An Experimental Study on Pharmacodynamics Interaction of Doramectin with Anticonvulsants and Anaesthetics in Mice

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Abstract The present study was conducted to examine the pharmacodynamics interactions between doramectin and general anesthetics like diethyl ether and anticonvulsants like phenytoin and diazepam. The methods employed were supramaximal electroshock test (for phenytoin), chemical convulsion test and unconditioned response avoidance test (for diazepam) and different stages of anesthesia and duration of anesthesia (for diethyl ether). In supramaximal electroshock test phenytoin was equally effective in all the groups in controlling grand mal seizures. In chemical convulsion test no response to diazepam was observed in all the groups pretreated with doramectin. And in unconditioned response avoidance test only one mouse showed response to shock in doramectin 200µg/kg group similar to the control 2 group. However, no mouse showed response to shock in doramectin 600 µg/kg groups. No difference was observed in the time taken for exhibiting various stages of anesthesia in different groups in inhalant anesthesia. However the total duration of anesthesia was increased in doramectin (200 µg/kg) treated group (781.67±24.82 sec) and the value was significantly high in doramectin (600 µg/kg) treated group (861.67±55.69 sec) as compared to 673.33±30.94 sec in control 2. The result suggests that doramectin prolongs the recovery from anesthesia but does not support the anticonvulsant activity.

Keywords Doramectin, Pharmacodynamics, Diethyl Ether, Diazepam and Phenytoin

1. Introduction

Avermectins are a group of fermentation products from a strain of Streptomyces avermitilis possessing potent anthelmintic and insecticidal activities. Doramectin is one of the members of this group and is proposed to act on the GABA receptor/chloride ionophore complex. Studies show that doramectin interferes with GABAergic-related behaviours [1, 2]. It is a macrocyclic lactone
disaccharide and is used as a potent broad spectrum veterinary endectocide [3, 4, 5]. It was suggested that doramectin has the pharmacological profile of an anxiolytic/anti-convulsant drug with GABAergic properties [6, 2].

Gamma-aminobutyric acid (GABA) is the primary mediator of inhibitory transmission in the mammalian central nervous system [8]. GABA and its GABA-A receptors are involved in the regulation of a number of normal and pathological brain mechanisms, such as sleep, epilepsy, memory, emotions and various behaviours [7, 8, 9]. Benzodiazepines (diazepam) are Positive modulators of GABA-A receptors [10, 11, 12, 13].

Phenytoin is an anticonvulsant drug which can be useful in the treatment of epilepsy. The primary site of action appears to be the motor cortex [14] where spread of seizure activity is inhibited. Phenytoin was observed to act through sodium dependent high affinity synaptosomal transport of both glutamate (Glu) and γ-aminobutyric acid (GABA) [15]. Phenytoin tends to stabilize the threshold against hyperexcitability caused by excessive stimulation or environmental changes capable of reducing membrane sodium gradient.

There are reports of preliminary investigations on pharmacodynamics interactions of ivermectin with the CNS acting drugs. But there are very few studies on the pharmacodynamics interactions of doramectin with anticonvulsant drugs like diazepam, phenytoin and inhalant anaesthetic like diethyl ether.

The objective of the present study was to understand the possible pharmacodynamics interaction of doramectin with the anticonvulsant drugs; phenytoin, diazepam and inhalant anaesthetic like diethyl ether.

2. Materials and Methods

2.1. Animals

Male albino mice, weighing 20-25 grams were procured from Central Animal Facility of NIPER, Mohali. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2°C; relative humidity 60-70%) in a 12 h light-dark cycle in the departmental animal facility. Animals were given an acclimatization period of seven days before commencing first experiment. Food and water were provided ad libitum. Food was withdrawn 12 h before and during the experiment. Experiments were performed during 8 AM to 12 PM. All experimental protocols were approved by the institutional animal ethics committee (IAEC).

2.2. Drugs

The following drugs were used: doramectin (Dectomax®, Pfizer Inc., USA), diazepam (Calmpose®, Ranbaxy, India), Phenytoin (Phenytoin®, Sigma, USA), Solvent ether (Diethyl ether® S.D. fine chem. ltd). Doramectin was injected subcutaneously at two doses 200 μg/kg (normal dose) and 600 μg/kg (three times the normal dose). The observations were recorded on the 5th and 6th day since doramectin has a half-life of 1 week.

2.3. Methods

A. Test for Phenytoin In this study, the animals was divided into four groups with 6 animals in each group as follows:
Control 1- No Doramectin + no drug,
Control 2- No Doramectin + Phenytoin @ 25 mg / kg b.wt. i.p.
Test 1 -Doramectin 200 µg/kg + Phenytoin @ 25 mg / kg b.wt. i.p.
Test 2 -Doramectin 600 µg/kg + Phenytoin @ 25 mg / kg b.wt. i.p.

