Shahid Bahonar University of Kerman

Veterinary and Comparative Biomedical Research

Online ISSN: 3060 – 7663

Original Research

Doi:10.22103/vcbr.2024.23833.1024

Investigating the analgesic effects of combined diphenhydramine and dipyrone in mice

M. Kalantari [1], F. Faedmaleki [2], A. Soneishargh [3]*

[1] Graduated from the Faculty of Veterinary Medicine, Babol branch, Islamic Azad University, Babol, Iran
[2] Department of Basic Sciences, Faculty of Veterinary Medicine, Babol branch, Islamic Azad University, Babol, Iran
[3] Graduated from the Faculty of Sciences, Neyshabour branch, Islamic Azad University, Neyshabour, Iran

***Correspondence:**

Author email:
Ffaedmaleki@baboliau.ac.ir

Article history:

Received: 03 June 2024
Revised: 06 July 2024
Accepted: 03 August 2024
Published:12 August 2024

Keywords:

Analgesia
Diphenhydramine
Dipyrone
Pain

Abstract Pain relief is a crucial aspect of modern surgery. The analgesic used should possess strong analgesic properties, a rapid onset of action, and minimal side effects to effectively reduce pain during and after surgical procedures. Therefore, the aim of the present study was to evaluate the analgesic effects of diphenhydramine and dipyrone (metamizole) in mice. For this study, 30 mice were randomly divided into five groups: the control group received normal saline; the standard group received 10 mg/kg morphine; treatment group 1 received 2.5 mg/kg diphenhydramine; treatment group 2 received 2.5 mg/kg dipyrone; and treatment group 3 received 2.5 mg/kg diphenhydramine combined with 2.5 mg/kg dipyrone. After injection, pain was assessed using the hot plate test and the formalin test. The results from both tests indicated that the combined use of dipyrone and diphenhydramine produced significant analgesic effects. Additionally, neither dipyrone (metamizole) nor diphenhydramine exhibited any side effects. Therefore, the use of dipyrone (metamizole) and diphenhydramine is recommended for safer surgical procedures.

Introduction

Pain is a universal human experience with a long history. Understanding and treating pain has been a focus of medicine since ancient times, leading to many important discoveries. Pain arises from various causes, including surgical procedures. It is a protective mechanism triggered by harmful stimuli that signals tissue damage. Diphenhydramine is an antihistamine drug widely used to relieve allergy symptoms like itching, eye/nose inflammation, nausea, dizziness, and insomnia. It also has local anesthetic properties. However, it should be used with caution in young children as it can cause side effects like fatigue and impaired coordination [1]. Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It involves sensory, motivational and cognitive components influenced by factors like genetics, culture, age, and gender. Pain management is a critical part of postoperative care. Reducing reliance on opioid painkillers due to side effects is an important goal, with alternatives like combining opioids with non-opioid drugs being explored. Pain serves a defensive purpose but can significantly impair life quality when chronic. Understanding pain

mechanisms aids development of new analgesic drugs [2, 3].

Metamizole (dipyrone) is a pain medication with fever-reducing, anti-inflammatory and muscle-relaxing effects similar to NSAIDs. It appears to be a safe drug with minimal toxicity. In addition to human use, it has applications in veterinary medicine for conditions like colic in horses. The study aimed to investigate the combined pain-relieving effects of diphenhydramine and metamizole in hamsters [1].

The way non-steroidal anti-inflammatory drugs (NSAIDs) work is distinct from that of steroid medications (corticosteroids). As indicated by their name, NSAIDs do not contain cortisone. These medications alleviate pain, inflammation, and fever by blocking enzymes known as cyclooxygenases (COX), which are involved in the production of prostaglandins. However, this inhibition can also result in various side effects, since prostaglandins perform numerous important physiological roles in the body. While the mechanism of action is consistent, the therapeutic effects and side effects of NSAIDs can differ among individuals [4].

