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Original Article

Ellagic acid alleviates inflammatory pain and paclitaxel-induced neuropathic pain in murine models

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Abstract: Ellagic acid is a healthy food and drug developed by foreign countries. It has been proven that it is not only anti-cancerous and anti-mutational, but also anti-microbial and antiviral. In this study, we examined the effect of ellagic acid alleviates inflammatory pain and paclitaxel-induced neuropathic pain in murine models. It was shown that the effect of ellagic acid could alleviate acetic acid-induced nociception, formalin-induced nociception and paclitaxel-induced neuropathy pain in mice. Meanwhile, we found that treatment with ellagic acid effectively suppressed the interleukin-6 (IL-6) and nuclear factor-kappa b (NF-κB) activities in acetic acid-induced inflammatory visceral nociception, formalin-induced nociception or paclitaxel-induced neuropathy pain of mice. Next, we researched the mechanism of ellagic acid down-regulated the P38 mitogen-activated protein kinase (p38 MAPK) in acetic acid, formalin or paclitaxel-induced mice. These investigations suggested that ellagic acid alleviates inflammatory pain and paclitaxel-induced neuropathic pain in murine models through suppression of inflammatory and down-regulated the p38 MAPK signal path.

Keywords: Ellagic acid, inflammatory pain, paclitaxel-induced neuropathic pain

Introduction

Pain, an unhappy feeling and emotional experience, is a complicated physiological and psychological activity [1]. As a common feature of most clinical diseases, pain is also one of the most common symptoms. From the point view of physiology, the physiological function of pain is protective and adaptive, which can improve the repair of body tissues and prevent further damage [2]. However, the feeling of pain is a special somatic sensation including feeling and emotion. The feeling of pain cannot or is difficult to produce adaptivity. If pain exists for long period, it could lead to the sensitization changes of pain perception center and result in chronic pain characterized by hyperpathia and douleur anormale [3]. Regarding chronic pain, inflammatory pain which can be considered as a chronic disease, is a common clinical symptom [4]. Patients suffering from inflammatory diseases also are accompanied by inflammatory pain characterized by hyperpathia and douleur anormale [5]. Recent research shows that during pathophysiological procedure of chronic inflammatory pain, after the activation of nociceptive neurons of spinal cord central system and spongiocyte (microglial cells and astrocytes) would release numerous inflammatory mediators which will combine with corresponding receptors, resulting in inflammation cascade amplification and "Waterfall Effect" [6].

Ellagic acidis provided with biological functions such as antioxidant function, anticancer and anti-mutation properties as well as the inhibition functions for HIV [7, 8]. Furthermore, ellagic acid is also a effective coagulant, which has inhibiting effects for a variety of bacteria and viruses, preventing the invasion of bacteria into wound surface to guard against infection and anabrosis [9]. Meanwhile, Ellagic acid has the functions of depressurization and sedation. The present study was designed to characterize the effect of ellagic acidis alleviates inflammatory pain and paclitaxel-induced neuropathic pain in murine models.

Materials and methods

Animals

Male ICR mice (20 to 25 g) were housed constant room temperature (maintained between

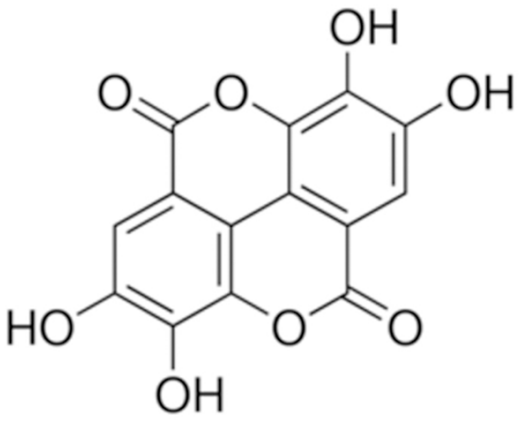

Figure 1. The chemical structure of ellagic acid.

20-25°C), 40-60% humidity and in a standard environment consisting of a 12 h light/dark cycle with Food and water ad libitum. All animal experimentation adheres to the policy of the Gansu Provincial Hospital Lanzhou regarding the use and care of animals.

