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ORIGINAL ARTICLE

Ellagic acid enhances the antinociceptive action of carbamazepine in the acetic acid writhing test with mice

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Abstract

Context: Ellagic acid (EA) produced antinociceptive and anti-inflammatory effects through the central and peripheral sites of action.
Objective: The objective of the current study was to examine the functional interaction between ellagic acid and carbamazepine (CBZ) on pain.
Materials and methods: Fourteen groups of mice (8–10 each) were used in this study. Pain was induced by intraperitoneal acetic acid in mice (writhing test) and the functional interaction was analyzed using the isobolographic method. EA at doses 0.3, 1, 3, and 10 mg/kg and carbamazepine at doses 3, 10, 20, and 30 mg/kg, alone and also in combination (1/2, 1/4, and 1/8 of the drug's ED_{50}) were intraperitoneally administered 30 min before acetic acid (0.6% v/v). Then, the abdominal writhes were counted during a 25-min period.
Results: EA (0.3–10 mg/kg, i.p.) and CBZ (3–30 mg/kg, i.p.) inhibited the writhing response evoked by acetic acid. Fifty percent effective dose (ED_{50}) values against this tonic pain were 1.02 mg/kg and 6.40 mg/kg for EA and CBZ, respectively. The antinociception induced by EA showed higher potency than that of carbamazepine. Co-administration of increasing fractional increments of ED_{50} values of EA and CBZ produced additive interaction against writhing responses, as revealed by isobolographic analysis.
Discussion and conclusion: These results suggest that a combination of carbamazepine and ellagic acid may be a new strategy for the management of neuropathic pain such as what occurs in trigeminal neuralgia, since the use of carbamazepine is often limited by its adverse effects and by reduction of its analgesic effect through microsomal enzyme induction.

Keywords

Analgesia, isobolographic analysis, visceral pain

History

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Introduction

The development of new pain strategy involves combining analgesic drugs to receive greater analgesia at reduced doses of individual drugs and lower side effects, both of which are very important in improving patient health (Tallarida, 2001).

Plant polyphenols are bioactive compounds which possess multiple neuroprotective actions in some central nervous pathophysiological conditions, especially the pain regulation (Ullah & Khan, 2008).

Ellagic acid (EA) (2,3,7,8-tetrahydroxy-[1]-benzopyranol-[5,4,3-cde]-[1]-benzopyran-5,10-dione) is a polyphenolic agent that occurs largely as ellagitannins in plants such as pomegranate juice, raspberries, the stem and bark of eucalyptus species, and nuts. This bioflavonoid compound has been reported to have antioxidant, antifibrotic, cardioprotective, anticancer (Girish & Pradhan, 2008), neuroprotective (Dolatshahi et al., 2015; Girish et al., 2012), anti-inflammatory, and antinociceptive properties (Gainok et al., 2011). It has been found to have antidepressant-like activity and its effect is dependent on the interaction with the central serotonergic (5-HT1, 5-HT2, and 5-HT3 receptors) and noradrenergic (α1 and α2 adrenoceptors) systems (Girish et al., 2012). Also, Girish et al. (2013) proved the involvement of the GABAergic system in the anxiolytic-like effect of EA. Recently, we have shown the central and peripheral antinociceptive activities of EA which were mediated by opioid and L-arginine/NO/cGMP/ATP-sensitive K^+ channel pathways in different experimental models of pain (Mansouri et al., 2013, 2014).

Antiepileptic drugs such as oxcarbazepine, gabapentin, and topiramate are likely to produce analgesia. In addition, these drugs have been reported to show antinociceptive or antihyperalgesic effects in animal models of neuropathic, inflammatory somatic, and visceral pain (Stepanovic-Petrovic et al., 2008). Carbamazepine is one of the therapeutic choices

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that has been used as an analgesic to treat neuropathic pain, especially pain caused by trigeminal neuralgia. However, the use of carbamazepine is often limited by its side effects such as dizziness, somnolence, gait, and hematological abnormalities (Backonja, 2002; Jensen, 2002). Furthermore, the chronic use of carbamazepine reduces its analgesic effect due to liver microsomal enzyme induction (Benedetti et al., 2005). It has been reported that the antinociceptive effect of carbamazepine is potentiated when combined with antidepressants such as fluvoxamine, imipramine, or milnacipran (Aoki et al., 2006).

