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Short communication

Eugenol exhibits antidepressant-like activity in mice and induces expression of metallothionein-III in the hippocampus

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Abstract

Here we show that eugenol has an antidepressant-like activity comparable to that of imipramine using a forced swim test and a tail suspension test in mice. Furthermore, we show that both eugenol and imipramine induce brain-derived neurotrophic factor (BDNF) in the hippocampus with and without induction of metallothionein-III (MT-III), respectively. It may be possible that MT-III expression is involved in the exhibition of antidepressant-like activity of eugenol, not of imipramine.

Theme: Disorders of the nervous system
Topic: Neuropsychiatric disorders

Keywords: Depression; Antidepressant; Eugenol; Metallothionein-III; Brain-derived neurotrophic factor; BDNF

Eugenol, an oil-like odorant substance, is contained in many medicinal herbs like cinnamon and clove [22]. It has activities of anti-convulsive [16] and hypothermic [24] agent. It also possesses properties of an antioxidant [6] and protects neurons in culture from toxic events [23]. Eugenol is also widely used as an analgesic in dentistry [13]. In a previous report, we demonstrated that eugenol is the major active principle of *rhizoma acori graminei* (RAG), a medicinal herb that has been used for epilepsy and forgetfulness in the East Asia for centuries, in decrease of $A\beta_{1-40}$-induced cytotoxicity on PC-12 cells in vitro [9]. These findings prompted us to further pursue the therapeutic and prophylactic potentials of eugenol for the management of other neuro- or psychiatric disorders.

Depression is a mood disorder the prevalence of which is about 10–20% of the general population and which causes common psychosocial problems [25]. After debut of imipramine as an antidepressant, various sorts of reagents have been developed such as tricyclic acids, monoamine oxidase (MAO) inhibitors, selective serotonin receptor inhibitors (SSRI) and used in the treatment of depression. However, recurrent and chronic depressions are still present at high prevalence [7]. Therefore, new antidepressants are desired. In this study, we tested eugenol for antidepressive activity using two established tests for antidepressants: the forced swim test (FST) [14] and the tail suspension test (TST) [19]. Furthermore, we examined if eugenol would change BDNF gene expression in the hippocampus that is responsible for antidepressive activity [18] using real-time RT-PCR. MT-III, a brain-predominant protein that has been of our interest in that it alleviates various neurotoxic events [3,8,20], was also included in the real-time RT-PCR study.

Fifty male ddY mice (6 weeks, weighing 25–30 g) were purchased from Japan SLC (Hamamatsu, Japan) and maintained (five in each cage) in our animal facility at 25 ± 1 °C with free access to food and drinking water. Light was on

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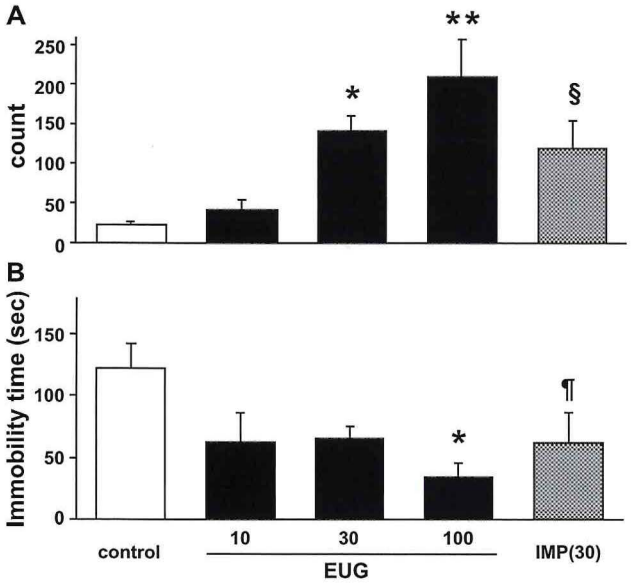

Fig. 1. (A) Forced swim test (FST). Treatment with imipramine (IMP: 30 mg/kg BW) or various doses of eugenol (EUG: 10, 30, and 100 mg/kg BW) for 14 days increased numbers of wheel rotation (N=5). (B) Tail suspension test (TST). The same treatment as above decreased immobility of the mice (N=5). *, $P<0.05$; **, $P<0.01$ compared with the control, respectively, by the Kruskal-Waliis test and the Tukey test. ¶, $P<0.05$; §, $P<0.01$ compared with the control, respectively, by the Student-*t* test. See text for details.

