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Evaluation the combination of chlorpheniramine and tramadol at a level of thermal and visceral antinociceptive in a mouse acute pain model

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Abstract

The possible benefits of employing combination treatment include the ability to increase antinociceptive effects while minimizing the occurrence of unfavorable side effects. As a result, they are combining drugs that provide analgesic synergism allowing for a reduction in needed dosage as well as lowering the occurrence of unwanted side effects. In the current study, we evaluated quantitatively and qualitatively the type of drug interaction between tramadol (a typical opioid analgesic) and chlorpheniramine (an H_1-antagonist) at the level of thermal (hot plate) and visceral (writhing reflex) nociceptive stimuli in mice model. The 50% antinociceptive effective dose (ED_{50}) of intraperitoneal administration for tramadol and chlorpheniramine was 12 and 18.4 mg/kg respectively, using the up and down approach and hot plate apparatus. The treated animals showed signs of sedation and immobility. At 0.5:0.5 and 1:1 ED_{50} ratios of each, the kind of pharmacological interaction between the two medications was synergism at the level of acute antinociceptive impact, using hot plate apparatus and isobolographic analysis. The reduction in ED_{50} value was significant for tramadol and chlorpheniramine by 58.8 and 58.8 % at 0.5:0.5 ratio while 53.5 and 53.5 % at 1:1 ratio respectively. The synergistic interaction between the two drugs was also confirmed using the double ED_{50} dose of each drug as simultaneously i.p. injected of these doses producing a synergism antinociceptive effect at visceral (writhing reflex) test. Which represented as prevent 100% writhing induced by i.p. injection of acetic acid compared to the control group and that with each drug alone at the same double doses. The present results concluded that simultaneous injection of tramadol and chlorpheniramine produced synergism, a potent and safe antinociceptive effect even at low doses which may be clinically useful in treating pain in the veterinary clinic.

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Introduction

Pain is defined by The International Association for the study of pain, as an unpleasant sensory and emotional experience linked to actual or potential tissue injury (1,2). Pain is divided into three categories nociceptive pain, neuropathic pain, and pain from musculoskeletal disorders, nociceptive pain resulting from tissue damage, neuropathic pain resulting from nerve injury, and neuroplastic pain is the third type of pain (2). Tramadol hydrochloride ((+)-trans-2-[(dimethylamino) methyl] -1 -(3-methoxy-phenyl)-cyclohexanol HCl) is a therapeutically effective and centrally acting analgesic (3,4). Used in both human (4) and veterinary medicine (5-7). It can be used to relieve acute, dental, labor, chronic, and cancer pain (1).Tramadol is a racemic combination of two enantiomers, each with a different but complementary mode of action in contrast to other opioid receptor agonists: the (+) enantiomer is a selective μ-receptor agonist that inhibits serotonin reuptake and increases serotonin efflux in the CNS, whereas the (-) enantiomer primarily inhibits norepinephrine reuptake (8,9).Chlorpheniramine is an alkylamine subtype utilized as

an antihistamine H_1 in both humans (10,11) and animals (12,13). It is commonly used to treat the symptoms and diseases of allergy (14-17). It also has analgesic properties by the mechanism which was previously investigated at the visceral (18,19) and cutaneous (20) levels. While their systemic analgesic was excluded (19). Different combinations of tramadol and other analgesics (20), were tested in order to provide good analgesia at low doses, minimize the side effects and extend the analgesia, as a combination of tramadol with naproxen (21), diclofenac (22), acetaminophen, and Ibuprofen (23), ketorolac (24) all of them produce a good analgesic effect. As the hyper nociception can be induced by activating histamine H_1 receptors (25) the antinociceptive impact of antihistamine H_1 receptors has been well documented, both in human and laboratory animals (26). Therefore, the combination of antihistamines with tramadol was also examined, but only to a limited extent (27). Diphenhydramine was one of the combinations combined with tramadol in mice but failed to provide adequate analgesia (28).

The goal of our research was to determine the efficacy of another H_1 antihistamine, Chlorpheniramine, in the antinociceptive effect of tramadol as a combination in mice as well as to assess the kind of pharmacological interaction between the two medicines at the level of visceral and thermal analgesia in order to achieve a novel analgesic combination, which had not been previously evaluated.

Materials and methods

Ethical approval

All mice in this research were handled according to institutional regulations on animal handling and use in research, we obtained official approval for the study protocol from the Committee of Postgraduate Studies at the College of Medicine, University of Mosul, Iraq.

Animals model

Adult (male and female) albino Swiss mice weighed between 22-34 g were utilized in all experiments. The mice were kept in the house of animals which is located in The College of Veterinary Medicine, University of Mosul. The animals were housed as 8 per cage with 12 h of light and 12 h of darkness, at a temperature of 20±2ºC. Food and drink were always available to the animals. All experiments were carried out in accordance with the ethical Guide Lines of the Ethical committee from animal Research and the study of pain (29). The scientific committee of the college of veterinary medicine, department of physiology, biochemistry, and pharmacology reviewed and approved the study protocol.

Drugs

The doses of tramadol (100 mg/2ml, DUOPHARMA, Malaysia) and a pure powder of chlorpheniramine (Pioneer pharmaceutical, Iraq) and their combinations were prepared freshly in a solution of physiological saline (Marksans pharma, India) and administered intraperitoneally in a final volume of injection at 5ml/kg. Acetic acid 99.99% (TEDIA, USA) diluted with distal water to 1%.

Detection the ED_{50} and the type of drug interaction

This experiment was designed according to the up-and-down procedure (30). To calculate the median effective dosage of ED_{50} of tramadol and chlorpheniramine each alone or as a combination in mice at ratios 1:1 and 0.5:0.5 of ED_{50} of each one. The kind of pharmacological interaction between two drugs was also discovered in this experimental protocol. All experiments were applied using a hot plate test as a quantitative method for evaluating the antinociceptive effect of drugs induced by thermal pain stimuli. The antinociceptive effect of each drug was evaluated 15 minutes, after intraperitoneal injection of every single dose of tramadol and chlorpheniramine by placing the individual animal on the surface of the hot plate apparatus at a temperature of 56±1ºC (28). The first hind paw response as paw withdrawal, licking, and/or jumping was measured as the latency time of response to heat painful. The cutoff point was 20 sec (31).