Supramaximal Electroshock Test

This test is employed to screen anticonvulsant drugs against grand mal epilepsy, using electroconvulsiometer. In this the mice were applied with pinna (ear) electrode after proper restraining. On the previous day of testing, animals were given sub maximal electrical shock of 6 mA for 0.2 sec duration and those mice, which exhibited extension of hind limbs, were chosen. Next day supramaximal electric shock of 27 mA for 0.2 seconds duration was applied using pinna electrode. The resultant seizures in normal mouse showed a tonic phase of limb flexion for roughly 1.5 seconds followed by full tonic extension for approximately 10 seconds and a few clonic jerks thereafter. This timing was noted in all the mice. The number of deaths due to asphyxia was also noted. The animal after recovery remained in opisthotonus condition and exhibited exaggerated response to stimuli such as clapping or noise. The animals were administered with standard phenytoin 20 min before the actual trials [16].

B. Test for Diazepam

In this study, twenty four animals were divided into four groups with 6 animals in each group as follows:

Control 1- No Doramectin + no drug,
Control 2- No Doramectin + Diazepam @ 4 mg/kg b.wt. i.p
Test 1 -Doramectin 200 µg/kg + Diazepam @ 4 mg/kg b.wt. i.p.
Test 2 -Doramectin 600 µg/kg + Diazepam @ 4 mg/kg b.wt. i.p.
Two tests were employed for testing the pharmacodynamics interaction of doramectin and diazepam.

Chemical Convulsion Test

In this test, the mice were injected Pentylenetetrazole (@ 70 mg/kg b.wt.) in the scruff of the neck and they were observed for convulsions for the next 15 minutes for clonic convulsions. Administration of Pentylenetetrazole in the scruff of the neck of mouse showed initial tonic phase of limb flexion followed by clonic phase. The clonic convulsions are characteristic of petit mal seizures. Convulsions in which the mouse showed repeated contractions of limbs and body followed by loss of equilibrium was considered as complete clonic convulsion. While short jerky movements were taken as incomplete clonic convulsion. The animals were administered standard Diazepam 20 min before the actual trials [17].

Unconditioned Response Avoidance Test

In this protocol, the mice were subjected directly to shock, without any pre-conditions. This was considered to be the instinctive response to the noxious stimuli, shock. Initial training for 3 days was given to the mice and only those mice that responded to the stimuli by climbing on the pole to avoid the shock were chosen. The standard diazepam was administered 20 minutes prior to trials and the mice were tested for response to shock. Failure of the mice to climb the pole after giving shock was considered as the end point.

C. Diethyl Ether

Diethyl ether was chosen for the present study since it exhibits all the stages of anesthesia.
In this study, twenty four animals were divided into four groups with 6 animals in each group as follows:

Control 1 - No Doramectin + no drug,
Control 2 - No Doramectin + Diethyl ether,
Test 1 - Doramectin 200 μg/kg + Diethyl ether,
Test 2 - Doramectin 600 μg/kg + Diethyl ether.

In this test the mice were induced general anesthesia by using diethyl ether in a closed glass observation chamber. The induction time and the recovery time were noted in both controls as well as in the test groups. The time required for transition to various stages of anesthesia was also observed by noting various behavioural signs viz., voluntary excitement (Stage I), involuntary excitement (Stage II) and anesthesia (Stage III) and reflexes such as corneal reflex, palpebral reflex, pedal reflex, etc. to evaluate the depth of anaesthesia. The animals were then placed in supine position and time taken to show righting reflex, three times, was taken as recovery time.

2.3. Statistics Analysis

Results are presented as mean ± standard error of mean (SEM). The level of significance was taken as: p<0.05 and p<0.01 as significant; p<0.001 highly significant.

3. Results

3.1. Supramaximal Electroshock Test (MES)

Supramaximal electroshock technique is used to screen anticonvulsant drugs against grand mal epilepsy, using electroconvulsiometer. The resultant seizures in normal mouse showed a tonic phase of limb flexion for roughly 1.3 seconds followed by full tonic extension for approximately 10 seconds and a few clonic jerks thereafter. This timing was noted in all the animals subjected to MES and presented in table 1. The number of deaths due to respiratory arrest (asphyxia) was also noted.

