Antidepressant Effect of Diclofenac against Experimental Parkinson’s Rat Model

Sadaf Naeem1*, Rahila Najam1, Nousheen Alam2

1Department of pharmacology, University of Karachi, Pakistan
2Department of pharmacology, Federal Urdu University of Karachi, Pakistan
*Corresponding author: ssadafnaeem@gmail.com

Received May 31, 2015; Revised June 17, 2015; Accepted July 05, 2015

Abstract Previous clinical studies confirm the presence of inflammatory mediators like tissue necrotic factor alpha (TNF-α) and interleukins-1β in depressive patients are the main cause of depression and the use of non-steroidal anti-inflammatory drugs (NSAIDs) especially cyclo-oxygenase-2 (COX-2) inhibitors are associated with a marked reduction in the symptoms of depression. In our study we investigated the role of diclofenac in ameliorating depression in chlorpromazine (CPZ) induced PD model. Forty Wistar albino rats were divided equally into 4 groups, Rats of group I (control) received only vehicles and had free access to food and water. Rats of groups II, III and IV were treated with chlorpromazine (3mg/kg/day) via the intraperitoneal route once a day for 21 days. Diclofenac (20mg/kg/day) was given orally to group III and standard drug L-dopa/carbidopa (30mg/kg/day) orally was given to group IV for 21 days. CPZ was administered 30 min before the administration of test drugs, antidepressant effects of diclofenac were examined by using open field activity, cage crossing and forced swimming test. In the open field, diclofenac and standard drug (L-dopa/carbidopa) animals showed significant (P<0.001) improved number of square crossed in 10 minutes, increased grooming period and relatively more time spent in center of open field arena after 10 and 21 days as compared to CPZ group. In cage crossing diclofenac showed highly significant (P<0.001) improved activity of cage crossing after 21 days just similar to standard group (L-dopa/carbidopa), while CPZ significantly showed immobility and depressive behavior. In FST diclofenac was able to restore significantly the immobility time and decrease swimming effort as well as climbing duration induced by CPZ. Taken together, the present work evidenced antidepressant effects of diclofenac. This may be through inhibiting depressive markers like TNF-α and IL-1β in brain compartments, and possibly nor-adrenergic mechanism could also play a role.

Keywords: non steroidal anti-inflammatory drugs, diclofenac, depression, TNF-α, IL-1β, chlorpromazine, FST


1. Introduction

Mood variations for example, dementia and depression are cardinal non motor symptoms of Parkinson's disease, just as tremors and rigidity, these non motor symptoms affects patients quality of life and burden to care giver. Depression and cognitive impairment is correlated and often present at the point when confronting a diagnosis of Parkinson's disease. Chronic neuroinflammation have been associated with the etiopathogenesis of different neurological problems especially parkinsons disease and may affects the complex brain functions like sleep, memory and depression [19]. In inflammatory condition brain resident cells like microglia releases number of inflammatory mediators including prostaglandins (PGs), interleukins (IL), TNF-alpha and other cytokines. In recent studies TNFα, IL-1β, IL-6 and C-reactive proteins were found elevated in parkinson disease associated depressive patients which indicates that inflammation is the major cause of depression [7]. These proinflammatory cytokines hypothalalus-pituitary-adrenal axis and sympathetic nervous system become activated and upregulate the levels of glucocorticoid and catecholamines [9]. Neurotransmitters such as serotonin and dopamine modify vital behaviors, especially learning and memory, eating patterns, mood variations, sleep and locomotion [10,11]. Depression is related to the levels of monoamines in the brain. Proinflammatory cytokines can affect monoamines regulation and uptake; thereby activate adrenal corticotrophin hormone and cortisol. The over expression of cortisol is the key link between the chronic stress and depression [3]. Regardless of advances in the treatment of parkinson's disease, there is a need for neuroprotection, treatment of motor and non motor symptoms, and treatment of Inflammation.

