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Effects of curcumin and vitamin C on visceral nociception induced by acetic acid in rats*)

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Summary

The present study investigates the effects of 14 days oral administration of curcumin and 7 days intraperitoneal injection of vitamin C in separate and combined treatments on visceral nociception in rats. Visceral nociceptive responses including the latency time to the beginning of the first abdominal wall contraction (first writhe) and the number of writhes were recorded for a period of 1 h after an intraperitoneal injection of acetic acid (1 cm^3, 2%). In chronic separate treatments with curcumin and vitamin C at the same doses of 12.5 and 25 mg/kg B.W., nociceptive responses were significantly ($p < 0.05$) attenuated. In chronic combined treatments with curcumin and vitamin C at the same dose of 12.5 mg/kg B.W., the suppression of pain was significantly ($p < 0.05$) greater than the one obtained with the same dose of 12.5 mg/kg B.W. of both agents used separately. Based on the results of this study, it is concluded that both curcumin and vitamin C are able to suppress visceral pain, and vitamin C may potentiate the antinociception induced by curcumin.

Keywords: curcumin, vitamin C, visceral nociception, rats

Pains arising from a distension, ischemia and inflammation of the viscera, such as the stomach, kidney, gallbladder, urinary bladder and intestines, constitute a large part of clinically treated pains (1). Over the past few years, a number of animal models have been developed that, to a large extent, mimic the nociception originating in the viscera (10, 12). In mice and rats an intraperitoneal injection of irritant agents, such as acetic acid and phenylquinone, produces a very stereotyped behavior characterized by abdominal muscles contraction accompanied by a hind limb extensor motion. This visceral nociceptive test has been known as the writhing test (10, 12).

Spices have been recognized to possess several medicinal properties and have been effectively used in indigenous systems of medicine (18). Curcumin is a major yellow-orange pigment extracted from turmeric, a commonly used spice, derived from the rhizome of the herb Curcuma longa. Curcumin has a wide range of biological and pharmacological effects including anti-carcinogenic, anti-mutagenic, anti-diabetic, antibacterial, antiviral, anti-inflammatory and antioxidantive effects (11). On the antinociceptive effect of curcumin, it was reported that curcumin produced antinociception in a diabetic mouse model of neuropathic pain (17). In addition, Tajik et al. (20) reported an antinociceptive effect of curcumin in the formalin test of rats.

Vitamin C (ascorbic acid) is a water-soluble vitamin and has various biological functions such as antiatherosclerotic, antihistaminic, anti-rheumatoid, anticancer, anti-cataract, anti-inflammatory and anti-oxidantive effects (9). It has been reported that vitamin C is able to attenuate back pain and pain from an inflamed vertebral disc (7). Moreover, it was found an intraperitoneal injection of vitamin C suppressed pain response induced by an intraplantar injection of formalin in mice (15).

The aim of this study was to investigate the effects of curcumin and vitamin C in separate and combined treatments on the visceral pain responses induced by acetic acid in rats.

Material and methods

Animals. Healthy adult male albino Wistar rats weighing 220-250 g were obtained from the Laboratory Animals Care and Use Center of Urmia University. Rats were maintained in polypropylene cages with six rats in each cage with food and water available *ad libitum*, in a laboratory with controlled ambient temperature (20-23°C) and under a 12 h light – dark cycle (lights on 07.00 h). Six rats were used in each treatment. The experimental protocol was approved by the Laboratory Animal Care and Use Center of Urmia University.

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Drugs and treatments. Drugs used in the present study were curcumin (Merck, Darmstadt, Germany) and vitamin C (Ascorbic acid, Sigma-Aldrich CO., Steinheim, Germany). Curcumin is insoluble in water but soluble in ethanol, alkalis, ketone, acetic acid and chloroform (2). Therefore, to prevent the effect of the solvent (ethanol and etc.) on pain, a curcumin suspension was freshly prepared in 0.15 M NaCl (normal saline) and was administered orally in a constant volume of 0.2 ml per rat over a period of 1-2 min once a day for a period of 14 days. Vitamin C was dissolved in normal saline, and the pH of the prepared solution was altered to 5-6 by adding a drop (40 µl) of KOH 1 M. An intraperitoneal injection of vitamin C was performed in a volume of 1 ml/kg B.W. using a 25-gauge injection needle once a day for a period of 7 days. In chronic separate treatments the doses of 6.25, 12.5 and 25 mg/kg B.W., and in the chronic combined treatment the dose of 12.5 mg/kg of the both agents were used. Intraperitoneal injections of vitamin C were initated on the 8th day of the curcumin therapy. In all treatments, visceral pain was induced 1 h after the latest administration of both agents.