All experimental mice were divided into the following seven groups: control group (n = 6), acetic acid-induced model group (n = 6), formalin-induced model group (n = 6), paclitaxel-induced model group (n = 6), formalin-induced + ellagic acid group (n = 6), formalin-induced + ellagic acid group (n = 6) and formalin-induced + ellagic acid group (n = 6).

Experimental design

Acetic acid-induced inflammatory visceral nociception in mice: Model and treated group of every mouse were injected subcutaneously with 2 ml saline (Model) or 50 mg/kg/2 ml ellagic acid for 5 days respectively. Model and treated group of every mouse were injected intraperitoneally with 0.2 ml/20 g body weight of 0.7% acetic acid solution in saline. The number of abdominal constrictions was recorded for 15 starting 10 min after acetic acid injection. Control group of every mice were injected saline.

Formalin-induced behavioral nociception in mice: All experimental mice were acclimated to the cylindrical acryl cages for 30 min. Model and treated group of every mouse were injected subcutaneously with 2 ml saline (Model) or 50 mg/kg/2 ml ellagic acid for 5 days respectively. Then, model and treated group of every mouse were injected subcutaneously with 20 ml of formalin (0.5%) from the right hind paw of the mice. Nociceptive response was quantified using the time spent of licking with 0-5 min (early phase, neurogenic) and 20-25 min (late phase, inflammatory).

Paclitaxel-induced neuropathic nociception in rat: Model and treated group of every mice were injected with 10 mg/kg of paclitaxel, and then with 2 ml saline (Model) or 50 mg/kg/2 ml ellagic acid for 5 days respectively. All experimental mice were placed on a plastic mesh floor covered with transparent cages.

Measuring of IL-6 and NF-κB level using Enzyme-linked immunosorbent assay (ELISA)

Bloods of very mice were extracted from eye socket and centrifuged at 2000 g for 10 min at room temperature. Blood serum IL-6 and NF-κB level were analyzed using commercial ELISA kits (Beyotime Institute of Biotechnology, Jiangsu, China) according to the manufacturer's instruction and used a Microplate Reader (Bio-Rad, Hercules, CA).

Western blotting analysis

All experimental mice were anesthetized with 50 mg/kg sodium pentobarbital. Saphenous nerves were dissected with slight modifications and homogenized on ice in Tris buffer containing proteinase inhibitors and phosphatase inhibitors (Beyotime Institute of Biotechnology, Jiangsu, China). The protein concentration of very group was determined using Bicinchoninic Acid kit (BCA, Beyotime Institute of Biotechnology, Jiangsu, China). Equivalent samples protein was separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene fluoride membrane (PVDF, BioRad). The blots were placed in block buffer for 1 h at room temperature and incubated with primary antibody against P38 mitogen-activated protein kinase (MAPK, 1:2000, Cell Signaling Technology, Danvers, MA, USA) and β-actin (Beyotime Institute of Biotechnology, Jiangsu, China) overnight at 4°C. The membrane was incubated with horseradish peroxidase-conjugated anti-rabbit antibody (1:2000, Calbiochem, Darmstadt, Germany) for 1 h at room temperature. Proteins were dete-

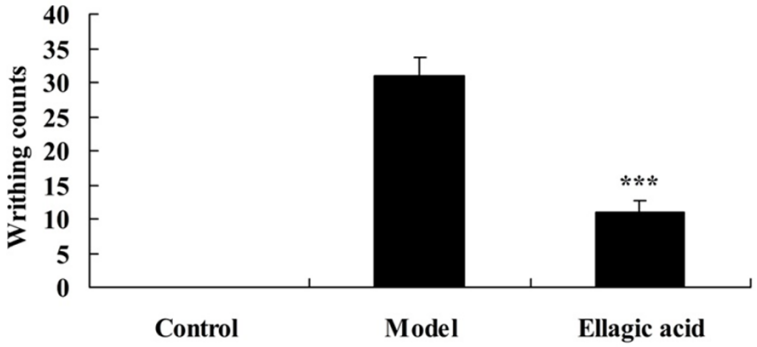

Figure 2. Effects of ellagic acid on acetic acid-induced writhing response. Control, control group; Model, induced model; Ellagic acid, ellagic acid treated group. ***P < 0.01 compared with model group.

cted using a chemiluminescence assay kit (Pharmacia Amersham, Freiburg, Germany).

