EFFECT OF AMITRIPTYLINE ON ANTICONVULSANT ACTIVITIES OF DIVALPROEX SODIUM ON MES AND PTZ INDUCED SEIZURES IN RATS

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ABSTRACT

The objective of this study was to investigate the type of interactions between Amitriptyline (AMI) and Divalproex (DVPX) against different animal model. Divalproex alone and in combination with AMI were examined in the rat model of maximal electroshock (MES test) and Pentylentetrazole (PTZ) induced convulsion. Combination of AMI and DVPX were significantly supraadditive (synergistic) in MES test and PTZ induced convulsion. AMI significantly decreased the duration of hind limb tonic extension (HLTE). The percentage prolongation of onset of clonic convulsion was enhanced to 86.76% in combination treatment. VPA pretreatment can block the N-methyl-D-aspartate (NMDA) receptor. The effect of VPA is synergistically potentiated by AMI, which acts as NMDA receptor antagonist. Increase in extracellular 5-HT level inhibits many types of seizure. AMI has significant role in increase in brain 5-HT concentration. GABA_B receptors are able to affect the development of the epileptic kindling state induced by pentylentetrazole. VPA and AMI both affect GABAergic neurotransmission by acting on GABA receptors, enhancing responses of GABA_B receptor. Synergistic interaction was found between the two interacting drugs. The combination of AMI and DVPX can be advantageous in epilepsy, and therefore should be recommended for further study in clinical conditions.

Keywords: Divalproex, Amitriptyline, Antiepileptic drugs, Electroshock seizure test, Pentylentetrazole induced convulsion.

INTRODUCTION

Depression is the most frequent co-morbid psychiatric disorder in epilepsy (Mazarati A, et al., 2008; Seethalakshmi R & Krishnamoorthy ES, 2005). Co-existing depression deteriorates
the quality of life of people with epilepsy. Antidepressants may display not only anticonvulsant, but also proconvulsant action. Therefore, the treatment of both disturbances requires the selection of appropriate drugs (Borowicz KK, B, Stepniak B, & Czuczwar SJ, 2012). Divalproex consist of sodium valproate and valproic acid in a 1:1 molar ratio ("Abbott Laboratories. Depakote ER® package insert," 2006) and used in treatment of epilepsy, maniac episodes associated with bipolar disorder and migraine (Calabrese JR, Woyshville MJ, Kimmel SE, & Rapport DJ, 1993; Davis R, Peters DH, & D, 1994; Pope HG, McElory SL, Keck PE, & Hudson JI, 1991; Silberstein & Wilmore, 1996). Valproic acid has shown to be an inhibitor of cytochrome P-450 (CYP) hepatic enzymes (Anderson G, 1998; Anderson G, Gidal BE, Kantor E, & Wilensky AJ, 1994; Levy R, Mattson H, & B, 2002) but VPA is primarily hepatically metabolized by UGT enzymes and β-oxidation and to a much lesser extent by CYP 2C9 and 2C19 (Luszczki, Krzyzanowski, & Swiader, 2009). Amitriptyline is a widely used tricyclic antidepressant (TCA) drug for the treatment of major depression (Barbui C, Guiaiana G, & Hotopf M, 2004) and migraine (Ziegler DK, et al., 1987). Numerous preclinical and clinical studies suggest that serotoninergic and noradrenergic neurotransmission systems play a crucial role in the pathogenesis of both epilepsy and depression (Hasler G, et al., 2007; Merrill MA, Clough RW, Dailey JW, Jobe PC, & Browning RA, 2007). The effect of divalproex on amitriptyline and nortriptyline has been studied pharmacokinetically (Wong, Cavanaugh, Shi, Awni, & Granneman, 1996), yet the pharmacodynamic effect of AMI to Divalproex had not been studied yet.

MATERIALS AND METHODS

Animals and experimental conditions All experiments were performed on adult male wistar albino rats weighing 180-220 g. The animals in a group of 6 were kept in cages with free access to food and ad libitum, and in standardized housing conditions (experimental temperature and natural light-dark cycle). After 7 days of adaptation to laboratory conditions, each animal was used only once. All experiments were conducted between 10:00 to 16:00 to minimize confounding effect of circadian rhythms. The experiment was approved by Office of Institutional Animal Ethical Committee (Reg. no. 1432/PO/a/11/CPCSEA dated 31.05.2012; Ref no. MCP/AIEC/Clear/003/2012-13)

Drugs The following drugs were used in this study: Amitriptyline (AMI; Times Pharmaceuticals, Nepal), Divalproex (DVPX; Yarrow Chemicals, Mumbai), Pentylentetrazole (PTZ; Yarrow Chemicals, Mumbai). AMI, DVPX and PTZ were
dissolved in sterile water. AMI (15 mg/kg) and DVPX (10 mg/kg) were administered orally where as PTZ was administered 80 mg/kg, i.p..