Nociceptive test. The induction of visceral nociception was performed using the writhing test. For this purpose, each rat was placed inside a plexiglass chamber (40 × 30 × 20 cm) for an acclimation period of 30 min. At the end of this period, and 1 h after the latest drug treatment, 1 ml of 2% acetic acid was injected intraperitoneally using a 25-gauge injection needle. Immediately after the injection of acetic acid, the behavior of the animal was videotaped for a total of 1 h. The latency time to the beginning of the first contraction of the abdominal musculature (first writhe) was measured and the number of writhes was counted from the recorded videotapes. A writhe was defined as a wave of the contraction of the abdominal musculature followed by extension of the hind limbs (10, 12). In control rats the intraperitoneal injection of appropriate amount of normal saline was performed.

Statistical method. Data were expressed as mean ± SEM. Differences between the 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 and discussion

An intraperitoneal injection of normal saline produced no writhes. Therefore, the results (0 ± 0) obtained from normal saline injections are not shown in the figures. After an intraperitoneal injection of acetic acid, the latency time to the beginning of the first writhe and the numbers of writhes observed were 8.2 ± 1.2 min and 47.2 ± 3.4 n/1 h, respectively (fig. 1 and fig. 2).

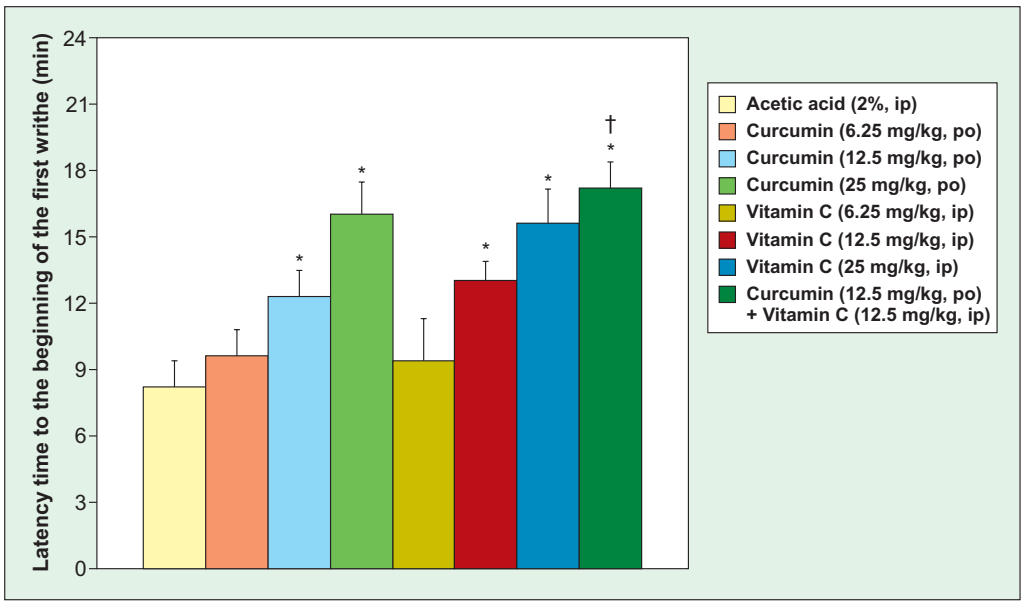

Fig. 1. Effect of chronic separate and combined administrations of curcumin and vitamin C on the latency time to the beginning of the first writhe-induced by acetic acid in rats
Explanations: * – $p < 0.05$ as compared with normal saline; † – $p < 0.05$ as compared with curcumin and vitamin C at the same dose of 12.5 mg/kg; po – *per oral*; ip – intraperitoneal; n = 6 rats in each group

