Evaluation of anxiolytic effect of zonisamide and its combination with bupropion in mice

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Anxiety disorders are common but serious illness that affects quality of life in humans. There is a need for safe and effective anxiolytic drugs. In the present study, we evaluated the anxiolytic effect of zonisamide and the combination of zonisamide with bupropion in male Swiss Albino mice. Animals were randomized to six different groups and received intraperitoneal treatment of normal saline: 1 DMSO (80:20), zonisamide (5 mg/kg), zonisamide (10 mg/kg), hydroxyzine (3 mg/kg), bupropion (10 mg/kg), zonisamide+bupropion (5 mg/kg), respectively. The animal models of anxiety were performed 30 min after treatment and relevant parameters were evaluated. Estimation of dopamine and serotonin levels in hippocampus, cerebral cortex, and whole brain were done using HPLC with FD method. The zonisamide treated group at 5 and 10 mg/kg was effective in in vivo models as compared to the control groups. It was more effective particularly at 5 mg/kg dose. Zonisamide showed significant increase in dopamine and serotonin levels in different brain regions than hydroxyzine, bupropion and combination treated groups. Overall, the anxiolytic effect was better with zonisamide monotherapy than other treated groups in the treatment of anxiety.

Keywords: Anxiety disorders, Biphasic effect, Brain monoamines, Elevated plus maze (EPM), Hydroxyzine

Anxiety is a common human emotional experience which is usually a response to fear and life-threatening situation. However, at excessive levels it causes distress and suffering, and thus it affects the quality of life of the person. According to Baxter et al., the prevalence rate of anxiety disorders in 2013 was between 0.9 and 28.3%. The current treatment options available for anxiety are limited. Antidepressants, particularly the selective serotonin reuptake inhibitors (SSRIs) and benzodiazepines, are the widely prescribed classes of drugs for the treatment of anxiety disorders. These agents are associated with side effects; such as benzodiazepines cause sedative effect, impaired cognition and coordination particularly on chronic use and SSRIs increase the risk of suicidality, whereas other antidepressants cause weight gain and sexual dysfunction, thus indicating an unmet need in the treatment of anxiety disorders.

Zonisamide is a synthetic 1, 2-benzisoxazole-3-methanesulfonamide with known anticonvulsant action. Its antiepileptic activity is produced by blocking voltage-dependent sodium channels and inhibition of T-type calcium channels in neurons. It also showed benefits in the treatment of Parkinsonism by enhancing the dopamine turnover and release. In addition, there was inhibition of dopaminergic oxidative stress and monoamine oxidase B (MAOB) activity. The dose reported to improve the motor symptoms in patients with Parkinson’s disease was 25-100 mg/day. Zonisamide therapy showed mood stabilizing properties and beneficial effects in treating bipolar disorders in patients with hypomanic or mixed symptoms. In another clinical assessment, adjunctive zonisamide therapy with naturalistic approach was effective in the treatment of refractory anxiety. Zonisamide produced centrally mediated antihyperalgesic and antiallodynic effects followed by partial nerve injury in mouse at a dose of (10 and 30 mg/kg; s.c.) . Zonisamide significantly reduced licking/biting behaviour at 3 and 10 mg/kg, (s.c.) doses during the second phase of the formalin test. It produced a centrally and peripherally mediated analgesic effect in the formalin test and suppressed the pain symptoms of formalin-induced inflammatory and streptozotocin-induced diabetic neuropathy.
was found to be effective in the treatment of neuropathic pain, including postherpetic neuralgia and painful diabetic neuropathy in mice. The related effect was independent of the descending monoaminergic pain inhibitory system and was due to the blockade of nitric oxide synthesis. Zonisamide showed a biphasic effect on the rat striatal dopaminergic and serotonergic system. It increased the dopamine and serotonin release at therapeutic doses of 20 and 50 mg/kg (i.p.). However, at supratherapeutic dose 100 mg/kg (i.p.) it decreased striatal dopamine and serotonin release.

Taking clues from the available literature, in the present study, we determined to assess the potential benefits of zonisamide in the treatment of anxiety due to its action on the dopaminergic and serotonergic release. In a short-term, open label study, the combination treatment of zonisamide and bupropion had an enhanced weight loss than zonisamide treatment alone. Bupropion, a dopamine and norepinephrine reuptake inhibitor and nicotinic receptor antagonist, is an atypical antidepressant, also used in the treatment of generalized anxiety disorder, smoking cessation and obesity. Bupropion at 10 mg/kg in mice showed decrease in immobility activity. In a case report, bupropion was effective in the treatment of panic disorder over a long follow-up period. As bupropion is known to enhance the activity of dopamine and norepinephrine, and zonisamide has enhanced the effect on dopamine and serotonin release, the present study hypothesized that the combination of zonisamide plus bupropion would have potential benefits in the treatment of anxiety disorders through the increase in major neurotransmitters.