**MES test** Wistar albino rats were chosen by preliminary screening. Animals which showed extension of hind limb were included for the study. In first part of experiment, animals were divided into two groups, six animals in each. First group received sterile water orally serving as control while second group received Divalproex (10 mg/kg, p.o.). After an hour of treatment, seizures were induced by maximal electroshock in albino rats with the help of electroconvulsionmeter by passing current of 150 mA for 0.2 sec using ear clip electrodes. The animals were observed for the extensor phase as well as its duration. The abolition of extensor (tonic phase) in drug treated group was taken as criteria for anticonvulsant activity. In second part of the experiment, after a wash out period of 15 days the same groups of animals were administered with Amitriptyline (15 mg/kg, p.o.) once a day for one week. On the 8th day, Divalproex sodium (10 mg/kg, p.o.) and Amitriptyline (15 mg/kg, p.o.) was administered. The test was repeated and the duration of extensor phase was measured.

**Pentylenetetrazole induced convulsion** Wistar albino rats were selected prior to conducting the experiment. In first part of experiment, animals were divided into two groups, six animals in each, first group which received PTZ (80 mg/kg, i.p.), served as a control while second group received 10 mg/kg of Divalproex sodium orally. After one hour of drug treatment, PTZ (80 mg/kg, i.p.) animals were observed for clonic convulsions in 30 minutes duration, number of convulsions and 24 hour mortality. Absence of clonic convulsions in drug treated groups was taken as criteria for anticonvulsant activity. In second part of the experiment, after a wash out period of 15 days the same animals were administered with Amitriptyline (15mg/kg, p.o.) once daily for a week. On the 8th day, Divalproex sodium (10 mg/kg p.o.) and Amitriptyline (15 mg/kg, p.o.) was administered. The test was repeated and onset to forelimb clonic, as well as hind limb extension was recorded. The onset and number of deaths after showing tonic hind limb extension were also recorded. Animals were kept individually in different cages under observation for 1h (Yemitan OK & Adeyemi OO, 2005).

**Statistical analysis:** The data were expressed as mean ± S.E.M or percentage. One-way analysis of variance (ANOVA) followed by post hoc Dunnett’s multiple comparison test was used for data expressed in mean ± S.E.M, using GraphPad Prism Software (version 6.0). Differences between means were considered to be significant at P<0.05. The statistical evaluation of drug treated vs. control values were performed with Student’s t test.
RESULTS AND DISCUSSION

Effect of Amitriptyline treatment on anticonvulsant activity of Divalproex sodium on Maximal Electroshock (MES) induced convulsion in Rats

MES-induced seizures in rodents provide a reliable model of generalized tonic-clonic convulsions (Löscher W & Schmidt D, 1988). Moreover, this experimental model was proved to be suitable for the evaluation of anticonvulsant efficacy of drugs used in combination (Czuczwar SJ & Borowicz KB, 2002). The duration of hind limb tonic extensor (HLTE) in rats treated with Divalproex sodium alone was 18 sec which decreased to 14.3 sec significantly after combined treatment. These results indicate that AMI significantly enhance anticonvulsant activity of divalproex sodium. Amitriptyline exhibit high affinity binding to N-methyl-D-aspartate (NMDA) receptors in vitro and inhibit NMDA activation which is synergistically potentiated by VPA, which blocks the NMDA induced increases in K+ (Basselin M, Chang L, Chen M, Bell JM, & Rapoport SI, 2008). In general, an increase in extracellular 5-HT level inhibits many types of seizure (Browning, Hoffmann, & Simonton, 1978; Buterbaugh, 1978; Kilian & Frey, 1973; Prendiville & Gale, 1993; Statnick, et al., 1996; Yan, Jobe, Cheong, Ko, & Dailey, 1994). AMI has significant role in increase in brain 5-HT concentration (Nakamura, Fukushima, & Kitagawa, 1976). When AMI is administered with DVPX, it synergistically avoids all types of seizures.

Effect of Amitriptyline treatment on anticonvulsant activity of Divalproex sodium by Pentylenetetrazole (PTZ) induced convulsion