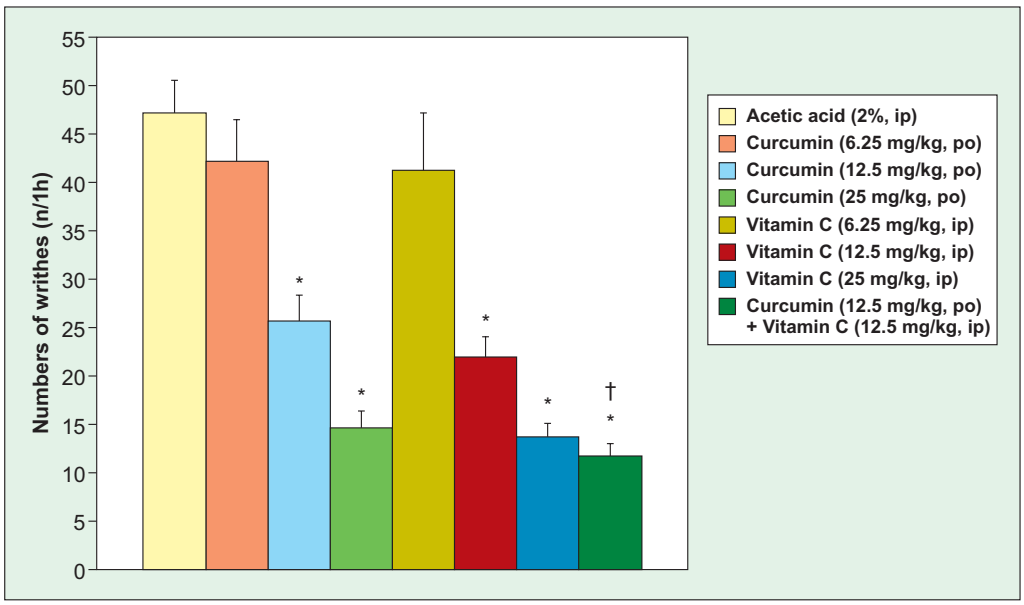

Fig. 2. Effect of chronic separate and combined administrations of curcumin and vitamin C on the numbers of writhes-induced by acetic acid in rats
Explanations: as in fig. 1.

Chronic separate administration of curcumin and vitamin C at the same dose of 6.25 mg/kg B.W. produced no effect on the latency time to the beginning of the first writhe. In the chronic separate administrations of curcumin and vitamin C at the same doses of 12.5 and 25 mg/kg B.W. and in the chronic combined treatment with curcumin and vitamin C at the same dose of 12.5 mg/kg B.W. the latency time to the beginning of the first writhe was significantly ($p < 0.05$) increased. The effect of the combined treatment on the latency time to the beginning of first writhe was significantly ($p < 0.05$) higher than the effect of separate treatments (fig. 1).

The number of writhes was not changed by the chronic separate treatments with the same dose of 6.25 mg/kg B.W. of curcumin and vitamin C. In the chronic separate administrations of curcumin and vitamin C at the same doses of 12.5 and 25 mg/kg B.W. and in the chronic combined treatment with curcumin and vitamin C at the same dose of 12.5 mg/kg B.W. the number of writhes was significantly ($p < 0.05$) decreased. The reduction in the number of writhes in the chronic combined treatment was significantly ($p < 0.05$) greater than the one achieved through the chronic separate treatments (fig. 2).

In the present study, the chronic separate administration of curcumin and vitamin C suppressed the visceral nociception induced by acetic acid, and in the chronic combined treatment, vitamin C potentiated the curcumin-induced antinociception. It has been found that after an intraperitoneal injection of acetic acid, inflammatory reactions develop in the peritoneum (6). Inflammatory reactions may contribute to the pain process through mechanisms involving the release of proinflammatory cytokines and nitric oxide (19), reactive oxygen species (8) and cyclooxygenase products (5). In addition, the acetic acid-induced visceral pain has been reported to be sensitive to opiate and non-opiate analgesics (14). In the diabetic model of neuropathic pain, it has been found that the antinociceptive effect of curcumin may operate through its inhibitory action on the tumor necrosis factor-α and nitric oxide release (17). In addition, in the inflammatory pain, the opioid-dependent and opioid-independent antinociceptive effect of the curcumin was reported (20). Vitamin C applied in large doses twice a week for three weeks produced a suppressive effect on the development of Freund's adjuvant-induced arthritis in rats by reducing the superoxide dismutase activity in inflammatory synovium (16). In addition, vitamin C was reported to suppress the pain induced by intraplantar injections of formalin and glutamate in mice, and it was suggested that ionotropic, but not metabotropic, glutamate receptors may be involved in vitamin C-induced antinociception (15). The effect of the combined treatment with curcumin and vitamin C on pain has not been reported. In the streptozotocin-induced diabetic rats, chronic separate and combined oral administrations of curcumin and vitamin C were reported to reduce blood glucose and prevent the endothelial disfunction in the iris tissue, and it was suggested that curcumin may increase the protecting effect of vitamin C in the function of endothelial cells (13). Considering the fact that both curcumin and vitamin C are able to inhibit the activation of inflammatory mediators such as cyclooxygenase products, nitric oxide, cytokines and reactive oxygen species (3, 4, 9), the contribution of several mechanisms in the antinociceptive effect of curcumin and vitamin C needs to be evaluated.

Conclusion

According to our data, visceral nociception induced by acetic acid was suppressed by separate application of curcumin and vitamin C. In a combined treatment, an additive effect of curcumin and vitamin C was observed in the suppression of the acetic acid-induced visceral pain.

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