NSAIDs readily cross blood brain barriers and inhibits microgial released cyclo-oxygenases and other cytokines. Recently Al-Amin et al [1] investigated that diclofenac along with sertraline significantly inhibit inflammatory
Physiological saline (0.9% NaCl) and given orally. Both were freshly prepared daily by dissolving in and Diclofenac sodium (Novartis Pharma (Pak) LTD) and Dopa/carbidopa 30mg/kg/day oral for 21 days. (4) standard group (n=10), received L-dopa/carbidopa 30mg/kg/day oral for 21 days, (4) standard group (n=10), received 3mg/kg/day i.p chlorpromazine only for 21 days, (3) diclofenac treated group (n=10), received 20mg/kg/day i.p chlorpromazine only for 21 days, (3) diclofenac treated group (n=10), received 20mg/kg/day i.p chlorpromazine only for 21 days, (2) negative control group (n=10), received saline water only (2) negative control group (n=10), received saline water only for 21 days, (1) control group (n=10), received saline water only for 30 minutes of drug administration. 

Therefore, the objective of the present work was to investigate the possible antidepressant effect of diclofenac in chlorpromazine induced rat model using the cage crossing, open field and forced swimming induced depression test after 10 and 21 days of diclofenac dosing.

2. Material and Method

2.1. Experimental Animals

Locally bred wistar rats (weighing 200–230 gram) of both sexes were obtained from Karachi university HEJ research institute of Karachi, Pakistan. Rats were housed in standard plastic cages (n=6 rats per cage) and maintained in a constant environmental temperature and light-controlled room (25±1°C, humidity 62%, a 12:12 hr light/dark cycle). All rats were kept in laboratory room conditions for 10 days before the experiment started to aclimatize the lab environment. Free excess of tap water and cubes of standard rat’s diet were given during whole experimental period. All rats were handled as per specification provided in Helsinki Resolution 1964 and experimental procedures were approved by university local ethics committee. The research plan followed for animal experiments were approved by BASR University of Karachi under resolution No .02(50).

2.2. Chemicals

Chlorpromazine (Sigma-Aldrich, MO, USA), (3 mg/kg/day, i.p.), L-dopa/carbidopa (OBS-Pharma Company, Pakistan) and Diclofenac sodium (Novartis Pharma (Pak) LTD) both were freshly prepared daily by dissolving in physiological saline (0.9% NaCl) and given orally.

2.3. Experimental protocol

Forty Wister rats (weighing 200-230g) were randomly assigned to four groups, each group contain 10 animals. Four groups were, (1) control group (n=10), received saline water only (2) negative control group (n=10), received 3mg/kg/day i.p chlorpromazine only for 21 days, (3) diclofenac treated group (n=10), received 20mg/kg/day oral for 21 days, (4) standard group (n=10), received L-dopa/carbidopa 30mg/kg/day oral for 21 days. Chlorpromazine was administered to all treated groups 30 min before the administration of test drugs [20]. Behavioral Activities of rats like cage crossing open field and forced swimming test in familiar environment was monitored on day 10 and 21 after 30 minutes of drug treatment.

3. Behavioral Assessment

3.1. Cage Crossing Behavior

Cage Crossing Activity was used to evaluate locomotor activity that consisted of a transparent cage (26cm x26cm x 26cm) and escaping was prevented by the surrounded walls. Wistar rats control and treated groups were placed in the transparent cage separately for 10 minutes and count the number of crossing after the administration of diclofenac, CPZ and standard drug after 10 and 21 day of dosing [17].

3.2. Open Field Activity

Open field test was used to assess behavioral and spontaneous locomotor activity in rats. The apparatus was a square open field arena consisted of wooden Board (76cm length x 76cm width x 40cm height) with 25 squares evenly in size and escaping was prevented by the surrounded walls which were painted in black. Albino Wistar rats control and treated groups were placed in the central square of the Open Field separately for 10 minutes for free exploration and count the number of squares crossed, time spent at center square and grooming time after the administration of diclofenac, CPZ and standard drug after 10 and 21 days of dosing [4].

3.3. Forced Swimming Induced Depression

Behavioureal activity for the assessment of depression in rodents, Swimming – induced Depression Test was used. It consisted of a acrylic glass cylinder (20cm in height, 6cm in diameters) filled with water (25±2°C) at specific level (12cm high) and escaping was prevented by the surrounded walls. Albino Wistar rats control and diclofenac and L-dopa/carbidopa treated groups were introduced in the cylinder separately and note the struggling time after 30 minutes of drug administration after 10 and 21 days of dosing [6].

