Antidepressant and anti-inflammatory effects of a combined fluoxetine and celecoxib treatment in a rat model of depression

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Background : An evidence suggests a potential therapeutic target of depression toward treating inflammation with nonsteroidal anti-inflammatory drugs (NSAIDs). However, the efficacy of the combined standard antidepressant with NSAID treatment still remains uncertain.

Objective : To examine the effects of fluoxetine (serotonin reuptake inhibitor) in adjunct with celecoxib (specific COX-2 inhibitor) on depression-like behavior and inflammation in rats with chronic mild stress (CMS) induced depression.

Methods : The rats were divided into 4 groups: control, CMS, CMS with fluoxetine treatment (5 mg/kg/d), and CMS with fluoxetine and celecoxib treatment (5 mg/kg/d each). All treatments were continued for 5 weeks. The depression-like behavior was examined using forced swimming test. Blood samples were collected for biochemical analysis of plasma levels of cortisol, prostaglandin E\textsubscript{2} (PGE\textsubscript{2}), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), and an oxidative stress marker malondialdehyde (MDA).
Results: The combined treatment decreased depression-like behavior to the same extent as fluoxetine treatment alone. However, the adjunctive celecoxib significantly reversed the effect of fluoxetine in lowering the plasma cortisol level which implied the aggravation of hypothalamic-pituitary-adrenal axis (HPA axis) dysregulation, and also increased body weight gain compared with fluoxetine, suggesting a possibility of body fluid retention. Both drug treatments showed a comparable degree in lowering the plasma levels of PGE₂, TNF-α, CRP and MDA. Nonetheless, fluoxetine showed no significant effect on plasma IL-1β level; whereas the adjunctive celecoxib treatment lowered this proinflammatory cytokine to almost normal.

Conclusion: The results suggest that the combined celecoxib treatment shows no synergistic antidepressant effect to fluoxetine, but may exacerbate the HPA axis hyperactivation.

Keywords: Depression, fluoxetine, celecoxib, antidepressant, anti-inflammation.
ผลของการให้ยา fluoxetine ร่วมกับ celecoxib ต่อการต้านซึมเศร้าและการต้านอักเสบในหนูขาวที่มีภาวะซึมเศร้า จุฬาลงกรณ์เวชสาร 2561 ก.ค. – ส.ค.; 62(4): 653 – 655

เหตุผลของการทำวิจัย: การศึกษาที่ผ่านมาได้เสนอแนะว่าการรักษาโรคซึมเศร้าอาจทำได้โดยการใช้ยาต้านอักเสบที่ไม่ใช่สเตียรอยด์ (NSAIDs) และประยุกต์ยาต้านซึมเศร้าที่มีเชิงรุก.

วัตถุประสงค์: เพื่อศึกษาผลของการให้ยา fluoxetine (serotonin reuptake inhibitor) ร่วมกับ celecoxib (specific COX-2 inhibitor) ต่อพฤติกรรมเหมือนอาการซึมเศร้าและการอักเสบในหนูขาวที่ถูกเหนี่ยวนำให้ซึมเศร้าด้วยความเครียดระดับต่ำเป็นเวลา 28 วัน.

วิธีการทำวิจัย: แบ่งหนูขาวออกเป็น 4 กลุ่ม ดังนี้ กลุ่มควบคุม กลุ่มที่ได้รับความเครียด กลุ่มที่ได้รับความเครียดร่วมกับยา fluoxetine (5 มก./กก./วัน) และกลุ่มที่ได้รับความเครียดร่วม fluoxetine กับ celecoxib (คือละ 5 มก./กก./วัน). ประเมินผลพฤติกรรมซึมเศร้าและระดับของ cortisols, prostaglandin E2, interleukin-1β, tumor necrosis factor-a (TNF-α), C-reactive protein (CRP), และ malondialdehyde (MDA) ซึ่งเป็นตัวบ่งชี้ภาวะเครียดออกซิเดชัน.

ผลการศึกษา: Fluoxetine และการทำร่วมสามารถลดพฤติกรรมซึมเศร้าได้เท่ากันแต่การให้ยาบนกลุ่มเพิ่มระดับ cortisols ในหลอดหากมีความผิดปกติของ hypothalamic-pituitary-adrenal axis (HPA axis) รุนแรง อาการซึมเศร้าจะเพิ่มหนักขึ้นได้มากกว่าการให้ fluoxetine อย่างเดียว ข้อเสียคือยาต้านซึมเศร้านี้ไม่สามารถลดระดับ IL-1β แต่เมื่อใช้ร่วมกับ celecoxib สามารถลดระดับสารนี้จนกลับปกติ.