Considering the studies mentioned above, the present study was designed to elucidate the interaction of EA with the analgesic activity of carbamazepine using isobolographic analysis in the mouse acetic acid-induced writhing test.

Materials and methods

Animals

Adult male Swiss albino mice weighing between 25 and 30 g were obtained from the animal house of Ahvaz Jundishapur University of Medical Sciences (Iran). The animals were housed at controlled temperature ($22 \pm 2\,^{\circ}C$) and allowed free access to food and drinking water. Testing took place in the middle of the light period of a 12 h light/dark cycle. All animal experiments were carried out in accordance with the NIH Guide for Care and Use of Laboratory Animals. The Institutional Animal Ethical Committee of Jundishapur University, formed under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Reg. no. PRC115) approved the pharmacological protocols. All behavioral observations were carried out as blinded studies.

Drugs and chemicals

Acetic acid was purchased from Merck Co (Darmstadt, Germany) and dissolved in physiologic saline solution (0.9% sodium chloride). Ellagic acid hydrochloride (Sigma-Aldrich, St. Louis, MO) and carbamazepine (Jalinous Pharmaceutical Co., Tehran, Iran) were dissolved in the physiologic saline solution containing 10% and 40% DMSO, respectively. Drug concentrations were freshly prepared in such a way that the necessary dose could be injected intraperitoneally in a volume of 5 ml/kg. Doses and drug administration schedules were selected based on the previous reports (Beltz et al., 2008; Rogerio et al., 2006) and our experiments in lab (Mansouri et al., 2013).

Measurement of analgesic activity

The writhing test was selected as a model of acute visceral pain, because it can be a model of clinical relevant intestinal pain in humans (Reichter et al., 2001). All animals were acclimatized to laboratory environment for at least 2 h before testing. Mice were injected i.p. with 10 ml/kg of 0.6% acetic acid according to the method described previously (Mansouri et al., 2013). The number of abdominal writhes was counted during a 25-min period, starting 5 min after the administration of acetic acid solution. A writhe was defined as a contraction of the abdominal muscles following by body elongation and hind limbs' extension. Drugs and their vehicles were given 30 min before acetic acid. Antinociceptive activity (reduction in writhes) was expressed as percent maximum possible effect (%MPE) that was calculated by the following equation: $\%MPE = [100 \times (\text{mean writhes in control group-mean writhes in drug(s) treated group})]/\text{mean of writhes in control group}$ (Jain et al., 2001). Eight to ten animals were used at each of the dose levels to determine the ED_{50} value for a drug. The antinociceptive effects of EA (0.3, 1, 3, and 10 mg/kg) and CBZ (3, 10, 20, and 30 mg/kg) administered either alone or in combination were studied.

Isobolographic analysis

A graphical assessment of synergy was carried out using isobolographic analysis. In the present study, the interaction of antinociceptive effect of EA with CBZ was evaluated by simultaneous administration of fixed proportions of EA with CBZ, as described by Tallarida et al. (1997). The isobologram was constructed by connecting the ED_{50} value (dose that produced 50% of antinociception) of CBZ, plotted on the ordinate with the ED_{50} value of EA plotted on the abscissa to obtain the additive line. For drug combination, ED_{50} value and an associated 95% confidence interval (CIs) were determined by linear regression analysis of the log dose–response data and the equation of the straight line. For interaction studies, fixed-ratio proportions were selected by first combining the ED_{50} value of each compound and then constructing a dose–response curve in which ED_{50} fractions (1/2, 1/4, and 1/8) of drug combinations were administered (Miranda & Pinardi, 2004; Pinardi et al., 2005). The variance of $ED_{50(add)}$ value was calculated from the fraction of the ED_{50} value (i.e., 0.5) in the combination as $\text{Var } ED_{50(add)} = 0.5^2 \times \text{Var } ED_{50}\ EA + 0.5^2 \times \text{Var } ED_{50}\ CBZ$. From these variances, 95% confidence intervals were calculated and resolved according to the ratio of the individual drugs in the combination. Supra-additivity or synergistic effect is defined as the effect of a drug combination that is higher and statistically different (ED_{50} significantly lower) than the theoretical calculated equi-effect of a drug combination with the same proportions. When the drug combination gives an experimental ED_{50} value not statistically different from the theoretically calculated ED_{50} value, the combination has an additive effect and additivity means that each constituent contributes with its own potency and the less potent drug is acting as though it is merely a diluted form of the other (Miranda & Pinardi, 2004; Pinardi et al., 2005; Tallarida et al., 1997).