Materials and Methods

Animals

Swiss Albino mice (male) weighing 25-30 g were procured from Bharat Serum Ltd., Thane. Perspex cages were used to house them while maintaining controlled conditions such as temperature (22-24°C), humidity (50-60 %) and 12 h light: dark illumination cycle in animal house. Standard food and water were easily accessible to them. The experimental work was executed between 10.00 h and 14.00 h. According to the experimental models, the animals were randomly distributed and separated into groups (n = 6/group). The present study related experimental protocols were approved by Institutional Animal Ethics Committee (Approval number: CPCSEA/IAEC/BNCP/P-24/2014).

Drug solutions and treatment

Animals received treatment 30 min before each test session through intra-peritoneal route. The drug solutions were prepared as follows: zonisamide (Watson Pharmaceuticals Ltd) was dissolved in saline/dimethylsulfoxide (DMSO) in the ratio 80:20 v/v, bupropion (AurobindoPharma Ltd.) was dissolved in saline. The treatment description for each group was as follows: Group I, control; Group II, hydroxyzine (3 mg/kg); Group III & IV, zonisamide @5 and 10 mg/kg; Group V, bupropion (10 mg/kg); and Group VI, zonisamide + bupropion @5 mg/kg each. The consideration of half-dose of each monotherapy (i.e. 5 + 5 mg/kg) instead of whole monotherapy dose (i.e. 10 + 10 mg/kg) in ‘combination treatment’ helps to avoid confusion between resultant additive effect and synergism. The outcome of combination approach consisting monotherapy doses (i.e. 10 + 10 mg/kg) may lead to additive effect, however consideration of half-dose of each monotherapy dose (i.e. 5 + 5 mg/kg) if given better outcome than respective monotherapy should indicate synergism.

Animal models for anxiety

Elevated plus maze

The elevated plus maze test is one of the most popular tests for anxiety. The apparatus comprised of two open arms (25×5×0.5 cm) across from each other and perpendicular to two closed arms and placed 50 cm above the floor. The walls of the closed arm were 16 cm in height. At the start of the activity, each animal was placed in the centre with its head directed towards the closed arm. The entire test session was video recorded for 5 min and the parameters, such as time spent in closed arm (CAT), time spent in open arm (OAT), frequency of entries in closed arm (CAE) and open arm (OAE) were recorded and analysed. The criteria for entry of mouse into an arm were considered when all its four paws were present inside an arm. In addition, the percentage of time spent in the open arms (%OAT= [(open arm time/300) ×100]) and the percentage of OAE (%OAE= [(open arm entries/open + closed arm entries) ×100]) were calculated. All the parameters were analyzed by a single trained observer using recorded video.

Light/Dark transition test

The apparatus used for the light/dark transition test consisted of a cage of dimensions 21×42×25 cm divided into two sections of equal size by a partition with door. The two sections were separated by
Estimation of brain monoamines (dopamine and serotonin) by HPLC with fluorescence detector (FD) method

HPLC (Shimadzu, LC-2010C HT, autosampler) with FD (RF-20A-prominence, Shimadzu) was used to estimate brain monoamine levels (dopamine and serotonin) in hippocampus, cerebral cortex and whole brain. The considered whole brain was the addition of cerebral cortex, hippocampus and remaining brain tissue. Animals were euthanized 1 h after treatment and brain was isolated and placed in ice cold 0.1 M perchloric acid. The isolated whole brain was weighed and further hippocampus, cerebral cortex and remaining brain parts were isolated, weighed separately and placed in 0.1 M Perchloric acid. Each sample was homogenized in 2 mL of ice cold 0.1 M Perchloric acid and centrifuged at 16356×g (Eppendorf 5810 R, Rotor F-45-30-11) for 20 min at 4°C. The supernatant fluid was filtered through 0.45 μm membrane and stored at −80°C until the time of analysis. Reverse-phase analytical column (INERTSIL100, C18, 5 μm, 25 cm × 0.46 μm) was used for the chromatographic separation and was carried out at room temperature. The data were processed using LC Solution® software. The mobile phase was prepared by dissolving 1.36 g of potassium dihydrogen phosphate in 1 litre of millipore water. The buffer consisted of Acetonitrile:water (10:90). Phosphoric acid (pH-3.92) was used to adjust pH of mobile phase with the flow rate of 1 mL/min. It was filtered through a 0.45 mm membrane. Monoamines were detected at an excitation wavelength of 280 nm and an emission wavelength of 315 nm. Retention time of standard and sample peaks were compared and used for identification. The concentrations of each monoamine in the samples were analyzed according to the area of the standard peak. The results were expressed as μg/g of wet weight of tissue

Statistical analysis

The statistical analysis was done by One-way ANOVA followed by Tukey’s honest post-hoc test using Graphpad InStat for 32 bit Windows version 3.06 (GraphPad Software, Inc) software. The data were represented as mean ± standard error (per group n = 6).