สรุป: ผลการทดลองนี้ชี้ว่าการให้ยา celecoxib ร่วมไม่สามารถเสริมฤทธิ์ด้านซึมเศร้าของ fluoxetine แต่อาจทำให้การกระตุ้น HPA axis ที่มากกว่าปกติมีความรุนแรง.

คำสำคัญ: ภาวะซึมเศร้า, ฟลูออกซิทีน, เซเลโคซิบ, ต้านซึมเศร้า, ต้านการอักเสบ.
Converging evidence indicates that the onset of depression is a stress-related disorder with hypothalamic-pituitary-adrenocortical (HPA) axis hyperactivity, and subsequently increase glucocorticoid level. The excess blood glucocorticoid found in mood disorders is involved in hippocampal damage and atrophy, causing attention and memory impairments. In addition, the degree of cognitive impairment in depressed patients was correlated with the 24-hour urinary cortisol excretion. Several studies have linked the activation of inflammatory pathways in the brain and the periphery with the pathogenesis of depression since elevated plasma inflammatory mediators in depressive patients were consistently observed. The excess proinflammatory cytokines in depressed patients were also correlated with the severity of depression and the HPA axis hyperactivity. A number of studies showed that the depletion of serotonin in depression was attributed to proinflammatory cytokines mediating in the degradation of its precursor tryptophan. A recent clinical study also found that TNF-α inhibitor therapy was able to reduce depressive symptoms in patients with illness. Therefore, the strong relationship between depression and inflammation suggests that the therapeutic approach for this illness could be done by directly treated inflammation.

Prostaglandin E₂ (PGE₂), an inflammatory mediator synthesized via the cyclooxygenase (COX) pathway, also has a role in the development of depression. Increased PGE₂ levels in the serum, cerebrospinal fluid and saliva of depressive patients were previously demonstrated. The involvement of PGE₂ in the activation of HPA axis is confirmed by increased ACTH secretion upon intrahypothalamic injection of this mediator. Consequently, the interference of PGs biosynthesis with nonsteroidal anti-inflammatory drugs (NSAIDs) has become a therapeutic target for depressive disorder. However, the antidepressant effect of NSAIDs in monotherapy or in combination with conventional antidepressants is still controversial. Clinical studies found that the uses of NSAIDs, in particular the COX-2 inhibitor, as an adjuvant to antidepressant with inhibition of norepinephrine or serotonin reuptake showed a better outcome in depressed patients. In contrast, a recent study in animal and human models demonstrated that concomitant use of NSAIDs, non-specific COX inhibitor, decreased the antidepressant effects of specific serotonin reuptake inhibitor (SSRI). Subsequently, a pharmacovigilance study also pointed to a significant association between NSAIDs exposure and poorer antidepressant treatment outcome in major depressive disorder. Further studies are thus needed to clarify the efficacy of NSAID as a therapeutic approach in depression.

The aim of the present study was to compare the treatment effects between SSRI fluoxetine alone and in combination with COX-2 inhibitor, celecoxib, in a rat model with chronic mild stress (CMS) induced depression. The antidepressant potential of the treatments was then evaluated by using depression-like behavior induced by CMS. The HPA axis activity (plasma cortisol), anti-inflammatory effect (plasma inflammatory mediators), and oxidative stress were also simultaneously examined.

Materials and Methods

Animal and Treatments

Thirty-six male Sprague-Dawley rats were
purchased from the National Laboratory Animal Center, Mahidol University, Thailand. The animals were housed in an individual cage and allowed to access food and water ad libitum, except in specific treatments as noted. All experiments in this study were complied with the standard protocols for the use of laboratory animals, and all experimental procedures were approved by the Institutional Animal Care and Use Committee, Burapha University (ID# 18/54).

After 1 week of acclimatization, the rats were divided into 4 groups: 1) non-treated control, 2) chronic mild stress (CMS) with vehicle, 3) CMS with fluoxetine (serotonin re-uptake inhibitor) 5 mg/kg/d (CMS-F), 4) CMS with fluoxetine and celecoxib (COX-2 inhibitor) 5 mg/kg/d each (CMS-FC). In the CMS procedure, the rats were exposed to the following mild and unpredictable stressors in a weekly random order: food and water deprivation (two 4-hour periods), cage tilt 40° (two 14-hour periods), paired housing (two 16-hour periods), damp bedding (three 4-hour periods), white noise (three 2-hour periods), empty water bottle (two 4-hour periods), and overnight water deprivation with continuous lighting (one 15-hour periods). The fluoxetine (Medic Pharma, Thailand), celecoxib (Celebrex®, Pfizer, Thailand) and vehicle (distilled water) were administered daily by gastric gavage. Both CMS and drug treatments commenced on the same day and continued for 5 weeks. The body weight of each rat was measured weekly.

