PAIN MODULATION BY CURCUMIN AND ASCORBIC ACID IN MICE

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PAIN MODULATION BY CURCUMIN AND ASCORBIC ACID IN MICE (Abstract) **Aim:** The present study aims to evaluate whether ascorbic acid (AA) and curcumin, two substances with redox properties, have similar effects on different models of pain in mice. **Materials and methods:** This study included a total of 28 mice that were divided into four groups. One group (AA) received intraperitoneally 500 mg/kg b.w. AA for 21 days and the 2-nd group (curcumin) received 120 mg/kg b.w. curcumin by gastric gavage for two weeks. Other two groups serve as control and received vehicle in a dose – time manner similar to that of the treated groups. The pain models (oro-facial formalin induced pain, paw formalin induced pain and visceral pain) were performed 24 h after the last dose. **Results:** when compared with control groups, curcumin significantly decreases pain perception in oro-facial (p=0.01 1-st phase, p=0.002 2-nd phase) and paw formalin induced pain (p=0.04 1-st and 2-nd phase) while AA stimulates pain perception in acid acetic induced visceral pain (p=0.05) and increases oro-facial inflammatory pain induced by formalin (p=0.02) but demonstrates analgesic effects on paw formalin induced pain (p=0.003 1-st phase, p=0.01 2-nd phase). **Conclusions:** ROS production is important in pain modulation. Structures involved in the process of pain have different antioxidant defense capacities. Curcumin and AA are able to modulate pain perception, but beside their antioxidant capacities, there are other mechanisms involved. **Keywords:** CURCUMIN, ASCORBIC ACID, PAIN, ANALGESIA.

According to recent studies, reactive oxygen species (ROS) are reported to be associated with chronic pain, especially neuropathic and inflammatory (1). They are also closely related to central sensitization and the elevated ROS in the spinal cord and colon have proven to be involved in visceral pain (2).

Recent studies suggest that oxidative stress and the balance between oxidants and antioxidants can be directly involved in certain diseases characterized by different types of pain or inflammation (3). Curcumin, a hydrophobic polyphenol, protects mitochondrial dysfunction and modulates endogenous antioxidant enzymes, scavenges ROS and NO-based radicals, neutralizes ROS and RNS-based radicals and increases the synthesis of glutathione. On the other hand, several studies demonstrate that curcumin has pro-oxidant capacities through its ability to increase oxidized glutathione (GSSG) and to also reduce glutathione (GSH), thus decreasing
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It was demonstrated that ROS are involved in the apoptosis induced by curcumin in A549 lung adenocarcinoma cells, but also that curcumin prevents the mitochondrial dysfunction and suppresses the ROS increase (5).

Curcumin seems to have analgesic properties, but the mechanism by which it induces antinociception is not clear. Activation of K (ATP) channels (6) or transient receptor potential vanilloid 1 (TRPV1) activation have been proposed (7). Ascorbic acid (AA) is an important antioxidant known to quench ROS, and its oxidase form dehydroascorbic acid (DHA), enters mitochondria via facilitative glucose transporter 1 (Glut1) and accumulates in the mitochondria as ascorbic acid (mtA). The accumulation of AA in the mitochondria inhibits the oxidative mitochondrial DNA damage and thus AA may be useful in generating therapies by antagonizing the effects of ROS (8).

Although AA has a controversial role in pain therapy, it has been shown that a combined antioxidant therapy can be safe and efficient in pain relief for patients with chronic diseases (9).

The purpose of our study was to evaluate if, by modulating ROS production through two antioxidant substances (AA and curcumin), similar effects will be obtained on different models of pain (orofacial formalin induced pain, paw formalin induced pain or the acetic acid induced visceral pain).

MATERIAL AND METHODS

Animals. The experiments were conducted using male BALB/c mice (28-34 g), housed at 21 ± 2 °C under a 12-h light/dark cycle, with access to food and water ad libitum. All animals were habituated to the testing laboratory for at least five days. The experimental protocols and procedures described in this article complied with the European Communities Council Directive 86/609/EEC and followed the International Association for the Study of Pain’s (10) and the University of Medicine and Pharmacy “Grigore T. Popa” ethical guidelines for investigations of experimental pain in conscious animals.

Drugs. The drugs used in these experiments were: vitamin C intravenous solution 750mg/5 ml (Arena, Romania), 37% formaldehyde (Fluka, Germany), acetic acid (Sigma, Germany) and Curcumin (Turmeric powder, Sigma, Germany). AA was freshly diluted in distilled water to avoid precipitation and was disposed after first use to avoid oxidation. Curcumin was freshly prepared in olive oil.

The acetic acid induced visceral pain. Writhing Test (WT) test was performed as described by Koster et al. (11). After acclimatization in an acrylic observation chamber for at least 20 minutes, the mice received 0.1 ml /10 mg kg b.w. i.p. injections with 1.0% (v/v) acetic acid (12). A writhes was defined as a contraction of the abdominal muscles accompanied by an extension of the forelimbs and elongation of the body.

