Original article

Effect of Allopregnenalone (AP) and 4′-Chlorodiazepam (4′CD) on the Lindane-induced acute and chronic convulsive behavior in rats

Krishna Tanwar¹, Pramod K Mediratta², Krishna K Sharma²

¹Department of Pharmacology, Deccan College of Medical Sciences, Hyderabad-500058, Andhra Pradesh, India.
²Department of Pharmacology, University College of Medical Sciences, Dilshad Garden, Delhi-110095, India.

Abstract

Neurosteroids (NS) are considered important modulators of brain functions. Lindane a pesticide has been shown to affect the nervous system adversely. The present study was designed to explore the modulation of the effects of lindane on convulsions by Allopregnenalone (AP), and 4′-Chlorodiazepam (4′CD), in both acute and chronic seizure models using Pentylenetetrazole (PTZ). We used acute and chronic models. In the acute model, seizures were induced by PTZ 90mg/kg, intra-peritoneal (i.p) injection, while in the chronic model, kindling was induced by injecting PTZ 30 mg/kg sub-cutaneous(s.c) on alternate days three times in a week. Lindane produced augmented effect on convulsions by decreasing the onset of preclonic convulsions and increased duration of clonic convulsions. AP (2.5mg/kg, i.p) and 4′-CD (0.5mg/kg, i.p) were able to attenuate the effect of acute as well as chronic exposure of lindane. They significantly increased the onset and decreased the duration of convulsions in lindane-treated rats. These results conclusively demonstrate the efficacy of the neurosteroids in lindane-induced convulsions in both acute as well as chronic models. Thus NS have a potential role as anticonvulsant in treatment of convulsions produced by pesticides like lindane.

Key words: convulsions, neurosteroids, pesticides, PTZ

© 2014 Deccan College of Medical Sciences. All rights reserved.
In the past few decades NS have been implicated in many CNS functions like behavior, stress, depression, anxiety, memory, neurodegenerative disorders and especially convulsions.

PROG and AP have been found to be efficacious in convulsions and neuroprotective activity in animal seizure models. AP is a metabolite of PROG which is responsible for its acute anticonvulsant effects, has immediate and pronounced effect on GABA mediated current. The serum level of AP is increased after epileptic seizures indicating that NS might be a possible serum marker of an epileptic event. It has been shown that endogenous neurosteroids protect against seizures susceptibility in mouse model of kindling. PROG and AP reduce brain edema and reactive gliosis in traumatic brain injury, via their anti-inflammatory and antioxidant activity. It is now well recognized that NS mediate their effects through genomic mechanisms, through steroid receptors, and through non-genomic mechanisms via GABA-A, nicotinic, muscarinic acetylcholine, sigma, N-methyl D-aspartate, serotonergic, kainate, glycine and neuropeptide receptors, voltage gated Ca2+ channel, microtubule-associated protein-2. So there is sufficient background for their unique broad-spectrum anti-seizure and neuroprotective effect of NS acting via various mechanisms.

Lindane, an organochlorine pesticide has been used as a broad-spectrum insecticide for agricultural and nonagricultural purposes. Brain is an important target for its toxicity mainly due to two reasons. First, it stimulates CNS by increasing neurotransmitter release from neurons and also alters activity of enzymes AChE, Na+K+-ATPase and Mg2+-ATPase. Second, brain is rich in polyunsaturated fatty acids and lindane is highly lipophilic, which make brain prone to its toxicity. It is also known to inhibit the conversion of cholesterol to PREG by inhibiting the enzyme P450scc in mice ovaries. It has been further claimed that lindane inhibits the activity of steriodogenic acute regulatory (StAR) protein, which mediates an important step in steroid genesis—the intra mitochondrial transfer of cholesterol to the P450scc enzyme. Lindane poisoning results in sudden seizures of the mixed type i.e. grand mal, petit mal and myoclonus. Experimental exposure of lindane to rats also enhances the rate of acquisition of seizure in kindled rats.

Thus, NS have been vastly reported to be anticonvulsant and neuroprotective by various mechanisms in scientific literature. Further, lindane is proposed to be a neurotoxicant by altering certain neurotransmitter release and producing oxidative stress. With the possibility that NS may modulate effect of lindane, the present study was done to evaluate the effect of AP and 4’ CD on lindane-induced convulsive behavior in acute as well as chronic models of epilepsy in rats.

