Original Research Article

Convenient method to induce orofacial dyskinesia: a novel animal model of tardive dyskinesia

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ABSTRACT

Tardive dyskinesia is a hyperkinetic movement disorder in which repetitive involuntary movements. Preclinical studies for tardive dyskinesia require use of large amount of animals. In order to reduce animal use, principle of refinement is used in the present research. Albino rats were used as animal model. Different drugs were used to induce orofacial dyskinesia. Locomotor activity, open field activity and motor co-ordination were also studied for all the drugs and their neuroprotective effects were evaluated using this new model.

KEYWORDS: Dyskinesia, animal model, tardive dyskinesia, ANOVA, haloperidol

INTRODUCTION

Tardive dyskinesia (TD) is a hyperkinetic movement disorder characterized by repetitive involuntary movements, usually involving mouth, face and tongue and sometimes limb and trunk musculature\textsuperscript{1}. It is considered to be a late onset adverse effect of prolonged administration of neuroleptic drugs\textsuperscript{2}. Rats treated chronically with neuroleptic drugs often develop spontaneous mouth movements, such movements have been described as "vacuous chewing movements" (VCMs), and reliable techniques have been described to rate them\textsuperscript{2} and is used as animal model of TD\textsuperscript{2}. Such increased behavior can be seen after a single dose, a few days, several weeks, or 1
year of neuroleptics in rodents \(^3\)\(^-\)\(^7\) and nonhuman primate \(^8\). Existing animal models of orofacial dyskinesia are very painful to animals and either takes longer duration of time or needs highly skilled or highly sophisticated procedures.

An important area in animal research is humane concepts in animal methodologies based on the 3 R's principles focusing on the replacement, reduction, and refinement of the use of alternatives in research \(^9\). Replacement is the application of alternative methods that do not require the use of animals within limits that allow scientific objectives to be achieved. Reduction is the use of as few animals as possible within limits that allow scientific objectives to be achieved, and Refinement is the application of methods that do not distress the animals or subject them to pain within limits required for use. These 3R principles are the ideology behind both animal experimentation and the handling of laboratory animals. Consequently, within the limits required to achieve the objectives of research, they should be taken into consideration and applied appropriately when conducting animal experiments \(^10\).

So following the principle of Refinement, the present study is undertaken to develop a convenient method to induce orofacial dyskinesia as an animal model of TD.

**MATERIALS AND METHODS:**

**ANIMALS**

Albino rats weighing 200-250 g were used in the study (n=6 in all groups). Animals were housed under standard laboratory conditions with free access to food and water. Animals were acclimatized to laboratory conditions before the experiments. All experiments were carried out between 09.00 and 15.00 hr. Each animal was used only once. The experimental protocols were approved by the Institutional Animal Ethics Committee (22/09/IAEC/ SOAU).

**EXPERIMENT FOR INDUCTION OF OROFACIAL DYSKINESIA**

Haloperidol (Serenace® inj., Searle India, India) was purchased from local chemist and injected to groups 2, 3 & 4 in the single dose of 3mg/kg, 4mg/kg, and 5mg/kg intraperitonealy respectively. Group 1 was kept as control (Saline 10 ml/kg) and group 5 was administered with Haloperidol 1mg/kg twice a day for 14 days which served as standard \(^20\). Groups 1, 2, 3 & 4 were observed after 48 hours of drug administration while group 5 was observed 24 hours after the last dose.

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To validate the new model developed, we have studied the effect of nifedipine 20 mg/kg (a Ca^{++} channel blocker) whose protective effect is already proved in the classical models of orofacial dyskinesia.11 In addition to that we have also evaluated the effects of 2 antiparkinsonian drugs (levodopa 50, 100, 150 mg/kg; atropine 0.5, 1, 1.5 mg/kg), a new Ca^{++} channel blocker (Lercanidipine 10, 15, 20 mg/kg) and a COX II inhibitor (Etoricoxib 10, 15, 20 mg/kg) on orofacial dyskinesia using our novel animal model. Locomotor activity, open field activity and motor co-ordination were also studied for all the drugs and their neuroprotective effects were evaluated using this new model.

