Detection of potential effects of orphenadrine upon anesthesia with propofol and/or thiopental in mice

Y.M. Albadrany, A.S. Naser and K.A. Shaaban

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Abstract

Orphenadrine is an anticholinergic drug used in clinics as a muscle relaxant. It acts on several receptors, including muscarinic, histaminic, and NMDA. This study aimed to evaluate the effect of pretreatment with orphenadrine on the anesthesia indices of two essential parenteral anesthetic drugs in mice. Three experiments were performed with 15 mice per experiment. All experiments used orphenadrine at 10 mg and 20 mg/kg/IP b.wt as a pretreatment drug. Propofol (100 mg/kg/IP), thiopental (50 mg/kg/IP), and their combinations were used in the first, second, and third experiments, respectively. The indices of anesthesia, represented by the latency to onset of anesthesia, duration of anesthesia, and recovery from anesthesia, were recorded. Orphenadrine at doses of 10 mg and 20 mg/kg/IP as a pretreatment drug showed a dose-dependent decrease in the latency to the onset of anesthesia and an increase in the duration of anesthesia compared to the control groups of all three experiments (propofol 100 mg/kg, thiopental 50 mg/kg, and combinations of propofol and thiopental) respectively. We conclude that orphenadrine affects anesthesia indices, which are recorded for the first time, laying the foundation for further studies and the possibility of using it as an anesthesia co-treatment.
muscarnic, histamine, and NMDA (12). It is also used as an analgesic, even though its particular mode of action is unknown (13).

This study aimed to evaluate the effects of two doses of orphenadrine on propofol and thiopental anesthesia indices. We anticipated that these combinations would lead to secure anesthetic protocols with prompt induction of anesthesia.

**Materials and methods**

**Ethical approval**

The animals handling ethics protocol of the College of Veterinary Medicine, University of Mosul, was followed. The Scientific Board of Physiology, Biochemistry, and Pharmacology department approved this study (Ref: UM.VET. 2023. 007, date: 25/3/2023).

**Animals**

Adult male albino mice (10-12 weeks old, 20-30 g) were used in the study. Five mice were housed per cage in a room kept at 25±1°C with a relative humidity of 55±5% and an alternate 12-hour light and dark sequence, with free access to food and water. The animals were used only once in all experiments. The animals were separated and housed in different cages after the IP injection. The experimenters observed the reactions of the mice during the experiments that had been followed from 8 a.m. to 2 p.m.

**Impact of orphenadrine on the anesthesia induced by propofol in mice**

Fifteen mice were randomized into three equal groups. Group 1 mice were pretreated with normal saline, after 15 min with propofol 100 mg/kg/IP (14). Group 2 mice were pretreated with orphenadrine 10 mg/kg /IP (15), after 15 min with propofol 100 mg/kg/IP. The mice of the third group were previously administered by orphenadrine at 20mg/kg intraperitoneal, followed by fifteen minutes administered by propofol at 100mg/kg intraperitoneal.

**Impact of orphenadrine on the anesthesia generated by thiopental in mice**

Fifteen mice were allocation for three groups. First group were administered by normal saline intraperitoneal, followed by fifteen minutes administered by thiopental at 50mg/kg, intraperitoneal (16). The second group were administered by orphenadrine at 10mg/kg, intraperitoneal, followed by fifteen minutes administered by thiopental at 50mg/kg, intraperitoneal. The third group were administered by orphenadrine at 20mg/kg, intraperitoneal, followed by fifteen minutes administered by thiopental at 50mg/kg, intraperitoneal.

**Impact of orphenadrine on anesthesia generated by propofol and thiopental in mice**

Fifteen mice were allocation for three groups. Group 1 mice were pretreated with normal saline, after 15 min with propofol 100 mg/kg/IP and thiopental 50 mg/kg/IP. Group 2 mice were pretreated with orphenadrine 10 mg/kg /IP, after 15 min with propofol 100 mg/kg/IP and thiopental 50 mg/kg/IP. Group 3 mice were pretreated with orphenadrine 20 mg/kg/IP, after 15 min with propofol 100 mg/kg/IP and thiopental 50 mg/kg/IP.

**Drugs**

Orphenadrine citrate injection 30 mg/ml Teva® (USA), propofol 100 mg/10 ml DIPRIVAN® (U.K), thiopental sodium injection 1 g powder THOWELL®(INDIA). The volume of administration was 10 ml/kg/IP of body weight (3). All drugs were injected after preparing the doses in different syringes, and then the latency to onset of anesthesia was noted, which is the interval between the injection and the loss of the righting reflex of mice. The duration of anesthesia was noted, which is the interval between the loss of the righting reflex and the time it took for the mice to correct their posture, and the recovery from anesthesia, which is the interval between the time the mouse had returned to normal position and the time to resumed movement (17).