Statistical analysis

All data were expressed as means ± SEM using SPSS 17.0 (SPSS, Chicago, IL) and one-way ANOVA followed by Tukey's post hoc test for inter group comparisons. The criterion for statistical significance was $P < 0.05$.

Results

Effects of ellagic acid on acetic acid-induced writhing response

The chemical structure of ellagic acid (**Figure 1**, ≥ 95%, HPLC, Sigma, USA). On the pretreatment day, we detected whether effects of ellagic acid on acetic acid-induced writhing response. Ellagic acid could weaken acetic acid-induced writhing response (**Figure 2**).

Effects of ellagic acid on IL-6 and NF-κB in acetic acid-induced inflammatory visceral nociception of mice

As shown in **Figure 3**, there was a consequential increase of IL-6 and NF-κB in bloods of acetic acid-induced mice, compared with control group. Acetic acid-induced IL-6 and NF-κB level weakened was observed in treated with ellagic acid (**Figure 3**).

Effects of ellagic acid on formalin-induced nociception

At starting or finishing treatment with ellagic acid, licking time of formalin-induced mode group was higher than that of control, in which showed that licking time of formalin-induced mode group at starting was higher than that of finishing (**Figure 4**). Meanwhile, treatment with ellagic acid inhibited the formalin-induced licking time in mice, in which indicated licking time of formalin-induced mode group at starting was higher than that of finishing (**Figure 4**).

Effects of ellagic acid on IL-6 and NF-κB in formalin-induced nociception of mice

To explore the effects of ellagic acid on IL-6 and NF-κB in bloods of formalin-induced nociception of mice, IL-6 and NF-κB levels were detected using ELISA kit. As shown in **Figure 5**, formalin observably accelerated the bloods IL-6 and NF-κB level in mice. There were shown in **Figure 5** on the effects of ellagic acid reduced the IL-6 and NF-κB levels in bloods of formalin-induced nociception of mice.

Effects of ellagic acid on paclitaxel-induced neuropathy pain in mice

We explore the effects of ellagic acid paclitaxel-induced neuropathy pain in mice. From 3 days to 5 day of treatment with ellagic acid, percentage of the mean pre-paclitaxel in paclitaxel-induced model was remarkable lower than that of control group (**Figure 6**). But, pretreatment with ellagic acid remarkably increased the percentage of the mean pre-paclitaxel in paclitaxel-induced neuropathy pain in mice after 4 days or 5 days (**Figure 6**).

Effects of ellagic acid on IL-6 and NF-κB in paclitaxel-induced neuropathy pain of mice

In order to confirm effects of ellagic acid on IL-6 and NF-κB in paclitaxel-induced neuropathy pain of mice. As shown in **Figure 7**, there was a remarkable increase in paclitaxel-induced IL-6 and NF-κB, compared with control group. However, treatment with ellagic acid remarkably inhibited paclitaxel-induced IL-6 and NF-κB levels in mice (**Figure 7**).

