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Effects of MK-886, a 5-lipoxygenase activating protein (FLAP) inhibitor, and 5-lipoxygenase deficiency on the forced swimming behavior of mice

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Abstract

A common biological pathway may contribute to the comorbidity of atherosclerosis and depression. Increased activity of the enzymatic 5-lipoxygenase (5-LOX; 5LO) pathway is a contributing factor in atherosclerosis and a 5-LOX inhibitor, MK-886, is beneficial in animal models of atherosclerosis. In the brain, MK-886 increases phosphorylation of the glutamate receptor subunit GluR1, and the increased phosphorylation of this receptor has been associated with antidepressant treatment. In this work, we evaluated the behavioral effects of MK-886 in an automated assay of mouse forced swimming, which identifies antidepressant activity as increased climbing behavior and/or decreased rest time. Whereas a single injection of MK-886 (3 and 10 mg/kg) did not affect forced swimming behaviors assayed 30 min later, 6 daily injections of 3 mg/kg MK-886 slightly increased climbing and significantly reduced rest time in wild-type mice but not in 5-LOX-deficient mice. A diet delivery of MK-886, 4 µg per 100 mg body-weight per day, required three weeks to affect forced swimming; it increased climbing behavior. Climbing behavior was also increased in naive 5-LOX-deficient mice compared to naive wild-type controls. These results suggest that 5-LOX inhibition and deficiency may be associated with antidepressant activity. Increased climbing in a forced swimming assay is a typical outcome of antidepressants that increase noradrenergic and dopaminergic activity. Interestingly, 5-LOX deficiency and MK-886 treatment have been shown to be capable of increasing the behavioral effects of a noradrenaline/dopamine-potentiating drug, cocaine. Future research is needed to evaluate the clinical relevance of our findings.

Keywords

5-lipoxygenase (5-LOX, 5LO); antidepressant; depression; atherosclerosis; GluR1 cardiovascular

Recent studies pointed out an association between depressive symptoms and the progression of atherosclerosis [7,26]. It was proposed that a common biological mechanism contributes to triggering and/or maintenance of these two pathological conditions [19].

A prominent role in the pathobiology of atherosclerosis is played by 5-lipoxygenase (5-LOX; 5LO), an enzyme involved in the metabolism of arachidonic acid in 5(*S*)-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HPETE) and leukotrienes [16,25]. Whereas an

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upregulated 5-LOX pathway promotes atherosclerosis, prolonged treatment with the 5-LOX inhibitor MK-886 decreased atherosclerosis in an in-vivo mouse model [13]. The 5-LOX pathway is also active in the mammalian brain [2,8]. 5-LOX inhibitors, including MK-886, stimulate phosphorylation and membrane insertion of the GluR1 subtype of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) glutamate receptors [12,23]. Drug-induced increases of GluR1 phosphorylation have been linked to antidepressant activity [5,18,21].

Thus, it was hypothesized that by interfering with GluR1 phosphorylation, 5-LOX might be involved in depression and also in antidepressant therapy [20]. In this study, we used a mouse model of forced swimming to evaluate the putative antidepressant action of MK-886. Since this drug may affect systems other than the 5-LOX pathway [22], we performed experiments in wild-type and 5-LOX-deficient (knockout) mice.

Male 5-LOX deficient mice and their wild type controls were purchased from Jackson Laboratories (B6.129S2-*Alox5^{tm1Fnn}/J*; Stock #004155; Bar Harbor, ME). Male mice were selected to reduce the confounding variables associated with the estrous cycle of females, including possible hormonal changes associated with chronic administration of 5-LOX inhibitors. Animals (2-3 months old) were housed in groups of three and had free access to laboratory chow and water except during the experiments. They were kept in a temperature-controlled room on a 14 h light/10 h dark cycle (lights off at 7 PM). All behavioral experiments were performed between 10 AM and 3 PM. The experimental protocol was approved by the Institutional Animal Care and Ethics Committee. MK-886 (Sigma Chemical St. Louis, MO, USA) was dissolved in sterile 5% dimethylsulfoxide (DMSO; Sigma) saline and 3 mg/kg (except in one experiment 10 mg/kg) of this drug was administered intraperitoneally (i.p.). This dose was selected based on prior results; administered repeatedly it increases GluR1 phosphorylation in the mouse brain [12]. Controls were treated with the vehicle. The food delivery of MK-886 was performed as reported previously [13]. Briefly, mice received a diet (TD.06348, Harlan-Teklad, Madison, WI, USA) mixed with MK-886 (Sigma) at a dosage of 4 µg per 100 mg body-weight per day for three weeks. The drug was mixed with the diet without heating by the manufacturer. Controls received the same diet without MK-886. Mice treated with MK-886 had a similar food intake and body-weight after the experiment as the control mice.