**Statistical analysis**

One-way analysis of variance (ANOVA) followed by the Least Significant Difference test was used to assess the results statistically. Data are expressed as mean±standard error. P≤0.05 was chosen as the minimal degree of significance. The analysis was conducted with the statistical software SPSS 17.

**Results**

**Effect of orphenadrine on the anesthesia induced by propofol in mice**

As shown in table 1, the latency to onset of propofol anesthesia within approximately four minutes, the duration of anesthesia continued for half an hour through the mice return to normal position, and the recovery period was approximately twenty-five minutes, which the mice represented a return to its normal movement and behavior. When treated with orphenadrine, there was a dose-dependent decrease in latency to the onset of anesthesia and an increase in the duration of anesthesia compared with the propofol group. It was noted that the orphenadrine prolonged the anesthesia duration time. In addition, there was an increase in the recovery period in the group treated with orphenadrine 20 mg/kg compared to the propofol group.
Table 1: Impact of orphenadrine on anesthesia induced by propofol

<table>
<thead>
<tr>
<th>Groups</th>
<th>Latency to onset (min)</th>
<th>Duration (min)</th>
<th>Recovery time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>4.16±0.22(^a)</td>
<td>37.33±1.94(^b)</td>
<td>25.44±1.14(^b)</td>
</tr>
<tr>
<td>Group 2</td>
<td>3.59±0.18(^a)</td>
<td>61.49±1.43(^b)</td>
<td>19.95±0.68(^b)</td>
</tr>
<tr>
<td>Group 3</td>
<td>2.39±0.25(^b)</td>
<td>92.27±2.55(^a)</td>
<td>35.55±1.00(^a)</td>
</tr>
</tbody>
</table>

As shown in table 2, latency to onset of thiopental anesthesia within approximately ten minutes, the duration of anesthesia continued for a third of an hour through the mice return to normal position, and the recovery period was approximately ten minutes, which the mice represented a return to its normal movement and behavior. When treated with orphenadrine, there was a dose-dependent decrease in latency to the onset of anesthesia and an increase in the duration of anesthesia compared with the thiopental group. It was noted that the orphenadrine prolonged the anesthesia duration time. In addition, there was an increase in the recovery period in the orphenadrine group compared to the propofol and thiopental group.

Table 2: Impact of orphenadrine on anesthesia induced by thiopental

<table>
<thead>
<tr>
<th>Groups</th>
<th>Latency to onset (min)</th>
<th>Duration (min)</th>
<th>Recovery time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>9.87±0.52(^a)</td>
<td>21.03±0.73(^c)</td>
<td>10.18±0.36(^c)</td>
</tr>
<tr>
<td>Group 2</td>
<td>4.74±0.17(^b)</td>
<td>40.58±1.01(^b)</td>
<td>20.31±0.65(^b)</td>
</tr>
<tr>
<td>Group 3</td>
<td>2.55±0.19(^c)</td>
<td>84.41±1.34(^c)</td>
<td>40.03±1.69(^c)</td>
</tr>
</tbody>
</table>

Table 3: Impact of orphenadrine on anesthesia induced by propofol and thiopental

<table>
<thead>
<tr>
<th>Groups</th>
<th>Latency to onset (min)</th>
<th>Duration (min)</th>
<th>Recovery time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1.26±0.12(^a)</td>
<td>86.88±0.88(^b)</td>
<td>26.01±0.75(^c)</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.93±0.06(^b)</td>
<td>127.55±1.91(^b)</td>
<td>39.28±1.88(^b)</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.73±0.05(^c)</td>
<td>177.43±5.92(^c)</td>
<td>72.37±2.63(^c)</td>
</tr>
</tbody>
</table>

Effect of orphenadrine on the anesthesia induced by thiopental in mice

As shown in table 2, latency to onset of thiopental anesthesia within approximately ten minutes, the duration of anesthesia continued for a third of an hour through the mice return to normal position, and the recovery period was approximately ten minutes, which the mice represented a return to its normal movement and behavior. When treated with orphenadrine, there was a dose-dependent decrease in latency to the onset of anesthesia and an increase in the duration of anesthesia compared with the thiopental group. It was noted that the orphenadrine prolonged the anesthesia duration time. In addition, there was an increase in the recovery period in the orphenadrine group compared to that in the propofol group.