**Forced swimming test**

The depression-like behavior was assessed by the forced swimming test (FST) described by Andreatini R. and Bacellar LF. with increasing water depth to gain sensitivity. Behavioral study was carried out between 9:00 - 11:00 a.m. to avoid the effect of circadian rhythm on emotional response. After the 5-week treatment period, rats were placed individually in an unescapable and transparent plastic cylinder filled with water (25°C) up to a depth of 40 cm. On the first day of the test, rats were forced to swim for 15 min to acclimatize to the experimental procedure. The animals were retested on the second day for 6 min and their behaviors were monitored by a video recorder which were further analyzed by three observers. Immobility time, a depression-like behavior, during the last 4 min of the second day test were recorded by an individual observer, and data were averaged for each rat. The immobility of rats was judged when they adopted a characteristic of immobile posture with their head emerging just above the water level while their body still remained floating. After the test, animals were dried up with towel and placed in a heated cage.

**Biochemical analysis**

At the end of the treatments, rats were euthanized with an overdose of thiopental sodium (100 mg/kg), and then their blood was taken out between 9:00 - 11:00 a.m. via cardiac puncture and collected into chilled tubes with heparin. The blood was centrifuged at 3,000 rpm at 4°C to obtain plasma. The plasma was separated in 100 μL aliquots and stored at –80°C for further biochemical analyses for plasma cortisol, prostaglandin E₂ (PGE₂), interleukin-1β (IL-1β), tumor necrosis factor-a (TNF-α), C-reactive protein (CRP), and oxidative stress marker malondialdehyde (MDA). The plasma cortisol, IL-1β, TNF-α, and CRP were measured by using a quantitative sandwich enzyme-linked immunosorbent
assay kit with monoclonal antibody specific to rat cortisol, IL-1β, TNF-α, and CRP (MyBioSource, California, USA); while the plasma PGE₂ was measured by a competitive enzyme immunoassay kit (Enzo Life Sciences, Inc., New York, USA). Finally, the plasma MDA was measured through a thiobarbituric acid reactive substance assay kit (Cayman Chemical, Michigan, USA). All assay protocols were performed according to the manufacturer’s instructions.

Data analyses

Values were expressed as mean ± standard error of the mean (SEM). Body weight gain was calculated as percentage of baseline values. Differences between groups were tested using one-way ANOVA followed by Tukey HSD test for post-hoc analysis. Differences were considered significant with \( P < 0.05 \). Analyses were performed using the SPSS 17.0 program.

Results

Depressive-like signs

The preventive effects of fluoxetine alone and in combination with celecoxib were investigated in depressive-like conditions. Figure 1 displays the weekly body weight gain of all animal groups during 5-week period of treatments. CMS caused a significant decrease in body weight gain at week 3 to week 5 of the test period \( (P < 0.05) \). Fluoxetine treatment was found preventing weight loss induced by CMS in the last two weeks of the test period \( (P < 0.05) \). The animals treated with fluoxetine plus celecoxib significantly gained higher body weight than those with fluoxetine treatment alone during weeks 4 and 5 \( (P < 0.05) \). Immobility time as a state of behavioral despair was evaluated in FST at the end of week 5. Total immobility time of CMS group was significantly longer than that of the control group as illustrated in Figure 2 \( (P < 0.05) \). Both fluoxetine alone and in combination with celecoxib partially reversed immobility time to a comparable degree compared with CMS group \( (P < 0.05) \).

Figure 1. Body weight gain presented in percentage of baseline values of all groups expressed as non-treated control, chronic mild stress (CMS), CMS with fluoxetine (CMS-F), and CMS with fluoxetine plus celecoxib (CMS-FC). \( n = 9 \) in all groups; * \( P < 0.05 \) vs control, # \( P < 0.05 \) vs CMS, @ \( P < 0.05 \) vs CMS-F.
ผลของการให้ยา fluoxetine ร่วมกับ celecoxib ต่อการต้านซึมเศร้าและการต้านอักเสบในหนูขาวที่มีภาวะซึมเศร้า

Plasma cortisol

CMS induced a significant increase in the plasma level of cortisol ($P < 0.05$). Fluoxetine significantly reduced the level of this hormone in CMS rats, but the adjunctive treatment with celecoxib returned it to the level that was significantly higher than that of fluoxetine treatment alone ($P < 0.05$, Figure 3).