Results

Elevated plus maze

Table 1 depicts the outcome of elevated plus maze test. The zonisamide treated groups III & IV (5 and 10 mg/kg) showed a significant decrease in CAT parameter when compared against the control group. Groups treated with bupropion (Gr V) and hydroxyzine (Gr II) also showed a significant decrease in CAT and significant increase in OAT and %OAT than control group. Group III showed a significant increase in OAT, %OAT and OAE as compared against the control group and a significant increase in OAT and %OAT when compared against Gr. II. Group IV showed a significant increase in OAT and %OAT than control group, whereas the Group VI (zonisamide+bupropion at 5 mg/kg, each) showed a significant increase in CAT compared to groups II-IV, however a significant decrease in OAT, %OAT, and OAE than zonisamide treated group III. The combination treated group VI showed a significant decrease in %OAT compared to Gr II and Gr. IV and significant decrease in CAE compared to Gr. V.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CAT (±SEM)</th>
<th>OAT (±SEM)</th>
<th>%OAT (±SEM)</th>
<th>OAE (±SEM)</th>
<th>%OAE (±SEM)</th>
<th>CAE (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>221.4±9.19</td>
<td>9.2±0.55</td>
<td>3.9±0.23</td>
<td>3±0.33</td>
<td>29.2±2.94</td>
<td>7±0.44</td>
</tr>
<tr>
<td>Hydroxyzine (3 mg/kg)</td>
<td>124.2±6.21***</td>
<td>21.4±1.71***</td>
<td>13.21±1.58***</td>
<td>4.17±0.54</td>
<td>37.25±3.16</td>
<td>6.53±0.68</td>
</tr>
<tr>
<td>Zonisamide (5 mg/kg)</td>
<td>140.4±8.93***</td>
<td>35.6±2.49***</td>
<td>19.8±1.38***</td>
<td>5.4±0.43**</td>
<td>44.20±2.81</td>
<td>6.6±0.66</td>
</tr>
<tr>
<td>Zonisamide (10 mg/kg)</td>
<td>140.2±9.20***</td>
<td>21.6±1.08***</td>
<td>14.26±1.28***</td>
<td>4.4±0.61</td>
<td>43.19±6.04</td>
<td>4.8±0.72</td>
</tr>
<tr>
<td>Bupropion (10 mg/kg)</td>
<td>160.4±10.24***</td>
<td>16.4±1.96</td>
<td>9.64±0.59**</td>
<td>4.2±0.48</td>
<td>32.05±2.94</td>
<td>9.6±1.20</td>
</tr>
<tr>
<td>Zonisamide+Bupropion (5 mg/kg+5 mg/kg)</td>
<td>193.8±9.26****</td>
<td>14.8±1.77****</td>
<td>7.29±0.54****</td>
<td>3.2±0.22*</td>
<td>36.9±4.103</td>
<td>5.6±0.61**</td>
</tr>
</tbody>
</table>

[Significant difference is denoted by *P <0.05, **P <0.01, ***P <0.001 as compared against the vehicle treated group; ^P <0.05, ^aP <0.01, ^aaaP <0.001 as compared against zonisamide treated group at 5 mg/kg; !!!P <0.01, !!!!P <0.001 as compared against zonisamide treated group at 10 mg/kg; &P <0.05, &&P <0.01, &&&P <0.001 when compared against Hydroxyzine treated group; ^^P <0.01 as compared against bupropion treated group. CAT=Time spent in closed arm, OAT=Time spent in open arm, % OAT=Percentage time spent in open arm, OAE= Entries in open arm, % OAE=Percentage open arm entries, CAE=Entries in closed arm]
The results of Light-Dark transition test are shown in Table 2. Zonisamide treated group III (5 mg/kg) and hydroxyzine treated group II showed a significant increase in time spent in light box and % time spent in light box and significant decrease in time spent in dark box and % time spent in dark box compared to the control group. Zonisamide treated group III showed a significant decrease in time spent in light box and % time in light box as compared against hydroxyzine treated Gr. II. Zonisamide treated group IV (10 mg/kg) and bupropion treated group V showed a significant decrease in time spent in light box and % time in light box and significant increase in time spent in dark box and % time spent in dark box as compared to the group II. Zonisamide treated group IV showed a significant decrease in time spent in dark box and % time spent in dark box as compared to control group. The group VI treated with combination of zonisamide+bupropion however showed a significant decrease in time spent in light box, % time in light box and significant increase in time spent in dark box and % time spent in dark box as compared to group III and a significant decrease in time spent in light box and significant increase in time spent in dark box compared to Gr. IV. The combination treated group VI also showed a significant decrease in time spent in light box and % time spent in light box and a significant increase in time spent in dark box and % time spent in dark box as compared to group II.