To calculate the individual ED_{50} of tramadol and chlorpheniramine, 11 mice used weighting 22-34 g. the first tramadol dose was administered intraperitoneally to the first mouse at 10 mg/kg (initial dose) whereas the initial chlorpheniramine dose was 20 mg/kg, the dose was selected dependent on initial trials and other studies of tramadol (32) and chlorpheniramine (33). The animals were used separately in two separate trials. 15 minutes after injection in each experiment, the mouse was put on the hot plate surface. The latency (in seconds) of response to heat stimuli was recorded using the stopwatch. The decrease and increase in the subsequent doses were at a fixed ratio of 2.5 mg/kg for tramadol and 5 mg/kg for chlorpheniramine which represented 25% of ED_{50} of each drug. Each animal had a baseline latency time before the injection of an individual dose of each drug and then recorded the latency time 15 minutes after each later dose of each drug. The ED_{50} value was detected finally by using the following equation $ED_{50}=xf+kd$ (34).

Kind of pharmacological interaction between chlorpheniramine and tramadol

11 mice were used to determine the kind of pharmacological interaction between the two medicines at the level of analgesia. In two separate trials, tramadol and chlorpheniramine were simultaneously injected into the first mice at 12 and 18.4 mg/kg intraperitoneally, respectively which represented the ED_{50} of each 1:1 ratio. While the first animal in the second trial was injected with tramadol and chlorpheniramine at 6 and 9.2 mg/kg intraperitoneally, respectively as half of the ED_{50} value of each 0.5:0.5 ratio.

The doses previously obtained in experiment one. 15 minutes after the injection of the dose in each trial the mice were subjected to evaluate the analgesic effect on the hot plate surface as in experiment one (31). The fixed up and down in subsequentED_{50}value of tramadol and chlorpheniramine was 3 and 4.6 mg/kg respectively at a 1:1 ratio. While in the 0.5:0.5 ratio trial the doses were at 1.5 and 2.3 mg/kg respectively. The effective 50% dose of each drug at each ratio was detected as in experiment one. To explain the type of interaction between tramadol and chlorpheniramine at 1:1 and 0.5:0.5 ratio of$ED_{50}$of each, the isobolographic analysis was used (34,35). Fixing individual ED_{50} of each drug on a different axis of graphic paper. Then connected the individual ED_{50} in a straight line. The ED_{50} of the combination at each ratio was plotted on the graphic paper. If located at the diagonal line, the interaction will be additive (no interaction). while falling above or below the line the interaction is considered as antagonism or synergism (34,35).

The interaction index was also used to obtain the value of Y as an additional confirmation to indicate the interaction between the two drugs, by using the following formula [Y=da/Da+db/Db], if Y value > 1 means pharmacological interaction is antagonism, while if $Y \leq 1$the interaction is synergism and no interaction respectively (35,36). We also obtained the percentage (%) of reduction in ED_{50} value of each drug at each ratio of combination by using the equation %reduction ofED_{50}=individual ED_{50}-ED_{50}(interaction)/ individual ED_{50}×100 (36).

The writhing reflex (visceral pain)

To additional support for the antinociceptive effect by hot plate (central and peripheral pain), tramadol and chlorpheniramine alone or together were evaluated for antinociception activity by writhing reflex test (visceral pain) (37). This procedure is considered as a quantitative method by recording the cumulative number of writhing between 0 and 30 minutes after injection of nociception stimuli (37). The mice were individually divided into four groups of six animals each. Group I was considered as a control, which was intraperitoneally injected with normal saline. Group Π was treated with tramadol at 24 mg/kg. Group Ш was treated with chlorpheniramine at 36.8 mg/kg, intraperitoneally. While the last group (IV) was administrated with tramadol and chlorpheniramine at24 and 36.8 mg/kg intraperitoneally, respectively. The dose of tramadol and chlorpheniramine was obtained from experiment one as a double dose of ED_{50} of each. 30 minutes after the injection of drugs each animal in all four groups was intraperitoneally injected with 0.1ml/10g b.w. of 1% acetic acid (37). Immediately after the injection of acetic acid, the mice in each group were observed individually for recording the latency time of the onset of the writhing reflex. The number of writhing reflexes (constriction of abdominal muscle accompanied by the extension of the hind limb) was also recorded for 30 minutes in each group (37). The percentage of reduction in writhing was also calculated in each group is compared with the control group (acetic acid alone) by using the equation. Writhing inhibition = mean No. of control - mean No. of test/mean No. of control × 100 (38).

Exploring the antinociceptive effect of a non-analgesic dose of tramadol and chlorpheniramine on hot plate

On the heated plate, the antinociceptive impact of a non-analgesic dose of tramadol and chlorpheniramine each alone or together was determined. We designed the experiment by randomly dividing 20 mice at a weight of 26-35 g into separated four groups of 5 mice each and intraperitoneally injected as follows: group I respected as the control group (normal saline), Group Π and Ш were injected with sedative non-analgesic doses of tramadol and chlorpheniramine at 6 and 9 mg/kg, respectively. While group IV is considered a combination group that received tramadol and chlorpheniramine together at 6 and 9 mg/kg. Each animal in each group had a baseline of latency time and then repeated 15 minutes after injection of each drug. All experiments were performed on the hot plate apparatus. The dose for each drug was obtained from experiment one (50% of ED_{50}) of each drug.

Statistical analysis

The parametric data in experiments three and four were statistically evaluated using One Way Analysis of Variance (ANOVA) via using Sigma plot software version 12.5 and the analysis between different treatment groups were performed by the least significant test (LSD). While Mann Whitney U test was applied to nonparametric data (scores) as the number of writhing (64). All data were presented as mean ± SE. The data were considered statistically significant at a P level less than or equal to 0.05.

Results

Estimation the individual ED_{50} of tramadol and chlorpheniramine in mice

By using the up and down method on the hot plate apparatus, the ED_{50} of tramadol and chlorpheniramine each alone were 12 and 18.4 mg/kg b.w respectively. These doses produced a 50% antinociceptive effect 15 minutes after intraperitoneal injection in mice (Table 1). All up and down doses of each drug were combined with signs of sedation, quiet, and immobility (Table 1).

The type of interaction between tramadol and chlorpheniramine

The combination ED_{50} value, which was determined by simultaneously intraperitoneal injection of tramadol and chlorpheniramine at ratios of 1:1 and 0.5:0.5 of ED_{50} of each alone was 5.58:8.56 mg/kg and 4.94:7.58 mg/kg, respectively (Table 1). The percentage of reduction in the

value of ED_{50} of tramadol and chlorpheniramine each alone was 53.5 and 53.5% respectively at 1:1 and 58.8 and 58.8% respectively at 0.5:0.5 in comparison with individual ED_{50} of each drug alone (Table 1). The kind of pharmacological interaction between two medications at 1:1 and 0.5:0.5 of individual$ED_{50}$of each was synergism dependent on isobolograpgic analysis method. The falling of combination ED_{50} at 1:1 and 0.5:0.5 under the diagonal straight line between the ED_{50} of each drug on graph paper indicated the synergism interaction between the two drugs (Figure 1). The results were confirmed by the estimation of the Y value from the interaction index equation. The Y values were 0.92 and 0.82 at 1:1 and 0.5:0.5 respectively (Table 1). This is considered more indicator of the synergism pharmacological interaction between two medications at the level of central analgesia as Y<1(Table 1).

Table 1: Determination of ED_{50} of tramadol and chlorpheniramine each alone or as combination in mice on hot plate

Variable	Tramadol	Chlorphen	Combination at0.5:0.5		Combination at1:1	
			Tramadol	Chlorphen	Tramadol	Chlorphen
ED_{50}(mg/kg)	12	18.4	4.94	7.58	5.58	8.56
Dose range (mg/kg)	15-10=5	20-15=5	6-4.5=1.5	9.2-6.9=2.3	12-3=9	18.4-4.6=13.8
First dose (mg/kg)	10	20	6	9.2	12	18.4
Last dose (mg/kg)	12.5	20	6	9.2	3	4.6
Dose change (mg/kg)	2.5	5	1.5	2.3	3	4.6
Number of mice	6(OOXXOX)*	5(OXXOX)*	5(XOXOX)*	5(XOXOX)*	6(XXOXXO)*	6(XXOXXO)*
% decrease in ED_{50}			58.8%	58.8%	53.5%	53.5%
Y value			0.82	0.82	0.92	0.92

*X: analgesic effect, O:no analgesic effect. The up and down approach used to calculate ED_{50} value.

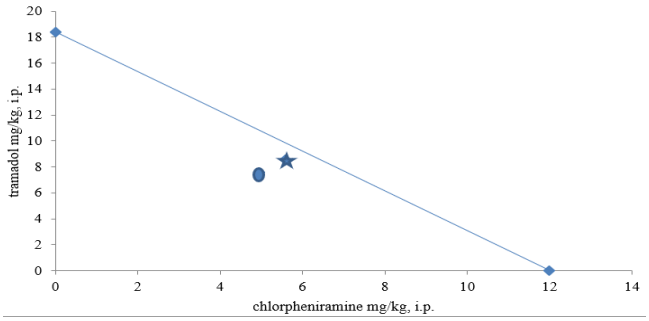

Figure 1: Isobolographic analysis of tramadol and chlorpheniramine interaction at 0.5:0.5 and 1:1 ratio in mice. The ED_{50} of each drug connected by diagonal line. (circle) 0.5:0.5 and (star) 1:1 point represented the ED_{50} combination of two drugs, fall down under the diagonal line, indicated synergism interaction.

Effect of doubleED_{50} dose of tramadol and chlorpheniramine on acetic acid produce writhing reflex (visceral pain)

Intraperitoneal injection of double ED_{50} dose of tramadol at 24 mg/kg and chlorpheniramine at 36.8 mg/kg each alone in mice 30 minutes before the intraperitoneal injection of acetic acid, significantly decreased the number of writhing induced by acetic acid in comparison with the control group (acetic acid alone) (Table 2), while co-administration of tramadol and chlorpheniramine at the same doses prevent the writhing reflex by 100% when compared with tramadol and chlorpheniramine each alone as well as with control group (Table 2 and Figure 2). There was a significant increase in the onset of writhing in the group treated with tramadol or chlorpheniramine alone compared with the control group, while the combination of the two drugs at the same double dose produce a significant increase in the onset time of writhing compared to the control group as well as with the two drugs each alone at the same doses (Table 2).

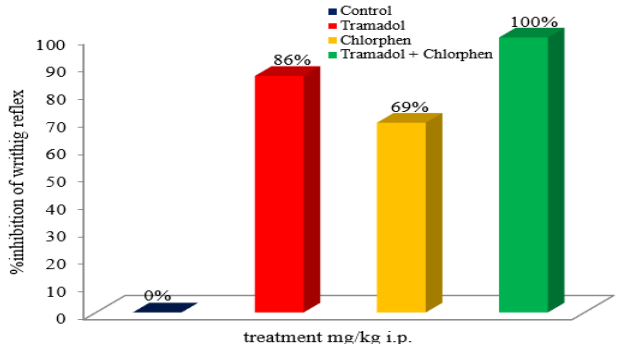

Figure 2: Effect of tramadol 24mg/kg i.p and chlorpheniramine 36.8 mg/kg i.p either individually or in combination on mice's visceral pain (writhing reflex).

Effect of sub analgesic dose of tramadol and chlorpheniramine on the thermal nociception

Sub-analgesic doses of tramadol and chlorpheniramine at 6 and 9 mg/kg intraperitoneally as ED_{25} failed to produce a significant analgesic effect on the hot plate surface (Table 3). Whereas simultaneously intraperitoneal injection of the same sub-analgesic doses of both drugs induced a complete antinociceptive effect 100% in comparison with control, tramadol, and chlorpheniramine each alone (Table 3), with no apparent side effects (as deep sedation).

Table 2: percentage of inhibition for tramadol and chlorpheniramine each alone or as combination by using writhing test

Treatment	Onset time (minute) of writhing	NO. of writhing (score)/30 min	Inhibition %
Control (acetic acid)	2.39 ± 0.17	108.8 ±2.7	0 %
Tramadol 24mg/kg, i.p.	8 ± 0.27*	1.8*a ± 14.6	↓ 86 %
Chlorpheniramine 36.8mg/kg i.p.	8.32 ± 0.44*	33 ± 4.2*	↓ 69 %
Tramadol and Chlorpheniramine	0.00^{*ab} ± 0.00	0.00^{*ab} ± 0.00	↓ 100%

Acetic acid was injected intraperitoneally 30 minutes after injection of tramadol and chlorpheniramine in each group. *Significant difference from the control group (P≤0.05). [a] significant difference from Chlorpheniramine 36.8mg/kg group (P≤0.05).[b] significantly differences from Tramadol 24mg/kg group (P≤0.05).

Table 3: Effect of tramadol and chlorpheniramine in sub-analgesic doses (low doses) on the thermal pain (hot plate) in mice

.	Latency time	Latency time After 30 minutes	Analgesia %
Control (normal saline 0.9%) i.p.	3.85 ± 0.081	3.64 ± 0.1	0 %
Tramadol 6 mg/kg i.p.	3.60 ± 0.15	3.40 ± 0.11	0 %
Chlorpheniramine 9 mg/kg i.p	3.854±0.20	3.71 ± 0.23	0 %
Tramadol + Chlorpheniramine	3.75 ± 0.13	$7.15 \pm 0.27^{\#ab}$	100 %

Chlorpheniramine was injected directly after tramadol. #: significant to control group at P≤0.05. [a]: significant to Tramadol 6 mg/kg group at P≤0.05. [b]: significant to Chlorpheniramine 9 mg/kg group at P≤0.05.

Discussion

We aimed to investigate the antinociceptive activity of chlorpheniramine alone or as a combination with the conventional medication tramadol. This study is considered the first research that evaluated qualitatively and quantitatively the kind of pharmacological interaction between the two medications at the level of acute pain in mice model using two types of nociceptive stimuli, thermal (hot plate) and visceral (writhing reflex),by using hot plate apparatus at 56℃ (39) and up and down method (30), we detected the individual ED_{50} of tramadol and chlorpheniramine by intraperitoneal injection of each in mice, the doses were 12 and 18.4 mg/kg respectively.

The ED_{50} of tramadol was in agreement with that in the previous study (40). While ED_{50} of chlorpheniramine is regarded as the first detection in our research as no previous research referred to or detected using the same method and apparatus in the mice model. There was only one study that referred to the analgesic dose of chlorpheniramine in mice on the hot plate but not as the up-and-down ED_{50} dose (41). Our current study confirmed the findings of previous studies that referred to the antinociceptive effect of chlorpheniramine (19) and tramadol (40) on the hot plate, which was illustrated by increasing the pain threshold.

We also tested the analgesic action of tramadol and chlorpheniramine each alone on visceral pain (writhing test) (37) and we revealed that the double ED_{50} of each alone succeeded in relieving visceral pain by significantly decreasing the number of writhing reflexes in mice. We used a double dose of chlorpheniramine 36.8 mg/kg in visceral pain perception, as the previous study showed that the dose of 20 mg/kg i.p. of chlorpheniramine failed in producing an antinociceptive effect in acetic acid-induced visceral pain (42). Our findings in the current study agreed with previous research for tramadol (21) and chlorpheniramine (18) as each of them alone produced a good visceral antinociceptive effect.

Combinations of antinociceptive medications are frequently used in the treatment of pain in order to increase or maintain analgesia, low doses needed as well as to reduce the adverse effects of each drug (43,44). When two or more medications are concomitantly used, they may be exerted a distinct effect producing additive or no interaction. Other combinations may be over or less than anticipated, which produced synergism or even antagonism combinations (44). Many different forms of combinations were introduced to the clinic which related to particular drug combinations and could not be extrapolated to other drug combinations (44). So that we always need to discover a novel combination to explore the mechanism by which these combinations affect. Tramadol as a good analgesic (45) and chlorpheniramine as a specific H_1-receptor antagonist (19) are widely used in clinics. Each of them has a mixed mechanism as analgesic action. In our study, we explored the kind of pharmacological interaction between tramadol and chlorpheniramine at different ED_{50} of 0.5:0.5 and 1:1 ratio at the level of analgesia using isobolographic analysis (34,35). The synergism was revealed as the decrease in the value of ED_{50} of tramadol and chlorpheniramine by 58.8 and 58.8% at 0.5:0.5 and 53.5 and 53.5% at 1:1 ratio respectively. We confirmed this result by calculating the Y value (interaction index) which was less than one (34,35).

We also used another sensitive test (writhing reflex) as a more confirm the synergism combination, which is regarded as a sensitive tool to assess the anti-inflammatory and analgesic effect of the new drug (46-48). This test also revealed a synergistic interaction between the two drugs in

the current study. These results are regarded as the first study that explored the kind of interaction between tramadol and chlorpheniramine on the level of visceral antinociceptive effect in mice as no previous study referred to the same effect.

We also examined the competence of the novel combination by simultaneously intraperitoneal injection of sub-analgesic doses (low doses) of each drug, which succeeded in producing a synergistic effect on the hot plate without overt side effects. We used the same procedure in our previous studies to prove the synergism interaction between different analgesics (49).

The mechanism by which the two drugs exerted their synergism interaction related to the mixed mechanism of action for each. Tramadol has a mixed mechanism as an analgesic by their central activation of μ-receptor as well as peripheral (on the gut) (49). The drug has two enantiomers, (+) dexo enantiomer, which acts as μ opioid receptor agonist as well as serotonin reuptake inhibitor. While (–) levo enantiomer acts as a noradrenalin reuptake inhibitor (50,51). The effect of drug on the $5HT_{2A}$ receptor was also involved in their analgesic effect (52). Related to the previous reports that referred to the critical role of serotonin in the modulation of pain perception (53). As the neurotransmitter (serotonin) activates the 5HT receptors and the presynaptic $5HT_3$ has a role in the antinociceptive effect (54). The stimulation of the adenosine A_1 receptor also has a role in the analgesic effect of tramadol (54). Tramadol also has an anticholinergic effect (anti-muscarinic) (55). As well as it has an H_1 receptor antagonist which is involved in its analgesic effect as previous research referred to the critical role of theH_1 receptor in pain perception (56) and the opioid-produced analgesic effect via their antagonism to this receptor as well as stimulation of the μ receptor (57).

Chlorpheniramine also produced its analgesic effect as an H_1 receptor antagonist (19). As histamine evoked their visceral and somatic pain via activation of the H_1 receptor (19). So that our findings agreed with previous research that discovered the role of chlorpheniramine (H_1 antagonist) in potentiating the antinociceptive effect of morphine (opioid) in mice using mechanical, thermal, and chemical nociceptive stimuli and related this effect as the two drugs act as H_1 receptor antagonist (57). Another study also revealed the role of chlorpheniramine in potentiating the antinociceptive effect of morphine on the visceral writhing reflex test by a mechanism related to their effect as activation of endogenous opioid receptors as well as H_1 blockers. So, histamine H_1 antagonists produced antinociceptive effects on their own or in combination with opiates (58).

The combination between tramadol (a synthetic opioid) and chlorpheniramine in our study also was synergistic, as the two drugs have an H_1 receptor antagonism effect as well as μ receptor activation (57). Chlorpheniramine potentiated the analgesic effect of tramadol may be also via their effect as serotonin reuptake inhibitor as tramadol. This is the characteristic that distinguishes it from most other 1st generation antihistamines (58).

The drug also has a G_i proteins stimulation (59) as inactivation of these proteins blocked the analgesic effect of GABA, and catecholamine and prevented the analgesic effect of tricyclic antidepressants and opioids (60-62). Chlorpheniramine also has an anti-muscarinic effect which may play a role in its analgesic effect (63-65). All these suggested mixed mechanisms of tramadol and chlorpheniramine, which may be involved in the synergism interaction between them at the level of thermal and visceral analgesia in mice in our current study, especially on the level of μ receptor activation, serotonin reuptake inhibitor as well as antagonism ofH_1 receptors (pharmacodynamic interaction).In the future, we need further studies on the pharmacokinetic level to explore if the interaction between the two drugs may be related to the effect of one on the kinetic of the other (66-68).

Conclusion

In our current study, we concluded that the interaction between tramadol and chlorpheniramine was synergistic at the level of the thermal and visceral test even using low (sub-analgesic) doses, which evoked a novel safe, and effective analgesic combination that may be useful in clinical practice. The novel combination has super advantages using low doses, producing strong analgesia without overt side effects.

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Conflict of interest

None.

References

1. Mana S, Shalini B, Maushm SK, Mayur YC. An overview of tramadol and its usage in pain management and future perspective. Biomed Pharmacother. 2019;3(11):443-451. DOI: 10.1016/j.biopha.2018.12.085
2. Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, Gibson S, Vader K. The revised IASP definition of pain: Concepts, challenges, and compromises. Pain. 2020;161(9):1976. DOI: 10.1097/j.pain.0000000000001939
3. DayerP, Collart L, Desmeules J. The pharmacology of tramadol. Drugs. 1994;47(1):3-7. DOI: 10.2165/00003495-199400471-00003
4. Scott LJ, Perry CM. Tramadol. Drugs. 2000;60(1):139-176. DOI: 10.2165/00003495-200060010-00008
5. Morgaz J, Navarrete R, Muñoz P, Domínguez JM, Fernández JA, Gómez RJ, Granados MM. Postoperative analgesic effects of dexketoprofen, buprenorphine and tramadol in dogs undergoing ovariohysterectomy. Res Vet Sci. 2013;95(1):278-282. DOI: 10.1016/j.rvsc.2013.03.003

6. Cagnardi P, Villa R, Zonca A, Gallo M, Beccaglia M, Luvoni GC, RavasioG. Intraoperative effect and postoperative analgesia of tramadol in cats. Res Vet Sci. 2011;90(3):503-509. DOI: 10.1016/j.rvsc.2010.07.015
7. Zeiler GE, Dzikiti BT, Fosgate GT, Stegmann FG, Venter FJ, Rioja E. Anaesthetic, analgesic and cardiorespiratory effects of intramuscular medetomidine-ketamine combination alone or with morphine or tramadol for orchiectomy in cats. Vet Anaesth Analg. 2014;41(4):411-420. DOI: 10.1111/vaa.12136
8. Codd EE, Shank RP, Schupsky JJ, Raffa RB. Serotonin and norepinephrine uptake inhibiting activity of centrally acting analgesics: Structural determinants and role in antinociception. J Pharmacol Exp Ther. 1995;274(3):1263-1270. [available at]
9. Frink MC, Hennies HH, Englberger W, Haurand M, Wilffert B. Influence of tramadol on neurotransmitter systems of the rat brain. Arzneimittelforschung. 1996;46(11):1029-1036. [available at]
10. Peets EA, Jackson M, Symchowicz S. Metabolism of chlorpheniramine maleate in man. J Pharmacol Exp Ther. 1972;180(2):464-474. [available at]
11. Moreno RA, Oliveira D, Sverdloff CE, Borges BC, Rebelo PA, Astigarraga RB, Borges NC. Determination of chlorpheniramine in human plasma by HPLC-ESI-MS/MS: Application to a dexchlorpheniramine comparative bioavailability study. J Chromatogr B Biomed Appl. 2010;24(7):774-781. DOI: 10.1002/bmc.1362
12. Sankar DV, Li EH, Santare M. Studies on antihistaminic action I effect of chlorpheniramine maleate on blood levels of 5-hydroxytryptamine and histamine in the rabbit. Res Commun Chem Pathol Pharmacol. 1974;7(3):513-518. [available at]
13. Kuroda T, Nagata SI, Takizawa Y, Tamura N, Kusano K, Mizobe F, Hariu K. Pharmacokinetics and pharmacodynamics of d-chlorpheniramine following intravenous and oral administration in healthy thoroughbred horses. Vet. 2013;197(2):433-437. DOI: 10.1016/j.tvjl.2013.02.003
14. Siegel C. The use of dexchlorpheniramine to treat nasal allergies. Curr Ther Res Clin Exp. 1964;6(12):714-715. [available at]
15. Vickers M. Dextro-chlorpheniramine (polaramine) in allergy: Preliminary report of 75 patients and comparison with racemic chlorpheniramine (chlor-trimeton) in 39 patients. J Maine Med Assoc. 1959;50(1):16-20. [available at]
16. Munro SJ, WallaceMG. A comparative study of clemastine ('Tavegil') and chlorpheniramine maleate in the treatment of hay fever. Curr Med Res Opin. 1976;4(4):245-249. DOI: 10.1185/03007997609109312
17. Sloan VS, Jones A, Maduka C, Bentz JW. A benefit risk review of pediatric use of hydrocodone/chlorpheniramine, a prescription opioid antitussive agent for the treatment of cough. Drugs Real World Outcomes. 2019;6(2):47-57. DOI: 10.1007/s40801-019-0152-6
18. Zanboori A, Tamaddonfard E, Mojtahedein A. Effect of chlorpheniramine and ranitidine on the visceral nociception induced by acetic acid in rats: Role of opioid system. Pak J Biol Sci. 2008;11(20):2428-32. DOI: 10.3923/pjbs.2008.2428.2432
19. Mobarakeh JI, Sakurada S, Katsuyama S, Kutsuwa M, Kuramasu A, Lin ZY, Yanai K. Role of histamine H1 receptor in pain perception: A study of the receptor gene knockout mice. Eur J Pharmacol. 2000;391(1-2):81-89. DOI: 10.1016/S0014-2999(00)00060-1
20. Chiu CC, Liu KS, Chen YW, Hung CH, Wang JJ. Chlorpheniramine produces cutaneous analgesia in rats. Pharmacol Rep. 2020;72(4):827-832. DOI: 10.1007/s43440-019-00028-7
21. Satyanarayana PS, Jain NK, Singh A, Kulkarni SK. Isobolographic analysis of interaction between cyclooxygenase inhibitors and tramadol in acetic acid-induced writhing in mice. Prog Neuropsychopharmacol Biol Psychiatry. 2004;28(4):641-649. DOI: 10.1016/j.pnpbp.2004.01.015
22. Shah DD, Sorathia ZH. Tramadol/diclofenac fixed-dose combination: A review of its use in severe acute pain. Pain Ther. 2020;9(1):113-128. DOI: 10.1007/s40122-020-00155-7
23. Hannam JA, Anderson BJ, Potts A. Acetaminophen, ibuprofen, and tramadol analgesic interactions after adenotonsillectomy. Paediatr Anaesth. 2018;28(10):841-851. DOI: 10.1111/pan.13464
24. López FJ, Díaz MI, Terrón JA, Campos MD. Analysis of the analgesic interactions between ketorolac and tramadol during arthritic nociception in rat. Eur J Pharmacol. 2004;484(2-3):157-165. DOI: 10.1016/j.ejphar.2003.11.005
25. Malmberg P, Lamberti C, Ipponi A, Bartolini A, Schunack W. Evidence for hypernociception induction following histamine H1 receptor activation in rodents. Life Sci. 1998;63(6):463-476. DOI: 10.1016/S0024-3205(98)00295-1
26. Oluyomi AO, Hart SL. Involvement of histamine in naloxone-resistant and naloxone-sensitive models of swim stress-induced antinociception in the mouse. Neuropharmacol. 1991;30(9):1021-1027. DOI: 10.1016/0028-3908(91)90115-R
27. Yang BR, Um HY, Lee MT, Kim MS, Jung SY. Characterizing tramadol users with potentially inappropriate co-medications: A latent class analysis among older adults. PloS One. 2021;16(2):e0246426. DOI: 10.1371/journal.pone.0246426
28. Al-Jader GM. Study the effects of diphenhydramine (H1-recepyor antagonist) on tramadol analgesic effect in mice [master's thesis]. Mosul: University of Mosul; 2011. 69 p.
29. Ghasemi M, Dehpour AR. Ethical considerations in animal studies. J Med Ethics Hist Med. 2009;2. [available at]
30. Dixon WJ. Efficient analysis of experimental observations. Annu Rev Pharmacol. 1980;20(1):441-462. DOI: 10.1146/annurev.pa.20.040180.002301
31. Hunskaar S, Berge OG, Hole KA. Modified hot-plate test sensitivie to mild analgesics. Behav Brain Res. 1986;21(2):101-108. DOI: 10.1016/0166-4328(86)90088-4
32. Mishra AK, Sinha RR, Tiwari RK, Dhone PG, Hishikar R, Rathod V. Experimental evaluation of analgesic activity of flupritine with opioids on Swiss albino mice. Acta Biomed Sci. 2020;7(2):53-56. DOI: 10.21276/abs.2020.7.2.2
33. Zendehdel M, Torabi Z, Pourrahimi M. Antinociceptive mechanisms of *Bunium persicum* essential oil in the mouse writhing test. Planta Med. 2011;77(12):PE2. DOI: 10.1055/s-0031-1282333
34. Puig MM, Pol O, Warner W. Interaction of morphine and clonidine on gastrointestinal transit in mice. Anesthesiol. 1996;85(6):1403-1412. DOI: 10.1097/00000542-199612000-00022
35. Tallarida RJ. The interaction index: a measure of drug synergism. Pain. 2002;98(1-2):163-168. DOI: 10.1016/S0304-3959(02)00041-6
36. Mbiantcha M, Almas J, Dawe A, Faheem A, Sidra Z. Analgesic, anti-inflammatory and anticancer activities of Combretin A and Combretin B isolated from *Combretum fragrans F*. HOFFM (Combretaceae) leaves. Inflammopharmacol. 2018;26(6):1429-1440. DOI: 10.1007/s10787-017-0421-5
37. Mota FV, Coutinho FN, de Carvalho VM, de Assis Correia JC, Bastos IV, Marcelino-Neto PP, Ximenes RM, Brondani DJ, de Faria AR, Marchand P, da Silva TG. Antinociceptive effects of aza-bicyclic isoxazoline-acylhydrazone derivatives in different models of nociception in mice. Curr Top Med Chem. 2022;22(4):247-258. DOI: 10.2174/1568026622666220105102508
38. Uddin M, Ali Reza AM, Abdullah M, Kabir MS, Nasrin M, Akhter S, Rahman M. Antinociceptive and anxiolytic and sedative effects of methanol extract of *Anisomeles indica*: An experimental assessment in mice and computer aided models. Front Pharmacol. 2018;9(8):246. DOI: 10.3389/fphar.2018.00246
39. Sampaio R, Nascimento EP, Menezes IA, Sales V, Pereira AB, Lacerda GM, Kerntopf MR. Antinociceptive activity of the *Psidium brownianum* Mart ex DC. leaf essential oil in mice. Food Chem Toxicol. 2020;135(4):111053. DOI: 10.1016/j.fct.2019.111053
40. Viviana N, Fernando S, Ramón SZ, Carlos PJ. Modulation of synergism COX-1 with COX-2 in the tail flick of mice. World J Adv Res Rev. 2020;6(3):222-228. DOI: 10.30574/wjarr.2020.6.2.0114
41. Bluhm R, Zsigmond EK, Winnie AP. Potentiation of opioid analgesia by H1 and H2 antagonists. Life Sci. 1982;31(12-13):1229-1232. DOI: 10.1016/0024-3205(82)90349-6

42. Zendehdel M, Torabi Z, Hassanpour S. Antinociceptive mechanisms of *Bunium persicum* essential oil in the mouse writhing test: Role of opioidergic and histaminergic systems. Vet Med. 2015;60(2):63-70. DOI: 10.17221/7988-VETMED
43. Maund E, McDaid C, Rice S, Wright, K, Jenkins B, Woolacott N. Paracetamol and selective and non-selective non-steroidal anti-inflammatory drugs for the reduction in morphine-related side-effects after major surgery: A systematic review. Br J Anaesth. 2011;106(3):292-297. DOI: 10.1093/bja/aeq406
44. Puig MM, Warner W, Pol O. Intestinal inflammation and morphine tolerance alter the interaction between morphine and clonidine on gastrointestinal transit in mice. Anesthesiol. 2000;93(1):219-230. DOI: 10.1097/00000542-200007000-00033
45. Subedi M, Bajaj S, Kumar MS, Mayur YC. An overview of tramadol and its usage in pain management and future perspective. Biomed Pharmacother. 2019;111:443-451. DOI: 10.1016/j.biopha.2018.12.085
46. Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol. 1968;32(2):295. DOI: 10.1111/j.1476-5381.1968.tb00973.x
47. Ness TJ. Models of visceral nociception. ILAR J. 1999;40(3):119-128. DOI: 10.1093/ilar.40.3.119
48. LeBars D, Gozariu M, Cadden SW. Animal models of nociception. Pharmacol Rev. 2001;53(4):597-652. [available at]
49. Raffa RB, Friderichs E, ReimannW, Shank RP, Codd EE, Vaught JL. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an'atypical'opioid analgesic. J Pharmacol Exp Ther. 1992;260(1):275-285. [available at]
50. Grond S, Sablotzki A. Clinical pharmacology of tramadol. Clin Pharmacokinet. 2004;43(13):879-923. DOI: 10.2165/00003088-200443130-00004
51. Raffa RB, Buschmann H, Christoph T, Eichenbaum G, Englberger W, Flores CM, Tzschentke TM. Mechanistic and functional differentiation of tapentadol and tramadol. Expert Opin Pharmacother. 2012;13(10):1437-1449. DOI: 10.1517/14656566.2012.696097
52. Xie H, Dong ZQ, Ma F, Bauer WR, Wang X, Wu GC. Involvement of serotonin 2A receptors in the analgesic effect of tramadol in mono-arthritic rats. Brain Res. 2008;1210:76-83. DOI: 10.1016/j.brainres.2008.02.049
53. Zendehdel M, Babapour V. Study of antinociceptive effects of *Ziziphora tenuior* and its interference on opioidergic and serotoninergic systems. J Vet Res. 2010;65(1):1-4. [available at]
54. Sawynok J, Reid AR, Liu J. Spinal and peripheral adenosine A1 receptors contribute to antinociception by tramadol in the formalin test in mice. Eur J Pharmacol. 2013;714(1-3):373-378. DOI: 10.1016/j.ejphar.2013.07.012
55. Nakamura M, Minami K, Uezono Y, Horishita T, Ogata J, Shiraishi M, Sata T. The effects of the tramadol metabolite O-desmethyl tramadol on muscarinic receptor-induced responses in Xenopus oocytes expressing cloned M1 or M3 receptors. Anesth Analg. 2005;(1):180-186. DOI: 10.1213/01.ANE.0000154303.93909.A3
56. Raffa RB. Antihistamines as analgesics. J Clin Pharm Ther. 2001;26(2):81-85. DOI: 10.1046/j.1365-2710.2001.00330.x
57. Mobarakeh JI, Sakurada S, Hayashi T, Orito T, Okuyama K, Sakurada T, Yanai K. Enhanced antinociception by intrathecally-administered morphine in histamine H1 receptor gene knockout mice. Neuropharmacol. 2002;42(8):1079-1088. DOI: 10.1016/S0028-3908(02)00058-8
58. Hellbom E. Chlorpheniramine, selective serotonin-reuptake inhibitors (SSRIs) and over-the-counter (OTC) treatment. Med Hypotheses. 2006;66(4):689-690. DOI: 10.1016/j.mehy.2005.12.006
59. Galeotti N, Ghelardini C, Bartolini A. Antihistamine antinociception is mediated by Gi-protein activation. Neurosci. 2002;109(4):811-818. DOI: 10.1016/S0306-4522(01)00537-1
60. Yeh SY. The effect of antihistaminic drugs on pentazocine antinociception in the rat. Pharmacol Biochem Behav. 1986;24(4):925-930. DOI: 10.1016/0091-3057(86)90438-7
61. Huh H, Lee SW, Cho JE, Kim HC. Effect of chlorpheniramine administration on postoperative catheter-related bladder discomfort in patients undergoing transurethral excision of bladder tumor: A prospective randomized study. J Anesth. 2021;35(5):646-653. DOI: 10.1007/s00540-021-02970-4
62. Shaban KA, Faris GA. Evaluation of the analgesic effect of xylazine, dipyrone and tramadol in a single dose or as a combination in chicks. Al-Anbar J Vet Sci. 2012;5(2):197-210.[available at]
63. Suh HW, Song DK, Choi YS, Kim YH. Effects of intrathecally injected histamine receptor antagonists on the antinociception induced by morphine, β-endorphin, and U50, 488H administered intrathecally in the mouse. Neuropeptides. 1996;30(5):485-490. DOI: 10.1016/S0143-4179(96)90014-1
64. Petrie A, Watson P. Statistics for veterinary and animal science. USA: Balckwell Science Ltd; 1999.
65. Millan MJ. Serotonin and pain: Evidence that activation of 5-HT1A receptors does not elicit antinociception against noxious thermal, mechanical and chemical stimuli in mice. Pain. 1994;58(1):45-61. DOI: 10.1016/0304-3959(94)90184-8
66. Mohammed QM, Albadrany YM. Pregabalin potentiates the analgesic effect of tramadol, diclofenac and paracetamol in chicks: Isobolographic analysis. Iraqi J Vet Sci. 2022;36(4):931-937. DOI: 10.33899/ijvs.2022.132586.2108
67. Mousa YJ, Mahmood MB. Pharmacodynamics and pharmacokinetics interaction between nefopam and tramadol in the broiler chicks model. Iraqi J Vet Sci. 2022;36(2):327-332. DOI: 10.33899/ijvs.2021.130163.1746
68. Jassim OY, Mousa YJ. Nefopam and ketorolac: Isobolographic analysis of analgesic effect and pharmacokinetic profile in chicks. Iraqi J Vet Sci. 2022;36(1):145-150. DOI: 10.33899/ijvs.2021.129540.1660

تقييم مزيج الكلورفينيرامين والترامادول على مستوى التسكين من الألم الحراري والحشوي في نموذج الألم الحاد في الفئران

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الخلاصة

تشمل الفوائد المحتملة لاستخدام الأدوية بشكل مركبات القدرة على زيادة التأثيرات المضادة للألم مع التقليل من الآثار الجانبية غير المرغوبة، نتيجة لذلك تم السماح بإعطاء الأدوية معا التي توفر تأثير تآزري مسكنا للألم لتقليل الجرع فضلا عن التخفيف من التأثيرات الجانبية الغير مرغوبة. في دراستنا الحالية قمنا بتقييم كمي ونوعي لنوع التداخل الدوائي بين الترامادول والكلورفينيرامين على مستوى الألم الحشوي (منعكس التلوي) والحراري (الصفيحة الساخنة) في نموذج الفئران. كانت الجرعة المسكنة الوسطية للترامادول والكلورفينيرامين اللذان تم حقنهما داخل الخلب ١٢ و ١٨,٤ ملغم/كغم من وزن الجسم، على التوالي، باستخدام طريقة الصعود والنزول بالجرعة وجهاز الصفيحة الساخنة، وأظهرت الحيوانات علامات التسدير وعدم الحركة. وكان التداخل الدوائي بين العقارين بشكل تآزري على مستوى التسكين من الألم الحاد عند حقنهما بنسبة ٠,٥:٠,٥ و ١:١ من الجرعة المسكنة الوسطية لكل منهما باستخدام الصفيحة الساخنة والتحليل الايزوبولوغرافك. وكان الانخفاض في قيمة الجرعة المسكنة الوسطية للترامادول والكلورفينيرامين ٥٨,٨ و ٥٨,٨% عند نسبة ٠,٥:٠,٥ و ٥٣,٥ و ٥٣,٥% عند نسبة ١:١ على التوالي. وتم تأكيد وجو تداخل

تأزري باستخدام جرع مضاعفة من قيمة الجرعة المسكنة الوسطية من كل عقار وإعطائهما معا للحيوان نفسه بالحقن داخل الخلب والذي أحدث تأثير مسكن تأزري على مستوى تحفيز الألم الحشوي والذي تمثل بالمنع النهائي ١٠٠% للتلوي المحدث بحمض الخليك المحقون داخل الخلب مقارنة مع مجموعة السيطرة والمجموعة المعاملة بكل عقار لوحده عند الجرع المضاعفة نفسها. استنتجت نتائجنا الحالية بان إعطاء الترامادول والكلورفينيرامين معا أحدث تأثير مسكن تأزري قوي وآمن حتى عند إعطائهما بجرع واطئة والذي قد يعد مفيد سريريا في معالجة الألم داخل العيادات البيطرية.