Possible Modulating Effects of Celecoxib (COX II Inhibitor) on Antidepressant Action of Duloxetine (SNRI) in Stressed Mice

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Abstract: Rationale: In recent years, a potential link between inflammation and depression has been shown and the role of proinflammatory cytokines in the pathophysiology of major depressive disorders has been observed. Celecoxib (selective COX-II inhibitor) has been reported to inhibit the production of PGE-II and proinflammatory cytokines and also increase tryptophan levels and serotonin availability in depressed patients. On the other hand, duloxetine (potent SNRI) has been shown to be efficacious in inflammatory and acute pain models in rodents and synergistic interaction with NSAIDs. However, the interactions of duloxetine with celecoxib are currently unknown.

Objective: We evaluated the antidepressant effect of celecoxib (15, 30 mg/kg/day for 15 days, ip) alone and in combination with duloxetine (5, 10 mg/kg/day for 15 days, ip) and also the biochemical parameters in stressed mice.

Results: Pretreatment of celecoxib (15, 30 mg/kg) for 15 days to forced swim-induced stressed mice produced significant antidepressant effect which has been evidenced by decreased in immobility time in tail suspension test (TST). Celecoxib (30 mg/kg) also showed significant increase in locomotor activity and protective effect on biochemical parameters of oxidative stress by reversing stress-induced increase in TBARS and reduction in GSH levels. Pretreatment with combination of celecoxib with duloxetine (5, 10 mg/kg) showed significant antidepressant and neuroprotective effects against stress induced depression and oxidative damage in mice at both dose levels.

Conclusions: This study demonstrated dose-dependent antidepressant action of celecoxib in stressed mice. The combination of celecoxib with duloxetine further enhanced its antidepressant effect on TST in stressed mice. The treatment reversed forced swim-induced elevation in TBARS levels and depleted glutathione activity, suggesting their antioxidant and protective role in brain.

Keywords: Celecoxib, duloxetine, forced swim induced stress, oxidative stress.

INTRODUCTION

Major depression is a severe psychiatric disorder that is the fourth leading cause of disability worldwide [1]. This makes depression a major concern to the personal and economic welfare [2]. With the recent developments in psychiatric research, it has been shown that inflammatory processes and brain-immune interactions are also involved in the pathogenesis of major depression, and may contribute to the dysfunction of serotonergic and noradrenergic systems. There exist a complex and bidirectional interaction between the immune and central nervous system in depression [3].

Proinflammatory cytokines play a causative role in major depressive disorders. Circulating cytokines influence brain activity by inducing the expression of cyclooxygenase (COX)-II and microsomal prostaglandin E synthase-1 (mPGES-1) in brain vascular cells, which transduces inflammatory signals into a prostaglandin signaling cascade [4,5]. Increased levels of pro-inflammatory cytokines e.g. interleukin (IL)-1, IL-6, IL-8, IL-12, interferon (IFN)-γ and tumor necrosis factor (TNF)-α have consistently been reported in patients with depression [6-8]. In depression, both cortisol and pro-inflammatory cytokine levels are increased due to dysregulation of hypothalamic-pituitary-adrenal (HPA) axis feedback mechanism [9]. Elevated levels of circulating corticosteroids found to mediate the critical role of IL-1β in chronic stress-induced depression and suppressed neurogenesis in mice [10]. The hypercortisolism and excessive inflammatory responses have been also reported to contribute to neuronal apoptosis and death [11,12].

Celecoxib (selective COX-II inhibitor) inhibits the production of PGE-II and proinflammatory cytokines and decreases indoleamine-2,3-dioxygenase (IDO) activity, resulting in increased tryptophan levels and serotonin availability in depressed patients [13]. Decreased IDO activity contributes to a reduction of the glutamatergic activity via decreasing the production of quinolinic acid (N-Methyl-D-aspartate agonist) [14], which exert antidepressant effects. It also prevents the dysregulation of HPA-axis, in particular the increase of cortisol, a key biological factor associated with depression [15]. Literature shows that chronic celecoxib treatment reversed stress-induced depressive like behavior in animal models [16]. Moreover, it has been shown to be effective as an adjunctive therapy for major depression [17] and as maintenance treatment with the patient obtaining long

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term full remission [18]. Such studies suggest that celecoxib may be an effective adjuvant/alternative agent in the management of patients of major depression.