Estimation of dopamine and serotonin in mice brain by HPLC-FD method

Zonisamide treated group III (5 mg/kg) showed a significant increase in dopamine (Fig. 1A) and serotonin (Fig. 1B) levels as compared to control group I and hydroxyzine treated group II in hippocampi, cerebral cortex and whole brain regions. Zonisamide treated group IV showed a significant increase in dopamine level in hippocampi and increase in serotonin level in hippocampi and whole brain regions when compared against control group. Also, when compared against hydroxyzine (Gr. II), zonisamide treated group IV showed a significant increased levels of dopamine in hippocampi, cerebral cortex and whole brain regions. Bupropion treated group V showed a significant increase in dopamine levels in whole brain and serotonin levels in cerebral
cortex and whole brain as compared to the control group. However, the combination treated group VI showed a significant decrease in dopamine and serotonin in cerebral cortices and whole brain as compared against zonisamide treated group III and significant decrease in dopamine levels in cerebral cortices and serotonin levels in cerebral cortex and whole brain regions as compared to Gr. IV. The combination showed significant increased anxiolytic effect of combination treated group as compared against hydroxyzine Gr. II. In addition, significant increase in dopamine and serotonin levels were observed in hippocampi and cerebral cortices, respectively when compared against hydroxyzine Gr. II.

Discussion

The present study outcome clearly indicates the benefit of zonisamide as an anxiolytic agent at both 5 mg/kg and 10 mg/kg. Interestingly, the lower dose of 5 mg/kg showed better anxiolytic effect. The brain monoamine profile of zonisamide treated group at 5 mg/kg and 10 mg/kg showed an increased dopamine and serotonin levels. It was more effective at 5 mg/kg dose than at 10 mg/kg dose. The increased levels, particularly at lower dose of 5 mg/kg of zonisamide are in line with the previously published reports. These studies showed an increased levels of dopamine and serotonin after receiving zonisamide treatment particularly at lower doses of 20 and 50 mg/kg (i.p.) and decreased levels of dopamine and serotonin levels at 100 mg/kg (i.p.) in rat striatum. Based on the potential of this drug to increase the dopamine and serotonin levels at lower doses, consideration of lower dose of zonisamide is justified in the present study. The anxiolytic effect of zonisamide was better than hydroxyzine, which is in line with the previously published reports. Bupropion treated Group V showed anxiolytic effect in elevated plus maze. Though not significant, the combination treated Group VI of zonisamide + bupropion at (5 mg/kg each) has shown benefit in time spent in light box and % time spent in light box in light and dark transition test and percentage entries in open arm (%OAE) in elevated plus maze as compared to the bupropion Group V. Otherwise, the combination was not beneficial as anxiolytic agent as compared to the monotherapies of zonisamide, hydroxyzine and bupropion. The results of brain monoamine estimation showed a significant decrease in dopamine levels and increase in serotonin levels with hydroxyzine treatment as compared to control group which are in conformity with the published reports. The lower dose of zonisamide was better in terms of in vitro outcomes among the drug treated groups.

The in vitro outcomes were better with the combination treated group as compared to hydroxyzine. The combination treatment showed an enhanced level of dopamine as compared to bupropion in hippocampus and an increased serotonin levels as compared to zonisamide and bupropion in hippocampus. Apart from these outcomes, there were no major anxiolytic benefits with combination treatment when compared against zonisamide and bupropion separately. These in vitro results may help in reasoning slight anxiolytic effect of combination treated group observed in the in vivo results.

The metabolism of zonisamide is primarily mediated by CYP3A4 and reduced to 2-sulfamoyl-acetyl-phenol (SMAP) and conjugated to SMAP-glucuronide that causes 50% of zonisamide elimination, whereas the metabolism of bupropion is mediated by CYP2B6. As the metabolism of zonisamide and bupropion is mediated by different enzyme systems, there may not be enzymatic interaction of the drugs combined. Still, a thorough study on the pharmacokinetic and pharmacodynamic drug-drug interaction of this combination is required.

Overall, zonisamide is effective anxiolytic agent particularly at lower dose. Its combination with bupropion is not better than the respective monotherapies. The present study outcome may help in selecting right combination therapy in clinics after further validation of present outcomes in different pre-clinical and clinical experimental settings.

References