Material and methods

Healthy male wistar rats weighing 150 to 180g were used. The animals were housed in polypropylene cages under standard laboratory conditions on natural light-dark cycle; 23±1°C temp, 50±2% humidity. Standard pellitized feed and tap water were provided ad libitum. All the experiments were conducted as per instructions provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) after the approval by Institutional Animal Ethics Committee (IAEC). Reagents: Lindane, AP, and PTZ were procured from Sigma chemicals, USA. 4’ CD was procured from Fluka, USA. All chemicals were prepared according to methods previously reported.

Groups for acute study

Petit mal convulsions were induced by PTZ at dose of 90mg/kg (i.p.) dissolved in normal saline solution. It produced clonic convulsions by stimulating the cortex and is an accepted model for clinical petit mal epilepsy.

Rats were divided in to six groups (n=10) and assigned to treatment in each group as follow:

- **Group 1**: Vehicle for lindane (ground nut oil, p.o.) + solvent for neurosteroids (Tween 80 in dist. water, i.p.) + PTZ
- **Group 2**: Lindane; (30mg/kg, p.o.) + solvent for neurosteroid (i.p.) + PTZ
- **Group 3**: Lindane; (30mg/kg, p.o.) + AP (2.5mg/kg,i.p.) + PTZ
- **Group 4**: Lindane (p.o.) + 4’-CD; (0.5mg/kg i.p.) + PTZ
- **Group 5**: Lindane; 30mg/kg (p.o.) + AP (2.5 mg/kg,i.p.) + PTZ
- **Group 6**: Lindane(30mg/kg,p.o.) + 4’ CD; (0.5 mg/kg,i.p.) + PTZ

Rats were observed for their behavior for one hr and the latency to pre-clonic convulsions and duration of clonic convulsions were recorded using stop watch.

Groups for chronic study

Kindling is an accepted model for epileptogenesis.

Rats were divided in to six groups (n=10) and assigned to treatment in each group as follow:
Results for acute model-PTZ convulsions method

The results of acute study show that lindane (30 mg/kg) administration orally decreased latency to onset of pre-clonic convulsions significantly (p<0.001) from 598.0 ± 15.23 sec to 333.0 ± 18.4 sec as compared to control group. Lindane increased duration of clonic convulsions. AP and 4’CD per se significantly delay (p<0.001) in onset of pre-clonic convulsions from 598.0 ± 15.23 sec to 1209.7 ± 30.88 sec and 1222.0 ± 29.58 sec respectively. Administration of AP and 4’CD prior to lindane administration, significantly (p<0.001) attenuated the effect of lindane on latency to onset of pre-clonic convulsions as well as duration of convolution (Table 1, 2).

Table 1: Effect of lindane and neurosteroids on PTZ-induced latency to onset of pre-clonic convulsions

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg, route)</th>
<th>Latency to onset of pre-clonic convulsions (sec) (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>598.00 ± 15.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lindane</td>
<td>30, p.o.</td>
<td>333.00 ± 18.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2.5, i.p.</td>
<td>1209.70 ± 30.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.5, i.p.</td>
<td>1222.00 ± 29.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lindane</td>
<td>+ AP 30, p.o.+2.5, i.p.</td>
<td>1166.70 ± 37.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>+ Lindane 30, p.o.+0.5, i.p.</td>
<td>1151.81 ± 39.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>p<0.001 as compared to control group  
<sup>b</sup>p<0.001 as compared to lindane treated group

Table 2: Effect of lindane and neurosteroids on duration of PTZ-induced clonic convulsions

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg, route)</th>
<th>Duration of clonic convulsions (sec) (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>28.38±0.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lindane</td>
<td>30, p.o.</td>
<td>45.43±1.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2.5, i.p.</td>
<td>13.01±0.59&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.5, i.p.</td>
<td>15.70±0.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lindane</td>
<td>+ AP 30, p.o.+2.5, i.p.</td>
<td>14.78±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>+ Lindane 30, p.o.+0.5, i.p.</td>
<td>18.01±0.56&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>p<0.001 as compared to lindane treated group

Results for chronic model–Kindling method

In the chronic model, the important finding of the present study was that administration of lindane along with PTZ in kindling model did not produce any change in time taken for development of kindling as compared to PTZ alone group. Animals in all the groups were kindled by 6 weeks duration. Lindane at dose of 15 mg/kg orally for 6 weeks produced significant decrease in latency to onset of clonic convulsions and increased the duration of the convulsions. AP and 4’CD per se delayed onset of clonic convolution as well as reduced duration of convolution significantly (p<0.001) as com-
pared to PTZ kindled rats. AP and 4’ CD single dose administration at the end of 6 weeks of lindane administration significantly antagonized the effect of lindane in kindled rats. AP and 4’CD, both delayed the onset of convulsion from 446.07±15.22 sec to 1322.50 ±42.43 sec and 1189.08± 31.55sec respectively as compare to lindane(Table 3). They also significantly decreased the duration of action from 50.20±1.58 sec to 20.25±0.99 sec and 22.10±0.61 sec respectively as compare to lindane group (Table 4)