Table 1: Effect of phenytoin in mice pretreated with doramectin. (Time taken in seconds)

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Tonic limb flexion</th>
<th>Tonic limb extension</th>
<th>Clonic jerks</th>
<th>Respiration regain</th>
<th>Recovery time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1.33 ± 0.21</td>
<td>10.83 ± 0.31</td>
<td>5.67 ± 0.56</td>
<td>11.00 ± 0.52</td>
<td>135.83 ± 16.45</td>
</tr>
</tbody>
</table>

Phenytoin treated (25 mg / kg b.wt. ip)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tonic limb flexion</th>
<th>Tonic limb extension</th>
<th>Clonic jerks</th>
<th>Respiration regain</th>
<th>Recovery time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2</td>
<td>No response</td>
<td>No response</td>
<td>5.17 ± 0.70</td>
<td>5.33 ± 0.42</td>
<td>88.17 ± 7.04</td>
</tr>
<tr>
<td>Group 3</td>
<td>No response</td>
<td>No response</td>
<td>4.50 ± 0.43</td>
<td>7.80 ± 1.06</td>
<td>83.33 ± 6.91</td>
</tr>
<tr>
<td>Group 4</td>
<td>No response</td>
<td>No response</td>
<td>4.50 ± 0.43</td>
<td>6.50 ± 0.43</td>
<td>81.17 ± 3.52</td>
</tr>
</tbody>
</table>

On phenytoin treatment the control and doramectin (200 μg/kg) groups showed no response to MES, except for some clonic jerks which lasted for approximately 5 seconds in the mice of all the groups. The time taken to regain respiration in group 2, 3 and 4 were 5.33±0.42, 7.80±1.06 and 6.50±0.43 sec. The recovery time from convulsions was similar in all the groups.
3.2. Chemical Convulsion Test

Pentylenetetrazole induced seizures were employed to screen diazepam against petit mal kind of epilepsy. The observations made are presented in Table 2. There was no significant change in the control and doramectin treated mice. Diazepam was able to protect the mice in all the groups from petit mal seizures effectively.

**Table 2: Effect of diazepam against petit mal seizures in mice treated with doramectin**

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Tonic phase (min)</th>
<th>No. of convulsions</th>
<th>Recovery time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Full</td>
<td>Jerky</td>
</tr>
<tr>
<td>Group 1</td>
<td>4.50 ± 0.43</td>
<td>5.67 ± 0.42</td>
<td>6.83 ± 0.79</td>
</tr>
<tr>
<td><strong>Diazepam treated (4 mg/kg b.wt. ip)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>No response</td>
<td>No response</td>
<td>No response</td>
</tr>
<tr>
<td>Group 3</td>
<td>No response</td>
<td>No response</td>
<td>No response</td>
</tr>
<tr>
<td>Group 4</td>
<td>No response</td>
<td>No response</td>
<td>No response</td>
</tr>
</tbody>
</table>

3.3. Unconditioned Response Avoidance Test

In this protocol, the rats were subjected directly to shock, without any pre-conditions. The mice from all the groups responded to this shock instinctively by climbing on the pole. Diazepam at anxiolytic dose was able to block this unconditioned response. Failure of the mice to climb the pole after giving shock was considered as the end point. The result is presented in Table 3. Pole climbing was observed in only one mouse in control group 2 and group 3 (doramectin 200 µg/kg) and no mouse was found to climb the pole in group 4.

**Table 3: Effect of diazepam on unconditioned response test (pole climbing) in rats treated with doramectin.**

(Value in parenthesis show number of rats responding to electric shock and climbing the pole)

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Response to shock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Diazepam treated (4 mg/kg b.wt. ip)</strong></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>Absent (1)</td>
</tr>
<tr>
<td>Group 3</td>
<td>Absent (1)</td>
</tr>
<tr>
<td>Group 4</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Altogether, it was observed that the effect of diazepam on unconditioned response was unaltered in doramectin treated mice.
3.4. General Anesthesia

Doramectin treatment did not alter the time taken for exhibiting various stages of anesthesia. However, the total duration of anesthesia was increased in doramectin (200 µg/kg) treated group (781.67±24.82 sec) and the value was significantly high in doramectin (600 µg/kg) treated group (861.67±55.69 sec) as compared to 673.33±30.94 sec in control. The result is presented in the table 4.

Table 4: Effect of inhalant anesthetics in mice treated with doramectin. (Time in seconds)

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
<th>Total duration of Anesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>125.53± 08.78</td>
<td>291.23±16.19</td>
<td>352.35±12.13</td>
<td>668.26±27.45</td>
</tr>
<tr>
<td><strong>Diethyl ether inhalant anesthesia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 2</td>
<td>126.67±14.98</td>
<td>286.67±23.33</td>
<td>346.67±23.33</td>
<td>673.33±30.94</td>
</tr>
<tr>
<td>Dora 200</td>
<td>105.00±16.68</td>
<td>280.00±12.11</td>
<td>340.00±12.11</td>
<td>781.67±24.82</td>
</tr>
<tr>
<td>Dora 600</td>
<td>103.33±7.71</td>
<td>320.00±9.04</td>
<td>380.00±9.04</td>
<td>861.67±55.69**</td>
</tr>
</tbody>
</table>

** p<0.01, indicates significant difference when compared with the respective control values

4. Discussion

Concurrent administration of doramectin and CNS depressants may result in pharmacodynamics interactions. Perusal of the literature indicates lack of any such systematic studies. Therefore, the present study was envisaged to investigate the possible pharmacodynamics interaction of doramectin treatment with the CNS depressants.