PARAMETERS

Orofacial Dyskinesia

After the prescribed time as described for different methods/groups above, animals were placed individually in a small (26 x 26 x 26 cm) observation cage (which consisted of mirrors at three sides and at floor) the observation chamber is designed especially for the purpose of vacuous chewing movements (VCM) counting. Before observation animals were acclimatized to laboratory condition for 1hr. Then each animal was allowed to acclimatize to the observation cage for a period of 10 min. For the sake of simplicity each chewing burst is counted as one and tongue protrusion is also scored for 10 min observation period. VCM were referred to as single mouth openings in the vertical plane not directed toward physical material. Individual tongue protrusions during a bout of oral dyskinesia were each preceded by visible retraction of the tongue. If tongue protrusions or vacuous chewing movements occurred during a period of grooming, they were not taken into account. All the scorers were blind to the treatment groups.

Open Field Activity

We have configured a novel open field activity box (OFB) especially for the measurement of movement disturbance due to TD. The box measuring 35x35x35 cm was divided in four equal sub squares separated by 1cm height which serves as hurdles for moving to the next square. The number of Rearing (standing on hind paw) and Ambulation (crossing of square with all four paws) were observed for 1 minute.
Motor Coordination

Motor coordination was assessed for all rats on a Rota-rod. The Rota-rod (INCO, India) has a 7 cm radius and a speed of 20 rpm. The surface is not glossy to avoid the slippery effect when animal was simply placed on it. Prior to any treatment rats were trained in a single session until they attained 150 seconds on Rota-rod. The trip of animals was taken as end point and the time in seconds were noted.

Locomotor Activity

The locomotor activity was monitored using Actophotometer (SHREEJI, India). The animals were individually placed in the instrument and the total activity count was registered for 10 min. The locomotor activity was expressed in terms of total photo beam counts/10 min per animal.

Statistical Analysis

One way ANOVA followed by Dunnet’s t-test was used. p<0.05 was considered as significant.

RESULTS

The VCM and TP count (mean±SD) after haloperidol administration in different models are given in Table-1. The standard treatment (Haloperidol 1 mg/kg twice a day for 14 days) and Haloperidol 5mg/kg single dose significantly increased both the VCM and TP count. Haloperidol 3mg/kg single dose and 4mg/kg single dose increased the VCM and TP count but not significant.

The effect of different drugs on Orofacial Dyskinesia induced by Haloperidol 5 mg/kg i.p. single dose is shown in Table-2. Nifedipine 20mg/kg p.o. protected/decreased the VCM count and tongue Protrusion (p<0.01), Lercanidipine 10mg/kg p.o. significantly (p<0.01) prevented the development of Orofacial dyskinesia. L-Dopa at doses 50 & 100mg/kg failed to prevent the Orofacial dyskinesia but at the dose of 150mg/kg significantly (p<0.01) prevented the development of Orofacial dyskinesia. Atropine at doses 0.5 & 1mg/kg failed to prevent the Orofacial dyskinesia but at the dose of 1.5mg/kg significantly (p<0.05) prevented the development of Orofacial dyskinesia. Etoricoxib at dose 10 mg/kg failed to prevent the Orofacial dyskinesia but at the dose of 15 & 20 mg/kg significantly (p<0.05) prevented the development of Orofacial dyskinesia.

