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Interaction Between Curcumin and Opioid System in the Formalin Test of Rats

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Abstract: In this study, the effect of curcumin on the formalin-induced pain was investigated in rats. Interaction between curcumin and opioid system using morphine and naloxone was also examined. A biphasic pain response was induced after intraplantar injection of formalin (50 μL, 1%). Curcumin, morphin and naloxone had no effect on the early phase of pain. Late phase of pain was suppressed by curcumin at the doses of 100 and 200 mg kg^{-1} body weigh. Morphine (1 mg kg^{-1} BW) reduced, whereas naloxone (1 mg kg^{-1} BW) did not affect the late phase of pain. Curcumin did not influence the morphine-induced antinociception, but reversed the effect of naloxone on pain. Present findings indicate that curcumin may produce antinociception by activation of both opioid and non opioid mechanisms of pain.

Key words: Curcumin, morphine, naloxone, formalin-induced pain, rats

INTRODUCTION

Curcumin is the prominent yellow pigment in turmeric (*Curcuma longa*), a widely used spice and food colouring agent with anti-inflammatory, anti-oxidant, anti-angiogenic, anti-bacterial and anti-cancer properties (Ammon and Wahl, 1991; Maheshwari *et al*., 2006). On the antinociceptive effect of curcumin, it was reported that cucumin produced antinociception using tail immersion and hot plate assays of pain in a diabetic mouse model of neuropathic pain (Sharma *et al*., 2006). JCICM-6 is an extract of an anti-arthritic herbal formula and Curcuma longa is one of its components. It was found that JCICM-6 produced antinociception in both tail flick of rats and writhing reflex of mice (Zhou *et al*., 2006).

The formalin test is an important animal model in the study of acute long-lasting inflammatory pain (Fu *et al*., 2001). In this model, intraplantar injection of formalin into a hindpaw elicits a biphasic pattern of pain-related behaviours, an early short-lasting neurogenic phase followed by a second and more sustained inflammatory phase (Tjolsen *et al*., 1992).

It was reported that S1627, an inhibitor of nuclear factor kappaB, reduced the late phase of formalin pain in rats (Tegeder *et al*., 2004). It was found that curcumin inhibited the activation of nuclear factor kappaB using the Panomics FkappaB Receptor Stable Cell Line (Weber *et al*., 2006).

To date, the effect of curcumin on the formalin-induced pain was not reported. Thus the present study was designed to investigate the effect of curcumin on the formalin-induced pain. Curcumin effect on the endogenous analgesic opioid system was also examined using morphine (an opioid agonist) and naloxone (an opioid antagonist).

MATERIALS AND METHODS

Animals: Healthy adult male albino wistar rats (220-250 g) obtained from the Animal Care and Use Center of Urmia University. Rats were maintained in polypropylene cages in four groups with food and water available *ad libitum*, with controlled ambient temperature (20-23) and under a 12 h light-dark cycle (lights on 0.700 h; lights off 19.00 h). Eight rats were used in each group. The experimental protocol was approved by the Laboratory of Animal Care and Use Center of Urmia University.

Drugs and treatments: Drugs used in the present study were curcumin (Merck, Darmstadt, Germany), morphine sulphate and naloxone hydrochloride (Temad, Tehran, Iran). Curcumin is insoluble in water, but is soluble in ethanol, alkalis, ketone, acetic acid and chloroform (Araujo and Leon, 2001). Therefore, curcumin suspension was prepared in 5% ethanol solution. Curcumin suspension was freshly prepared and administered orally at the doses of 25, 50, 100 and 200 mg kg^{-1} body weight 45 min before formalin injection. Curcumin administration was made in a constant volume of 1 mL^{-1} for each rat over a period of 3-5 min. Morphine and naloxone were dissolved in normal saline and were injected intraperitonealy and subcutaneously 30 min and 1 h

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before formalin injection, respectively. In combined treatments, morphine and naloxone were injected 15 min after or before curcumin administration, respectively.

Formalin test: Each rat was placed inside a plexiglass observation chamber for an acclimation period of 30 min. At the end of this period, drug treatment was performed according to the time schedule for each treatment and then 50 μL of 1% formalin was subcutaneously injected into the plantar region of the right hindpaw using a 29-gauge injection needle. Control group was intraplantarly injected with normal saline. Immediately after formalin and normal saline injections, the time spent licking and biting the injected paw was recorded in 5 min intervals for 1 h (Tjolsen *et al*., 1992). In present study, data collected between 0 and 5 min post-formalin injection represented phase one (early phase) and data collected between 16-45 min after injection of formalin represented phase two (late phase).

Statistical analysis: Data were expressed as means±SEM. Differences among treated groups were statistically evaluated using the one-way analysis of variance (ANOVA) followed by Duncan's test. Differences were considered significant at $p<0.05$.

RESULTS

Figure 1 shows the effect of curcumin on the formalin-induced pain response. Intraplantar injection of formalin produced a marked biphasic pain (early phase: 59.5±7.4 s; late phase: 222.7±18.4 s). Curcumin at the doses of 25, 50, 100 and 200 mg kg^{-1} body weight had no effect on the early phase of pain. Late phase of pain was not affected by curcumin at the doses of 25 and 50 mg kg^{-1} body weight, whereas curcumin at the dose of 100 and 200 mg kg^{-1} body weight significantly suppressed the late phase of pain ($p<0.05$). No significant difference was observed between the effects of 100 and 200 mg kg^{-1} body weight of curcumin.