Metamizole, also known as dipyrone, is a non-opioid pain reliever and fever reducer derived from pyrazolones. It comes in injectable, oral, and rectal forms. Due to toxic side effects, metamizole use is banned in the US and other developed countries. However, some argue it has fewer side effects than other drugs in its class. Based on this, metamizole has been used as a strong pain and fever medication since 1992. When exposed to liquids, metamizole slowly breaks down into 4-methylaminoantipyrine, which is the active pharmacological metabolite. After oral dosing, metamizole is rapidly absorbed, with peak levels in dogs at 90-120 minutes. Its metabolites are mainly excreted by the kidneys and don't bind much to plasma proteins. Like other NSAIDs, metamizole reduces inflammation by inhibiting prostaglandin production in the central and peripheral nervous systems. Its peripheral effects depend more on dose than prostaglandin inhibition in injured tissues. Its active metabolites disrupt the cyclooxygenase enzyme via an iron-dependent mechanism. Metamizole's peripheral actions involve energy-sensitive potassium channels and the L-arginine pathway in sensory neurons, similar to opioid painkillers. It has greater muscle relaxant effects compared to other drugs in its class, confirmed in lab and clinical studies. At therapeutic doses, metamizole has fewer side effects than other pyrazolone derivatives. High doses can cause increased saliva, nausea, appetite loss, and elevated liver and kidney tests in dogs. Unlike some NSAIDs, it does not appear to cause gastrointestinal ulcers in rats even with varying doses. Compared to diclofenac, metamizole and acetaminophen cause less bleeding, anemia, and kidney problems [5]. So, current study conducted to Investigate the analgesic effects of combined diphenhydramine and dipyrone in Mice. Because, according to the best knowledge of the authors,there is no published data about combined use of diphenhydramine and dipyrone in mice.

Materials and Methods

Animals

This research involved mice weighing between 25 and 35 g, which were kept under standard conditions featuring a 12-hour light-dark cycle and a temperature of 25 °C. The mice had access to free water, food, and suitable bedding. The study adhered to ethical guidelines for laboratory animal use, with the mice randomly assigned to five groups of six individuals each, as defined in the grouping.

Grouping

The control group received normal saline; the standard group received 10 mg/kg morphine; treatment group 1 received 2.5 mg/kg diphenhydramine; treatment group 2 received 2.5 mg/kg dipyrone; and treatment group 3 received 2.5 mg/kg diphenhydramine combined with 2.5 mg/kg dipyrone. After injection, pain was assessed using the hot plate and the formalin tests.

Experimental Method

For the formalin test and to record analgesic behaviors, either an extract or distilled water was administered 30 min prior to the experiment. The animals were then placed in the formalin test chamber to acclimate and reduce stress. After a 15-min period, 20 ml of a 2.5% formalin solution was injected subcutaneously into the right hind paw using an insulin syringe, and the animals were immediately placed back in the formalin test chamber. Observations of the animals' behavior were made in a plexiglass chamber measuring 30x30x30 cm, equipped with mirrors at a 45-degree angle to the horizontal surface. Pain-related motor responses were recorded every 15 seconds and scored as 0, 1, 2, or 3: score 0: The animal maintains full balance while walking, with body weight evenly distributed on both legs. Score 1: The animal does not bear weight on the injected leg but has no difficulty walking. Score 2: The animal lifts the painful paw and does not make contact with the chamber floor. Score 3: The animal licks or shakes the painful leg vigorously. The pain score was assessed over 90-min period.

Statistical Analysis

The data collected in this study were analyzed using Prism statistical software version 9.1.0, employing descriptive statistics along with one-way and repeated measures ANOVA and Tukey's HSD post hoc for statistical analysis and P-value < 0.05 was considered statistically significant.

Results

In this study, 30 mice were randomly assigned to 5 groups, each consisting of 6 mice, based on predefined categories. Following the intraperitoneal injection of the drugs, pain evaluation for each group was carried out using the hot plate and formalin tests.