between 0700 and 1900 h. After 7 days of habituation, treatment of eugenol or imipramine (St. Louis, MO, USA) was started. These compounds were supplied in the drinking water containing 1% DMSO as vehicle in light-protected bottles. After 14 days of treatment, 25 mice were subjected to the FST. The FST was performed in a WW-3002 apparatus designed for mice (O'hara, Tokyo, Japan) that consists of a clear plastic water tank with a steel wheel attached at the level of water surface and a clear cover on the top [12]. The tank was filled with fresh distilled water (25 °C). A mouse placed in the water would start swimming desperately to escape, and as a consequence, the wheel would rotate vigorously that was counted automatically by the system (1 rotation = 3 counts). As depressed, it would discontinue the rotation of the wheel. Each mouse was subjected to a pre-trial for 15 min, and the real trial on the next day for 5 min (recorded).

The TST enrolled the rest 25 mice. Each mouse was acoustically and visually isolated and suspended by the tail.

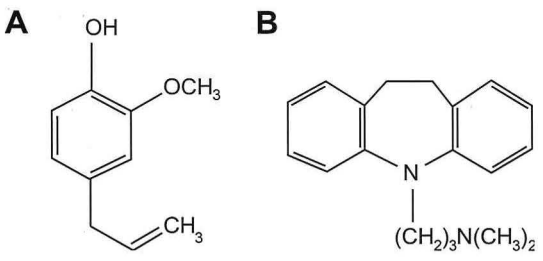

Fig. 2. Molecular structures of eugenol (A) and imipramine (B).

Tails were fixed using an adhesive tape placed at about 20 mm from the extremity of its tail on a steel bar placed horizontally at the height of 60 cm from the floor. The sum of immobility (depressed) time during a 5-min session was recorded. All animal experiments were conducted between 0900 and 1100 h according to the guideline approved by our institute in accordance with the NIH Guide for Care and Use of Laboratories Animals.

For real-time RT-PCR, total RNA was extracted from the hippocampi of the mice enrolled in the TST with an Absolutely RNA RT-PCR Miniprep Kit (Stratagene, La Jolla, CA, USA) and used as the templates. Reactions including data

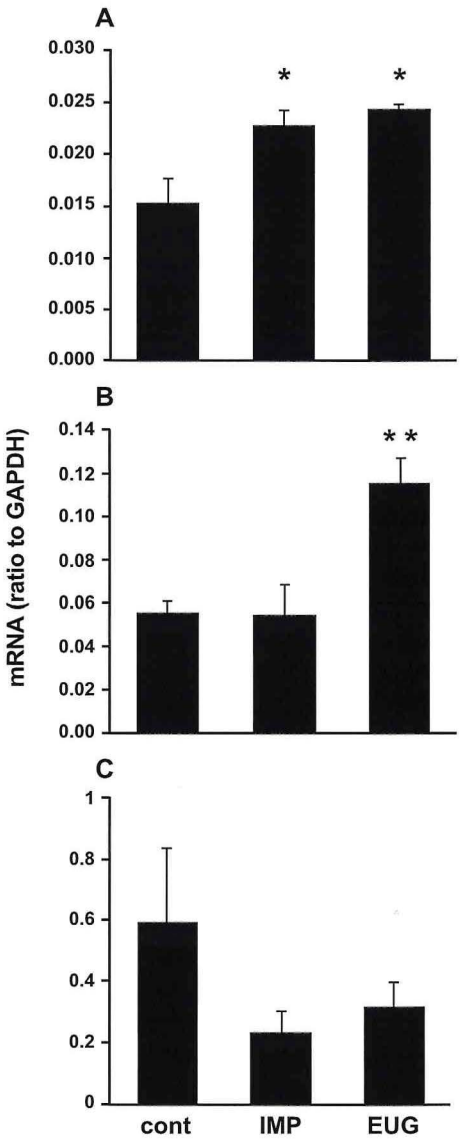

Fig. 3. Quantification analysis of the expression changes of BDNF (A), MT-III (B), and MT-I (C) genes by real-time RT-PCR in the hippocampi of mice treated with vehicle, imipramine (30mg/kg/day), and eugenol (100mg/kg/day), respectively (N=5). Bars (± S.E.M.) indicate mRNA ratio of each gene to that of GAPDH. *, $P<0.05$; **, $P<0.01$ compared with the control, respectively, by the Student-t test.