Table 3: Kindling method-Effect of lindane and neurosteroids on latency to onset of pre-clonic convulsions

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg, route)</th>
<th>Latency to onset of pre-clonic convulsions (sec)(Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>906.00 ± 28.74b</td>
</tr>
<tr>
<td>Lindane</td>
<td>15, p.o.</td>
<td>446.07±15.22a</td>
</tr>
<tr>
<td>AP</td>
<td>2.5, i.p.</td>
<td>1444.09 ± 36.87ab</td>
</tr>
<tr>
<td>4’CD</td>
<td>0.5, i.p.</td>
<td>1219.20 ± 36.71ab</td>
</tr>
<tr>
<td>Lindane</td>
<td>15, p.o. + AP</td>
<td>1322.50 ± 42.43ab</td>
</tr>
<tr>
<td>Lindane</td>
<td>15, p.o. + 4’CD</td>
<td>1189.08 ± 31.55ab</td>
</tr>
</tbody>
</table>

*a,b p<0.001 as compared to control group
b p<0.001 as compared to lindane treated group

Table 4: Kindling method-Effect of lindane and neurosteroids on duration of clonic convulsions in PTZ-kindled rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg, route)</th>
<th>Duration of convulsions (sec)(Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>26.58±0.98a</td>
</tr>
<tr>
<td>Lindane</td>
<td>15, p.o.</td>
<td>50.20±1.58</td>
</tr>
<tr>
<td>AP</td>
<td>2.5, i.p.</td>
<td>14.47±0.69a</td>
</tr>
<tr>
<td>4’CD</td>
<td>0.5, i.p.</td>
<td>20.15±0.78a</td>
</tr>
<tr>
<td>Lindane</td>
<td>15, p.o. + AP</td>
<td>20.25±0.99a</td>
</tr>
<tr>
<td>Lindane</td>
<td>15, p.o. + 4’CD</td>
<td>22.10±0.61a</td>
</tr>
</tbody>
</table>

*p<0.001 as compared to lindane treated group

Discussion

Our study was done to investigate modulatory effect of NS (AP, 4’CD) on lindane-induced convulsions using PTZ acute seizures model and chemical kindling model. In acute as well as in chronic model the significant delay in onset of convulsion and decrease in duration of seizures was observed with AP and 4’CD. The observed effect is in accordance with earlier reports. NS have been proposed to act through multiple modes of action. PROG and AP have been demonstrated to influence epileptiform activity and inhibition of kindling in models. However they also have been shown to be neuroprotective by antioxidant effect and to promote neurogenesis. Now it is well known that cyclic changes in the PROG and AP contribute to change in seizure susceptibility (e.g. catamenial epilepsy). It is surprising that among the various classes of compounds known to inhibit seizure activity steroids have been studied least, even though their anticonvulsant properties have been known to be distinct from their hormonal effects.

4’-Chlordiazepam (4’CD) is agonist of mitochondrial diazepam binding inhibitor receptor complex. It has been shown to increase the brain PREG synthesis without any effect on the blood PREG concentration. The ligands of this receptor facilitate the intramitochondrial flux of cholesterol thereby increasing the availability of cholesterol to cytochrome P450scc leading to an increased NS biosynthesis. 4’-CD has been implicated in various functions, including steroidogenesis, mitochondrial respiration, cell growth and differentiation. It indirectly modulates GABAergic transmission via increasing NS production. This has been reported, that 4’CD at high doses inhibits the GABA_A receptor channel and may induce seizures. However, the low dose of 4’CD (0.5mg/kg) was used to render such action unlikely. The anticonvulsant action of 4’CD on the proconvulsant effect of lindane in this study is supported by the previous reports of neuroprotective and anticonvulsant effect of 4’CD.

