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Original research article

Ellagic acid enhances the antinociceptive action of venlafaxine in mouse acetic acid-induced pain: An isobolographic analysis

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ABSTRACT

Background: Several antidepressants have been used to treat severe pain in clinics. Recently, it has been shown that ellagic acid (EA), a major constituent of pomegranate juice, produced antinociceptive and anti-inflammatory effect through the central and peripheral sites of action.
Materials and methods: In this study, we examined the interaction between EA and venlafaxine (VLF) on pain induced by intraperitoneal acetic acid in mice using isobolographic analysis.
Results: EA (0.3–10 mg/kg, *ip*) and VLF (3–60 mg/kg, *ip*) produced a dose-dependent inhibition of the writhing response evoked by acetic acid. Fifty percent effective dose (ED_{50}) values against writhing behaviors were 1.02 (0.86–1.19) mg/kg and 12.37 (9.74–15.37) mg/kg for EA and VLF, respectively, and also with higher potency than that of VLF. Co-administration of increasing fractional increments of ED_{50} doses of EA and VLF produced synergistic interaction against writhing behaviors, as revealed by isobolographic analysis.
Conclusion: The synergistic action of EA and VLF provides valuable tool referring to the management of visceral pain.

Introduction

The development of new pain strategies involves combining analgesics to deliver greater analgesia at reduced doses of individual drugs and lower adverse effects, both of which are extremely important in improving patient health. When a combination of drugs is administered, effectiveness is altered in different ways: the final effect may be equal to the sum of the effects of all drugs administered separately (additive effect), it may be less than the sum of the effects of drugs administered independently of each other (antagonistic effect), and optimally, the final effect may be greater than the sum of the effects of separately administered drugs, which is known as synergism [1].

Plant polyphenols play an important role in human nutrition and are implicated with numerous biological properties including antioxidant, anti-inflammatory, anticancer and antiatherosclerotic activities. Ellagic acid (EA, 2,3,7,8 tetrahydroxy[1]benzopyranol [5,4,3-cde][1]benzopyran-5,10-dione) is a polyphenolic compound occur largely as ellagitannins in plants such as raspberries, the stem and bark of eucalyptus species and nuts. This bioflavonoid compound has been reported to have antioxidant, antifibrotic, cardioprotective, anticancer [2], anti-inflammatory and antinociceptive properties [3]. It has been found to have antidepressant-like activity and its effect is dependent on the interaction with the serotonergic (5-HT1, 5-HT2, 5-HT3 receptors) and noradrenergic (α1 and α2 adrenoceptors) systems [4]. Also, Girish et al. proved the involvement of the GABAergic system in the anxiolytic-like effect of EA [5]. Recently, we have shown the central and peripheral antinociceptive activities of EA which were mediated by opioid receptors and L-arginine/NO/cGMP/ATP-sensitive K^+ channel pathway in different experimental models of pain [6,7].

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Antidepressants that increase neuronal transmission in the serotoninergic and/or noradrenergic systems are likely to produce analgesia, because an increase in monoamine levels in the descending serotonin and noradrenaline pathways likely plays a key role in the nociceptive regulation. Indeed, several antidepressants including tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), and serotonin noradrenaline reuptake inhibitors (SNRIs) have been used to treat several types of pain in clinic [8,9].

Venlafaxine (VLF) is a structurally novel phenylethylamine atypical anti-depressant drug with the serotonin and noradrenaline reuptake inhibitor action. It has been reported to have antinociceptive activity in different animal models [10,11] and humans [12,13]. It increases the inhibiting tone of serotonergic and norepinephrine descending pathways in the brain [11]. Also, it has been shown that VLF antinociception could be mediated through the κ- and δ-opioid [14], α_2-adrenergic [15] and 5-HT1A receptor [16] systems.

Considering the mentioned above, the present study was designed to characterize the interaction of EA with the analgesic activity of VLF using isobolographic analysis in the acetic acid-induced writhing test in mice.

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. The animals were housed at controlled temperature (22 ± 2 °C) and allowed free access to food and drinking water. Testing took place in the middle of the light period of a 12 h/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. The animals were used only once and then euthanized.

Drugs and chemicals

VLF hydrochloride (Poursina Pharmaceutical, Iran) and acetic acid (Merck, Germany) were dissolved in physiologic saline solution (0.9% sodium chloride). EA hydrochloride (Sigma-Aldrich, USA) was dissolved in 10% solution of DMSO. EA and VLF were administered intraperitoneally, and control animals received physiological saline or DMSO, respectively. Drug concentrations were freshly prepared in such a way that the necessary dose could be injected in a volume of 5 ml/kg by intraperitoneal route.

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 [17]. Mice were injected *ip* with 10 ml/kg of 0.6% acetic acid according to the method described by Mogil et al. [18]. 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 an elongation of the body and extension of the hind limbs. Drugs were given 30 min before acetic acid, and their antinociceptive activities were recorded as the percentage relative to the number of abdominal writhes of a control group (% of maximal possible effect, MPE). %MPE = [100 × (mean writhes in control group − mean writhes in drug(s)-treated group)]/mean of writhes in control group [6,10,11,19]. 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 VLF (3, 10, 30 and 60 mg/kg) administered either alone or in combination were studied in the mouse acetic acid-induced writhing test.

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 VLF was evaluated by simultaneous administration of fixed proportions of EA with VLF, as described by Tallarida [20]. The isobologram was constructed by connecting the ED_{50} (dose that produced 50% of antinociception) of VLF, plotted on the ordinate with the ED_{50} of EA plotted on the abscissa to obtain the additive line. For drug combination, ED_{50} and an associated 95% confidence interval (CIs) were determined by linear regression analysis of the log dose response data (the percentage of MPE values) and the equation of the straight line (8–10 animals for each dose). For interaction studies, fixed-ratio proportions were selected by first combining the ED_{50} 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. Variance of $ED_{50(add)}$ was calculated from the fraction of the ED_{50} (i.e., 0.5) in the combination as: Var $ED_{50(add)} = 0.5^2 \times$ Var ED_{50} EA + $0.5^2 \times$ Var ED_{50} VLF. 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 equieffect of a drug combination with the same proportions. When the drug combination gives an experimental ED_{50} not statistically different from the theoretically calculated ED_{50}, 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 [20,38–40].

Statistical analysis

Results are presented as mean values ± SEM or as ED_{50} values and 95% CIs. The antinociceptive effects of EA and VLF was examined by one-way analysis of variance (ANOVA) followed Tukey's *post hoc* test. A two-way ANOVA (administration, treatment, and their interaction) followed by a Bonferroni's test was used to compare the observed effects of the combination of EA plus morphine and the expected sum of individual effects at each administration [21]. The mean responses of two independent experimental groups were compared using an unpaired Student's *t*-test. The $p < 0.05$ was considered statistically significant. All data calculations and statistical analysis were done by using the GraphPad Prism Version 5.01 (GraphPad Software Inc., San Diego, CA, USA).

Results

Antinociceptive effects of the drugs

Both EA and VLF demonstrated the antinociceptive efficacy in the mouse acetic acid-induced writhing assay. The intraperitoneal administration of EA and VLF produced dose-dependent antinociception (Fig. 1A and B, respectively). Table 1 shows ED_{50} values with 95% confidence intervals (CIs) for the antinociceptive effect of EA and VLF on the test. The value of ED_{50} for EA was lower than ED_{50} for VLF indicating greater potency in antinociceptive effect of EA in the test (ED_{50s} were 1.02 (0.86–1.19) and 12.37