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Original Article

INVESTIGATION OF ANALGESIC ACTIVITY OF XANTHINE OXIDASE INHIBITOR ALLOPURINOL: AN EXPERIMENTAL STUDY

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ABSTRACT

Objectives: To investigate the analgesic activity of xanthine oxidase inhibitor, allopurinol in three graded doses and compare it with control and aspirin.

Methods: Analgesic effect of allopurinol in three graded doses (100, 200, 400 mg/kg I. P.), aspirin in analgesic dose (100mg/kg I. P.) and tramadol was evaluated by using Radiant heat Tail-flick method and Acetic-acid induced writhing method in albino rats and mice respectively. The results were analyzed statistically by ANOVA followed by Dunnet's test.

Results: Allopurinol possesses significant analgesic activity in both models of analgesia**.** In Tail-flick method, in doses of 100mg/kg the percentage of tail flick elongation time with allopurinol is comparable with aspirin at all time intervals with maximum activity at 60 minutes. In doses of 200mg/kg, the percentage increase in reaction time is more than aspirin at 30, 90 & 120 minutes but this difference is not statistically significant. In doses of 400mg/kg, the percent increase in reaction time is more than aspirin but the difference is not statistically significant.

In acetic acid induced writhing method, allopurinol in dose of 100mg/kg differ significantly than aspirin, latter being more effective. The analgesic activity of allopurinol in doses of 200mg/kg & 400mg/kg was comparable to aspirin.

Conclusion: Allopurinol possesses significant analgesic activity comparable with aspirin in tail flick model as well as acetic acid induced model of nociception. And this drug should be further evaluated for this indication

Keywords: Analgesic, Allopurinol, Purines, Tail-flick method, Xanthine oxidase inhibitor.

INTRODUCTION

Purines modulate the various physiological responses in body. Adenosine and ATP are the major neuromodulators involved in the purinergic system [1]. Adenosine regulates the pain transmission in spinal cord and peripherally by acting on adenosine/ P^1 receptors which are classified into A_1, A_{2a}, A_{2b}, and A_3 receptors. It is known fact that adenosine and its analogue exert multiple effects on pain pathways. Activation of adenosine receptor A_1 at the peripheral nerve terminal produces analgesic response in rodents while A_2 receptor activation produces pro-nociception [2]. The analgesic effect of adenosine may be due to attenuation by NMDA-induced production of nitric oxide or by inhibition of intrinsic neuron by an increase in potassium conduction [3]. The drugs which may alter adenosine concentration in body may modulate pain transmission.

Allopurinol is a potent inhibitor of enzyme xanthine oxidase, which catalyses the conversation of hypoxanthine to xanthine and to uric acid [4]. Subsequently there is the decrease in the concentration uric acid along with increase in the concentration of precursors hypoxanthine and xanthine. These hypoxanthine can be converted to inosine and ultimately to adenosine and guanosine [5]. Allopurinol is primarily used for treatment of gout and hyperuricemia [4]. Thus, allopurinol by inhibiting enzyme xanthine oxidase increases the concentration of endogenous adenosine and its analogues thereby may have the effect on pain transmission. So enhancing purinergic transmission by decreasing the degradation of purines may be a valid strategy to decrease the pain transmission in the body. There are some evidences that allopurinol may have analgesic activity [6].

Therefore we conducted this study to further evaluate analgesic activity of Xanthine Oxidase Inhibitor Allopurinol graded doses.

MATERIALS AND METHODS

General

The present study was carried out in SRTR Government Medical College, Ambajogai. Dist-Beed, Maharashtra. The study was carried out after prior approval from the Institutional Animal Ethics Committee of our institute and animals were handled as per CPCSEA guidelines.

Animals

Wister albino rats of either sex weighing between 200-250 mg and albino mice weighing 20-25 grams were used for the study. Animals were divided into six groups with six animals in each group for tail flick method and into five, groups with six animals in each group for acetic acid induced writhing method. Animals were acclimatized to laboratory conditions at least 24 hours prior to study. Animals were housed in separate plastic cages (six animals per cage), with tap water and commercial food ad libitum. The analgesic activity was assessed by tail flick method and acetic acid induced writhing method.

Chemicals

Pure form of drug allopurinol was obtained from Cipla Laboratories Pvt. Ltd. Mumbai as a gift sample for investigation purpose. Aspirin, Tramadol and acetic acid was purchased from Pharmacy College. Allopurinol was dissolved in a 10% Tween solution.

