Severe Toxicity of Citalopram Hydrobromide in Three Mouse Models of Seizures

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SUMMARY. Citalopram hydrobromide (CH) is known to have many side effects, but its risks in patients with epilepsy has not been evaluated. The aim of this study was to evaluate the risks of CH treatment in epilepsy using three different animal models of seizures. Seizures were induced in mice by maximal electroshock, pentylenetetrazol and isoniazid. The effect of a pretreatment with CH one hour before the seizure test was tested to investigate the toxicity and anticonvulsant activity of CH. We found that, although CH is an effective drug in the treatment of depression, it had harmful toxic effects in the tested seizure models. These findings suggest that CH should be used with care in epileptic patients with depression.

INTRODUCTION

The common antidepressant drug citalopram is a selective serotonin reuptake inhibitor (SSRI) with minimal effects on neuronal norepinephrine and dopamine reuptake. It is a racemic bicyclic phthalane derivative that is unrelated to the tricyclic and tetracyclic antidepressant drugs. Citalopram is administered orally as a salt, citalopram hydrobromide (CH), and its chemical name is (±)-1-(3-dimethyl-amino-propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrobromide.\(^1,2\) (Fig. 1).

The use of antidepressant medications—in particular SSRIs—is widespread, but there is little evidence that the increased use of antidepressants during the past decade has reduced the prevalence of suicidal ideation or suicide attempts.\(^3,4\) Common side effects of CH include drowsiness, insomnia, nausea, weight changes, frequent urination, decreased sex drive, anorgasmia, and dry mouth.\(^5-7\) While the risk of seizures during SSRI treatment has not been systematically evaluated, caution is currently recommended in patients with a history of seizures.

Initially, we were interested in the possible use of CH as a treatment of mice with seizures. To our surprise, we found that the maximal electroshock (MES) test killed many of the mice. We first thought that the mortality was caused by the electroshock itself, but after further observations we realized that CH may induce severe toxicity. In this study, we used three seizure models to characterize the toxicity of CH.

KEY WORDS: Citalopram, Isoniazid, Maximal electroshock test, Pentylenetetrazole, Toxicity.

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Li-Jun Yu and Rui Fan contribute equal to the work.
MATERIALS AND METHODS

Animals

The experiments were performed on adult (18-22 g) KunMing mice of either sex. Males and females were used in equal numbers in each group. Mice were housed in a quiet room with a 12 h light/dark cycle, at 22 ± 2 °C and 60 ± 5% humidity. All experiments were performed at the same time of the day, during the light period. Each mouse was used only once. All procedures in the present study were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adapted by the National Institutes of Health (Washington DC, USA, 1996). Approval from the local ethical committee was also obtained. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Chemicals

CH was purchased from Sigma, and suspended in 0.5% carboxymethyl cellulose sodium saline solution. Pentylentetrazole (PTZ) and isoniazid (ISO) were purchased from Sigma, and dissolved in saline. The dose, route, and timing of drug administration were based on preliminary experiments and pharmacokinetic considerations.

MES test

The MES test was carried out as described by the Antiepileptic Drug Development Program of the National Institutes of Health, and in accordance with previously described procedures (USA) 8. Convulsions were produced by an electric stimulation generator (JTC-1, ChengDu, China). Seizures were elicited by application of a 60 Hz alternating current at an intensity of 50 mA for 0.2 s, through ear-clip electrodes. The criterion for the onset of seizure activity was tonic hindlimb extension 9. The MES test was performed 1 h after the oral administration of CH 0.2 mL/20 g body weight.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Activity</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>144</td>
<td>8/8</td>
<td>8/8</td>
</tr>
<tr>
<td>120</td>
<td>6/8</td>
<td>8/8</td>
</tr>
<tr>
<td>100</td>
<td>5/8</td>
<td>8/8</td>
</tr>
<tr>
<td>83.4</td>
<td>3/8</td>
<td>8/8</td>
</tr>
<tr>
<td>69.5</td>
<td>1/8</td>
<td>8/8</td>
</tr>
<tr>
<td>59.7</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>48.3</td>
<td>0/8</td>
<td>0/8</td>
</tr>
</tbody>
</table>

Table 1. Effect of CH on MES-induced seizure. a The MES test was performed 1 h after oral administration of CH. b The dose were calculated which prevented 50% of the treated animals from tonic convulsion. c The dose were calculated which induced 50% of the treated animals into death.