Duloxetine, an antidepressant, is a potent serotonin and nor epinephrine reuptake inhibitor (SNRI) in the central nervous system (CNS) and possess an earlier onset of action, superior remission abilities, and better efficacy in treating the physical symptoms of depression. There are reports of its efficacy in inflammatory and acute pain models in rodents and synergistic interaction with non-steroidal anti-inflammatory drug (NSAID) [19,20]. It is also reported to modulate depression induced oxidative stress [21,22]. However, the interactions of duloxetine with celecoxib are currently unknown and warranted further study.

Numerous studies indicates that reactive oxygen species (ROS) induced neuronal damage has an important role in the pathophysiology of depression [23]. Physiological stress, which accompanies severe depression, may increase lipid peroxidation [24]. COX-II leads to release of inflammatory prostaglandins (PGs), such as PGE-2 that accounts for the accumulation of oxidative mediators in stressed brain. Thus inhibiting COX isoenzymes could reduce the levels of lipid peroxidation and nitrite, and restore the reduced glutathione level and catalase activity, suggesting their neuroprotective roles against oxidative stress [25,26]. Mounting data indicate that increased production of pro-inflammatory cytokines during stress, immune, or inflammatory responses can exert powerful influences on the CNS.

Based on the potential role of cytokines in the pathophysiology of depression, opportunities exist for translational research strategies that focus on the management of depressive symptoms using novel therapies targeted at the pathways by which inflammation may contribute to depression. Hence, the present study was planned to investigate the antidepressant effect of celecoxib (COX-II inhibitor) alone and with duloxetine (SNRI) at different dose levels in stressed mice. The effect of above mentioned combination on the biochemical parameters in stressed mice was also estimated.

**MATERIALS AND METHODS**

Animals: Swiss strain male albino mice (25-35 g) raised at the Central Animal House Facility of Jamia Hamdard were used (temperature 25±2°C, relative humidity 50±15%). The animals were housed in polypropylene cages (10/cage) with a 12 h light-dark cycle. The mice were fed on a standard pellet diet (Amrut rat and mice feed, Pune, India) and water *ad libitum*. The project was undertaken with prior approval from the University Animals Ethics Committee. All experiments were performed during the daytime on healthy animals.

**Drugs and Dosing Schedule**

Celecoxib and duloxetine were received as a gift sample from Ranbaxy Laboratories Limited, Gurgaon, India. Celecoxib was administered 15 or 30 mg/kg, p.o [27] and duloxetine in a dose of 5 or 10 mg/kg, p.o [21,22] for 15 days. Drugs were suspended in 0.25% CMC and administered orally, 30 mins prior to the forced swimming session for 15 consecutive days. After 15 days, various behaviour assess-ments followed by biochemical estimation were conducted, on the subsequent day 16th.

**Forced Swimming Induced stress [28]**

The animals were forced to swim individually in a glass jar (25×12×25 cm³) at (25±2) °C for 6 min every day for 15 consecutive days. The water was 15 cm in height. After an initial period of vigorous activity, each animal attained a typical immobile posture. The animal was considered to be immobile when it ceased to struggle and the limbs seldom moved to keep the head above the water. This test session was repeated for 15 days.

**Tail Suspension test [29]**

The tail suspension test (TST) was employed for assessing antidepressant activity. The mouse was suspended by the tail with tape from the strain gauge and the total duration of immobility was calculated for 6 minutes period.

**Locomotor Activity [30]**

Animal was kept in photoactometer for the first 3 min and then locomotor activity was recorded using photoactometer for a period of 5 min. The apparatus was placed in darkened, light-sound attenuated and ventilated testing room. Each animal was observed over a period of 5 min in a square (30 cm) closed arena equipped with infrared light sensitive photocells using digital photoactometer and values expressed as counts per 5 min.

**Biochemical Parameters**

After the behavioural testing, the animals were immediately decapitated and their brains were removed, weighed and homogenized in ice-cold KCl-phosphate buffer (0.1 M, pH 7.4). Firstly, tissue protein was estimated. 0.1 ml of 10% homogenate was diluted with 0.9 ml of distilled water. The resultant mixture was mixed with 5 ml of alkaline solution and allowed to stand at room temperature for 10 minutes. This was followed by addition of 0.5 ml F-C reagent into each of the test tube. The tubes were shaken immediately for a thorough mixing. After 30 min, the absorbance was read at 750 nm against a reagent blank. The protein content was expressed in mg [31].