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Haloperidol (5mg/kg single dose) significantly *(p<0.01)* decreases open field activity (ambulation and rearing), locomotion and motor co-ordination, the effects of different drugs on these parameters are given in Table-2. The ambulation and rearing are significantly *(p<0.01)* improved by Nifedipine 20mg/kg, Lercandipine 10mg/kg, Levodopa 100mg/kg and Atropine 0.5mg/kg, Etoricoxib 15mg/kg significantly *(p<0.05)* improved the ambulation but not rearing. All the drugs significantly improved locomotion and motor co-ordination.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>VCM (mean±SD)</th>
<th>TP (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Saline 10ml/kg)</td>
<td>0.33±0.51</td>
<td>0.16±0.40</td>
</tr>
<tr>
<td>Haloperidol 3mg/kg i.p. single dose</td>
<td>2.16±1.47</td>
<td>1±0.89</td>
</tr>
<tr>
<td>Haloperidol 4mg/kg i.p. single dose</td>
<td>3±1.41</td>
<td>1.83±0.75</td>
</tr>
<tr>
<td>Haloperidol 5mg/kg i.p. single dose</td>
<td>31.67±1.75*</td>
<td>8.83±1.60*</td>
</tr>
<tr>
<td>Haloperidol 1mg/kg p.o. twice a day for 14 days</td>
<td>50.67±2.94*</td>
<td>11.83±2.22*</td>
</tr>
<tr>
<td>F value(4,25)</td>
<td>946.74*</td>
<td>91.342*</td>
</tr>
</tbody>
</table>

**p<0.01 vs. control**

Table 1: Effect of different doses/ models of Haloperidol on vacuous chewing movements (VCM) and tongue protrusion (TP) in rats
<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Orofacial dyskinesia</th>
<th>Open field activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VCM</td>
<td>TP</td>
</tr>
<tr>
<td>Control (Saline 10ml/kg)</td>
<td>0.33±0.51</td>
<td>0.33±0.51</td>
</tr>
<tr>
<td>Haloperidol 5mg/kg i.p.</td>
<td>33.16±1.72**</td>
<td>8.16±0.75**</td>
</tr>
<tr>
<td>Nifedipine 20mg/kg p.o.</td>
<td>10±1.78**</td>
<td>1.16±1.16**</td>
</tr>
<tr>
<td>Lercanidipine 10mg/kg p.o.</td>
<td>26.16±2.04**</td>
<td>7.33±1.21**</td>
</tr>
<tr>
<td>Lercanidipine 15mg/kg p.o.</td>
<td>20±2.09**</td>
<td>5.67±1.21**</td>
</tr>
<tr>
<td>Lercanidipine 20mg/kg p.o.</td>
<td>12.83±2.04**</td>
<td>2±0.89**</td>
</tr>
<tr>
<td>Levodopa 50 mg/kg p.o.</td>
<td>31.83±1.72</td>
<td>7.67±1.21</td>
</tr>
<tr>
<td>Levodopa 100 mg/kg p.o</td>
<td>30.33±2.16</td>
<td>7±0.89</td>
</tr>
<tr>
<td>Levodopa 150 mg/kg p.o</td>
<td>25.16±2.85**</td>
<td>6.33±0.81*</td>
</tr>
<tr>
<td>Atropine 0.5mg/kg p.o</td>
<td>32.5±1.04</td>
<td>7.5±1.04</td>
</tr>
<tr>
<td>Atropine 1mg/kg p.o</td>
<td>31.66±2.13</td>
<td>7.16±1.47</td>
</tr>
<tr>
<td>Atropine</td>
<td>29.33±2.06*</td>
<td>6.16±0.98*</td>
</tr>
</tbody>
</table>

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DISCUSSION

Refinement is one of the principles of 3R concept in alternatives in research which focuses on the application of methods that do not distress the animals or subject them to pain within limits required for use. Hence the present study is undertaken to develop a novel animal model for induction of orofacial dyskinesia and to validate it under the principle of Refinement.

There exist various animal models for TD and most of them take longer duration for induction of orofacial dyskinesia. However there are model which give instantaneous results, but use intracerebroventricular route for induction of orofacial dyskinesia requires highly sophisticated skills and techniques, hence we undertook this study to develop an animal model to demonstrate the Vacuous Chewing movement on lab scale for students and reduce pain and suffering to animals during as well as after the experiment.
Orofacial dyskinesia is characterised by vacuous chewing movement and tongue protrusion[1]. In our study haloperidol at a dose of 5mg/kg i.p shows both these characteristics significantly (p<0.01) as compared to control but at 3mg/kg i.p. & 4mg/kg i.p. the VCM and TP were not significant. Hence we support the theory, that the development of orofacial dyskinesia in rats is dose dependent[15]. Like other studies[16], the standard (Haloperidol 1mg/kg twice a day for 14 days) significantly (p<0.01) increased the VCM and TP. This validates our model.