Statistical analysis

Results are presented as mean values ± SEM or as ED_{50} values and 95% CIs. The antinociceptive effects of EA and CBZ were examined by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Unpaired Student's *t-test* was used to compare the experimental and theoretical ED_{50} value to determine the mechanisms of the interaction between EA and CBZ. The difference was considered significant at 5% level. All data calculations and statistical analysis were done by using the GraphPad Prism Version 5.01 (GraphPad Software Inc., San Diego, CA).

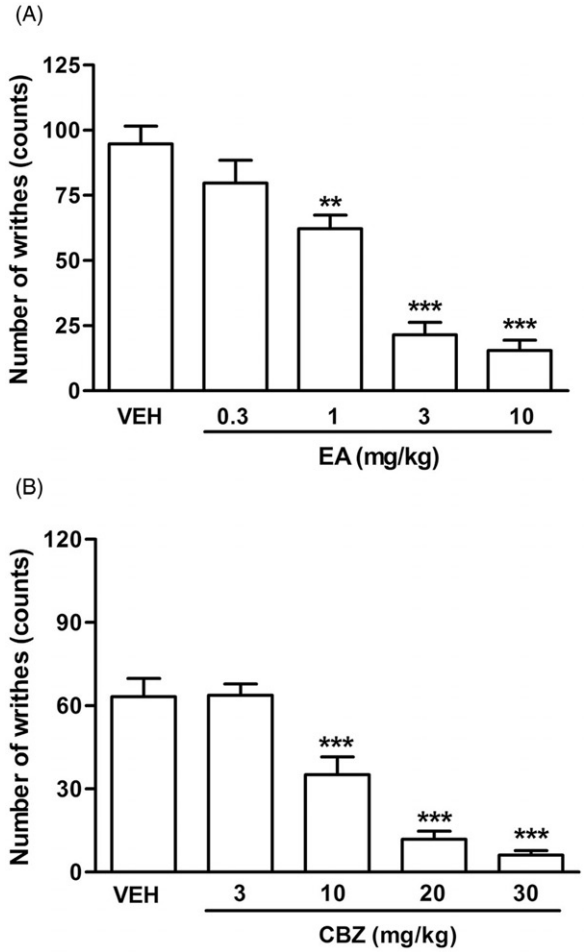

Figure 1. Effects of intraperitoneal (A) ellagic acid (EA; 0.3–10 mg/kg) or (B) carbamazepine (CBZ; 3–30 mg/kg) in the mouse acetic acid-induced writhing test. Data are the mean ± SEM of 8–10 animals per group (one-way ANOVA followed by Tukey's test). $^{**}p < 0.01$, $^{***}p < 0.001$ versus the vehicle (VEH) control group.

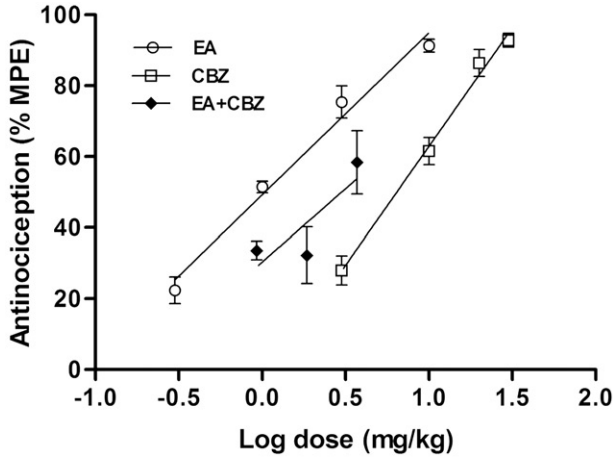

Figure 2. Dose–response curves for the antinociception induced by ellagic acid (EA; i.p.), carbamazepine (CBZ; i.p.) alone and in combination in the mouse acetic acid-induced writhing test. Data are the mean ± SEM of 10–12 animals per group.