Discussion

Researchers conducted a study comparing the

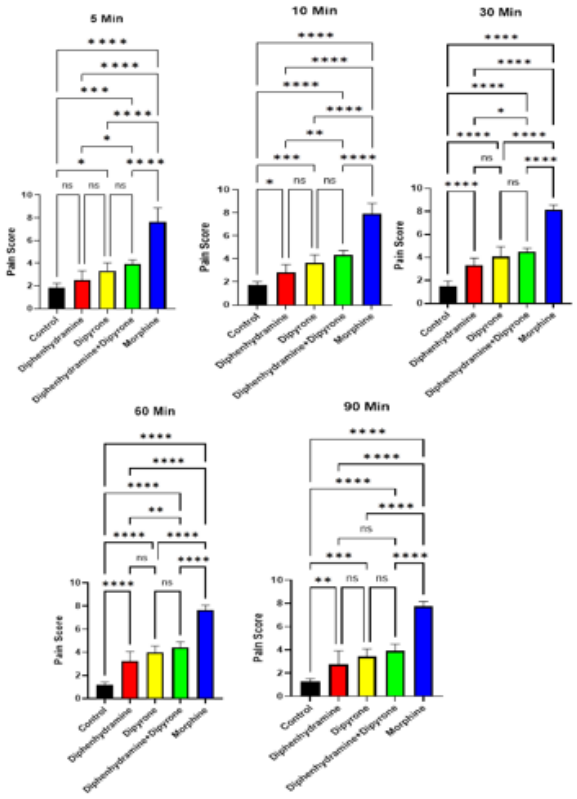

Fig 1. Hot plate Test, 5, 10, 30, 60 and 90 min after injection, on 5 groups of 6 mice, $p < 0.05$,

*: number of astricks shows significancy
NS: Not Significant

clinical and histopathological effects of metamizole (dipyrone) and midazolam as anesthetic premedication in pigeons. The results from the histopathological examination of liver and kidney tissues indicated that pigeons treated with metamizole exhibited the least amount of tissue changes compared to those treated with midazolam. Other researchers found that metamizole (dipyrone) had no toxic effects on neuronal cells and was even effective in treating neuronal cell damage [6, 7].

Jadzyniak et al. (2013) and Naga et al. (2012) proposed a rapid method to determine the effects of metamizole on ruminant muscle through a technique called Liquid Chromatography - Tendon Spectrometry.

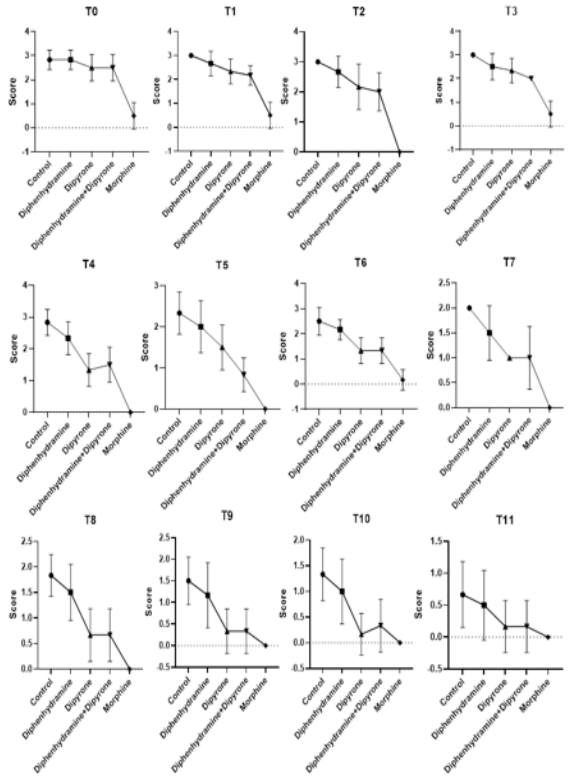

Fig 2. Formalin Test, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 min after injection, on 5 groups of 6 mice, $p < 0.05$,

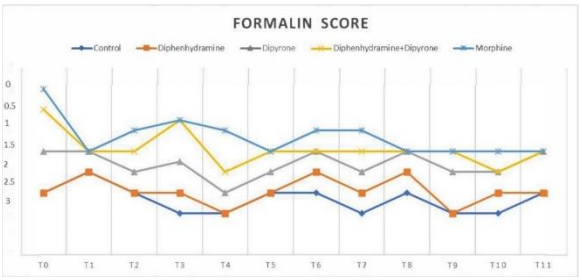

Fig 3. The results of formalin test in different groups and between time 0 and 11 of the study, on 5 groups of 6 mice, $p < 0.05$

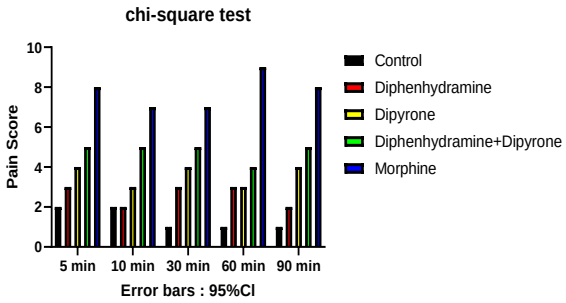

Fig 4. Comparison of pain score between groups by Chi- square test, 5 groups of 6 mice, $p < 0.05$