3.4. Statistical Analysis

Data were expressed as mean ± SD values. One-way ANOVA with Bonferroni’s post hoc test was performed using SPSS version 21 for Windows. A probability level of 0.05 or less than 0.05 was considered as significant.

4. Results

4.1. Cage Crossing Activity

In Figure 1, analysis by One way Anova shows that animals after 10 and 21 days of dosing of chlorpromazine showed highly significant decrease (P<0.001) in no. of crossing the Cage in comparison to control and diclofenac treated animals group . Significant (P<0.01) increase in cage crossing activity on day 10 by diclofenac (20 mg/kg/day), whereas highly significant (P<0.001) improved activity of cage crossing was observed after 21 days just similar to standard group (L-dopa/carbidopa).

4.2. Open Field Activity

Table 1 shows open field activity performed after 30 min of the administration of chlorpromazine, diclofenac and standard drug. The chlorpromazine treated rats significantly (P<0.05) reduced the number of square crossed in open field, decreased grooming time and increased time spent in the center of open field after 10 days. There was highly significant (P<0.001) decreased...
number of squares crossed, grooming time and increased time spent in center after 21 days as compared to control and diclofenac treated groups. In contrast, diclofenac and standard group animals showed significant (P<0.001) improved number of squares crossed in 10 minutes, increased grooming period and relatively more time spent in center of open field arena after 10 and 21 days as compared to chlorpromazine group.

**Figure 1.** shows the effect of NSAIDs and chlorpromazine on Open field activity as compared to control group after 10 and 21 days. Values are mean ± S.D. (n=10). Significant differences by Bonferroni test *p<0.05, **p<0.01, as compared to control group, # p<0.05, ##p<0.01, as compared to Chlorpromazine group, following data analyzed by One Way Anova, df (1,54)

**Figure 2.** shows the effect of NSAIDs and chlorpromazine on Open field activity as compared to control group after 10 and 21 days. Values are mean ± S.D. (n=10). Significant differences by Bonferroni test *p<0.05, **p<0.01, as compared to control group, # p<0.05, ##p<0.01, as compared to Chlorpromazine group, following data analyzed by One Way Anova, df (1,54)
Several studies explain neuroleptic drugs that cause increase degradation of dopamine which could cerebral cortex and mesolimbic area [21]. The decreased concentration of serotonin, dopamine and noradrenaline in central nervous system depression by decreasing activity of adrenergic system in the centre of the field. Decreased grooming behavior could be due to decreased activity of adrenergic system in the amygdaloidal cortex and shows that animals are depressed. Previous studies also prove that CPZ showed immobile and depressive behavior in rats [15]. Diclofenac and standard group treated rats reversed CPZ induced depressive behavior towards normal exploring activity after 10 days and showed immobility and anxious behavior after 21 days of treatment. It has been suggested that diclofenac inhibit PGE2, IL-1, TNF-α and other inflammatory cytokines. These cytokines are involved in the degradation and can cause decrease in the availability of dopamine and serotonin neurotransmitters in hypothalamus and mesolimbic areas of the brain [22]. Diclofenac produce increased levels of anti inflammatory cytokines by activating PPAR Ɣ which induces the release of chatochoamines and dopamine [2]. Johansson et al [13] indicated in a clinical study that celecoxib use can improve depression in depressed patients by restoring serotonin levels in the brain. These findings suggest that diclofenac, by reducing oxidative stress and retaining dopamine and serotonin levels shows antidepressant effect. Present study also suggests the effects of diclofenac on rat’s performance in forced swimming induced depression test following 7 and 21 days of treatment. In forced swimming test passivity of animals was present when focus to inescapable stress such as forced was explored. In preliminary studies suggested that cytokines especially IL-1 alter the metabolic process of neurotransmitters such as serotonin and dopamine and induces behavioral changes [7,13,23].

