Analgesic effect of chlorpheniramine in rat anesthesia with acepromazine – ketamine combination

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Received: 2016/20/03 Revised: 2016/12/08 Accepted: 2016/6/09

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Abstract

Introduction:
Recently, antihistamines have been considered as analgesic agents. The present study aimed to investigate the analgesic function of chlorpheniramine combined with acepromazine-ketamine during anesthesia and surgery in rats.

Methods & Materials:
Twenty adult rats were randomly assigned to two groups. The first group received chlorpheniramine and 30 minutes later acepromazine–ketamine. The second group received normal saline initially and 30 minutes later acepromazine-ketamine. Pain reflexes were assessed by toe pinch test, tail pinch test and rats’ reflex to laparotomy during anesthesia. Induction time, light anesthesia duration, surgical anesthesia time, walking time, heart rate and respiratory rate were measured.

Results:
Duration of light anesthesia was shorter in group 2 than in group 1 (P < 0.05). Laparotomy pain score was significantly less in group 1. Toe pinch and tail pinch tests were not significantly different between the two groups. Heart rate was not significantly different between the two groups.

Conclusion:
As a pre-anesthesia medication, chlorpheniramine induces appropriate anesthesia in rats in combination with acepromazine-ketamine. Thus, chlorpheniramine can be used in rats as a pre-anesthesia medication for surgical operations in combination with acepromazine-ketamine.

Keywords: Chlorpheniramine, Acepromazine, Ketamine, Anesthesia, Rat, Antinociception

Introduction

Histamine is a monoamine known as a neurotransmitter or neuroregulator in the brain of mammals (1). With the broadest scope of activity in different physiological and pathological conditions such as cell proliferation and differentiation, the process of hematopoiesis, embryonic growth and development, regeneration, wound healing, and aminergic neurotransmissions, histamine plays multiple roles in the brain including processes such as sleeping, pain perception, food intake and aggressive behaviors. Histamine has also an important role in the secretion of pituitary hormones, regulation of digestive system and circulatory system function, regulation of the cardiovascular system, inflammatory responses, immune...
responses modulation, energy of endocrine glands, and homeostasis. There is much evidence that histamine plays an important role in allergic and inflammatory reactions (2). Histamine has an important role in responses to many skin allergic conditions. This substance is important in stimulating vasodilation and increased capillary permeability (1). The central H1 receptors are the main location for sedative effects of antihistamines (3). Histamine exerts its biological effects by binding to specific receptors on the cell membrane. Currently, four different receptors are known for histamine called Histamine H1, H2, H3 and H4 receptors. These receptors are expressed in response to histamine in various target tissues and their activation stimulate phospholipase C and cAMP increase (2).

The origin of the pain might be in superficial tissue damages (e.g. skin and mucosa) or deep tissue damages (e.g. muscles, joints, or viscera). The greatest pain is usually felt at the time of tissue damage occurrence and then it reduces. Pain has a complex physiology. In general, after tissue damages, certain receptors called nociceptors, which are free nerve endings, are stimulated and the received message is transmitted to the central nervous system through various nerves, which will eventually stimulate the cerebral cortex and lead to the feeling of pain. Therefore, the activity of the cerebral cortex is necessary for the perception of pain. Pain, as a defensive phenomenon, causes the individual to move away from the source of pain and makes the patient not move or work with the affected organ. The nociceptors are sensitive to mechanical, thermal and chemical stimuli. Following tissue damages, different materials are produced by the damaged tissue, the most important of which are bradykinin, serotonin, histamine and prostaglandins E. These are in fact pain-causing substances and lead to pain by stimulating nociceptor. The release of these substances stimulate chemical receptors and decrease the stimulation threshold of other receptors (e.g. mechanical and thermal) (4, 5).

Considering that some antihistamines that inhibit H1 receptor show sedative effects in clinical and pre-clinical models (6), they have recently been considered as pain relievers. The intraperitoneal injection of mepyramine (H1 antagonist) in rats reduced the pain of plantar injection of formalin (7). Recent studies revealed the analgesic effect or synergistic effect of antihistamines with morphine. Repeated doses of H1 antagonist antihistamines such as diphenhydramine, promethazine or pyrilamine in Syrian mice did not reduce the effect or cause resistance, but easily induced analgesia similar to morphine or other opioids (6). Balanced anesthesia is used in order to achieve a proper and safe anesthesia in humans as well as laboratory animals. The aim of balanced anesthesia is creating analgesia, eliminating the memory of surgery, loss of consciousness, and immobility. No anesthetic alone can meet all these goals and provide adequate general anesthesia, so a combination of pre-anesthetic, analgesic, sedative and general anesthetic drugs can provide a well-balanced anesthesia (4, 5).