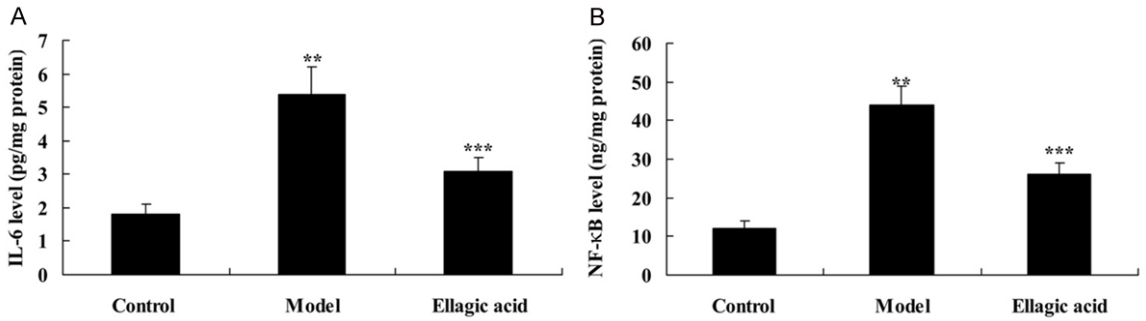

Figure 3. Effects of ellagic acid on IL-6 and NF-κB in acetic acid-induced inflammatory visceral nociception of mice. Effects of ellagic acid on IL-6 (A) and NF-κB (B) in acetic acid-induced inflammatory visceral nociception of mice. Control, control group; Model, induced model; Ellagic acid, ellagic acid treated group. **P < 0.01 compared with control group; ***P < 0.01 compared with model group.

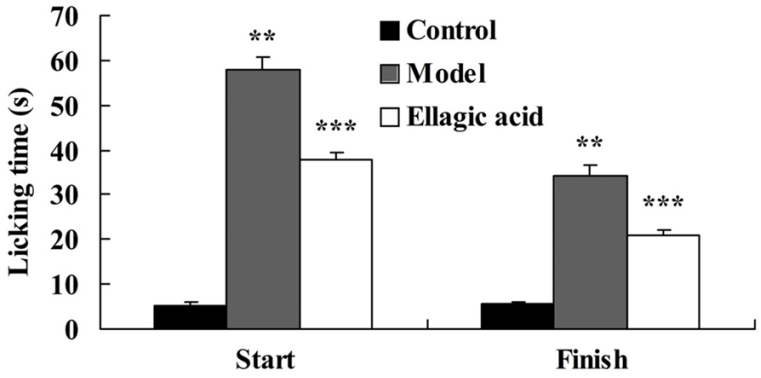

Figure 4. Effects of ellagic acid on formalin-induced nociception. Control, control group; Model, induced model; Ellagic acid, ellagic acid treated group. **P < 0.01 compared with control group; ***P < 0.01 compared with model group.

Effects of ellagic acid on P38 MAPK in paclitaxel-induced neuropathy pain of mice

To examine the mechanism of ellagic acid on paclitaxel-induced neuropathy pain of mice, we measured P38 MAPK protein expression using western blot. As shown in **Figure 8**, P38 MAPK protein expression of paclitaxel-induced group was still higher than that of control group. Treatment with ellagic acid suppressed the activation of P38 MAPK in paclitaxel-induced mice (**Figure 8**).

Discussion

Pain is the most primitive and general feeling. It is a complicated physiological and psychological activity. As a common feature of most clinical diseases, pain is also one of the most common symptoms and a feeling resulting from unhappy and emotional experience [10]. The International Association for the Study of Pain's widely used definition states: "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". Algesia is a highly integrated synaesthesia interwoven with motion response, autonomic nervous system activity, psychological and emotional response [11]. Pain threshold is the minimum perception of pain, which has high repeatability and relatively stability [12]. Pain tolerance is the maximum tolerance of pain endured or the threshold value individuals' avoidance of the stimulus of pain [13]. As closely related with individuals' growth en-vironment and their characteristics, pain tolerance has great variability [14]. In the present study, the effect of ellagic acid inhibited acetic acid-induced writhing response, reduced formalin-induced nociception and suppressed paclitaxel-induced neuropathy pain in mice.

From the point view of physiology, the physiological function of pain is protective and adaptive, which can improve the repair of body tissues and prevent further damage [15]. However, the feeling of pain is a special somatic sensation including feeling and emotion. The feeling of pain cannot or is difficult to produce adaptivity. If pain exists for long period, it could lead to