In this study, supramaximal electroshock (MES) technique was used to screen the effect of doramectin pretreatment on phenytoin against grand mal epilepsy, using electroconvulsiometer. From the table 1 it can be said that there was no significant difference in the various observations in different groups. Phenytoin was equally effective in all the groups in controlling MES induced grand mal seizures indicating no pharmacodynamics interaction.

There are contrary views on the pharmacodynamics of phenytoin [18]. Several reports have described that phenytoin causes drug-induced elevations of threshold for activation of the sodium action potential or partial blockade of sodium influx into neurons [19, 20, 21, 22, 23, 24]. There are other reports that say that phenytoin can interfere with voltage-dependent calcium entry [25, 26, 21]. In another report [15] phenytoin was observed to inhibit competitively the sodium dependent high affinity synaptosomal transport of both glutamate (Glu) and γ-aminobutyric acid (GABA). This contrasted with previous reports; the uptakes of glutamate and GABA were enhanced by phenytoin. In another study, [27] Segal and Douglas showed that the anticonvulsant phenytoin diminished late sodium channel openings underlying epileptiform activity.

In our study the results show that there was no interaction between phenytoin and doramectin. This could be reasoned out to the contrary pharmacodynamics of the two drugs. Further study is required to assess the pharmacodynamic interaction of phenytoin with doramectin.
While evaluating the pharmacodynamic interaction between doramectin and diazepam, in both chemical convulsion test and Unconditioned response avoidance test, it was found that there was no significant difference in control and doramectin treated groups. It can be inferred here also that doramectin treatment did not affect the anticonvulsant action of diazepam. Again this was a contrasting result that we have got. Because, benzodiazepines (diazepam) are known to be positive modulators of GABA-A receptors [10, 11, 12, 13, 28]. This could be due to different site of action of doramectin from that of diazepam [6].

There is a report that states the biphasic mechanism of action of diazepam [29] which is not seen in doramectin. Again a contrasting finding in the mechanism of action of diazepam by Bratati and Arvid [30]. The results of the study carried by these two scientists do not support the hypothesis that benzodiazepines act by enhancing GABAergic transmission. It rather suggests that benzodiazepines exert an inhibitory action on transmitter synthesis and utilization at the synaptic level, which is not necessarily bearing any direct relationship to GABA.

Previous studies by Spinosa and coworkers reported that doramectin treatment protected animals from convulsant effect of picrotoxin. They further reported that doramectin has a pharmacological profile of an anxiolytic/anticonvulsant drug with GABAergic properties. In a similar study by the same workers (Spinosa et al) on ivermectin, a structural analog of doramectin, reported that ivermectin protected the animals from convulsant effects of pentylentetrazole but not from those of picrotoxin. They suggested that the lack of effects on seizures induced by picrotoxin could be due to different site of action of ivermectin from that of benzodiazepine drugs, such as diazepam.

There are contrasting reports on the mechanism of action of doramectin (avermectins in general) as well. There are reports which state that avermectins act by binding to γ-Aminobutyric acid (GABA) receptor [31, 1, 2, 32]. There are reports stating that avermectins act by binding to Glutamate-gated chloride channels [33, 34, 35]. There are reports which state the antagonistic activity of avermectins at GABA receptors [36]. There are reports stating the dual effects of avermectins on GABA gated chloride channels [37]. These reports could be reasoned out for the difference in the pharmacological action of doramectin and diazepam.

To further assess the putative CNS depressant activity of doramectin, experiments for general anaesthesia was done. Our study revealed that doramectin treatment did not alter the time taken for exhibiting various stages of anesthesia induced by diethyl ether. However the total duration of anesthesia was increased in doramectin treated groups and the value was significantly high in doramectin (600 µg/kg) treated group. This suggests a potentiation action of doramectin on diethyl ether induced anesthesia. This finding is of clinical significance in surgical cases where inhalant anesthetics are used.

Our findings do not support the anticonvulsant/anxiolytic activity of doramectin in mice. Further study is needed to assess the exact pharmacological action of doramectin and anticonvulsants and the pharmacological interaction between doramectin and anticonvulsants at different multiple doses.

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