Further in the present study CPZ treated rats showed passive behavior and depression after 21 days of treatment in cage crossing test as compare to control and diclofenac group. The depressive behavior is due to decreased level of serotonin and dopamine. These neurotransmitters regulate animal’s mobility and their innate instinct to explore new environment. Previous studies also prove that CPZ showed immobile and depressive behavior in rats [15]. Diclofenac and standard group treated rats reversed CPZ induced depressive behavior towards normal exploring activity after 10 days and showed immobility and anxious behavior after 21 days of treatment. It has been suggested that diclofenac inhibit PGE2, IL-1, TNF-α and other inflammatory cytokines. These cytokines are involved in the degradation and can cause decrease in the availability of dopamine and serotonin neurotransmitters in hypothalamus and mesolimbic areas of the brain [22]. Diclofenac produce increased levels of anti inflammatory cytokines by activating PPAR Ɣ which induces the release of chatochoamines and dopamine [2]. Johansson et al [13] indicated in a clinical study that celecoxib use can improve depression in depressed patients by restoring serotonin levels in the brain. These findings suggest that diclofenac, by reducing oxidative stress and retaining dopamine and serotonin levels shows antidepressant effect. Present study also suggests the effects of diclofenac on rat’s performance in forced swimming induced depression test following 7 and 21 days of treatment. In forced swimming test passivity of animals was present when focus to inescapable stress such as forced was explored. In our study, swimming time of animals was markedly reduced in CPZ treated rats. It could be due to decrease muscle strength and it might be related to general excitement level of the brain which is due to decreased serotonin level. CPZ developed immobility and depression like symptoms by altering brain amines especially dopamine and 5HT concentrations [12]. After 21 day treatment animal was not anxious and did not show

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>Total number of square crossed in 10 minutes</th>
<th>Time spent in center area (seconds)</th>
<th>Grooming time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Day 10</td>
<td>81.60±2.47</td>
<td>120±1</td>
<td>180±3</td>
</tr>
<tr>
<td></td>
<td>Day 21</td>
<td>80.50±2.47</td>
<td>90±2</td>
<td>182±3</td>
</tr>
<tr>
<td>CPZ</td>
<td>Day 10</td>
<td>29.10±6.11</td>
<td>300±1</td>
<td>120±3</td>
</tr>
<tr>
<td></td>
<td>Day 21</td>
<td>6.60±3.77</td>
<td>492±4</td>
<td>20±2</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Day 10</td>
<td>24.50±5.66</td>
<td>238±4</td>
<td>63±3</td>
</tr>
<tr>
<td></td>
<td>Day 21</td>
<td>38.40±6.43</td>
<td>131±10</td>
<td>120±10</td>
</tr>
<tr>
<td>L-dopa/carbidopa</td>
<td>Day 10</td>
<td>23.30±6.51</td>
<td>240±4</td>
<td>72±4</td>
</tr>
<tr>
<td></td>
<td>Day 21</td>
<td>42.7±13.1</td>
<td>122±10</td>
<td>131±10</td>
</tr>
</tbody>
</table>

Table 1. Effect of diclofenac in the open field test in rats

4.3. Forced Swimming Test

In Figure 2, analysis by One Way Anova shows that animals after 10 days of dosing of chlorpromazine showed moderately significant decrease (P<0.01) in swimming and climbing time after 21 days of treatment in comparison to control animals group and diclofenac group. Diclofenac showed significant increase and improve (P<0.05) in swimming and climbing time after 10 days of dosing in comparison to chlorpromazone group. On day 21 highly significant increase (P<0.001) in struggling time in comparison to chlorpromazone group was observed, This effect is similar to standard group (L-dopa/carbidopa).