Chemically induced seizures

To induce seizures, PTZ was administered sc in the scruff of the neck, or ISO was administered ip. At 1 h after the oral administration of various doses of CH 0.2 mL/20 g body weight, the animals were given various doses of PTZ or ISO 0.1 mL/20 g body weight.

Statistics

Groups of 8 mice were given a range of intraperitoneal doses of the tested compound until at least three points were established in the range of 10-90% seizure protection or minimal observed neurotoxicity. From the plot of this data, the respective ED50 and TD50 values, 95% confidence intervals, slope of the regression line, and the standard error of the slope were calculated by means of a computer program written at National Institute of Neurological Disorders and Stroke 11,12.

RESULTS

Effects of CH in the MES model

CH had a dose-dependent protective effect against seizures in the MES model (Table 1) with an ED50 of 93.4 mg/kg. At the same time we find many animals were dead a moment later after MES test. As shown in Table 1, we also gave the dose were calculated which induced 50% of the treated animals into death. It was 53.6 mg/kg.

Convulsions and mortality in the PTZ and ISO models

In the PTZ and ISO models, we could test the rates of clonic convulsion, tonic convulsion and death. All the results were showed in Table 2. When gave animals PTZ singly, the 50% animals could be induced into clonic convulsion, tonic convulsion and death at the doses of 67.8, 80.2, and 74.5 mg/kg separately, but when PTZ combined with the CH of ED50 dose, the dose of 50% animals could be induced into clonic convulsion.
convulsion, tonic convulsion and death down to 22.3, 63.7, and 41.5 mg/kg separately. The same condition appeared in the ISO test.

**DISCUSSION**

The possible neurobiological relationship between epilepsy and affective disorders has been receiving increasing attention 13,14. Co-morbid depression correlates with poor quality of life in epileptic patients 15, and suicide is one of the leading causes of death in this group 16. Major depression is common in patients with epilepsy 17,18, and many of these patients require treatment with antidepressants.

CH has been approved by the U.S. Food and Drug Administration for the treatment of major depression, and is also prescribed off-label for a number of anxiety conditions. In this study, we estimated the toxicity of CH in three different seizure models.

The protective effect of CH against MES-induced seizures was dose-related in the same manner as the mortality rate, indicating that toxicity increased as the anticonvulsant activity improved. Thus, CH appeared to have dual actions –both anticonvulsant and toxic. To estimate the toxicity of CH in three different seizure models.

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**Table 2.** PTZ and ISO-induced seizures and death. 

<table>
<thead>
<tr>
<th></th>
<th>TC50 (mg/kg)</th>
<th>TT50 (mg/kg)</th>
<th>TD50 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTZ</td>
<td>67.8</td>
<td>80.2</td>
<td>74.5</td>
</tr>
<tr>
<td>PTZ+ED50</td>
<td>22.3***</td>
<td>63.7**</td>
<td>41.5***</td>
</tr>
<tr>
<td>ISO</td>
<td>113.6</td>
<td>255.1</td>
<td>126.3</td>
</tr>
<tr>
<td>ISO+ED50</td>
<td>46.5###</td>
<td>116.4###</td>
<td>58.4###</td>
</tr>
</tbody>
</table>

The dose were calculated which induced 50% of the treated animals into clonic convulsion. b The dose were calculated which induced 50% of the treated animals into tonic convulsion. c The dose were calculated which induced 50% of the treated animals into death. PTZ+ED50 Compared with PTZ, ***: p < 0.01, **: p < 0.001. ISO+ED50 Compared with ISO, ###: p < 0.001.

may be mediated by enhancement of GABAergic neurotransmission. However, CH did not protect animals from seizures in these models.

Epilepsy is a common neurological disorder affecting approximately 50 million people worldwide according to the World Health Organization 22. Depression is the most frequent comorbid condition in people with epilepsy 23. A threefold increase in the diagnosis of depression and a fourfold increase in suicide rate have been reported in patients with epilepsy compared with the general population 25,26. Some reports have suggested that most antidepressant agents have a tendency to lower seizure threshold, and that some are associated with a clinical risk of seizures 27-29. Consistent with this, our findings indicate that using antidepressants to treat epilepsy in mice may bring about some acute adverse effects, and suggest that CH should be used with care in epilepsy patients with concurrent depression.

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**REFERENCES**