For glutathione, 2 ml of 10% homogenate was mixed with 2.5 ml of 0.02 M EDTA (ethylene diamine tetra acetic acid). 2 ml of resultant mixture was further mixed with 4 ml of distilled water and 1 ml of 50% TCA (trichloroacetic acid) solution. The tubes were shaken intermittently for 10–15 minutes. This was followed by centrifugation at 3000 rpm for 10 minutes. 2 ml of the supernatant was mixed with 4 ml of tris buffer (0.4 M, pH 8.9) and 0.1 ml DTNB (5, 5'-dithiobis-(2-nitrobenzoic acid) solution and shaken. The absorbance was read at 412 nm within 5 min of addition of DNTB solution against a blank with no homogenate. The results were expressed in µ moles of GSH / mg of protein [32].

For TBARS, 0.2 ml brain homogenate was diluted to 4 ml of 0.15 M KCl solution. Then the resultant solution was mixed with 1 ml of 30% TCA and 1 ml of 0.8% TBA. All
tubes were then covered with aluminium foils and placed on a shaking water bath maintained at 80°C for 30 min. The tubes were then shifted to crushed ice bath. This was followed by centrifugation at 3000 rpm for 10 min. The amount of MDA formed in each of the samples was assessed by measuring the absorbance of supernatant at 535 nm using spectrophotometer against a reagent blank. Results were measured in n moles of MDA/ mg of protein [33].

**OBSERVATIONS AND RESULTS**

1. **Effect of Celecoxib and Duloxetine on Tail Suspension Test in Stressed Mice**

   As shown in Table 1, the 15-days forced swimming (6 min each day) significantly increased immobility period as compared to the naïve group (p<0.01). Pre-treatment with celecoxib (30mg/kg) and duloxetine (10mg/kg) significantly reduced the immobility period (p<0.05). The combination of celecoxib and duloxetine (15mg/kg, 5mg/ kg) and celecoxib and duloxetine (30mg/kg, 10mg/kg) showed significant decrease in the immobility time (p<0.01, F (7, 40) = 4.224).

2. **Effect of Celecoxib and Duloxetine on Locomotor Activity in Stressed Mice**

   Table 2 depicts the effect of celecoxib on locomotor activity in stressed and normal mice. The 15-days forced swimming (6 min each day) caused significant locomotor activity impairment (as shown by decreased ambulatory movements) as compared to naïve group (p < 0.01). No significant change in locomotor activity was observed with celecoxib (15 mg/kg) and duloxetine (5mg/kg), whereas celecoxib (30mg/kg) and duloxetine (10mg/kg) showed significant increase in locomotor activity. The combination of celecoxib and duloxetine (15mg/kg, 5mg/ kg) and (30mg/kg, 20mg/ kg) could all significantly reverse the locomotor activity in stressed mice (p < 0.01, F (7,40)= 11.123).

3. **Effects of Celecoxib and Duloxetine on the Levels of Brain Lipid Peroxidation and Reduced Glutathione**

   Chronic forced swimming for 15-days produced significant oxidative damage as shown by a significant increase in whole brain MDA levels and reduced GSH levels as compared to naïve group (p <0.01) as shown in Table 3. No sig-

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Table 1. Effect of Celecoxib and Duloxetine on Tail Suspension Test in Stressed Mice

<table>
<thead>
<tr>
<th>Group No. (n=6)</th>
<th>Treatment</th>
<th>Dose</th>
<th>Immobility Period (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Naive</td>
<td>Saline 10ml/kg</td>
<td>118.21 ± 7.60**</td>
</tr>
<tr>
<td>2</td>
<td>Control (stressed group)</td>
<td>Saline 10ml/kg</td>
<td>200.15 ± 18.94##</td>
</tr>
<tr>
<td>3</td>
<td>Celecoxib</td>
<td>15mg/kg</td>
<td>165.31 ± 8.98</td>
</tr>
<tr>
<td>4</td>
<td>Celecoxib</td>
<td>30mg/kg</td>
<td>126.66 ± 5.29</td>
</tr>
<tr>
<td>5</td>
<td>Duloxetine</td>
<td>5mg/kg</td>
<td>156.92 ± 15.01</td>
</tr>
<tr>
<td>6</td>
<td>Duloxetine</td>
<td>10mg/kg</td>
<td>133.41 ± 4.20</td>
</tr>
<tr>
<td>7</td>
<td>Celecoxib + Duloxetine</td>
<td>15mg/kg + 5mg/kg</td>
<td>112.58 ± 7.05**</td>
</tr>
<tr>
<td>8</td>
<td>Celecoxib + Duloxetine</td>
<td>30mg/kg + 10mg/kg</td>
<td>106.45 ± 3.91**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=number of animals  
*p < 0.05, **p < 0.01 Vs control (stressed group), ##p < 0.01 Vs naïve  
F (7, 40) = 4.224