5. Discussion

In the present study, antidepressant effects of diclofenac were studied in chlorpromazine induced Parkinson model. CPZ belongs to the class of neuroleptics, and blocks postsynaptic dopamine receptor in the mesolimibic system. It also increases dopamine turnover by blocking dopamine autoreceptors. This dopamine receptor blockade and increase degradation of dopamine is related to the development of pseudo-parkinsonism and extrapyrimidal effects [21]. Depressive behavior and mobility was first studied using the open field activity which gives a better clue of the rodent’s emotional state. The administration of 3mg/kg/day i.p CPZ produced a significant reduction in the number of square crossed in 10 minutes, decreased grooming time and an increase in the time spent in the centre of the field. Decreased grooming behavior could be due to decreased activity of adrenergic system in the amygdaloidal cortex and shows that animals are depressed. Several studies explain neuroleptic drugs that cause central nervous system depression by decreasing concentration of serotonin, dopamine and noradrenaline in cerebral cortex and mesolimibic area [21]. The decreased number of squares crossed by CPZ treated rats explains nigral dopaminergic neuronal degradation which could result in hypolocomotion and sedative effect [16]. Increase time spent in the center area of open arena clearly indicates that the CPZ has anxiolytic effect while diclofenac and standard drug (L-dopa/carbidopa) showed increased number of square crossed in open field and less time spent in central area, which is an indication of the central nervous system stimulant (antidepressant) effect. This finding is in accordance with previous research which shows that NSAIDs increases dopamine concentration by preventing dopaminergic neuronal loss through inhibition of inflammatory cytokines [5]. Recent studies suggested that cytokines especially IL-1 alter the metabolic process of neurotransmitters such as serotonin and dopamine and induces behavioral changes [7,13,23].

Further in the present study CPZ treated rats showed passive behavior and depression after 21 days of treatment in cage crossing test as compare to control and diclofenac group. The depressive behavior is due to decreased level of serotonin and dopamine. These neurotransmitters regulate animal’s mobility and their innate instinct to explore new environment. Previous studies also prove that CPZ showed immobile and depressive behavior in rats [15]. Diclofenac and standard group treated rats reversed CPZ induced depressive behavior towards normal exploring activity after 10 days and showed immobility and anxious behavior after 21 days of treatment. It has been suggested that diclofenac inhibit PGE2, IL-1, TNF-α and other inflammatory cytokines. These cytokines are involved in the degradation and can cause decrease in the availability of dopamine and serotonin neurotransmitters in hypothalamus and mesolimibic areas of the brain [22]. Diclofenac produce increased levels of anti inflammatory cytokines by activating PPAR Ɣ which induces the release of chatochoamines and dopamine [2]. Johansson et al [13] indicated in a clinical study that celecoxib use can improve depression in depressed patients by restoring serotonin levels in the brain. These findings suggest that diclofenac, by reducing oxidative stress and retaining dopamine and serotonin levels shows antidepressant effect. Present study also suggests the effects of diclofenac on rat’s performance in forced swimming induced depression test following 7 and 21 days of treatment. In forced swimming test passivity of animals was present when focus to inescapable stress such as forced was explored. In our study, swimming time of animals was markedly reduced in CPZ treated rats. It could be due to decrease muscle strength and it might be related to general excitement level of the brain which is due to decreased serotonin level. CPZ developed immobility and depression like symptoms by altering brain amines especially dopamine and 5HT concentrations [12]. After 21 day treatment animal was not anxious and did not show
despair to struggle out of the water. While diclofenac and L-dopa/carbidopa treated groups showed increased struggling time in forced swimming test after 21 day treatment due to increased muscle strength and improved depression, just like control rats. This result is consistent with previous studies. Episcopo et al, [8] reported that treatment due to increased muscle strength and improved depression, just like control rats. This result is consistent with previous studies. Episcopo et al, [8] reported that depression, just like control rats. This result is consistent with previous studies. Episcopo et al, [8] reported that NSAIDs act as antioxidant and scavenges nitrous oxide (NO) and reactive oxygen species (ROS) which decreases oxidative stress and lead to reduction in neuroinflammation and associated depression is relieved. Taken this into account, the results of our study explained that diclofenac, by inhibiting dopaminergic neuronal loss through its anti-inflammatory effect, regulate sympathetic neurotransmitters especially serotonin pathway which is linked to its antidepressant effect.

6. Conclusion

Our results suggest that prolong treatment with diclofenac shows antidepressant effect in chlorpromazine induced animal Parkinson’s model. Therefore there is a need that diclofenac should be further investigated in vivo and an in vitro model to explore its use in Parkinson’s associated depression.

Acknowledgement

The present research data was abstracted from a PhD thesis, submitted by Sadaf Naeem the Department of Pharmacology, faculty of pharmacy, university of Karachi Pakistan. The authors are thankful to University of Karachi, faculty of pharmacy for supporting the PhD project.

References