They confirmed this method by calculating a muscle performance recovery rate of 30-7% and a recovery rate of 95-45%, indicating that the drug is safe for somatic cells. Another study examined the effects of metamizole (dipyrone) and tramadol on severe renal pain, revealing that metamizole provided better analgesic effects compared to tramadol, significantly reducing acute renal pain at 20, 30, and 50 minutes post-administration [8, 9, 10]. Researchers such as Patel & Kopikar (2014) stated that using metamizole for managing pain from abdominal surgeries does not lead to any adverse side effects. Their findings are consistent with the current results. They also investigated the effects of metamizole on bone healing from tibia fractures in rats, reporting that metamizole positively influenced fracture pain without interfering with bone healing [11]. Additionally, other researchers have reported that the use of metamizole significantly improves gastrointestinal function and is safe in this regard [12].

Researchers conducted a study examining the effect of a single dose of dipyrone (metamizole) for acute postoperative pain in adults and reported that in more than 70% of participants, at least a 50% reduction in pain was experienced within four to six hours with oral dipyrone 500 mg compared to 30% with placebo. With dipyrone 500 mg compared to placebo, fewer participants required rescue medication within four to six hours (7% with dipyrone versus 34% with placebo: four studies; 248 participants). Also, no serious adverse events or withdrawals due to adverse events were reported.Dipyrone or metamizole is a non-steroidal anti-inflammatory drug used for pain management in some countries (postoperative, colic, cancer, and migraine); its administration is prohibited in other countries due to its association with potentially life-threatening blood disorders. This review is a replacement for the Cochrane review that was withdrawn in 2010 [6]. Farzin and Asghari investigated the effects of various histamine receptor agonists and antagonists on pain threshold induced by hot plate and abdominal writhing in mice. They reported that

intracerebroventricular injection of the histamine H1 receptor agonist 2-methylhistamine (50 µg/mouse) caused significant Hyperalgesia in hot plate and writhing tests. This dose of 2-methylhistamine had no significant effect on motor coordination in the Rota-rod test. Intraperitoneal injection of histamine H1 receptor antagonists, dexchlorpheniramine (30 and 40 mg/kg) and diphenhydramine (20 and 40 mg/kg), caused a dose-dependent antinociception in both hot plate and writhing tests, but since all doses of diphenhydramine used in this experiment caused motor impairment in the Rota-rod test, it seems that the antinociceptive effect of diphenhydramine is not a true antinociceptive effect [13]. It is noteworthy that histamine is a neurotransmitter in mammalian brains that exerts its physiological effects on target cells by stimulating three types of receptors (H1, H2, and H3). There are now reports showing that histamine plays a role in modulating pain transmission. For example, intracerebroventricular injection of histamine produces dose- or site-dependent antinociceptive or hyperalgesic effects. Intracerebroventricular injection of low doses of histamine induces Hyperalgesia, while high doses produce Analgesia. Injection of histamine into the Dorsal raphe nucleus and Periaqueductal grey areas produces antinociceptive effects, while injection into the Median raphe nucleus lowers the pain threshold. These results suggest that the opposing central effects of histamine on pain threshold may be due to stimulation of different histamine receptors [14].In a study, researchers compared the analgesic effects of diphenhydramine and acetaminophen and reported that prophylactic diphenhydramine 0.4 mg/kg during induction of general anesthesia along with acetaminophen 1 g and ondansetron 4 mg at the end of laparoscopic sleeve gastrectomy surgery reduced the incidence of postoperative pain severity , which is consistent with the findings of the present study regarding the effect of diphenhydramine on reducing analgesia. In another study, researchers investigated the rapid analgesic effect of diphenhydramine antihistamine ointment compared to indomethacin (a common non-steroidal anti-inflammatory drug) on bone-joint-muscle pain and reported that diphenhydramine ointment had a rapid and distinct analgesic effect that lasted for several hours, as assessed by skin impedance or subjective pain evaluation. In contrast, the analgesic effect of indomethacin ointment was marginal and only noticeable an hour or more after diphenhydramine. These results suggest that diphenhydramine ointment may be useful for relieving musculoskeletal pains common in the elderly. It is noteworthy that pain is sensed, transmitted, and modulated through various mediators and their receptors. Histamine is a known pain mediator. Classic antihistamines, in addition to their antagonistic effects on histamine, have varying degrees of anticholinergic, antiserotonergic, antiadrenergic, local anesthetic, membrane stabilizing, and other drug effects. Although considerable efforts have been made to use classic antihistamines as analgesics or analgesic adjuvants, the emergence of non-steroidal anti-inflammatory drugs has halted such efforts [15].