Despite sleeping and analgesic properties of H1 antagonists of histamines (6), they have not been used as pre-anesthetic medication in combination with injected anesthetics. Their impact on general anesthesia of laboratory animals has not been clinically studied, either. Hence, the present study was conducted to evaluate the analgesic effects of subcutaneous injection of chlorpheniramine on the anesthesia of male rats (intramuscular injection of ketamine-acepromazine combination) during laparotomy. According to the painfulness of laparotomy, it was used along with foot pad press and tail flick test to evaluate the analgesic properties and pre-anesthetic effects of chlorpheniramine during anesthesia of rats.

**Materials and Methods**

The present experimental study used an electrocardiogram (Sylmar, CA91342, USA), electric heater, desk lamp, alcohol
thermometer, max-min thermometer (to adjust the animals’ room temperature) and digital thermometer (MT16A1, Microlife, Switzerland). The following drugs were used: Ketamine (Ketamine, 100 mg/ml, Aesculaap, Boxtel, Holland), Acepromazine (Castran, Acepromazine 20 mg/ml, Interchemie, Holland) and Chlorpheniramine (Histadic, Chlorpheniramine 10mg/ml, Caspian, Iran). The animals were procured from the center of breeding and keeping laboratory animals at Ardebil University of Medical Sciences and the research ethics guidelines on laboratory animals were observed at all stages of the study. After the initial study, resources were reviewed and using the Dixon's method in order to determine the appropriate dose of subcutaneous injection of chlorpheniramine, 20 male adult Sprague-Dawley rats weighing 341±23 g (mean ± SD) were used to carry out anesthesia and compare the effects of drugs and compounds on pain and physiological parameters in two groups of 10. Laboratory animals were kept in standard conditions and in accordance with the National Institute for Health and Work. They were used following the keeping guidelines and ethical principles of using laboratory animals. Dose and injection routes of drugs are presented in Table 1.

Table 1: Anesthetic compounds used in intervention groups

<table>
<thead>
<tr>
<th>group</th>
<th>Used compounds</th>
<th>Injection route</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chlorpheniramine (10 mg/kg) Acepromazine (2.5 mg/kg) - ketamine (75 mg kg)</td>
<td>Subcutaneous Intramuscular</td>
<td>30 minutes after the injection of chlorpheniramine</td>
</tr>
<tr>
<td>2</td>
<td>Normal saline (1 ml) Acepromazine (2.5 mg/kg) - ketamine (75 mg kg)</td>
<td>Subcutaneous Intramuscular</td>
<td>30 minutes after the injection of saline</td>
</tr>
</tbody>
</table>

The rats were weighed individually with laboratory weighing scales and the amount of drugs was determined according to the intended dose and body weight of rats. Finally, using insulin needles, the calculated amount of drugs or the combination of the intended drug was injected intramuscularly in the right or left thigh muscle. All injections were performed by one particular person. In the first group, after weighing the animals, 10 mg/kg of chlorpheniramine was injected subcutaneously and after 30 minutes, 2.5 mg/kg acepromazine pre-anesthetic combination was injected intramuscularly (8-11). In the intervals between the injections, the rats were kept in cages in a quiet environment. In the second group, injections and their order were similar to the first group, but 1 ml/kg of normal saline was injected subcutaneously instead of chlorpheniramine. After injection, the mice in both groups were transferred to separate cages to induce anesthesia at peace. Injection duration, weight, the number of rats, date, drugs groups, information related to anesthesia period and recovery, induction time, injection doses, duration of anesthesia and all physiological data of the animals were recorded for each animal separately.