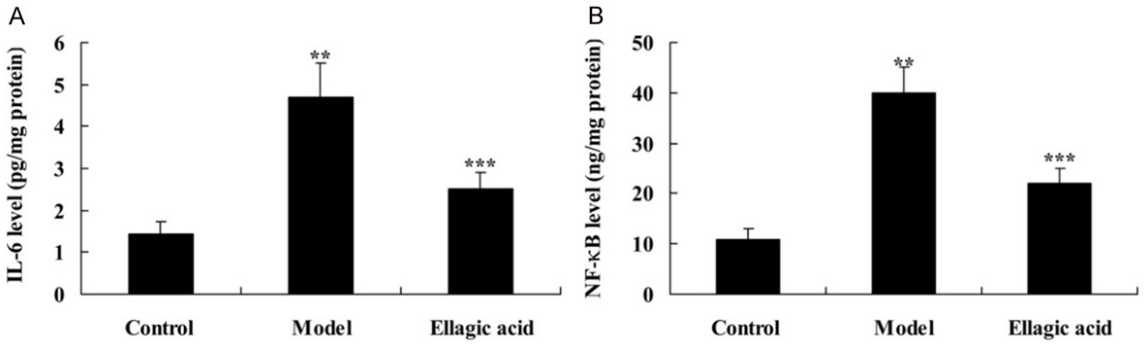

Figure 5. Effects of ellagic acid on IL-6 and NF-κB in formalin-induced nociception of mice. Effects of ellagic acid on IL-6 (A) and NF-κB (B) in formalin-induced nociception of mice. Control, control group; Model, induced model; Ellagic acid, ellagic acid treated group. **P < 0.01 compared with control group; ***P < 0.01 compared with model group.

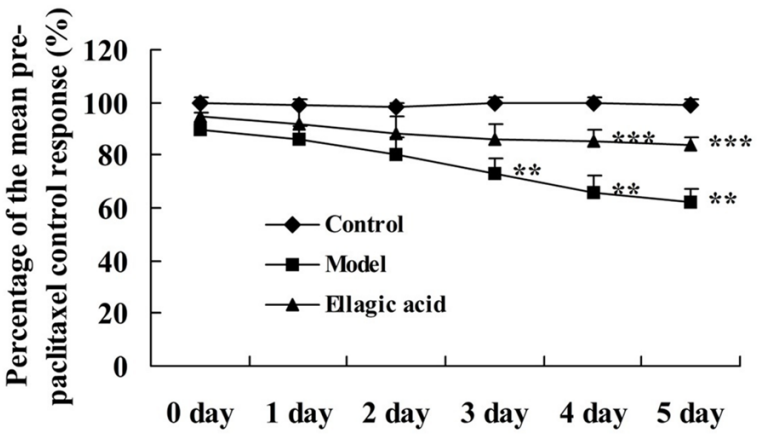

Figure 6. Effects of ellagic acid on paclitaxel-induced neuropathy pain in mice. Control, control group; Model, induced model; Ellagic acid, ellagic acid treated group. **P < 0.01 compared with control group; ***P < 0.01 compared with model group.

the sensitization changes of pain perception center and result in chronic pain characterized by hyperpathia and douleur anormale [16].

The increase of inflammatory factors of animal models of pain suggests that inflammatory factors play an important role in strengthening and maintenance of pain, however, it is not proven yet [17]. If it is confirmed, implementation of inflammatory factors around spinal cord would enhance pain and sealing of activation of these factors could reverse the intensified responses of pain [18]. Actually, administration of exogenous IL-6 or IL-1 around spinal cord may cause hypersensitivity of mechanical pain and irritability of thermalgesia [18]. Moreover, the application of IL-1 around spinal cord would increase the nerve excitability for destructive stimulus. Intrathecal injection of TNF antagonist would inhibit or reverse enhancement effects of pain, trisection of spinal nerves and ischiatitis [19]. In the present study, the effects of ellagic acid could inhibited acetic acid or formalin or paclitaxel-induced IL-6 and NF-κB levels in mice. Umesalma et al. demonstrated ellagic acid suppressed NF-κB and IL-6 in 1,2-dimethylhydrazine-induced rat colon carcinogenesis [20]. Favarin et al. reported that ellagic acid restrained acute lung injury through suppression of inflammatory in mice [21].