Automated forced swimming experiments were conducted using the computer controlled Hamilton-Kinder forced swim test device (Hamilton-Kinder; Poway, CA, USA), which consists of two clear water-filled tanks, each equipped with two photobeam arrays that allow monitoring of swimming and climbing behaviors [14]. Two mice at a time were individually dropped from approximately 10 cm above the water surface into tanks filled with 11 of water (24°C) and left there for six minutes. The water was replaced between each test. The device was operated with the manufacturer's software (MotorMonitor version 4.11). The collected data included: a) basic movements, which correspond to any kind of horizontal beam block, b) climbing, which corresponds to movements vertical to the water surface, and c) rest time, which is the time the animal remains inactive (i.e., did not change any beam status). The values for each of the above-noted parameter are automatically calculated and expressed as units/session (a and b) and sec/6 min (c). In this assay, antidepressant drugs increase climbing and/or shorten rest time. Data (mean ± S.E.M.) obtained from automated swim tests were evaluated by an independent-samples t-test. Significance was accepted at $p < 0.05$.

A single injection of MK-886 (3 and 10 mg/kg) did not alter the behavior of mice in the swim test (Fig. 1). Repeated injections of 3 mg/kg produced a slight (21 %) increase of climbing and a significant (45 %) reduction of rest time in wild type mice but not in 5-LOX-deficient mice (Fig. 2). In these experiments, vehicle-injected 5-LOX-deficient mice showed a trend to increased climbing compared to vehicle-treated wild-type controls. To investigate the

contribution of a 5-LOX deficiency to the behavior of mice in the swim test, we used naive 5-LOX-deficient and wild type mice and found significantly elevated climbing in the 5-LOX deficient group (Fig. 3). Previous research in atherosclerosis has established a diet MK-886 delivery schedule and dose for prolonged treatment [13]. We used this treatment schedule for 3 weeks. Mice were tested at the end of weeks 2 and 3. After 3 weeks on the diet containing MK-886, climbing behavior was significantly increased compared to mice on the control diet (Fig. 4).

The main finding of this study is that prolonged MK-886 treatment alters the behavior of mice in the forced swimming assay in a manner indicative of antidepressant activity. Antidepressant drugs that primarily increase noradrenergic and dopaminergic activity increase climbing whereas drugs acting primarily on serotonergic transmission affect swimming [4]. Both long-term diet delivery of MK-886 and 5-LOX gene disruption significantly increased the climbing behavior, suggesting that a long-lasting 5-LOX deficiency may promote noradrenergic and dopaminergic activity. Interestingly, it has been noted that 5-LOX deficiency potentiates the behavioral effects of cocaine, a drug that primarily stimulates the noradrenergic/dopaminergic neurotransmitter systems [15].

Activation of dopamine D1 receptors contributes to mouse antidepressant-like behaviors induced by drugs such as imipramine [11] and also increases the phosphorylation of GluR1 receptors [27]. Recent studies suggest that a drug-induced increase of GluR1 phosphorylation may contribute to antidepressant activity [5,18,21]. A single MK-886 injection, which does not affect brain GluR1 phosphorylation [12], did not produce antidepressant-like behavior, whereas this behavior was induced by repeated injections of MK-886, which also increase GluR1 phosphorylation [12]. These findings, along with our observation that several weeks of diet-delivery of MK-886 are needed to alter behavior, suggest that protracted 5-LOX inhibition or certain brain levels of MK-886 are a prerequisite for increased GluR1 phosphorylation and antidepressant-like effects. Although it has been reported earlier that a systemic administration of MK-886 is capable of delivering this drug into the brain [3], further studies are needed to evaluate the pharmacokinetics and bioavailability of MK-886.

The lack of antidepressant-like action of MK-886 in 5-LOX-deficient mice indicates that this behavior may involve MK-886-mediated inhibition of 5-LOX activity. Interestingly, in contrast to the stimulation of GluR1 phosphorylation in wild-type mice [12], repeated injections of MK-886 did not increase brain GluR1 phosphorylation in 5-LOX-deficient mice (unpublished observation). Hence, it is possible that 5-LOX inhibition produces behavioral effects by increasing GluR1 phosphorylation via the dopaminergic system and/or through dopamine-independent [12,23] mechanisms.

Our previous research found that glucocorticoids stimulate 5-LOX expression and activation in the brain [28]. Hence, it is possible that stress-induced glucocorticoid surge, i.e., due to forced swimming, could stimulate the 5-LOX system. 5-LOX activity leads to conversion of arachidonic acid into 5-HPETE and leukotrienes, and MK-886 disrupts this process by inhibiting the 5-lipoxygenase-activating protein (FLAP) [9]. 5-HPETE is a potent inhibitor of neuronal Na⁺, K⁺-ATPase activity [10] and it remains to be clarified whether MK-886 treatment and/or 5-LOX gene disruption alter Na⁺, K⁺-ATPase activity in brain regions involved in antidepressant effects. On the other hand, leukotrienes act through specific leukotriene receptors, some of which are expressed in the brain [6,29]. The exact function of brain leukotriene receptors is poorly understood and their putative role in the antidepressant action of 5-LOX inhibition is currently unclear. For example, in endothelial cells, activation of cysteinyl leukotriene receptor 2 by 5-LOX products leads to significant upregulation of cyclooxygenase-2 (COX-2) [17]. If similar action occurs in the brain, 5-LOX inhibition could lead to antidepressant effects by preventing COX-2 upregulation. Namely, hippocampal

COX-2 is upregulated in a rat model of depression [1] and a COX-2 inhibitor, celecoxib, demonstrated therapeutic effects in major depression [24].

In conclusion, our results suggest that the pharmacological inhibition of 5-LOX and 5-LOX deficiency created by 5-LOX gene disruption trigger antidepressant-like behaviors in mice. It remains to be investigated whether our findings have clinical value. Interestingly, a dietary administration of MK-886 to mice, which previously was shown capable of decreasing atherosclerosis [13], also produced antidepressant-like behavior. Considering the known comorbidity of atherosclerosis and depression [7], we propose that focusing on mechanisms common to both disorders, e.g., 5-LOX, could lead to discovery of novel and better therapeutic options.

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Fig. 1.

Effects of a single MK-886 injection on mouse behavior in the forced swimming test. Mice ($n = 6$) received i.p. injections of 3 and 10 mg/kg MK-886 or vehicle 30 min prior to testing. Results are shown as mean $\pm$ S.E.M.; open bars = vehicle (Veh), closed bars = MK-886 (MK). (A) basic movements, (B) climbing, (C) rest time. There were no significant differences between drug treated groups and vehicle treated controls.

Fig. 2.

Effects of repeated MK-886 injections on mouse behavior in the forced swimming test. Mice (wild-type and 5-LOX-deficient; $n = 8$) received either 3 mg/kg MK-886 or vehicle (saline with 5% DMSO) i.p. once a day for six days. Thirty min after the last injection, mice were assayed in an automated swim test apparatus. Results are shown as mean $\pm$ S.E.M.; open bars = vehicle (Veh), closed bars = MK-886 (MK). (A) basic movements, (B) climbing, (C) rest time. Rest time was significantly reduced after drug treatment only in wild-type mice ($p < 0.01$).

Fig. 3.

Effects of 5-LOX deficiency on mouse behavior in the forced swimming test. Naive wild-type and 5-LOX-deficient mice ($n = 26$) were assayed in an automated swim test apparatus. Data are shown as mean $\pm$ S.E.M.; open bars = wild-type, closed bars = 5-LOX-deficient. (A) basic movements, (B) climbing, (C) rest time. Climbing behavior was significantly greater in 5-LOX-deficient mice ($p < 0.01$).

Fig. 4.

Effects of prolonged diet delivery of MK-886 on mouse behavior in the forced swimming test. Mice ($n = 12$) on TD.06348 Harlan-Teklad diet with and without MK-886 were assayed in an automated swim test apparatus after two and three weeks of exposure to this diet. Results are shown as mean $\pm$ S.E.M.; open bars = vehicle (Veh), closed bars = MK-886 (MK). (A) basic movements, (B) climbing, (C) rest time. Climbing behavior was significantly increased in mice on a diet supplemented with MK-886 for three weeks compared to mice on a drug-free diet ($p < 0.05$).