Effect of orphenadrine on the anesthesia induced by propofol and thiopental combination in mice

As shown in table 3, concomitant treatment with propofol and thiopental resulted in the latency to onset of anesthesia within approximately one minute, the duration of anesthesia continued for almost two hours through the mice returned to the normal position, and the recovery period was approximately half an hour, which the mice represented a return to its normal movement and behavior. When treated with orphenadrine, there was a dose-dependent decrease in the latency to the onset of anesthesia and an increase in the duration of anesthesia compared to the propofol and thiopental group. It was noted that the orphenadrine prolonged the anesthesia duration time. In addition, there was an increase in the recovery period in the orphenadrine group compared to the propofol and thiopental group.

Discussion

A logical method for multimodal general anesthesia should include the following steps: administration of combinations of antinociceptive drugs, each targeting a distinct circuit in the nociceptive system; continuous monitoring of levels of antinociception and unconsciousness; expressly using the sedative effects of antinociceptive medicines to minimize the doses of hypnotic medications and inhaled anesthetics used to maintain unconsciousness; and maintaining multimodal pain control during the in-hospital postoperative period and after discharge (18,19). No anesthetic medication can supply all the constituents of general anesthesia without interfering with essential organ roles. As a result, a multi-drug method (balanced anesthesia) is used to decrease sensory, motor, sympathetic, and parasympathetic response actions, as well as distinct constituents of the anesthetic state (20-22). Therefore, this study aimed to estimate a new combination of anesthetic drugs with orphenadrine, a muscle-relaxant drug, and its effect on anesthesia indices.

Among the significant results noted in our research is that the administration of orphenadrine in doses 10 and 20 mg/kg reduced the period of onset of anesthesia and increased the induction period of anesthesia (period of surgical operation). This effect is recorded for the first time and needs further study and research to reveal the mechanism of this synergistic effect at the level of anesthesia. GABA is the core inhibitory neurotransmitter in the mammalian central nervous system (23,24). Glutamate is the primary excitatory amino acid neurotransmitter acting on NMDA and non-NMDA receptors, which play a main part in physiological processes such as memory and learning (and thus consciousness under anesthesia) and principal pain transduction mechanisms (25-27).

Propofol inhibits postsynaptic neurons by preventing GABA from dissociating from its receptors. This increases chloride entry via channels, leading to hyperpolarization of postsynaptic cell membranes and inhibition of postsynaptic neurons (28,29). Propofol also inhibits the NMDA subtype...
of the glutamate receptor, probably by modulation in the channel gating (30). Propofol inhibits voltage-gated sodium currents and controls calcium influx via slow calcium channels (31,32).

Thiopental works in a manner analogous to barbiturate drugs. It acts as an agonist of (the GABA_5) receptor, increasing inhibitory neurotransmission. It increases GABA binding to its receptor (an inhibitory neurotransmitter) and then increases transmembrane chloride entrances, causing hyperpolarization of the postsynaptic cell and inhibition of the postsynaptic neurons (33,34). Thiopental inhibitory activity on NMDA receptors may not be mediated via the secondary effects of the GABA_5 receptor agonist. These findings suggest that NMDA receptors play a role in mice's thiopental-induced anesthesia (35).

Notably, orphenadrine has no anesthetic effects; however, studies have indicated its effects on different receptors (36). Orphenadrine is a diphenylmethane analog of diphenhydramine with antimuscarinic, NMDA antagonistic, and antihistaminic characteristics to a lesser extent (37). The synergistic action of orphenadrine on propofol and thiopental anesthesia indices can be explained by its action on different receptors. Nevertheless, the hypothesis closest to explaining this synergistic effect may be through NMDA antagonism, as N-methyl-D-aspartate (NMDA) receptors are essential in the nervous system's excitatory neurotransmission. Some general anesthetics preferentially block them, so they have been implicated in mediating their effects (38). Hypnotic effects of ketamine occur by blocking NMDA receptors in nervous tissue (39). Blocking NMDA receptors is related to the immobilization effect of inhalational anesthetics (40,41).

Conclusion

It could be concluded that pretreatment with orphenadrine affects anesthesia indices for both propofol and thiopental alone or together. These results provide a foundation for further research and investigation. Orphenadrine can be used in clinical studies on different animals to investigate its safety and its possible use in various surgical procedures.

Acknowledgment

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Conflict of interest

No conflict of interest.

References


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