analysis were performed using the QuantiTect SYBR® Green RT-PCR Kit (Qiagen, Tokyo, Japan) with an iCycler iQ™ Real-Time PCR Detection System (Bio-Rad Laboratories, Tokyo, Japan) to amplify the cDNA fragments of BDNF [5], MT-III [20], MT-I [20], glyceraldehydes-3-phosphate dehydrogenase (GAPDH) [2] as described in each reference. The threshold cycles (Ct) were used to quantify the mRNA levels of each gene. Relative mRNA was calculated as $2^{-\Delta Ct}$, where ΔCt is the difference in threshold cycles for the target and GAPDH genes.

In the FST (Fig. 1A), mice treated with imipramine registered higher counts of wheel rotation as compared to the controls. Eugenol exhibited a similar activity in a dose-dependent manner (Fig. 1A). In the TST, decrease of immobility time by imipramine and eugenol was observed (Fig. 1B), which was inversely related to the result from the FST. Thus, antidepressant-like activities of imipramine and eugenol were confirmed by the two different tests. Vehicle (1% DMSO) alone had no effect in these tests. In these cases, eugenol should be able to pass the blood–brain barrier, enter the brain and act in situ. Our preliminary data (not shown) supports this possibility. The molecular structures of eugenol and imipramine are displayed in Fig. 2.

The pathogenesis of depression is still unclear; however, some possible mechanisms have been hypothesized. It is widely believed that depression is the result of a decrease in monoamines in the brain and that supplementation and/or restoration of these monoamines should alleviate the symptom (monoamine theory). Recently, it is known that hippocampus is retracted in a patient with depression [1,17] and this retraction caused by neuronal loss can be reversed by antidepressants [4]. A series of reports by Duman's lab revealed that antidepressants have activities of regenerating neurons via induction of BDNF in the hippocampus and that disruption of this neurogenesis abolishes those antidepressive activities [11,15,18]. Thus, here we examined if eugenol induces BDNF in the hippocampus by real-time RT-PCR. As shown in Fig. 3A, both imipramine and eugenol induced BDNF compared to control. This result indicates that the antidepressive-like activity of eugenol (Fig. 1) is exerted in a common pathway with that of imipramine, i.e. BDNF induction.

MT-III is a brain-specific member of MTs that is expressed highly in astrocytes [10,21] and in a lesser amount in neurons [10]. MT-III is believed to act in growth inhibition of neuronal cells [21] and protect neurons from various toxic events [3,8,20]. Hence we raised a hypothesis that MT-III may be involved in the action mechanism of antidepressants. MT-III expression in the mice hippocampi was observed by real-time RT-PCR. As shown in Fig. 3, eugenol but not imipramine induced MT-III. The expression of MT-I, a counterpart of MT-III, was not altered by eugenol and imipramine (Fig. 3C). These results obtained with real-time RT-PCR together with those from the FST and TST suggest that MT-III may be involved in the antidepressive action pathway of eugenol but not of imipramine. In this case, eugenol appears to exhibit its antidepressive activity in a different manner from that of imipramine, which may provide an alternative treatment to patients who are resistant to typical antidepressants. It is further suggested that expression of BDNF may be a result of induction of MT-III. Otherwise, the induction of MT-III by eugenol is an unrelated event to expression of BDNF, although it should mediate some effects of eugenol. To further understand this, it should be shown if disruption of MT-III abolishes antidepressive activity of eugenol. In the future, the experiments done in the present study should be repeated using depressed mice instead.

Together with our previous finding that eugenol inhibits Aβ-induced cell death by blocking abnormal Ca influx caused by Aβ [9], it is suggested that eugenol has a broader potency to protect cells from death inducing agents. Youdim and Weinstock [26] showed that MAO inhibitors selegiline and its derivative rasagiline have neuroprotective activity and that such activity might be independent of their MAO inhibitory activity [26]. Therefore, it would not be surprising if eugenol could also have dual (anti-Aβ and antidepressant) functions mediated by seemingly unrelated action mechanisms. This possibility is now being investigated in vivo in our laboratories.

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