Plasma inflammatory mediators and malondialdehyde

Circulating PGE$_2$, IL-1$\beta$, TNF-$\alpha$ and CRP were found significantly elevated in CMS group as illustrated in Figure 4 ($P < 0.05$). Treatments with fluoxetine alone and in combination with celecoxib significantly reversed the CMS-induced increase in the plasma PGE$_2$ ($P < 0.05$) to the same degree (Figure 4A). Combined drug treatment almost fully returned the plasma IL-1$\beta$ in CMS rats to the same level as that in the control group, whereas fluoxetine alone showed no effect on IL-1$\beta$ in depressive-like conditions (Figure 4B). Either fluoxetine or adjunctive celecoxib treatment significantly decreased the CMS-increased plasma TNF-$\alpha$ levels ($P < 0.05$, Figure 4C). The two drugs treatments significantly lowered the plasma level of CRP in CMS rats to a comparable degree ($P < 0.05$; Figure D). Oxidative damage to lipid was also examined by measuring the plasma level of an oxidative stress marker, MDA. Figure 5 shows that the plasma MDA was significantly elevated in CMS group, and significantly lowered by fluoxetine treatment ($P < 0.05$), but not in combination of fluoxetine and celecoxib group (Figure 5).
Figure 4. Effects of 5-week treatments with chronic mild stress (CMS), CMS with fluoxetine (CMS-F), and CMS with fluoxetine plus celecoxib (CMS-FC) on plasma levels of PGE\(_2\) (A), IL-1\(\beta\) (B), TNF-\(\alpha\) (C), and CRP (D). \(n = 7\) in all groups; \(^* P < 0.05\) vs control, \(^# P < 0.05\) vs CMS.

Figure 5. Plasma MDA levels of all groups after 5-week treatments with chronic mild stress (CMS), CMS with fluoxetine (CMS-F), and CMS with fluoxetine plus celecoxib (CMS-FC). \(n = 9\) in all groups; \(^* P < 0.05\) vs control, \(^# P < 0.05\) vs CMS.
Discussion

CMS in rat has been considered as a highly valid and robust model for studying the efficacy of antidepressants since the stress exposure causes behavioral and neurohormonal alterations which mimic the signs and symptoms of depression in human. (20, 21) Hence, the present study utilized CMS model to assess the antidepressant, anti-inflammatory, and antioxidant effects of fluoxetine treatment alone and in combination with a specific COX-2 inhibitor, celecoxib. After a 5-week exposure to CMS, the rats manifested increased immobility in FST which can be interpreted as a passive stress-coping strategy or behavioral despair. (19) CMS rats also displayed a loss in body weight compared to the control, suggesting poor appetite. These depression-like signs in the CMS group were in line with the elevated plasma cortisol level implying HPA axis hyperactivity. The fluoxetine treatment was able to improve the depression-like behavior, decreased immobility time, and the increased cortisol level induced by CMS. The concomitant treatment with celecoxib attenuated behavioral despair in CMS rats to a similar extent as fluoxetine treatment alone; however, it caused a rebound of the plasma cortisol. This finding suggested that the add-on celecoxib might aggravate the HPA axis dysregulation. In addition, the present study demonstrated that fluoxetine partially prevented the loss of body weight induced by CMS, corresponding with the attenuated depressive despair and decreased plasma cortisol found in this group. A recent study showed that COX-2 inhibition reduced basal and stress induced anxiety-like behavior in mice. (22) As a result of COX-2 inhibition, the combined treatment with celecoxib improved behavioral despair concurrent with increase in body weight nearly at the same rate as that of the control group. However, the regaining of weight in the add-on celecoxib group was inconsistent with the observed higher immobility and plasma cortisol compared to the control group. It is possible that the regained body weight might not relevant to improved poor appetite by celecoxib. This drug can affect renal function and causes sodium and water retention that has been persistently reported in both animal and clinical studies. (23, 24) Our parallel study also observed the association of celecoxib-increased systolic blood pressure with higher body weight. (25) The altered body weight thus may not be a good depressive index for evaluating antidepressant drugs with decreased renal function. Based on the present data, the adjunctive treatment with celecoxib showed no synergistic antidepressant effect to fluoxetine, but might cause unfavorable effects on the HPA axis function and renal salt excretion.