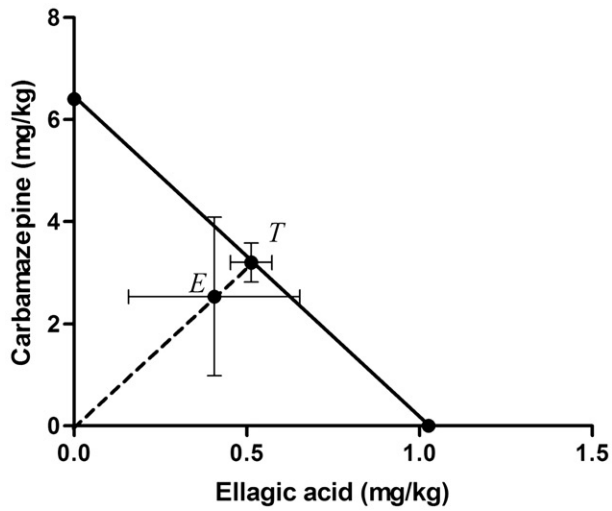

Figure 3. Isobologram for the co-administration of ellagic acid (EA, i.p.) and carbamazepine (CBZ; i.p.) on acetic acid-induced writhing in mice. Horizontal lines in the abscissa and the ordinate represent the S.E.M. of the corresponding ED50. Filled circles correspond to the theoretical and experimental ED50 values with 95% confidence intervals.

Results

Antinociceptive effects of EA and carbamazepine

Results showed that the intraperitoneal administration of ellagic acid (EA) and carbamazepine (CBZ) produced dose-dependent antinociception (Figure 1A and B, respectively). The value of ED_{50} with 95% confidence intervals (CIs) for EA was lower than the ED_{50} value for carbamazepine indicating greater potency in antinociceptive effect of ellagic acid in the test [ED_{50} values were 1.02 (0.86–1.19) and 6.40 (5.47–7.32) mg/kg for EA and CBZ, respectively]. Animals treated with ellagic acid did not show any significant behavioral or motor dysfunctions in locomotor activity assessment (data not shown).

Analysis of interaction

The antinociceptive activity induced by the co-administration of fixed ratios of ED_{50} fractions for EA and CBZ was examined by isobolographic analysis. Figure 2 shows a log dose–response curve obtained for EA, CBZ, and their combination administered. The theoretical ED_{50} values for the combination and their 95% confidence intervals were calculated at 3.71 (3.27–4.15) mg/kg. Co-administration of EA with CBZ revealed that the experimental ED_{50} was 2.94 (1.14–4.74) mg/kg. This experimental ED_{50} value was below the additive line, and also statistical analysis demonstrated that it was not significantly different from its corresponding theoretical ED_{50} value ($p > 0.05$). Therefore, EA and CBZ interacted in an additive fashion in the writhing test (Figure 3).

Discussion

An effective control of pain is difficult to attain when using one drug alone. So, the development of new pain relief strategies involves multiple combinations of analgesics that point both central and peripheral nociceptive pathways to produce greater analgesia at reduced and more tolerable doses of individual drugs (Kehlet & Dahl, 1993; Mehlisch, 2002; Tallarida, 2001). A combination of small-dose carbamazepine

with other analgesics could result in analgesia without the adverse effect associated with large-dose used. In the present study, carbamazepine and ellagic acid alone exhibited comparable dose-dependent antinociceptive activity in the acetic acid-induced writhing test. Also, co-administration of a carbamazepine with ellagic acid resulted in an additive antinociceptive interaction.

Previous studies suggested that the CNS depression and the non-specific muscle relaxation effect can reduce the response of motor coordination and might invalidate the nociceptive test results (Santos et al., 2005). Ellagic acid has little or no effect on motor function even at a dose as high as 30 mg/kg, when tested with both the open-field and also rotarod tests (Mansouri et al., 2013, 2014). Therefore, the present result indicates that ellagic acid may have antinociceptive activity in mice.

In this study, we used the acetic acid writhing test as an inflammatory pain model stimulus for acute nociception. In this test, acetic acid causes tissue damage and releases pain-producing substances, including prostaglandins, which activate peripheral nociceptors on the terminals of sensory nerve fibers. Painful stimuli caused by acetic acid reach higher centers by a number of spinal nerve pathways. Thus, this nociceptive test is used for determining both central and peripheral analgesia (Le Bars et al., 2001; Satyanarayana et al., 2004).