Pentylenetetrazole is a GABA-antagonist (Swinyard EA, Woodhead JH, White HS, & Franklin MR, 1989). PTZ induces the bilaterally synchronous spike wave discharges that typify absence seizures in rodent. Antiepileptics drugs which increase the threshold for the onset of myoclonic jerks and tonic extensor are known to prevent seizure generation and propagation, respectively (Löscher W, Hönack D, Fassbender CP, & Nolting B, 1991). Rats administered with Divalproex sodium (10 mg/kg, p.o.) alone exhibits latency period of clonic convulsion of 650 ± 14.0 seconds. The latency period of Amitriptyline (15 mg/kg, p.o.) administered for a week followed Divalproex (10 mg/kg, p.o.) was 545 ± 20.8 seconds. The nature of clonic convulsion was less jerky as compared with control. The percentage prolongation of onset of clonic convulsion was enhanced to 86.76%. The number of convulsion in combination of DVPX and AMI had been significantly decreased. A role for GABA_B-related mechanisms is suggested for the pathogenesis of generalized absence seizures (Snead Iii, 1992). Pretreatment with GABA_B-receptor agonists resulted in...
generalized absence status epilepticus lasting for hours. These data confirm the concept that specific GABA_B-receptor antagonist activity confers antiabsence seizure activity (Snead, 1996). A study by De Sarro, revealed that GABA_B receptor are able to affect the development of the epileptic kindling state induced by pentylenetetrazole (De Sarro, et al., 2000). In mice, chronic (but not acute) amitriptyline and electroconvulsive therapy increases the function of the gamma-aminobutyric acid (GABA_B) receptor in the frontal cortex modulating 5-HT release (Gray & Green, 1987). VPA affects GABAergic neurotransmission by acting on GABA receptors too, enhancing responses of both GABA-A and GABA_B receptors (Cunningham MO, Woodhall GL, & Jones RS, 2003; Harrison NL & Simmonds MA, 1982; Motohashi N, 1992). Moreover, NMDA receptor appear to be involved in the initiation and generalization of the PTZ-induced seizures (Nevins ME & Arnold SM, 1989; Velisek L, Kusa R, Kulovana M, & Mares P, 1990) where valproic acid and amitriptyline has its effect in NMDA receptors (Deckers CLP, et al., 2000; Eisenach & Gebhart, 1995).

Table 1 Effect of Amitriptyline treatment on anticonvulsant activity of Divalproex sodium on Maximal Electroshock (MES) induced convulsion in Rats

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Duration of Extension Tonus (sec)</th>
<th>Duration of Clonus (sec)</th>
<th>Duration of Stupor (sec)</th>
<th>Death/Recovery</th>
<th>% Incidence of convulsions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.5 ± 1.23</td>
<td>32.5 ± 5.09</td>
<td>71.2 ± 13.2</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Divalproex sodium (10 mg/kg, p.o.)</td>
<td>18.0 ± 0.816**</td>
<td>30.8 ± 1.26</td>
<td>50.5 ± 2.38</td>
<td>100%</td>
<td>65.45%</td>
</tr>
<tr>
<td>Amitriptyline (15 mg/kg, p.o.) + Divalproex sodium (10 mg/kg, p.o.)</td>
<td>14.3 ± 0.479**</td>
<td>25.0 ± 2.83</td>
<td>53.3 ± 2.06</td>
<td>100%</td>
<td>52%</td>
</tr>
</tbody>
</table>

All data are expressed in mean ± SEM, n=6. One-way ANOVA followed by Dunnett’s test was used for data expressed in mean ± S.E.M and mean difference was considered significant at the 0.01 level. *P<0.01, **P<0.001 compared to control.
Table 2 Effect of Amitriptyline treatment on anticonvulsant activity of Divalproex sodium by Pentylentetrazole (PTZ) induced convulsion

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Onset of clonic convulsion (sec)</th>
<th>% prolongation of onset of clonic convulsion (sec)</th>
<th>Duration of convulsion (sec)</th>
<th>No. of convulsion</th>
<th>Nature and severity</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTZ (80mg/kg, i.p.)</td>
<td>68 ± 10.5</td>
<td>-</td>
<td>1069 ± 24.0</td>
<td>38.7 ± 2.22</td>
<td>Straub’s tail, continuous jerky movements</td>
<td>0%</td>
</tr>
<tr>
<td>Divalproex sodium (10 mg/kg, p.o.) + PTZ (80 mg/kg, i.p.)</td>
<td>127 ± 9.98**</td>
<td>86.76%</td>
<td>650 ± 14.0**</td>
<td>17.3 ± 1.49**</td>
<td>Interrupted Jerky movements</td>
<td>84%</td>
</tr>
<tr>
<td>Amitriptyline (15 mg/kg, p.o.) + Divalproex sodium (10 mg/kg, p.o.) + PTZ (80 mg/kg, i.p.)</td>
<td>161 ± 14.9**</td>
<td>136.76%</td>
<td>545 ± 20.8**</td>
<td>13.5 ± 0.645**</td>
<td>Less Jerky movements</td>
<td>100%</td>
</tr>
</tbody>
</table>

All data are expressed in mean ± SEM, n=6. One-way ANOVA followed by Dunnett’s test was used for data expressed in mean ± S.E.M and mean difference was considered significant at the 0.01 level. *P<0.01, **P<0.001 compared to control.

REFERENCES


