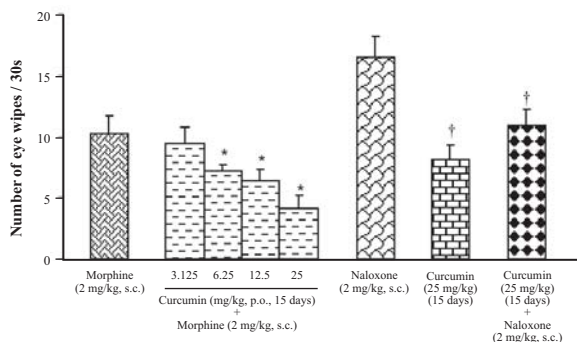
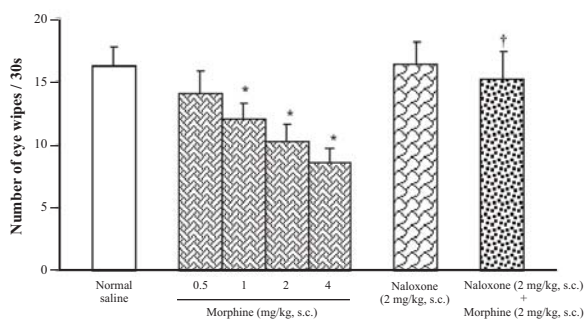


Figure 1: Effects of morphine and naloxone on hypertonic saline-induced corneal pain in rats. Values are expressed as the mean \pm SEM, (n=8 rats in each treatment group). Rats within the control group had 5 M NaCl applied to the corneal surface after the subcutaneous injection of normal saline. * $p < 0.05$ compared with control; sc: subcutaneous; SEM: standard error of the mean; NaCl: sodium chloride.



Morphine acts through μ -opioid receptors, and naloxone is a competitive antagonist of μ -, κ - and σ -receptors, with high affinity for the μ -receptors (Helm *et al.*, 2008). In a model of acute chemical injury to the rat cornea, the local application of a drop of morphine reduced hyperalgesia and naloxone prevented the morphine-induced analgesia (Wenk *et al.*, 2003). Moreover, in the eye-wiping test in rats, an ip injection of morphine attenuated the pain response that was induced by the local corneal application of 5 M NaCl (Farazifard *et al.*, 2005). In addition, systemic or local application of morphine decreased the firing rates of neurons in the caudalis/upper cervical spinal cord transition region that was induced by application of pulses of CO₂ to the cornea (Hirata *et al.*, 2000). Therefore, it seems the naloxone-sensitive antinociception induced by morphine that was observed in the present study may be associated with the action of morphine on the peripheral and central pathways involved in the transmission of corneal pain (Farazifard *et al.*, 2005; Dubner and Bennet, 1983; Hirata *et al.*, 2000).

In the present study, acute oral administration of curcumin at the highest dose produced antinociception, whereas long-term treatment with curcumin at doses of 6.25, 12.5 and 25 mg/kg produced the suppression of corneal pain. Acute oral administration of curcumin produced no effect on licking and biting behaviors in the first phase of formalin-induced pain in rats (Tajik *et al.*, 2007), whereas an ip injection of curcumin suppressed both phases of orofacial pain induced by formalin (Mittal *et al.*, 2009). The first phase of formalin-induced pain is an acute (neurogenic) phase and due to a direct algogenic effect of formalin on nociceptors (Tjolsen *et al.*, 1992; Robaïsson *et al.*, 2004).

Hypertonic saline-induced corneal pain has been introduced as a model of acute pain for study of mechanisms of pain in the trigeminal system in rats (Farazifard *et al.*, 2005). However, it has been reported that curcumin is poorly absorbed in the intestinal tract. Oral doses are largely excreted in feces and only trace amounts appear in the blood (Ravindranath and Chandrasekhara, 1980; Ammon and Wahl, 1991). On the other hand, chronic oral administration of curcumin at doses of 20 and 40 mg/kg suppressed acetic acid-induced visceral nociception (Tajik *et al.*, 2008). In addition, four weeks of oral treatment with curcumin at doses of 15, 30 and 60 mg/kg reduced hyperalgesia in a diabetic mouse model of neuropathic pain (Sharma *et al.*, 2006). In patients with chronic anterior uveitis, chronic treatment with curcumin induced a subjective and objective improvement in vision, as well as in pain relief, redness and lacrimation (Lal *et al.*, 1999). These findings and the results of the present study confirm the fact that curcumin may show an antinociceptive effect when used over a long period orally.

In this study, acute and chronic oral administrations of curcumin enhanced the antinociceptive effect of morphine and naloxone did not reverse the antinociception that was induced by curcumin. This indicates that curcumin may use of a number of different mechanisms in producing analgesia. In the formalin-induced pain in rats, acute oral administration of curcumin failed to change morphine-induced antinociception, but reversed the effect of naloxone on pain (Tagik *et al.*, 2007). Chronic oral administration of curcumin enhanced the suppressive effect of morphine on acetic acid-induced visceral pain, but it did not reverse the effect of naloxone (Tajik *et al.*, 2008). Curcumin potentiated the suppressive effect of morphine but did not reverse the effect of naloxone on formalin-induced paw edema in rats (Tamaddonfard *et al.*, 2009). It has been reported that curcumin has the ability to inhibit the activation of inflammatory mediators such as cyclooxygenase-2 (COX-2), lipooxygenase, inducible nitric oxide synthase products and nuclear factor kappa-B (Bengmark, 2006). The roles of oxygen free radicals, cytokines, arachidonic acid products and nuclear factor kappa-B in pain and analgesia mechanisms have been reported (Gao *et al.*, 2007; Zhang and An, 2007; Bujalska and Gumulka, 2008; Neiderberge and Geisslinger, 2008). The co-administration of curcumin with an antioxidant, vitamin C, produced a synergistic effect in reducing acetic-acid-induced abdominal wall contractions (Tamaddonfard *et al.*, 2008). Through its inhibitory effects on the release of nitric oxide and tumor necrosis factor alpha (TNF α), curcumin attenuated thermal hyperalgesia in a diabetic mouse model of neuropathic pain (Sharma *et al.*, 2006). Hyperalgesia, oxidative stress and TNF α levels were reduced by the long-term oral administration of curcumin in mice (Gupta *et al.*, 2009). It has been reported that curcumin produces a synergistic effect with a COX-2 and prostaglandin synthesis inhibitor, diclofenac, in the reduction of orofacial pain in rats (Mittal *et al.*, 2009).

In conclusion, the results of the present study demonstrate the antinociceptive activity of curcumin in a rat model of acute corneal pain and its synergistic interaction with morphine. Since naloxone did not affect of curcumin-induced antinociception that was observed in the present study, several other studies will be necessary to elucidate the specific mechanisms involved in curcumin-induced analgesia.

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