Methods

Tail flick Method [7]

Analgesic activity was assessed by tail flick method as described by D'Amour and Smith (1941) [7]. The temperature of nichrome wire was kept constant at 52±0.5 °C. The time taken by rat to withdraw its tail from noxious stimulus is measured. The cut off time of 10 seconds is set to prevent the injury to animal. The animals were divided into six groups with six animals in each group. Group one serves as control and received only normal saline. Group two received tramadol in doses of 10mg/kg as standard treatment. Group three received aspirin in doses of 100 mg/kg. Group four, five and six received allopurinol in doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg. On the first day, the animals were habituated to the tail flick apparatus taking 3 different measurements. On day two, the base line tail flick latency was

measured before an administration of drugs. The animals which have shown readings more than 6 seconds were discarded. The test drugs were given intraperitonially to the animals immediately after basal readings. The animals were subjected to tail flick apparatus 30 minutes after administration of drugs. Further readings were recorded at 60 minutes, 90 minutes and 120 minutes. Data for tail flick method was expressed as mean ± standard error of mean. The percentage maximum possible effect was calculated using formula [7].

$$\%\text{MPE} = \frac{\text{Test drug latency} - \text{basal latency}}{\text{Maximum latency} - \text{basal latency}} \times 100$$

Acetic Acid Induced writhing method [8, 9]

In acetic acid induced writhing method, swiss albino mice of either sex were divided into five groups. Group I (control group) was given normal saline 0.2 ml i. p. Group II (standard group) was given aspirin at the dose of 100 mg/kg intraperitoneally. Group III, IV, and V (test group) received allopurinol in doses of 100, 200, and 400 mg/kg i. p. All the drugs were given 30 min before the experiment. After 30 minutes, 0.1 ml 0.6% solution acetic acid was given i. p. The mice were placed individually into glass beakers and five mins were allowed to elapse. The mice were then observed over the period of 10 minutes and the total number of writhes counted. For a scoring purpose a writhe was indicated by stretching of abdomen with simultaneous stretching of one of the hind limb. % inhibition of writhing was counted using formula:

$$\%\ \text{Inhibition} = \frac{\text{No. of writhings in control group} - \text{No. of writhings in test group}}{\text{No. of writhings in control group}} \times 100$$

Statistical analysis

The data was expressed as Mean± standard error of mean. The data was evaluated by one way analysis of variance (ANOVA) followed by Dunnet's post test. The p-value <0.05 was considered statistical significant.

RESULTS

Tail flick method

The results of analgesic activity by tail flick method are shown in table 1**.** The basal latency in all groups was comparable. Analgesic activity profile of allopurinol in doses of 100 mg/kg showed that it possesses significant analgesic activity when compared to control group at all time interval. The analgesic activity of allopurinol in doses of 100 mg/kg was comparable to aspirin treated group with no statistical difference with maximum activity at 60 minutes. In doses of 200 mg/kg allopurinol possesses significant analgesic activity as compared to control. The analgesic activity at the doses 200 mg was comparable to aspirin at all time intervals with maximum activity at 90 minutes. At 90 minutes interval the analgesic activity of allopurinol in doses of 200 mg/kg was significantly more as compared to 100 mg/kg. With increase in doses at 400 mg/kg, there was no statistical increase in the analgesic activity of allopurinol. The analgesic activity of allopurinol in dose of 400mg/kg was comparable to allopurinol 200 mg/kg and aspirin at all time interval. Tramadol possesses maximum analgesic activity in all groups at all time interval in this method of analgesia.

Table 1: Analgesic activity of allopurinol in tail flick method

Group/Time	Basal	30 minutes	60 minutes	90 minutes	120 minutes
Control	3.54±0.17	3.65±0.23	3.87±0.27	3.97±0.26	3.67±0.29
Aspirin	3.62±0.34	6.96±0.22*	8.00±0.34*	7.38±0.34*	6.55±0.32*
Tramadol	3.44±0.02	8.39±0.32*##@@	9.19±0.32*#@@@	7.96±0.12*	6.93±0.05*
Allopurinol 100 mg/kg	3.30±0.27	6.98±0.30*	7.02±0.23*	6.33±0.20*	6.03±0.19*
Allopurinol 200 mg/kg	3.59±0.26	7.07±0.22*	7.70±0.15*	7.93±0.12*@@@	6.65±0.21*
Allopurinol 400 mg/kg	3.40±0.18	7.13±0.17*	7.60±0.10*	7.95±0.12*@@@	6.51±0.20*

* p value <0.001 compared to control, # p value <0.05 as compared to aspirin, ## p value <0.01 as compared to aspirin, @ p value <0.05 as compared to allopurinol 100 mg, @@ p value <0.01 as compared to allopurinol 100 mg, @@@ p value <0.001 as compared to allopurinol 100 mg.

The effect of various drugs in maximum possible effect is as shown in the table 2**.**

At 30 minutes, the maximum possible effect of allopurinol was comparable to aspirin groups but significantly less than tramadol group. At 60 minute intervals, mean Maximum Possible Effect of allopurinol at doses of 100 mg was less than that of aspirin and allopurinol in doses of 200 mg and 400 mg, the difference was statistically significant. At 60 minute, the Maximum Possible Effect of tramadol was the largest. At 90 minute interval, the mean of Maximum Possible Effect of allopurinol 100 mg was significantly less than aspirin. The mean of Maximum Possible Effect of allopurinol 200mg & 400 mg was comparable to aspirin. At 120 minute interval, the difference between mean Maximum Possible Effect of all the groups was decreased. It was comparable to all groups. The Analgesic Activity of allopurinol in tail flick method is as shown in fig. 1.