Conclusion

The findings obtained in the present study indicate a significant analgesic effect from the combined use of diphenhydramine and dipyrone (metamizole). It is also noteworthy that these two drugs with this dose used, the consumption consequences are the least, whereas the complications associated with common opioids like morphine have been well established for years. Therefore, for safer surgical procedures, the combined use of diphenhydramine and dipyrone (metamizole) is recommended.

Acknowledgements

Not applicable

Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

1. Lin HC, Riddell MG. (2003). Preliminary study of the effects of xylazine or detomidine with or without butorphanol for standing sedation in dairy cattle, Vet Ther; 4(3):285-91.
2. Borra RC, de Mesquita Barros F, de Andrade Lotufo M, Villanova FE, Andrade PM. (2009). Toll-like receptor activity in recurrent aphthous ulceration. J Oral Pathol Med, 38(3):289-98.
3. Guimarães AL, Correia-Silva Jde F, Sa AR, Victória JM, Diniz MG, Costa Fde O, et al. (2007). Investigation of functional gene polymorphisms IL-1beta, IL-6, IL-10 and TNF-alpha in individuals with recurrent aphthous stomatitis, Arch Oral Biol; 52(3):268-72.
4. Rezende, R.M. Franca, D.S. Menezes, G.B. and Bakhie, Y.S. (2007). Different mechanisms underlie the analgesic actions of paracetamol and dipyrone in a rat model, British Veterinary Journal of Pharmacology, 153(4): 760-768.
5. Sánchez, S.; De La Lastra, CA. Ortiz, P.; Motilva, V. & Martín, MJ. (2002). Gastrointestinal tolerability of metamizol, acetaminophen, and diclofenac in subchronic treatment in rats, Digestive Diseases and Sciences, 47(12), 2791- 2798.
6. Sokolov, A.Y. and Sivachenko, I. and Panteleev, S.S. (2014). Effect of intravenous metamizol on ongoing and evoked activity of dura–sensitive thalamic neurons in rats, Journal of Veterinary Anesthesia and Analgesia, 4(1): 31-32.
7. Zhang, Y. and Wang, X. (2014). Dipyrone inhibits neuron cell death. Journal of Pet Medicine, 69(4): 942-956.
8. Jedziniak, P., Pietruk, K. and Olejnik, M. (2013). Rapid method for the determination of metamizol residues in bovine muscle by LC – MS / MS, Journal of Veterinary Anesthesia and Analgesia, 2(6): 82-84.
9. Naga, R., Anjaneyulu, N. and Canesh, M.N. (2012). Evaluated the analgesic activity of metamizole and paracetamol, Journal of Advanced Pharmacology Researches, 3(1): 1-4.
10. Flor, B., Yazbek, V.B. and Keila, K. (2013). Tramadol plus metamizol combined or not with NSAIDS is clinically effective for moderate to severe chronic pain, Journal of Veterinary Anesthesia, 11(10): 21-23.
11. Patel, C.V. and Koppikar, M.G. (2014). Management of pain after abdominal surgery.Journal of Clinical Sciences, 10(2): 351-353.
12. Japr, A. (2012). Monitoring of anesthesia depth in birds and reported that the corneal reflex in birds, Journal of Poultry Science, 27(5): 5-9.
13. Farzin D, Saadati R. (2004). The effect of diphenhydramine on the neuromuscular transmission of the chick biventer cervicis muscle preparation, J Mazandaran Univ Med Sci; 14 (44):1-13.
14. Liu W., Yang X., Li C., Mo A. Adverse drug reactions to local anesthetics: a systematic review, Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2013; 115(3):319–327.
15. Fujita T, Ohue M, Fujii Y, Jotoku T, Miyauchi A, Takagi Y, Tsuchiya M, Endo Y. Prompt analgesic effect of antihistaminic diphenhydramine ointment on bone-joint-muscle pain as assessed by skin impedance, Pharmacology. 2013; 92(3-4):158-66. Doi: 10.1159/000354151. Epub 2013 Sep 5. PMID: 24008505.

How to cite this article:
Kalantar, M., Faedmaleki, F., Soneishargh, A. Investigating the analgesic effects of combined diphenhydramine and dipyrone in Mice. Veterinary and Comparative Biomedical Research, 2024, 1(2): 66 – 71. http://doi.org/10.22103/vcbr.2024.23833.1024