A common feature of depressive patient is the activation of inflammatory pathway resulting in elevated plasma levels of proinflammatory cytokines which subsequently induce acute phase proteins, such as CRP. These inflammatory mediators are associated with risk and severity of depression. (26, 27) Our CMS paradigm was able to induce inflammation as indicated by increases in plasma levels of PGE$_2$, IL-1$\beta$, TNF-$\alpha$ and CRP. The fluoxetine treatment appeared to have a potential anti-inflammatory effect on PGE$_2$, TNF-$\alpha$ and CRP, but was unable to return the elevated plasma IL-1$\beta$ to normal. The finding is in agreement with a clinical study revealing an unchanged level of IL-1$\beta$ in depressive patients treated with fluoxetine. (28) A previous study showed that peripheral IL-1$\beta$ infusion in experimental animals
caused sickness behavior, elevated plasma corticosterone, and enhanced proinflammatory cytokine expression in the prefrontal cortex and hippocampus.\(^{(29)}\) Thus, the observed partial antidepressant effect of fluoxetine in the present finding might be partly attributed to the elevated systemic IL-1\(\beta\) activating the central inflammation. This would partly explain fluoxetine resistance since a study showing that IL-1\(\beta\) antagonist was able to restore the effectiveness of fluoxetine in a rat model of depression.\(^{(30)}\) The add-on celecoxib produced a better anti-inflammatory effect in lowering the plasma IL-1\(\beta\), but it might aggravate the HPA axis dysregulation as indicated by the observed simultaneous higher level of plasma cortisol compared with fluoxetine treatment alone. Our finding is in agreement with previous studies which showed the poorer antidepressant outcome in the use of NSAIDs as adjunctive therapy.\(^{(17,18)}\) Based on the data of this study, it is still difficult to explain how inhibition of COX-2 antagonizes the effect of fluoxetine on the overdriven HPA axis. According to the classical view, COX-2 is induced by inflammatory stimuli, so it is the target for anti-inflammatory drug.\(^{(31)}\) However, evidence suggested that both isoforms of COX may have an opposite role in causing inflammation of the central.\(^{(32)}\) Furthermore, studies employing rodents with genetic deletion of COX-1 or COX-2 suggested that the central neuroinflammation may be mediated by COX-1; whereas COX-2 may play a role in the resolution phase of inflammation.\(^{(32,33)}\) It would, therefore, be possible that the elevated plasma cortisol upon COX-2 inhibition in the current study might be due to increased central neuroinflammation, and increased proinflammatory cytokines driven by COX-1 would activate the HPA axis. This notion needs to be clarified, perhaps by the use of a higher selective COX-1 NSAID as an adjunctive treatment.

The links between inflammation and oxidative stress in the pathogenesis of depression has been described, and both interact in a bidirectional manner.\(^{(34)}\) Enhanced circulatory oxidative stress markers and increased renal excretion of oxidative metabolite in depressive patients have been demonstrated.\(^{(34, 35)}\) The present study evaluated the plasma levels of a lipid degradation product MDA at the end of the treatment period. Fluoxetine showed a partial antioxidant effect by lowering the elevated MDA level induced by CMS. The MDA level in fluoxetine treatment remained markedly higher than that of the control, which is in line with a more recent study which found the F2-isoprostane level, a marker and mediator of oxidative stress, remained significantly high in non-responders to SSRI therapy.\(^{(36)}\) The additional treatment with celecoxib caused a slightly enhanced plasma MDA to the level which was not significantly different from that of CMS group. This finding is in line with an early study finding that celecoxib did not change MDA levels in patients with osteoarthritis.\(^{(37)}\) It was demonstrated that COX-2 inhibition with celecoxib exacerbated neuroinflammatory response to inflammatory stimuli and increased brain oxidative stress markers.\(^{(38)}\) Consequently, the slightly elevated plasma MDA in the add-on celecoxib group observed in our study might represent a spillover from increased central neuroinflammation and oxidative stress upon COX-2 inhibition. Taken together, it seems possible that the adjunct of COX-2 inhibitor NSAID up to the standard SSRI therapy might intensify central neuroinflammation and oxidative stress via COX-1.
enzyme, and central releases of both inflammatory mediators and oxidative stress would aggravate the HPA axis dysregulation in depressive state. However, this assumption needs further investigation, for instance, the uses of a highly selective COX-1 inhibitor and other classes of specific COX-2 inhibitors in adjunctive treatment with other classical antidepressants should be explored.

**Conclusion**

In conclusion, despite its more pronounced anti-inflammatory action, the add-on COX-2 inhibitor celecoxib augmented the plasma cortisol level which might attribute to the aggravation of the HPA axis hyperactivation. Consequently, in clinical use of concomitant medication with a specific NSAID celecoxib and SSRI in depression, caution should be exercised and its adverse medical outcome should be taken into consideration.

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