Table 2. Effect of Celecoxib and Duloxetine on Locomotor Activity in Stressed Mice

<table>
<thead>
<tr>
<th>Group No. (n=6)</th>
<th>Treatment</th>
<th>Dose</th>
<th>Locomotor Activity (Photo-beam Counts for 5mins Per Animal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Naive</td>
<td>Saline 10ml/kg</td>
<td>396.83 ± 14.79**</td>
</tr>
<tr>
<td>2</td>
<td>Control (stressed group)</td>
<td>Saline 10ml/kg</td>
<td>254.17 ± 22.13**</td>
</tr>
<tr>
<td>3</td>
<td>Celecoxib</td>
<td>15mg/kg</td>
<td>334.00 ± 30.44</td>
</tr>
<tr>
<td>4</td>
<td>Celecoxib</td>
<td>30mg/kg</td>
<td>363.17 ± 23.11</td>
</tr>
<tr>
<td>5</td>
<td>Duloxetine</td>
<td>5mg/kg</td>
<td>322.60 ± 32.43</td>
</tr>
<tr>
<td>6</td>
<td>Duloxetine</td>
<td>10mg/kg</td>
<td>438.33 ± 22.55</td>
</tr>
<tr>
<td>7</td>
<td>Celecoxib + Duloxetine</td>
<td>15mg/kg + 5mg/kg</td>
<td>458.17 ± 25.08**</td>
</tr>
<tr>
<td>8</td>
<td>Celecoxib + Duloxetine</td>
<td>30mg/kg + 10mg/kg</td>
<td>478.33 ± 18.34**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=number of animals  
*p < 0.05, **p < 0.01 Vs control (stressed group), ##p < 0.01 Vs naïve  
F (7, 40) = 11.123
significant change in MDA and GSH levels were found with celecoxib (15 mg/kg), Duloxetine (5 and 10mg/kg) and celecoxib (30mg/kg) showed significant decrease in TBARS levels. However, chronic administration of celecoxib and duloxetine combination significantly attenuated the increase in lipid peroxidation (TBARS) as compared to the control level (p < 0.01, F (7,40)= 10.039). The combination also restore the GSH levels in stressed mice (p < 0.01, F (7,40)= 5.717).

**DISCUSSION**

Literature shows that 15-days forced swimming in rodents could induce depression like symptoms in rodents, as revealed by increase in despair (increased immobility period) and reduction in locomotor activity [34]. In the present study, we investigated the effect of celecoxib alone and in combination with duloxetine on tail suspension test (TST), locomotor activity and oxidative stress parameters in stressed mice. We report that celecoxib (15 and 30 mg/kg) reduced immobility on TST in stressed mice. These antidepressant effects of celecoxib are in agreement with other studies in various animal models of depression [16]. Duloxetine being potent serotonin noradrenaline reuptake inhibitor (SNRI) also reduced the immobility time on TST and increased locomotor activity in stressed mice. We found that the combination of these two drugs (celecoxib and duloxetine) at different dose levels also showed highly significant antidepressant effect on TST and a significant increase in locomotor activity. In our study, it was observed that repetitive sessions of forced swimming for 6-mins for 15-days caused significant increase in immobility time and also decreased locomotor activity. Similar findings are reported with other related studies where chronic stress has been shown to induce depressive behaviour by influencing the immobility time, exercise and physical activity [28].

The suggested mechanism for antidepressant activity for COX-II inhibitors is complicated. A correlation between symptom severity and blood levels of proinflammatory cytokines has been demonstrated [35]. The role of IDO that breaks down tryptophan, the primary precursor of 5-HT into quinolinic acid has also been implicated. As decreased 5-HT availability and increased activity of the glutamatergic system are associated with depression, the pro-inflammatory cytokines such as IL-1, IL-6 and PGE-II can activate IDO, which may decrease the availability of 5-HT and enhances NMDA agonism, thereby depression results [13]. Since COX-II inhibitors such as celecoxib and others inhibit the production of PGE-2 and proinflammatory cytokines, they can exert antidepressant effect by multiple mechanisms: (a) by inhibiting PGE-2 production (b) by reducing glutamatergic activity via decreasing the production of quinolinic acid (a potent NMDA agonist) [13] and (c) by preventing the dysregulation of HPA axis [15].