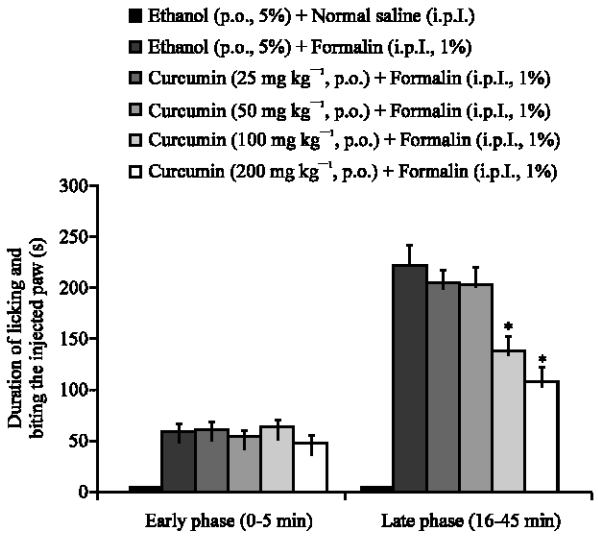

Fig. 1: Effect of curcumin on the formalin-induced pain in rats. Values are mean±SEM, *$p<0.05$ vs. other groups in the late phase, p.o: per oral, i.p.l: intraplantar

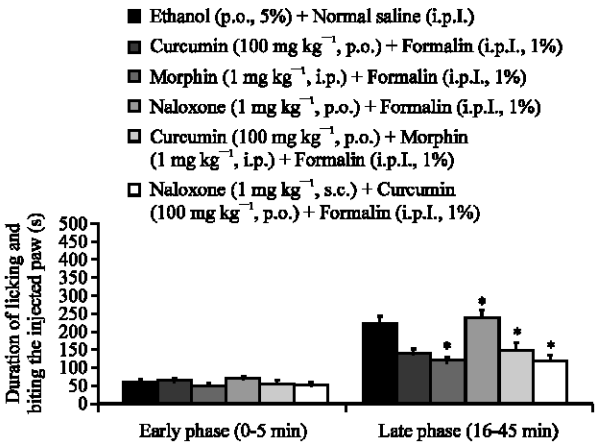

Fig. 2: Effect of curcumin on the morphine and naloxone-induced pain changes in rats. Values are mean±SEM, *$p<0.05$ vs. ethanol+formalin and naloxone+formalin groups in the late phase, p.o: per oral, i.p.l: intraplantar, s.c: subcutaneous

Figure 2 shows the effects of morphine, naloxone alone and with curcumin on the formalin-induced pain. Early phase of pain was not affected with alone application of curcumin, morphine and naloxone. Post-and pre-curcumin treatments with morphine and naloxone, respectively, did not change this phase, too. Curcumin (100 mg kg^{-1} BW) and morphine (1 mg kg^{-1} BW) significantly reduced the late phase of pain ($p<0.05$). No significant difference was observed between the effects of curcumin and morphine. Naloxone (1 mg kg^{-1} BW) did not change the late phase of pain. The effect of morphine on the late phase was not influenced by curcumin pre-treatment. Post-treatment with curcumin significantly decreased the effect of naloxone on pain ($p<0.05$).

DISCUSSION

In the present study, it was found that curcumin suppressed inflammatory pain. The lack of effect of curcumin, morphine and naloxone on the early phase of

pain observed in the present study, may be related to the fact that the early phase of formalin pain is a neurogenic pain and produces due to direct activation of C-fiber nociceptors (Tjolsen *et al*., 1992). It was reported that anti-inflammatory drugs did not affect the first phase of formalin pain even when a very low formalin concentration used (Rosland *et al*., 1990). Moreover, it was found that the potency of morphine in the formalin test affected by both the way of administration and formalin concentration (Servostianova *et al*., 2003).

It this study, curcumin at the doses of 25 and 50 mg kg^{-1} body weight did not influence pain. It has been reported that curcumin is poorly absorbed in the intestinal tract. Oral doses are largely excreted in faeces and only trace amounts appears in the blood (Ravindranath and Chandrasekhara, 1980; Ammon and Wahl, 1991).

The antinociceptive effect of curcumin on the inflammatory pain observed in this study may be related to its anti-inflammatory property. It has been reported that curcumin has ability to inhibit the activation of inflammatory mediators, such as cyclooxygenase 2, lipooxygenase, inducible nitric oxide synthase and nuclear factor kappaB (Bengmark, 2006). On the other hand, the contribution of histamine, serotonin, bradykinin, cytokines, sympathetic amines, lipooxygenase, cyclooxygenase one and two, inducible nitric oxide synthase to one or both phases of formalin-induced pain is firmly established (Tjolsen *et al*., 1992; Doak and Sawynok, 1997; Doursout *et al*., 2003; Chichorro *et al*., 2004).

In this study, curcumin with no effect on the morphine-nduced antinociception, attenuated the naloxone effect. This indicates that curcumin produces antinociception by activation of both opioid and non opioid pain mediated systems. The sensation of pain is well know to be modified by endogenous opioid and non opioid systems. Morphine (an opioid agonist) and naloxone (an opioid antagonist) are used to explore the involvement of endogenous analgesic systems activated by novel analgesics (Yoshimatsu and Furue, 2006; Ananthan, 2006). On the other hand, in bilateral Olfactory Bulbuectomy (OB) model of depression in rats, it was reported that curcumin changed the monoaminergic neurotransmitter levels such as 5-hydroxytriptamine, noradrenalin and dopamine and their metabolites in the hippocampus and frontal cortex of brain (Xu *et al*., 2005). These neurotransmitters comprise the important part of non opioid endogenous pain modulating system (Yoshimatsu and Furue, 2006).

Finally, it seems that several mechanisms are involved in the analgesic effect of curcumin in the formalin test. Further studies are needed to indentify mechanisms.

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