Haloperidol induces calcium ion influx via L-type calcium channel and elevates the calcium ions appears to render hippocampus cells more susceptible to oxidative stress which is one of the cause for development of orofacial dyskinesia[17]. The protective effect of L-type Ca^{++} channel blockers like nifedipine, verapamil and nimodipine in haloperidol induced orofacial dyskinesia is well documented[11]. In our study Nifedipine significantly (p<0.01) reduced the VCM and TP count. Another calcium channel blocker, Lercanidipine also dose dependently reduce orofacial dyskinesia significantly (p<0.01). Hence this also validates our new model and suggests that ions are involved in haloperidol induced neurotoxicity.

The Receptor Sensitivity Modification Hypothesis postulates that dopamine agonists in relatively high doses may reverse postsynaptic dopamine-receptor hypersensitivity[18]. In the present study it has been found that L-Dopa in low doses (50&100mg/kg) were not significant which supports the previous studies[19], but in the dose of 150mg/kg (p<0.01) it significantly reduced the VCM and TP but the value was far away from STD drug treatment. This supports the above hypothesis and further validates our model.

The existence of a so-called counterbalancing dopaminergic-cholinergic transmitter system in the central nervous system for maintenance of normal movement and behavior is well documented in pharmacological studies on animals[20,21]. In the present study Atropine significantly reduced the VCM and TP count in animals treated concomitantly with Haloperidol which supports the previous reports, that striatum is involved in the motor control of oral activities, and is rich in cholinergic neurons and muscarinic receptors[22]. So this reduction in VCM and TP count by atropine in our novel model validates our model.

Prostaglandins modulate the dopamine release in the striatum, the principle area involved in the pathophysiology of tardive dyskinesia[23]. Prostaglandins increases free radical production & oxidative stress both causes tardive dyskinesia[24], Selective COX-2 inhibition showed improving effects on the catalepsy followed by decreasing the striatum glutamatergic - GABAergic and

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enhancing the dopaminergic neurotransmission. In the present study Etoricoxib, a COX2 inhibitor dose dependently prevented the VCM count and TP hence, our findings support the report that significant increase in dopaminergic neurotransmission after selective COX-2 inhibition in the striatum (principle area involved in TD) of normal and hemiparkinsonian rats.

Our study shows that Nifedipine, Lercanidipine, L-Dopa, Atropine and Etoricoxib dose dependently prevented motor incoordination, impairment in loco motor activity; and enhanced open field activity, hence we suggest the neuroprotective effect of all tested drugs in haloperidol induced motor impairment.

So haloperidol 5 mg/kg i.p. can be used as novel animal model for inducing orofacial dyskinesia. This uses the principle of refinement and is a simple and less time taking animal model.

However, due to lack of biochemical & neurochemical findings in our studies, it should be regarded as a drug response model rather than a pathophysiologic model. As demonstrated in this study, the chewing movements were most sensitively affected by drugs and thus, they might serve as a good indicator of the effects of future therapeutic agents on TD.

**CONCLUSION**

Refinement is one of the principles of 3R concept in alternatives in research which focuses on the application of methods that do not distress the animals or subject them to pain within limits required for use. Hence the present study is undertaken to develop a novel animal model for induction of orofacial dyskinesia and to validate it under the principle of Refinement. Findings in study showed that Nifedipine, Lercanidipine, L-Dopa, Atropine and Etoricoxib dose dependently prevented motor incoordination, impairment in loco motor activity; and enhanced open field activity. As demonstrated in this study, the chewing movements were most sensitively affected by drugs and thus, they might serve as a good indicator of the effects of future therapeutic agents on tardive dyskinesia.
REFERENCES


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