The mechanism of antidepressant action of duloxetine is well established. It is considered as a balanced serotonergic and noradrenergic reuptake inhibitor with efficacy in the treatment of major depressive disorder [36]. Its effectiveness in reversing mechanical allodynia in nerve injury models of neuropathic pain and models of persistent pain in rats [37] and clinically in the treatment of inflammatory and persistent pain syndromes [22] has also been demonstrated. The synergistic interaction between duloxetine and ibuprofen (NSAID) in inflammatory pain in rodents has also been found [21]. Locomotor activity responses to stress are used as an output parameter for depression. Both acute and chronic stress has been reported to alter the locomotor activity and behavioural changes which might be due to alterations in the brain regions controlling motor activity leading to stress-induced depression in rodents [38]. In the present study, administration of celecoxib alone and its combination with duloxetine significantly reversed the impairment in locomotor activity and produced remarkable neuroprotective effect against chronic stress in mice.

Oxidative stress has been implicated in the pathophysiology of many neurological disorders including alzheimer’s disease, huntington’s disease, anxiety, depression etc [39,40]. Experimental data from several studies indicate the co-existence of oxidative stress with symptoms of depression as evidenced by defective plasma antioxidant defences and enhanced susceptibility to lipid peroxidation [41]. Significant correlation were found between the alteration in superoxide
dismutase (SOD) activity and MDA levels and severity of depression, as well as the length of index episode and duration of illness [27]. In our study, mice exposed to forced-swimming induced stress exhibited oxidative stress and increase in free radical generation as evidenced by a significant reduction in GSH levels and increase in TBARS levels. Pretreatment with celecoxib (30mg/kg) and duloxetine (5,10mg/kg) significantly reversed forced swim-induced increase in TBARS. When combined, a significant decrease in TBARS was observed even at celecoxib low dose (15mg/kg). On the other hand, celecoxib (at both dose levels) was unable to reverse forced swim-induced reduction in GSH levels; however duloxetine (10mg/kg) reversed the same. Their combination significantly reversed forced swim-induced reduced in GSH levels in stressed mice. Hence, the combined treatment exhibited a protective effect on brain by reducing TBARS levels and by increasing GSH levels.

The role of COX-II enzyme in stress-induced depressive symptoms has been recently implicated [28]. COX-II leads to the release of inflammatory PGs such as PGE-2 that causes accumulation of oxidative mediators in the brain, mitochondrial inhibition [42], increased excitatory amino acid release [43,44], activation of second messenger systems [43] and decreased efficiency of antioxidant defensive mechanism [28,45]. COX-isoforms also lead to the formation of hydroxyl free radicals and form peroxynitrite free radicals due to peroxidase activity. Therefore, inhibiting COX-isoenzymes has been proposed to inhibit induction & accumulation of oxidants. Expression of inducible nitric oxide synthase and COX-II enzyme increase in response to acute stress and production of oxygen and nitrogen free radicals causes oxidation of cellular components in the brain [46,47]. In the present study, pre-treatment with celecoxib, duloxetine and their combination exerted antioxidant and protective effect in brain against forced swim-induced stress in mice.

In conclusion, celecoxib exhibited antidepressant activity by decreasing immobility time and increasing locomotor activity in stressed mice. Combination with duloxetine further enhanced its antidepressant effect on TST in stressed mice. The treatment reversed forced swim-induced elevation in TBARS levels and depleted glutathione activity, suggesting their antioxidant and protective role in brain.

LIST OF ABBREVIATIONS

µm = micromoles
5-HT = 5 Hydroxy-tryptamine
ANOVA = Analysis of Variance
CMC = Carboxy methyl cellulose
CNS = Central nervous system
COX = Cyclooxygenase
DTNB = 5,5”-dithiobis-(2-nitrobenzoic acid)
EDTA = Ethylene diamine tetra acetic acid
F-C reagent = Folin-Ciocalteau reagent
GSH = Glutathione

HPA = Hypothalamic Pituitary Adrenal Axis
IDO = Indoleamine2,3- dioxygenase
IFN-γ = Interferon-alpha
IL = Interleukin
KCl = Potassium Chloride
KCI = Potassium chloride
MDA = malondialdehyde
mPGES = microsomal prostaglandin E synthase
NMDA = N-Methyl-D-aspartate
nmol = nano moles
NSAID = Non steroidal anti-inflammatory drug
PGE-2 = Prostaglandin E-2
ROS = Reactive oxygen species
SNRI = Serotonin-nor epinephrine reuptake inhibitor
SOD = Superoxide dismutase
TBA = Thiobarbituric Acid
TBARS = Thiobarbituric acid reactive substances
TCA = Trichloroacetic acid
TNF-γ = Tumor necrosis factor-alpha

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CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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