The induction of anesthesia for each compound was precisely controlled, and any imbalance, rotation in the cages, rotary movements of limbs, or smooth induction of anesthesia were recorded. After the induction of anesthesia, the time that animals slept on their side was considered the beginning of light anesthesia. The time between the time of injection until the animal lay on one side, was considered as anesthesia induction time. After the
induction of anesthesia and sleeping, the animal was slowly transferred to the ECG table. The ECG device was first calibrated at the sensitivity of 2 mV, the speed of 50 mm/s and lead II. All data related to anesthesia, including obtaining ECG was recorded once every 5 minutes during anesthesia. In order to record rats’ respiratory rate, their breathing was counted for 20 seconds and multiplied by 3 to achieve the number of breaths per minute. The number of heartbeats per minute was obtained from ECG (12). Footpad press and tail flick were tested to evaluate pain reflexes. Plastic atraumatic forceps were used to evaluate the reaction of paw and finger pressures were used to create tail reflex. Any reaction of animals, including breath holding, moaning, or sudden movement was considered a positive response to pain. Anima ls’ response to pain was scored from 0 to 4 (13). That is, no response to pain was 0 and severe reaction to pain, as the response of a conscious animal, was 4. All pain reactions were evaluated by a particular person. After anesthesia and connection to the ECG device, animals were laid in a supine position and midline abdominal laparotomy was carried out on the skin and muscles from the sternum to the pelvic and pubic bone. During surgery, possible animal movements or any responses (moaning) was recorded. After the laparotomy, abdominal muscles were sutured with Vicryl USP 2-0 and simple continuous suture. The skin was sutured with silk USP 3-0 and simple interrupted suture. From the beginning of anesthesia until recovery, a digital paraffinated thermometer was placed 2-3 cm inside the rectum of rats in order to control body temperature changes precisely. A desk lamp was used to prevent a decrease in body temperature of the rats and an alcohol thermometer was used to control the ambient temperature the animals. The ambient temperature at the time of anesthesia ranged 25-27 °C. Rats’ body temperature was measured by a digital thermometer and it was kept at 37-38.5 °C using heating lamps during anesthesia. Animals’ roll over from the supine position and lying on the chest was considered as the recovery time of righting reflex. The period between the loss of righting reflex and its recovery was considered as light anesthesia time. When the animals were able to move their legs and walk was also recorded. The animals’ quality of recovery from anesthesia in a volatile and violent form (imbalance, rotation in the cage and rowing movements of arms or legs) or a smooth form was recorded. The period between the loss of pain response of paw and tail until its recovery was defined as the surgical anesthesia time. The lack of reaction at the time of laparotomy was a sign of surgical anesthesia. Thus, in the case of animals’ reaction to painful stimuli of paw and tail and operation, they were only recorded at the time of light anesthesia.

Statistical analysis
Data was analyzed using SPSS statistical software version 16. According to the insignificance of Kolmogorov-Smirnov test, statistical analysis related to induction time, recovery time, sleeping period, light or surgical anesthesia period, and walking time were performed using the t-test. Repeated measures ANOVA was used to compare heart rate and respiratory rate physiological data. Pain responses were statistically analyzed using Kruskal–Wallis test, and induction quality and recovery from anesthesia using the Chi-square. All results were expressed as mean±SD. The statistical significance level was considered as P<0.05.

Results
Both drug combinations used in the groups created complete immobilization and the righting reflex in the second and first group disappeared up to 4.9 and 1.4 minutes after intramuscular injection of the anesthetic compound, respectively. Duration of anesthesia induction in the first and second groups had a significant difference (P<0.05). Induction of anesthesia was faster
in the first group than the second group (Table 2).
Duration of surgical anesthesia and starting to walk time in the two groups were not statistically significantly different. The duration of light anesthesia was significantly higher in the first group than the second group (Table 2).
The quality of anesthesia induction in the first group was calmer and better than the second group (without imbalance, hand and foot rowing motion and rotation in the cage) (P<0.05). The quality of recovery from anesthesia was the same in both groups (Table 3).
During anesthesia in both groups first, the paw reflex disappeared and then the tail reflexes, and surgical anesthesia depth was created. The recovery of pain reactions was vice versa, i.e. first paw reflexes and then tail reflexes recovered. During this study, the responses of animals to laparotomy was performed to assess painful reactions. According to Figure 1, reaction to the pain of laparotomy in the first group (1.7±0.48) was less than the second group (2.9±0.38) (P=0.001). Paw and tail reflexes were not significantly different between the two groups.
Heart rate after induction of anesthesia in both groups showed no significant changes (Figure 2). While, the number of breaths per minute in the two groups at 20th, 30th, and 35th minutes were statistically different (Figure 3) they were lower in the first group than in the second group (P<0.05).

Table 2: Induction time, surgical anesthesia duration, starting to walk time, and duration of light anesthesia in male rats (time in minutes)

<table>
<thead>
<tr>
<th>Group</th>
<th>Induction of anesthesia Mean±SD</th>
<th>Surgical anesthesia Mean±SD</th>
<th>Light anesthesia Mean±SD</th>
<th>Starting to walk time Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2±0.2</td>
<td>44±5.7</td>
<td>67.6±10.5</td>
<td>73.2±10</td>
</tr>
<tr>
<td>2</td>
<td>4.4±0.5</td>
<td>34.8±8</td>
<td>37.6±5.8</td>
<td>67.6±10.5</td>
</tr>
<tr>
<td>P-value</td>
<td>0.001</td>
<td>0.111</td>
<td>0.003</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3: Quality of induction of and recovery from anesthesia in the two groups, mean±SD, male rats

