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Acetylsalicylic acid as an augmentation agent in fluoxetine treatment resistant depressive rats

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ABSTRACT

Clinical studies have reported that adjunctive acetylsalicylic acid (aspirin) therapy is beneficial for patients with treatment resistant depression (TRD). However, there still exist negative epidemiological data on the link between aspirin and depression. Therefore, this study aimed to further investigate whether aspirin can be used as an augmentation agent in fluoxetine treatment resistant depressive rats induced by chronic unpredictable mild stress (CUMS). In this study, the effects of CUMS regimen and antidepressant treatment were assessed by behavioral testing, hippocampal expression of cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2). 4-week fluoxetine treatment reversed the behavioral changes in approximately 70–80% depressive rats. That is, 20–30% depressive rats were resistant to fluoxetine. In the hippocampus of fluoxetine treatment resistant depressive rats, a significant upregulation of COX-2 level and PGE_2 concentration was observed. However, in these rats adjunctive aspirin treatment significantly improved the depressive behaviors and downregulated the COX-2 level and PGE_2 concentration in the hippocampus. Thus, our results suggest that aspirin can be served as an effective adjunctive agent in the treatment resistant depression mediated by inhibition of the COX-2 level and PGE_2 concentration.

Major depression is a common and sometimes fatal disorder [20]. It is estimated that by 2020 major depression will be the second most disabling condition in the world [8,22]. Despite considerable strides have been made over the years, treatment resistant depression (TRD) remains a common condition which accounts for approximately 30% of the depressed population [32]. For those patients, therapeutic strategies including multiple trials of high-dose antidepressants, varying combinations of antidepressants, augmenting agents and psychotherapy, are less than ideal. A large body of evidence supports the acute efficacy of ECT in TRD. However, memory loss and the need for repeated treatments to maintain efficacy preclude the use of ECT as a long-term treatment option. The illness not only affects quality of life but is also a major cause of suicide [10,13,33]. Meanwhile, it contributes to increased mortality in the context of comorbid disorders including diabetes and cardiovascular disease [24]. Thus, these have encouraged the further research for more effective agents [4,18].

Studies suggest that TRD is accompanied by inflammatory dysregulation. Major depressive patients with a history of nonresponsiveness to antidepressants have been found to demonstrate increased plasma concentration of IL-6, TNF-α and acute phase reactants when compared with treatment responsive patients [17,30]. Similarly, patients with increased inflammatory cytokines before treatment have been reported to be less responsive to antidepressant treatment [2,14,30]. Arachidonic acid derivatives, such as prostaglandins, play an important role in the inflammatory response [23]. Cyclooxygenase (COX) is a rate-limiting enzyme in the metabolism of arachidonic acid to prostaglandins. COX exists in two distinct isoforms (COX-1 and COX-2). The importance of COX-2 in depressive pathology is highlighted by recent findings. In rat, chronic administration of lithium can decrease the expression of COX-2 in brain, whereas COX-1 protein is not altered [6]. Studies also indicate that COX-2 activity can be increased by proinflammatory cytokines and it also activates the release IL-1β and TNF-α [21].

Aspirin is a non-steroidal anti-inflammatory drug with a wide spectrum of pharmacological activities. Its anti-inflammatory benefit has been reported to be related to the fact that aspirin acetylates COX-2 and increases the synthesis of anti-inflammatory mediators [29]. Aspirin has antidepressant properties and accelerates antidepressant effect in preclinical models [7]. Clinically, Aspirin has been suggested to shorten the onset of action of selective reuptake inhibitors (SSRI) and to increase remission rates when added to fluoxetine in an open-label study of depressed patients previously nonresponsive to fluoxetine alone [19].

Based on the above demonstrations, aspirin may be served as an effective adjunctive agent for TRD. However, there still exist negative epidemiological data on the link between aspirin and

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depression. For example, a previous study has demonstrated that aspirin usage does not decrease, but increase the prevalence of depression [1]. This contradiction may be explained by the facts that co-existing medical morbidity or complications arise from the use of the medication.

To further investigate the adjunctive antidepressant action of aspirin, we chose fluoxetine treatment resistant depressive rats induced by CUMS in the present study, and then evaluated the adjunctive antidepressant-like potential of aspirin by using behavioral testing (sucrose preference test, forced swimming test, novelty suppressed feeding test). Furthermore, levels of COX-2 protein and COX-2 immunohistochemistry and prostaglandin E_2 (PGE_2, a major COX-2 mediated inflammatory mediator) concentration in rat hippocampus were then measured.

Two-month-old male Sprague-Dawley rats (220–250 g) were obtained from the Fourth Military Medical University animal center at the time of first treatment in this study. All animals were maintained under standard laboratory conditions (12/12 h light/dark cycle with lights on at 8:00 A.M., 22 ± 2 °C with a relative humidity at 50 ± 10%, food and water ad libitum). The animals were allowed to adapt to laboratory conditions for at least one week. All stress-exposed rats were singly housed and non-stressed rats grouped (five/cage). All procedures were in strict accordance with the guidelines established by the NIH in the US and approved by the Fourth Military Medical University Animal Care Committee.