Flavonoids are shown to have neuroprotective, anxiolytic, sedative, and anticonvulsant activities. The neuroprotective effects of flavonoids, including ellagic acid, may be mediated through direct actions on enzymes, receptors, and signaling pathways (Spencer, 2009).

A growing pieces of evidence indicated that ellagic acid is effective for altering acute visceral pain (Mansouri et al., 2013; Rogerio et al., 2006). Ellagic acid-induced antinociception in the writhing test is also consistent with the ability of this compound to reduce inflammation-induced nociception caused by a variety of inflammatory agents, such as carrageenan and formalin (Gainok et al., 2011; Mansouri et al., 2014). The analgesic action of ellagic acid has been explained by the inhibition of cyclooxygenase, which synthesizes prostaglandins at the peripheral cell-damage sites (El-Shitany et al., 2014; Rogerio et al., 2006). Moreover, Beltz et al. (2008) demonstrated the antinociceptive effects of EA in the hot-plate test. In our previous studies, we have shown the central and peripheral antinociceptive effects of EA which were mediated by opioid receptors and L-arginine/NO/cGMP/ATP-sensitive K^+ channel pathway using tail-flick, formalin, and writhing tests (Ghorbanzadeh et al., 2014; Mansouri et al., 2013, 2014). Further studies are needed to elucidate the mechanism of the antinociceptive action of ellagic acid.

Carbamazepine is a well-known anticonvulsant drug which is used for the management of bipolar affective disorders, as well as trigeminal and glossopharyngeal neuralgias (Stepanovic-Petrovic et al., 2008). Carbamazepine-induced antinociception in the writhing test is consistent with the ability of this drug, in a paw pressure test, to reduce somatic nociception caused by proinflammatory agents (Bianchi et al., 1995; Kawaura et al., 2011; Stepanovic-Petrovic et al., 2008). Although our results do not clarify the mechanism of the antinociceptive effects of this drug in the visceral pain model, it may suggest that the inflammatory nature of nociception in writhing and paw pressure tests leading to a facilitated state may be similar. It has been shown that activation of central adenosine A1 (Stepanovic-Petrovic et al., 2008) and adrenergic $\alpha 2$ (Vucvkovic et al., 2006) receptors and also inhibition of tetrodotoxin-resistant sodium channels in dorsal root ganglion neurons (Brau et al., 2001) produced antinociception in the writhing test. Therefore, it could be suggested that the peripheral and/or central component of nociceptive pathways was involved in the antinociception produced by carbamazepine in writhing test.

In the present study, an isobolographic analysis revealed additive interaction between carbamazepine and ellagic acid. There is no scientific evidence available to support the results obtained in this work; however, such interaction has not been studied previously in a valuable experiment. It should be noticed that an additive interaction might be exhibited if fundamentally the activation of common mechanisms is simultaneously engaged in the antinociception of a combination (Solomon & Gebhart, 1994). Therefore, ellagic acid and carbamazepine may act through a common pathway.

Our study did not investigate the mechanisms involved in the nature of interaction between ellagic acid and carbamazepine. The observed addition may be related to a pharmacokinetic or pharmacodynamics interaction.

Although synergism is optimal, to be clinically useful, a combination of drugs need not necessarily be synergistic but needs only to allow exhibition of an increased desired clinical result with the same or a lower level of side effects compared with a single agent. Therefore, the results observed here may provide a theoretical basis for the development of a multi-targeted drug strategy to augment antinociception while preventing or reducing the untoward side effects of each individual drug, thus enhancing analgesic efficacy. Further studies are needed to elucidate the detailed mechanisms of the potentiating effect of the combination of ellagic acid and carbamazepine on the antinociceptive effect in mice, including studies on the pharmacokinetic interactions between carbamazepine and ellagic acid.

Conclusion

In conclusion, isobolographic analysis indicated an additive antinociceptive interaction between ellagic acid and carbamazepine when administered systemically in an inflammatory visceral pain model. Therefore, these findings suggest that co-administration of ellagic acid with carbamazepine may be a new strategy for managing neuropathic pain such as pain caused by trigeminal neuralgia, although further investigations are necessary to elucidate this combination effect using other methods evaluating antinociceptive actions.

Declaration of interest

The authors report that they have no conflicts of interest. This research was financially supported by grants (PRC-115) from the Physiology Research Center, funded by the Vice Chancellor of Research, Ahvaz Jundishapur University of Medical Sciences (Iran).

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