Lindane has been shown to influence brain functions adversely. This is because it rapidly crosses blood brain barrier (BBB) and alters activity of various neuronal enzymes. It has also been found to bind with picrotoxin/TBPS receptors of GABA_A activated Cl channel. This could explain convulsant activity of lindane. Lindane has been shown to influence steroidogenesis by inhibiting the enzyme P450scc leading to an increased NS biosynthesis. It indirectly modulates GABAergic transmission via increasing NS production. This has been reported, that 4’CD at high doses inhibits the GABA_A receptor channel and may induce seizures. However, the low dose of 4’CD (0.5mg/kg) was used to render such action unlikely. The anticonvulsant action of 4’CD on the proconvulsant effect of lindane in this study is supported by the previous reports of neuroprotective and anticonvulsant effect of 4’CD.

Discussion

Our study was done to investigate modulatory effect of NS (AP, 4’CD) on lindane-induced convulsions using PTZ acute seizures model and chemical kindling model. In acute as well as in chronic model the significant delay in onset of convulsion and decrease in duration of seizures was observed with AP and 4’CD. The observed effect is in accordance with earlier reports. NS have been proposed to act through multiple modes of action. PROG and AP have been demonstrated to influence epileptiform activity and inhibition of kindling in models. However they also have been shown to be neuroprotective by antioxidant effect and to promote neurogenesis. Now it is well known that cyclic changes in the PROG and AP contribute to change in seizure susceptibility (e.g. catamenial epilepsy). It is surprising that among the various classes of compounds known to inhibit seizure activity steroids have been studied least, even though their anticonvulsant properties have been known to be distinct from their hormonal effects.

4’-Chlordiazepam (4’CD) is agonist of mitochondrial diazepam binding inhibitor receptor complex. It has been shown to increase the brain PREG synthesis without any effect on the blood PREG concentration. The ligands of this receptor facilitate the intramitochondrial flux of cholesterol thereby increasing the availability of cholesterol to cytochrome P450scc leading to an increased NS biosynthesis. 4’-CD has been implicated in various functions, including steroidogenesis, mitochondrial respiration, cell growth and differentiation. It indirectly modulates GABAergic transmission via increasing NS production. This has been reported, that 4’CD at high doses inhibits the GABA_A receptor channel and may induce seizures. However, the low dose of 4’CD (0.5mg/kg) was used to render such action unlikely. The anticonvulsant action of 4’CD on the proconvulsant effect of lindane in this study is supported by the previous reports of neuroprotective and anticonvulsant effect of 4’CD.

Lindane has been shown to influence brain functions adversely. This is because it rapidly crosses blood brain barrier (BBB) and alters activity of various neuronal enzymes. It has also been found to bind with picrotoxin/TBPS receptors of GABA_A activated Cl channel. This could explain convulsant activity of lindane. Lindane has been shown to influence steroidogenesis by inhibiting the enzyme P450scc thus inhibit the conversion of cholesterol to PREG. Further, lindane has been demonstrated to inhibit intra-mitochondrial transfer of cholesterol to the P450scc enzyme which gives an important link for convulsant action of lindane by decreasing the NS biosynthesis. It has been found to produce sudden seizures of the mixed type. Other effects include intention tremors, memory impairment, irritability and aggression. We observed lindane (30mg/kg) along with PTZ did not show any change in the time taken for the kindling to develop as compared to only PTZ treated group. However lindane produced marked decrease in latency and increase in the duration of convulsions in kindled rats, confirming convulsant action of lindane. This can be explained by the fact
that lindane may induce seizure by interfering with GABAergic inhibition. The steroid hormones circulating in blood rapidly attain unrestricted access to all parts of nervous system where hormone may be metabolized and the metabolite interact with receptors to produce effect. Since NS have been reported to show a free radical scavenging action also, it is possible that these agents would reduce the damage due to lindane and PTZ administration, by its free radical extinguishing action. The anticonvulsant actions of neurosteroids and their ability to act via multiple mechanisms are also known. In this study, the rapid effect of AP on kindled rats coincides with the hypothesis that anticonvulsant activity of NS is being mediated through the membrane-bound GABA<sub>A</sub>/BZD receptor ionophore complex.

**Conclusion**

The present data provides compelling evidence in support of the anticonvulsant potential of the AP and 4′CD in lindane-induced seizures. It can be concluded that NS are effective in lindane-induced convulsions. So it is proposed that NS deserve further investigation for their exact mechanism of action for future use in antagonizing lindane neurotoxicity.

**Conflict of interest:** None

**References**

6. Frye CA. The neurosteroid 3α,5α-THP has anti-seizure and possible neuroprotective effects in an animal model of epilepsy. Brain Res. 1995; 696:113-120.