Fluoxetine (FLX; 10 mg/kg, Sigma, St Louis, MO) was dissolved in distilled water. Aspirin was purchased from Sigma (Sigma Chemical Co., MO, USA). All drugs were injected i.p., and the final injection volume was 5 mL/kg. All drugs were freshly prepared. Fluoxetine and vehicle were injected between 8:00 AM and 10:00 AM; aspirin was administered between 4:00 PM and 6:00 PM, irrespective of the stress schedule. The dose of fluoxetine employed was chosen according to the previous studies [9,15].

In experiment 1, rats were divided into three groups: saline-treated non-stressed group (Con, $n = 12$), saline-treated CUMS-exposed group (CUMS, $n = 12$) and fluoxetine-treated CUMS-exposed group (FLX-CUMS, $n = 60$). The sucrose preference was assessed weekly. At the end of 4 week stress period, behavioral tests were performed and the fluoxetine treatment resistant depressive rats were chosen for experiment 2. Then, in experiment 2, aspirin (20 mg/kg/day) was administered in conjunction with fluoxetine in treatment resistant depressive rats. After 3 weeks, the behavioral changes, COX-2 expression and PGE_2 level were examined.

The CUMS protocol used in our study was slightly modified from Willner et al. [34]. In brief, rats were subjected to alterations of the bedding, cage-tilting (45°), stroboscopic light, intermittent white noise (80 dB), food and water deprivation and alteration of the light/dark cycle (see Table 1).

Non-stressed rats were isolated 3 h before the behavioral testing, and the cages of stressed rats were changed at the same time. Rats were habituated to the testing room for 30 min before behavioral analysis. All tests were conducted between 2:00 PM and 5:00 PM.

Rats were placed individually in the testing plastic cylinder (54 cm high × 35 cm in diameter) containing a 38 cm water column (22 ± 1 °C) according to the previous studies [11,12]. Water was replaced between every trail. An initial 15-min pretest, followed by a 6-min test 24 h later was examined. Two observers blind to the treatment conditions scored the time spent immobile manually from the last 4-min test session [25].

Rats were deprived of water and food for 20 h, water and 2% sucrose were then placed in pre-weighed bottles, and animals were allowed to consume the fluid freely for 1 h. The sucrose preference was calculated as the sucrose preference (%) = sucrose consumption/(sucrose consumption + water consumption). The definition of a fluoxetine responder was a minimum 10% increase in sucrose intake compared to anhedonic level [3].

Table 1
Schedule of chronic unpredictable mild stress.

Saturday	Light on overnight	Strobe light on Room light off Strobe light off Tilt cages	8:00 16:00
Sunday	Light off overnight	Untilt cages Room light on Food and water deprivation Damp cages Dry cages	9:00 17:00
Monday	Light off overnight	Restore food and water Room light on White noise on White noise off	9:00 15:30
Tuesday	Light on overnight	Sawdust changes Room light off	8:00 16:00
Wednesday	Light off overnight	Room light on Tilt cages Untilt cages	10:00 16:00
Thursday	Strobe light on	Strobe light off Food and water deprivation White noise on White noise off	8:00 15:00
Friday	Light off overnight	Room light on Add water bottle Offer restricted food Remove water bottle Restore food and water	9:00 10:00 16:00

Novelty suppressed feeding test was performed according to previously described protocols [5,12]. The whole process was videotaped and the latency to feed in the novel environment was measured by 2 experimenters blind to the treatment conditions.

After behavioral testing, rats were anesthetized with sodium pentobarbital (50 mg/kg, IP) and perfused transcardially with 0.9% saline (about 100 mL), followed by 200 mL of 4% paraformaldehyde in 0.1 M PBS (pH 7.4). The brains were removed, post-fixed for 2 h in the same fixative at 4 °C, and then immersed in 30% sucrose potassium phosphate buffered saline (PBS) until they sank. Serial coronal sections (30 μm thickness) through the entire hippocampus were cut on a sliding microtome, and every six section from each brain was collected. For staining of COX-2, free floating sections were incubated with a polyclonal goat anti-COX-2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in a dilution of 1:100 overnight at 4 °C. After washing with PBS, sections were incubated with secondary antibody (biotinylated rabbit anti-goat antibody; Dianova, Hamburg, Germany; dilution 1:500) for 2 h at room temperature, followed by wash with PBS again and incubation in avidin–biotin–peroxidase complex for 2 h. After a final wash, sections were colorized by the peroxidase substrate 3,3′-diaminobenzidine. Sections were washed with PBS and distilled water, air-dried and coverslipped with Entellan (Merck, Darmstadt, Germany).

Following the behavioral tests, rats were sacrificed. Then the brain was dissected, one hippocampus was used for COX-2 protein analysis, the other for analysis of PGE_2 (see below). The hippocampus was put into chilled tubes treated with lysis buffer (50 mM Tris–HCl, pH 8.0; 20 mM EDTA; 1% SDS; and 100 mM NaCl), Protein concentration was measured using a protein assay according to the manufacturer's procedure. Western blot analysis was carried out by COX-2 (1:1000) and β-actin (1:10,000, Santa Cruz Biotechnology, Santa Cruz, CA). A secondary antibody conjugated with horseradish peroxidase (HRP, 1:5000, Bio-Rad) was used. Immunoblots were visualized on X-ray film by chemiluminescence reaction (Pierce),