Another solution aims at specific intra-cellular pathway involving in generation and transmission of inflammatory factors. As the key part of the pathway, P38 MAPK is activated (phosphorylation) when gets immunostimulation [22]. In addition, the phosphorylation in pathological models increases, including spinal nerve liagation, subcutaneous injection of formalin and CCI [23]. Furthermore, p38 MAPK is activated by glutamic acid which is a classical pain neurotransmitter. Consequently, inhibition of p38 MAPK could hold back inflammatory factors to stop the enhancement of pain [24]. Indeed, p38 MAPK inhibitor can prevent pain enhancement in various animal models, including myelitis and peripheral neuritis [25]. We found that the effect of ellagic acid reduced the paclitaxel-induced P38 MAPK in mice. Lee et al. suggest that ellagic acid protects against T-cell-mediated hepatitis through TLR

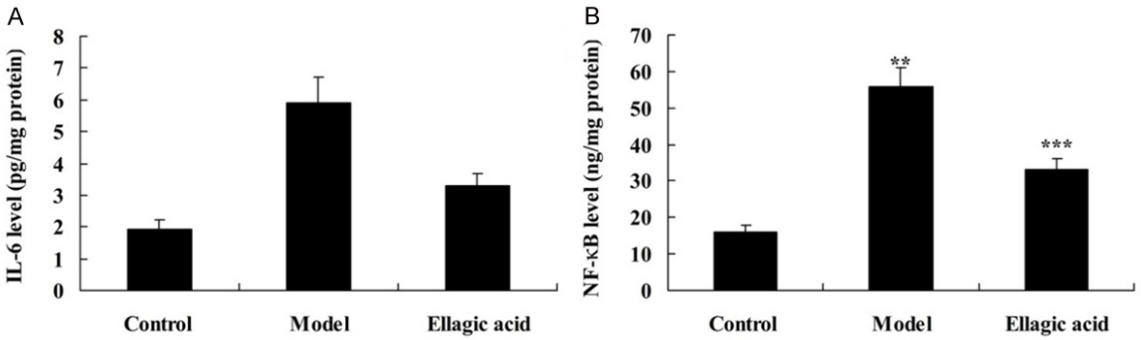

Figure 7. Effects of ellagic acid on IL-6 and NF-κB in paclitaxel-induced neuropathy pain of mice. Effects of ellagic acid on IL-6 (A) and NF-κB (B) in paclitaxel-induced neuropathy pain of mice. Control, control group; Model, induced model; Ellagic acid, ellagic acid treated group. **P < 0.01 compared with control group; ***P < 0.01 compared with model group.

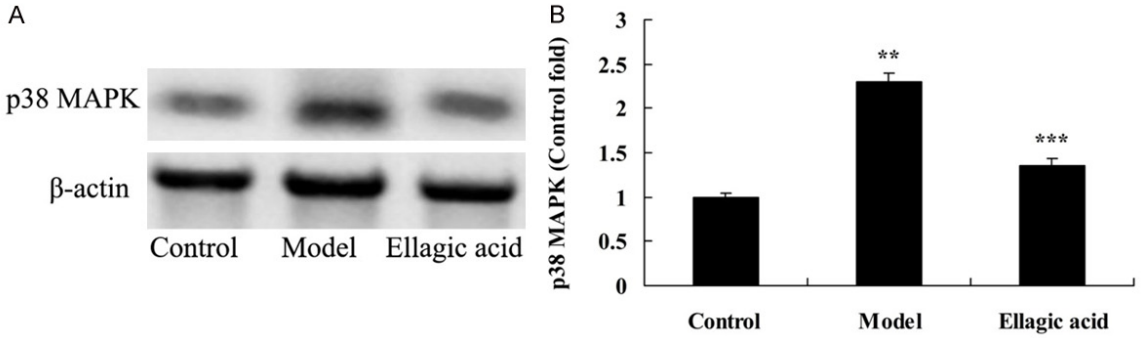

Figure 8. Effects of ellagic acid on P38 MAPK in paclitaxel-induced neuropathy pain of mice. Effects of ellagic acid on P38 MAPK protein expression using western blot (A) and statistics analyzed P38 MAPK protein expression (B). Control, control group; Model, induced model; Ellagic acid, ellagic acid treated group. **P < 0.01 compared with control group; ***P < 0.01 compared with model group.

and p38/NF-κB signaling pathways [26]. In conclusion, ellagic acid alleviates acetic acid or formalin or paclitaxel-induced neuropathy pain in mice. Meanwhile, ellagic acid alleviates acetic acid or formalin or paclitaxel-induced inflammation reaction and p38 MAPK in mice.

Disclosure of conflict of interest

None.

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