<table>
<thead>
<tr>
<th>Group(s)</th>
<th>Quality of anesthesia induction (Rank 1-4)</th>
<th>Quality of recovery from anesthesia (Rank 1-4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a 3.6±0.2</td>
<td>2.3±0.3</td>
</tr>
<tr>
<td>2</td>
<td>b 2.4±0.3</td>
<td>2±0.4</td>
</tr>
<tr>
<td>P-value</td>
<td>0.004</td>
<td>0.449</td>
</tr>
</tbody>
</table>
Figure 1: Ranking of responses to painful stimuli in male rats

Figure 2: Heart rate at different times in the two groups of male rats

Figure 3: Breathing rates at different times in the intervention groups
Discussion

The results showed that subcutaneous injection of chlorpheniramine (H1 antagonist of histamine) along with the combination of acepromazine-ketamine in male rats increased the duration of anesthesia and in other words enhanced the anesthetic effects of that combination. The average induction period in Syrian mice with acepromazine (0.75 mg/kg) - Ketamine (44 mg/kg) was reported as 4.7 minutes (14). In similar studies for anesthesia of rats with a combination of acepromazine (2.5 mg/kg) - Ketamine (75 mg/kg), the time of anesthesia induction was near the second group in this study (11). In this study, the rapid induction of anesthesia in the first group compared to the second group was due to the sedative effect of chlorpheniramine and enhancing the effect of acepromazine-ketamine combination (15). There are different parts with different sizes in the human cerebral cortex of the body for the perception of somatic pain stimuli. There are also such areas in the cerebral cortex of rats. The different number of pain-sensitive nerve endings in these areas explains the different degrees of reaction to pain. Despite poor response to somatic pains in the trunk, there are large areas in the cerebral cortex for the perception of the pain stimuli in lips, face, thumbs, and limb ends. This explains higher sensitivity to pain in paw than the pain caused by tail, ear or back skin reflex (16).

The histaminergic nervous system plays an important role in pain control (17, 18). In fact, it has been found that H1 histamine receptors which exist both in the environment and in the center, play an important role in regulating pain perception (19, 20). Accordingly, the H1 histamine receptor antagonists are involved in pain control in humans and laboratory animals (21, 22). These materials modify opioid-induced analgesia (23, 24). The interference between the opioid-induced analgesia and histamines have been raised in other studies, too (25-27). Given the close functional relationship between H1 histamine receptor antagonists and the opioid system, these factors significantly influenced the level of pain in increased androgen opioid tone model (26, 27). In the present study, a significant increase of pain tolerance threshold in the first group that used an H1 histamine antagonist was evident compared to the second group in abdominal laparotomy (Figure 1), which is in line with recent studies and matches the findings of Lamberti and other researchers in this case (28, 29). However, the paw pain test was not significant at the time of surgical anesthesia between the two groups (Table 2 and Figure 1). According to reports on the amount and rate of subcutaneous and intraperitoneal absorption of drug compounds, it appears that the low absorption rate of chlorpheniramine in subcutaneous injection method have affected the low effects of the compound in the first group on tail and paw pain responses in this study (30). Although, acepromazine is effective in reducing the adverse effects of ketamine such as muscle stiffness and emotional state, but it has little effect on boosting ketamine anesthesia effect. The low effect of the acepromazine-ketamine combination on paw and tail pain response (Table 2) was because the combination always creates light anesthesia in rats and is only suitable for chemical restraining (31).

Phenothiazines such as acepromazine did not lead to significant cardiovascular weakness derivatives and had little effect on the pulmonary ventilation (5), so in Figure 3, reduced number of breaths in the first group was due to respiratory depression by chlorpheniramine (32, 33). It is stated that the intravenous administration of ketamine creates a negligible increase in blood pressure, heart rate and cardiac output in rats due to increased sympathetic activity (31), on the other hand, H1 histamine antagonists decreased heart rate (34); In this study,
changes in heart rate between the two groups was not significant (Figure 2). It appears that there is a need to increase the dose of chlorpheniramine to study the effects of the above-mentioned anesthetic combinations on rats, and chlorpheniramine at doses used in this study did not cause significant cardiovascular changes.

Conclusion
According to the results, chlorpheniramine has sedative effects on rats’ anesthesia and enhances the effect of acepromazine-ketamine combination. Analgesic effects of chlorpheniramine in combination with acepromazine-ketamine was not significant for paws and tail pain perception. In other words, chlorpheniramine is not good for analgesia or to increase the pain tolerance threshold in the spinal cord, while considering creating analgesia in laparotomy, it is good for somatic and visceral nerve analgesia.

Acknowledgments
This study was funded by the Social and Cultural Department of Ardabil University who is hereby sincerely thanked. The authors express their appreciation of the help from all those participated in this study.

Conflict of interest
The authors declare no conflicts of interest.

References: