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Study on Antinociceptive effect of Antihistaminics in albino mice

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Abstract

Objectives: To study the antinociceptive effect of promethazine and cetrizine in albino mice and to compare its antinociceptive effect with the standard drug morphine. Lastly to rule out the influence of sedation contributing to antinociceptive action, lorazepam a known sedative was used.

Materials and Method: A total of 84 albino mice of either sex were used in the study and were divided into two sets with each set containing 7 groups. Each group in each set contained 6 animals. Group 1 in both sets was administered with Normal saline. Group 2 in both sets was administered with Morphine. Group 3 in both sets was administered with promethazine. Group 4 in both sets was administered with promethazine. Group 5 in both sets was administered with cetrizine. Group 6 in both sets was administered with promethazine + cetirizine. Group 7 in both sets were administered with lorazepam. First set of animals were subjected to Hot plate test and second set of animals were subjected to Tail flick test.

Results: Results were evaluated by ANOVA followed by post hoc analysis. Promethazine in both tests in both doses showed significant antinociceptive effect when compared to control. Cetrizine and lorazepam on the other hand did not show significant antinociceptive effect when compared to control.

Conclusion: This study shows that promethazine has statistically significant antinociceptive activity when compared to control whereas cetrizine has no antinociceptive effect. Lorazepam did not show antinociceptive effect proving us that antinociceptive effect of promethazine is not due to its sedative properties.

Keywords: Antinociceptive, Promethazine, Cetirizine, Morphine, Lorazepam

Introduction

Pain is one of the most ancient, common suffering endured by human beings and yet most elusive to complete and effective treatment. Though pain acts as a protective mechanism to alert us to get rid of causative stimulus, treatment of pain is imperative. Currently we have two major class of analgesics namely the Opioid and NSAID's. Although these drugs have stood the test of time as far as there efficacy is concerned, they come with a lot of side effects like dependence, gastric intolerance, constipation, bleeding disorders to name a few. So there is a constant effort to develop novel analgesics which not only is effective in wide variety of pain disorders but more importantly has a better safety profile. Histaminergic system plays an important role in central nociception.(1) Though several mechanisms of antinocieptive actions of antihistamines are described, exact role of histamine and its receptors in pain still eludes us.(2) Tissue damage releases histamine which acts on four subtypes of Histaminergic receptors that are known till now. These belong to the family of G-protein coupled receptors. H1 receptors are mainly present in smooth muscle cells, endothelial cells and CNS, H2 receptors are mainly expressed on gastric parietal cells, cardiac muscle, mast cells and also in CNS. H3 receptors which are predominantly presynaptic in location are mainly present in CNS. H4 receptors are present on cells of hematopoietic origin.(3) Contraction of the smooth muscle and an increase in vascular permeability are the main actions of H1 receptors. H2 receptors play a major role in the modulation of gastric acid secretion. H3 receptors are located in nerve endings. They inhibit Ca2+ conductance, decreasing neuronal depolarization and histamine release. H4 receptors are very similar to H3 receptors, but they are expressed in the hematopoietic cell line, especially eosinophils, mast cells, and basophils. Very little is known on the biologic role of H4 receptors.(4) The role of histamine in pain is different in the central and peripheral nervous systems. Central histamine has both pro and anti-nociceptive actions. H1 receptors are pro-nociceptive while H2 receptors seem to be anti-nociceptive.(5,6) Many studies have shown common proposed mechanisms for the analgesic action of H1 receptors which involves a supraspinal action on pre-synaptic receptors(5) located on the dorsal raphe nucleus or around the periaqueductal gray matter.(7) So this study was undertaken to study the antinociceptive activity of promethazine which is a sedative/first generation antihistaminic and cetrizine which is a non-sedative/second generation type of antihistaminic on albino mice using tail flick test and hot plate test. To remove the bias of sedation by promethazine contributing to antinociceptive action, lorazepam was used as control.

Objectives of study

1. To study the antinociceptive effect of promethazine and cetrizine in albino mice.
2. To compare the antinociceptive effect of promethazine, cetrizine and with the standard drug morphine.
3. Compare the antinociceptive effect of promethazine with lorazepam so as to remove the confounding factor that sedation can contribute to antinociceptive action of first generation antihistaminic.

Materials and Method

The study was conducted after obtaining the approval from Institutional animal ethical committee.

Source of data: Albino mice of either sex of average weight of 20-25g and aged 3-4 months which were inbred in central animal house of J.J.M Medical College, Davangere were used. Animals were randomly housed at an ambient temperature and humidity, with a 12 hour light and 12 hour dark cycle. The animals had free access to food and water.

Drugs and Chemicals: Morphine (2 mg/kg), Normal saline (5 ml/kg), Promethazine (6mg/kg), Promethazine (9mg/kg), Cetrizine (6.5 mg/kg) and Lorazepam (0.26 mg/kg)

Methods: A total of 84 animals (N=84) were divided into 7 groups of 6 (n=6) animals each and subjected to the following tests.

1. Tail flick test (radiant heat stimulus)
2. Hot plate method (thermal stimulus)

Different set of animals were used in each of the above mentioned test. The animals were divided into following groups in each of the above mentioned tests. Group 1 in both sets was administered with Normal saline 5 ml/kg body weight, intraperitoneally. Group 2 in both sets was administered with Morphine 2 mg/kg body weight, subcutaneously. Group 3 in both sets was administered with promethazine 6 mg/kg body weight, intraperitoneally. Group 4 in both sets was administered with promethazine 9 mg/kg body weight, intraperitoneally. Group 5 in both sets was administered with cetrizine 6.5 mg/kg orally. Group 6 in both sets was administered with promethazine6 mg/kg body weight, intraperitoneally+ 6.5 mg/kg cetrizine orally. Group 7 in both sets were administered with Lorazepam 0.26mg/kg body weight intraperitoneally.

1. **Tail Flick test:** This method involves use of an analgesiometer in which mouse tail is exposed to heated nichrome wire and the reaction time of the animal is recorded using a stop watch. Reaction time is defined as "Time interval between exposure to radiant heat and the tail flick". Cut off time for mouse in this test was taken as 15 seconds to avoid injury to the tail.
2. **Hot plate method:** The hot plate, which is commercially available, consists of electrically heated surface. The temperature is controlled for 55° to 56 °C. The animal was placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch. Cut off time for mouse in this test was taken as 15 seconds to avoid injury to the paw.

The above mentioned tests were done 15 minutes after the injection of drugs and 30 min after oral administration.

Statistical analysis: was done by one way analysis of variance (ANOVA) followed by Tukey HSD Test for group wise comparison.

Results

Table 1: Tail flick method showing Mean+/-SD of reaction time in different groups

Groups	N	Mean	Std. Deviation
Control	6	1.55	.45056
Morphine 2mg/kg	6	5.45	.43704
Promethazine 6mg/kg	6	3.58	.76790
Promethazine 9mg/kg	6	3.98	.96003
Cetrizine 6.5mg/kg	6	1.47	.37771
Prometg 6mg+cetz 6.5mg	6	3.07	.91368
Lorazepam 0.26mg/kg	6	1.69	.32898
Total	42	2.96	1.52836

Graph 1: Tail flick method showing Mean+/-SD of different groups

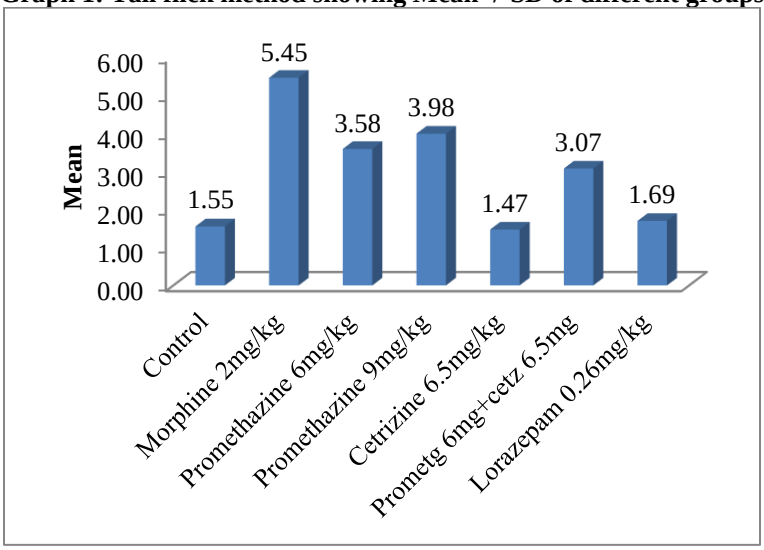

Table 2: ANOVA followed by a Post hoc Tukey's HSD test for tail flick method

Groups		Mean Difference	Adjusted P Value	Significant	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Morphine 2mg/kg	-3.90*	<0.0001	Yes	-5.083	-2.717
	Promethazine 6mg/kg	-2.03*	<0.0001	Yes	-3.217	-0.8499
	Promethazine 9mg/kg	-2.43*	<0.0001	Yes	-3.617	-1.25
	Cetrizine 6.5mg/kg	0.08	>0.9999	No	-1.1	1.267
	Promethazine 6mg+cetz 6.5mg	-1.51*	0.0058	Yes	-2.683	-0.3166
	Lorazepam 0.26mg/kg	-0.12	>0.9999	No	-1.3	1.067
Morphine 2mg/kg	Promethazine 6mg/kg	1.867*	0.0004	Yes	0.6833	3.05
	Promethazine 9mg/kg	1.467*	0.0074	Yes	0.2833	2.65
	Cetrizine 6.5mg/kg	3.983*	<0.0001	Yes	2.8	5.167
	Promethazine 6mg+cetz 6.5mg	2.4*	<0.0001	Yes	1.217	3.583
	Lorazepam 0.26mg/kg	3.783*	<0.0001	Yes	2.6	4.967
Promethazine 6mg/kg	Promethazine 9mg/kg	-0.4	0.9364	No	-1.583	0.7834
	Cetrizine 6.5mg/kg	2.117	<0.0001	Yes	0.9333	3.3
	Promethazine 6mg+cetz 6.5mg	0.5333	0.7935	No	-0.6501	1.717
	Lorazepam 0.26mg/kg	1.917	0.0002	Yes	0.7333	3.1
Promethazine 9mg/kg	Cetrizine 6.5mg/kg	2.517	<0.0001	Yes	1.333	3.7
	Promethazine 6mg+cetz 6.5mg	0.9333	0.2031	No	-0.2501	2.117
	Lorazepam 0.26mg/kg	2.317	<0.0001	Yes	1.133	3.5

*. The mean difference is significant at the 0.05 level.

Table 3: Hot plate method showing Mean+/-SD of different groups

Groups	N	Mean	Std. Deviation
Control	6	1.56	.47610
Morphine 2mg/kg	6	8.71	1.37611
Promethazine 6mg/kg	6	5.74	.73666
Promethazine 9mg/kg	6	6.20	.35777
Cetrizine 6.5mg/kg	6	3.48	.99482
Promethazine 6mg+cetz 6.5mg	6	5.45	2.14546
Lorazepam 0.26mg/kg	6	1.67	.47090
Total	42	4.68	2.62049

Graph 2: Hot plate method showing Mean+/-SD of different groups

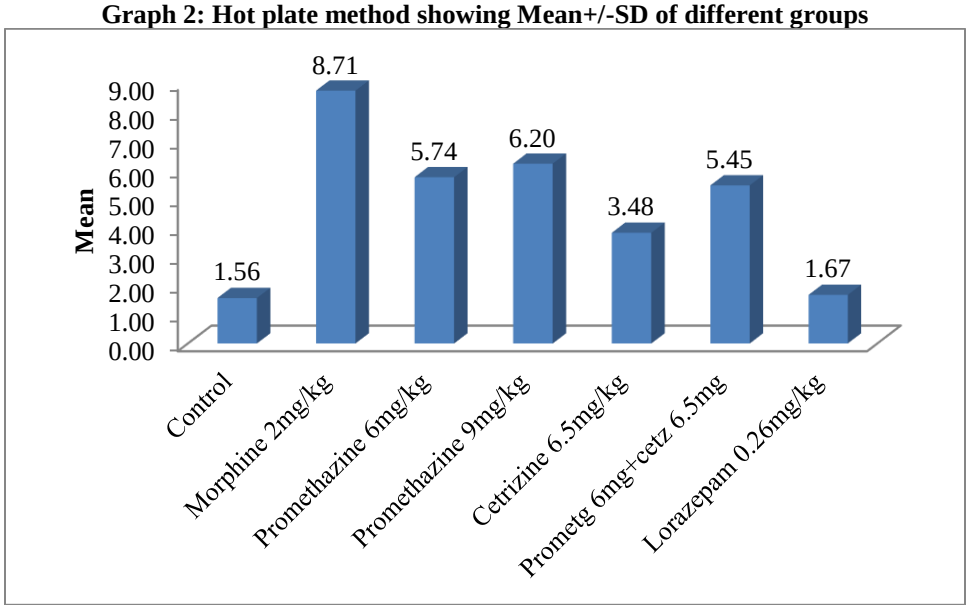

Table 4: Anova followed by Tukey HSD Test for hot plate method

Groups		Mean Difference	Adjusted P value	Significant	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Morphine 2mg/kg	-7.15*	<0.0001	Yes	-9.086	-5.214
	Promethazine 6mg/kg	-4.167*	<0.0001	Yes	-6.103	-2.231
	Promethazine 9mg/kg	-4.633*	<0.0001	Yes	-6.569	-2.697
	Cetrizine 6.5mg/kg	-1.917*	0.0538	No	-3.853	-0.01924
	Promethazine 6mg+cetz 6.5mg	-3.883*	<0.0001	Yes	-5.819	-1.947
	Lorazepam 0.26mg/kg	-0.1	>0.9999	No	-2.036	1.836
Morphine 2mg/kg	Promethazine 6mg/kg	2.983*	0.0005	Yes	1.047	4.919

	Promethazine 9mg/kg	2.517*	0.0044	Yes	0.5808	4.453
	Cetrizine 6.5mg/kg	5.233*	<0.0001	Yes	3.297	7.169
	Promethazine 6mg+cetz6.5mg	3.267*	0.0001	Yes	1.331	5.203
	Lorazepam 0.26mg/kg	7.05*	<0.0001	Yes	5.114	8.986
Promethazine 6mg/kg	Promethazine 9mg/kg	-0.4667	0.9878	No	-2.403	1.469
	Cetrizine 6.5mg/kg	2.25	0.0141	Yes	0.3141	4.186
	Promethazine 6mg+cetz6.5mg	0.2833	0.9992	No	-1.653	2.219
	Lorazepam 0.26mg/kg	4.067	<0.0001	Yes	2.131	6.003
Promethazine 9mg/kg	Cetrizine 6.5mg/kg	2.717	0.0018	Yes	0.7808	4.653
	Promethazine 6mg+cetz6.5mg	0.75	0.8852	No	-1.186	2.686
	Lorazepam 0.26mg/kg	4.533	<0.0001	Yes	2.597	6.469

*. The mean difference is significant at the 0.05 level.

Result Interpretation

Tail Flick Test and Hot Plate Test: Table 1 shows the mean values and standard deviation of group 1 to group 7 as obtained from tail flick test. It clearly shows significant changes in the mean difference in reaction time between control group (1.55) and promethazine treated groups (3.58 and 3.98) and also with morphine group (5.45). Whereas there were no significant changes in the mean difference in reaction time between control group (1.55), cetrizine (1.47) and lorazepam (1.69).This shows that morphine and promethazine in both doses of 6mg/kg and 9mg/kg has significant antinociceptive activity as compared to control group treated with normal saline. The same is interpreted in Bar Graph 1.

Table 3 shows the mean values and standard deviation of group 1 to group 7 as obtained from hot plate test. It clearly shows significant changes in the mean difference in reaction time between control group (1.56) and promethazine treated groups (5.74 and 6.20) and also with morphine group (8.71). Whereas there were no significant changes in the mean difference in reaction time between control group (1.56), cetrizine (3.48) and lorazepam (1.67). This shows that morphine and promethazine in both doses of 6mg/kg and 9mg/kg has significant antinociceptive activity as compared to control group treated with normal saline. The same is interpreted in Bar Graph 2.

Table 2 and Table 4 shows application of Tukey HSD test for results obtained from tail flick test and hot plate test respectivelywherein mean difference of reaction time of intergroup are compared statistically. Interpretation of Table 2 and Table 4 can be done in 4 parts.

Part 1 involves comparison between control group i.e. group 1 with group 2 through group 7. Mean differences between control group and groups treated with morphine (2mg/kg), promethazine (6mg/kg), promethazine (9mg/kg) and combination of promethazine 6mg/kg and cetrizine 6.5mg/kg clearly shows a significant difference indicating that morphine, promethazine in both doses and combination of cetrizine and promethazine has significant antinociceptive activity. It is also important to note in table 2 that lorazepam in dose of 0.26mg/kg and cetrizine in dose of 6.5mg/kg shows no significant antinociceptive activity as compared to control.

Part 2 involves comparison of morphine i.e. group 2 with group 3 through 7. Statistically analysis of mean differences clearly shows that morphine is having significant antinociceptive activity as compared to promethazine in both doses, cetrizine, lorazepam and combination of cetrizine and promethazine.

Part 3 of Table 2 shows comparison between promethazine 6mg/kg i.e. group 3 with groups 4 through 7. Statistical analysis clearly indicates that there is no difference in antinociceptive activity between 6mg/kg and 9mg/kg of promethazine and combination of promethazine and cetrizine. It also shows that promethazine 6mg/kg is having significant antinocieptive activity when compared with cetrizine and lorazepam.

Part 4 of Table 2 compares promethazine 9mg/kg i.e. group 4 with groups 5 through 7. Statistical analysis clearly indicates that there is no difference in antinociceptive activity between 9mg/kg of promethazine and combination of promethazine and cetrizine. It also shows that promethazine 9mg/kg is having significant antinocieptive activity when compared with cetrizine and lorazepam.

To summarize, promethazine in doses of 6mg/kg and 9mg/kg shows statistically significant antinociceptive activity as compared to control but failed to show significant antinociceptive activity when compared to standard drug morphine. Whereas cetrizine and lorazepam failed to show any antinociceptive activity when compared with both control and standard drug morphine.

Discussion

Present study was conducted to evaluate antinociceptive action of first generation H1 antihistaminic promethazine and second generation antihistaminic cetrizine in albino mice using tail flick test and hot plate test. Since first generation Antihistaminics cross blood brain barrier and are sedative in nature, lorazepam which is a known sedative was used to rule out any influence of sedation on antinociceptive action. Cetrizine was combined with promethazine to evaluate whether the combination produced any better antinociceptive action as compared to promethazine alone. Results from our present study in both tests clearly indicated that promethazine being a first generation sedative type of H1 antagonist in doses of 6mg/kg and 9mg/kg showed antinocieptive effect when compared with control, whereas cetrizine being a second generation non sedative type of H1 antagonist in dose of 6.5mg/kg failed to show any antinociceptive affect when compared with control. It is also important to note that combining cetrizine with promethazine did not improve the antinociceptive effect of promethazine in both tests. Lorazepam being a sedative did not show any antinociceptive activity as compared to control indicating that sedation by first generation antihistaminic had not influenced its antinociceptive activity.

Besides enkephalins, Pain modulation can occur via different neuronal systems like acetyl choline, GABA, catecholamines, and serotonin.[8-11] Exact role of histamine in pain has long eluded us because studies have shown histamine to be both pronociceptive and antinociceptive. Role of histamine in pain is said to be different in central and peripheral nervous system. As indicted earlier, central histamine has both pro and anti-nociceptive action whereas peripheral histamine stimulates nociceptive fibers.[12] Studies have shown that both pyrilamine an H1 antagonist and cimetidine an H2 antagonist has shown antinociceptive effects in formalin pain model in rats indicating that histamine in periphery has nociceptive action. Whether this peripheral antinociceptive action of antihistaminics is drug specific or class specific (H1 or H2) is not known. To study the role of central H1 receptors, highly selective agonist, 2-(3-trifluoromethylphenyl)histamine dihydrogenmaleate (FMPH), and of the better known H1 agonist, 2-thiazolylethylamine (2-TEA)was injected intracerebroventricularly into rats and were subjected to hot plate test, abdominal constriction test and paw pressure tests. Both of these substances produced significant hypernociceptive activity. A selective H1 receptor antagonist pyrilamine maleate showed significant antinociceptive activity in all three tests and both FMPH and 2-TEA prevented its effect, but not that of morphine, thus indicating action on H1 receptors.[13] Study conducted by Glick and Crane showed that injection of histamine into rat dorsal raphe nucleus and periaqueductal grey matter showed antinociceptive effect whereas histamine injection into median raphe nucleus caused hyperalgesia.[14] A study was conducted by Thoburn KK et al in which histamine was injected in the periaqueductal grey or the nearby dorsal raphe. They observed that Intracerebral microinjections of 1 mcg of histamine into median raphe and into periaqueductal grey and dorsal raphe evoked a mild, reversible antinociceptive response whereas injections into lateral or dorsal midbrain evoked either a delayed response or no response, respectively. Histamine dose response curve obtained from periaqueductal grey or the nearby dorsal raphe showed an inverted U shape showing that HA can induce both antinociceptive (0.3-3 micrograms) and pro-nociceptive (10-30 micrograms) responses. Whereas larger doses produced irreversible and highly variable antinociceptive responses that were accompanied by behavioral and histopathological changes indicative of toxicity.[15] Our present study is based on the model that H1 antagonist produces antinociception by acting supraspinally on presynaptic receptors on the dorsal raphe nucleus or around the periaqueductal gray matter. H1 antagonists by acting on presynaptic receptors, increases histamine release in periaquiductal grey and dorsal raphe nucleus and causes antinocicpetion. As said earlier, increase in histamine content actually causes nociception but since H1 antagonist is present, it also acts post synaptically and counteracts the excess histamine action. So even thought presynaptic action increases histamine content in the synaptic cleft, unoccupied receptors are few postsynaptically for histamine to act on so it mimics low dose histamine action i.e. antinociception.

Cetrizine did not show any antinociceptive effect probably because it doesn't significantly cross the blood brain barrier.

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