The paw formalin test (PFT). The mice were injected with 20 µL of 5% formalin (from 37% formaldehyde) subcutaneously into the plantar surface of the right hind paw using a microsyringe. Pain and inflammatory response was measured by the total time (in seconds) spent by mice licking and/or biting the injected paw during periods of 0–9 min (first / neurogenic phase) and 10–40 min (second / inflammatory phase) (13).
The orofacial formalin test (OFT). Nociception induced by formalin was performed as described by Luccarini et al. (14). Animals received 20 µL of 5% formalin into the right whisker pad. The nociceptive score was determined by recording the number of seconds the animals spent grooming the injected area with the ipsilateral hind paw or forepaw, often accompanied by the contralateral forepaw, for the first 6 min (first / neurogenic phase) and from 7 to 40 min (second / inflammatory phase).(15)

Study design. The study used a total of 28 mice that were divided into four groups: group AA mice receiving intraperitoneal 500 mg/kg b.w. AA for 21 days. Group Curcumin receiving two weeks curcumin in combination with oil by intragastric gavage 120 mg/kg b.w. The other two groups that served as control received an equal volume of distilled water (AA Control), olive oil (Curcumin control) respectively in the same time manner. All tests were performed 24 h after the last dose.

Statistical analysis. The data obtained were expressed as the mean value ± SEM. Statistical evaluations were performed using SPSS 20.0 software One-way analysis of variance (ANOVA) and Student’s t-test for independent variable were used to assess within and between groups variability. The significance level of the results was set at p<0.05.

RESULTS

The effects on paw formalin induced pain. Curcumin has shown an analgesic effect on both phases on the formalin induced pain (phase 1 p=0.04 respectively phase 2 p=0.04) when compared with control group (fig. 1). AA has also demonstrated an analgesic effect on both phases (p=0.01) as compared with control group (fig. 2). No significant differences were observed between curcumin and AA groups.

The effects on orofacial formalin induced pain. The analgesic effect of curcumin was statistically significant in both phases (phase 1 p=0.01, phase 2 p=0.01 respectively) when compared with control group (fig. 1).

![Fig.1. Effects of 14 days treatment with curcumin on the writhing test and phases 1&2 of the orofacial (FOF) and paw (FP) formalin tests. The results are expressed as mean± S.E.M. * p < 0.05 as compared with control group](image-url)
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Fig. 2. Effects of 21 days treatment with AA on the writhing test and phases 1&2 of the orofacial (FOF) and paw (FP) formalin tests. The results are expressed as mean± S.E.M.* p < 0.05 as compared with control group

By contrary, AA has no effect on the first phase, increases pain perception on the second phase (p=0.01), as compared to the distilled water group. Significant differences were observed between curcumin and AA groups in the first phase. (p =0.007).

The effects on visceral pain. Although curcumin has shown potential on orofacial and paw formalin induced pain, the effect on visceral pain had no statistical significance (fig. 1). However, AA stimulates pain perception compared to the control group (p=0.05) (fig. 2).

DISCUSSION

In our study, we demonstrated that long term administration of curcumin and AA modulate pain perception in formalin and acetic acid induced pain. Thus, curcumin significantly decreases pain perception in oro-facial and paw formalin induced pain, while AA has an analgesic effect on paw formalin induced pain. On the contrary, AA increases the oro-facial inflammatory pain induced by formalin and visceral pain.

Under physiological conditions, AA mainly exists in its reduced form and in trace quantities in the oxidized form, dehydroascorbic acid (DHA). Beside its antioxidant capacities, AA seems to down-modulate NF-KB signaling (15), as well as norepinephrine production (16).

Chen and collaborators have shown that AA might generate H2O2-dependent cytotoxicity toward a variety of cancer cells in vitro and in vivo, without adversely affecting normal cells (17), while Martinovich et al suggested that Ca (2+) release in HEP-2 cancer cell line is the mechanism through which AA increases mitochondrial ROS production (18). Thus there are enough evidences that AA has both pro and antioxidant capacities.

At the dose used in our study, AA seems to exert proprieties, having an antioxidant effect paw formalin induced pain and acting as a pro-oxidant on the inflammatory phase in orofacial formalin induced pain and the acetic acid visceral induced pain. Besides redox proprieties, we cannot exclude, that changes in epinephrine levels secondary to AA administration might be
involved in pain modulation. Indeed, it is well-documented that norepinephrine induces both antinociception by activation of the NO/cGMP/KATP pathway (19) and nociception by enhancing pain facilitation from the brain (20).

Our results suggest that AA has different effects on pain at the same dose. These effects depend probably on cells resistance to ROS production and on pain structures sensitivity to epinephrine stimulation.

Similar to vitamin C, curcumin, a mitochondrial modulator, has both pro- and antioxidant activities. The underlay mechanisms of curcumin effects are not clarified, but regulation of various molecular targets, such as nuclear factor-κB, inflammatory cytokines and other enzymes are implied (21).

In our study, curcumin had a significant antinociceptive effect on both phases of formalin induced pain, with no effect on visceral pain. Indeed, peripheral and central pathways for visceral pain are unique and some receptors such as acid sensing ion channels, sodium channels, calcium channels, kappa opioid receptor, appear to be particularly important to modulating pain. In addition to the acetic acid induced pain, nociceptive messages from the inflammed peritoneum involve neurokinin A rather than substance P as a mediator. By contrast, inflammatory phase in formalin induced pain is mainly mediated by substance P(22). Thus, the curcumin antinociceptive effects are not only a consequence of its antioxidant activity; other mechanism seems to be also involved. Considering everything, we have demonstrated that: ROS production might have a place in pain modulation; neuroanatomical structures involved in pain modulation have different antioxidant defense capacities and curcumin and AA are able to modulate pain perception, but there are other mechanisms involved, beside their antioxidant capacities.

CONCLUSIONS
By using two ROS modulators (ascorbic acid and curcumin), we demonstrated that oxidative stress might have an important place in pain pathophysiology. Further studies are however, however necessary to further explain the involvement of curcumin and ascorbic acid in pain modulation.

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