Table 2: Percentage Maximum Possible Effects (%MPE) in tail flick method

Drugs and doses	Percentage maximum possible effects (%MPE)			
	After 30 min	After 60 min	After 90 min	After 120 min
Control	----------	----------	----------	----------
Aspirin	51.48± 4.68	66.55± 5.97@	55.71± 6.39@	44.28± 6.86
Tramadol	74.39± 5.15##@@	87.06±4.73##@@	65.75.±2.92@@	51.10± 2.46
Allopurinol (100 mg/kg)	51.96± 5.37	50.85± 4.44	38.37± 5.11	36.52± 4.94
Allopurinol (200 mg/kg)	53.68± 3.35	62.53± 1.55	65.66± 1.68@@	46.99± 2.89
Allopurinol (400 mg/Kg)	54.51± 3.21	60.61± 2.03	65.68± 2.62@@	44.42± 4.16

p value <0.01 as compared to aspirin, @ p value <0.05 as compared to Allopurinol 100 mg, @@ p value <0.001 as compared to Allopurinol 100 mg

Acetic acid induced writhing method

The results of acetic acid induced writhing method at various doses of allopurinol are shown in table 3. In doses of 100 mg/kg, the allopurinol significantly decreases the number of writhes as compared to control. At the doses of 100mg/kg, the percentage inhibition was found to be 56%. At doses of 200mg/kg and 400 mg/kg the allopurinol has significant analgesic activity as compared to control and comparable with that of standard drug aspirin. The % inhibition in writhing was found to be 66% and 67% for allopurinol at the doses of 200mg/kg and 400 mg/kg respectively while that of aspirin was found to be 79%. The time of onset of writhes was also

significantly increased in the allopurinol treated group as compared to control group with maximum latency at the doses of 400mg/kg.

fig. 2 shows the analgesic activity of allopurinol in Acetic Acid Induced Writhing Method

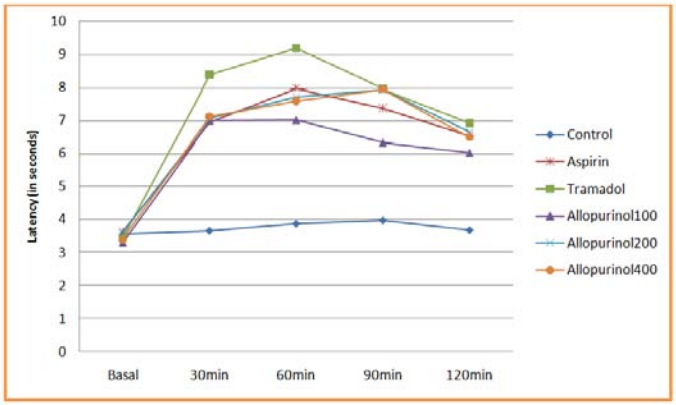

Fig. 1: Analgesic activity of allopurinol in Tail-flick Method

Table 3: Analgesic activity of allopurinol in acetic acid induced writhing method

Group	Time of onset (min)	Total no. of writhings	Percentage inhibition
Control	3.13±0.9	23.83±1.08	--------
Aspirin	10.30±0.8*	04.83±0.30*	79
Allopurinol (100 mg/kg)	7.10±0.2*	10.50±0.43*	56
Allopurinol (200mg/kg)	6.70±0.7*	08.00±0.37*	66
Allopurinol (400mg/kg)	6.80±0.6*	07.83±0.65*	67

* p value <0.001 as compared to control

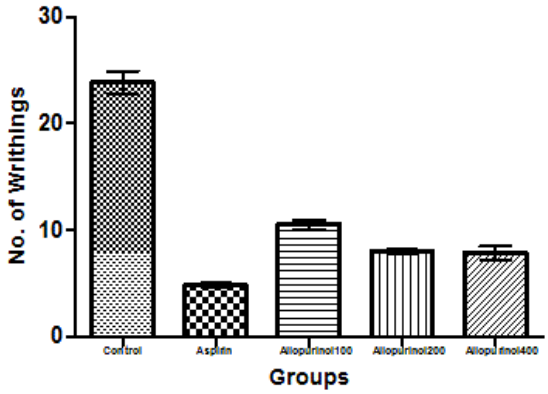

Fig. 2: Analgesic activity of allopurinol in Acetic acid induced writhing method

DISCUSSION

In this study, the analgesic activity of xanthine oxidase inhibitor allopurinol was evaluated. Allopurinol is primarily used for the treatment of hyperuricemia and gout. It acts by inhibiting the enzyme xanthine oxidase which is responsible for degradation of purines. Thus inhibition of this enzyme may increase in purine concentration and may have various effects on pain transmission. Previously very few studies have been carried out to evaluate its analgesic activity. So we carried out this study to find out whether allopurinol possesses any analgesic activity in various graded doses (100, 200, 400 mg/kg) and to compare it with standard and control.

In this study we evaluate analgesic activity of allopurinol by tail flick method and acetic acid induced writhing method to assess the central and peripheral mechanism of action. These models are essentially based on acute and short lasting noxious stimuli with some differences. These are well established methods for the evaluation of analgesic properties of drugs. Interestingly we found out that it do possess analgesic activity in both animal models of nociception.

In tail flick method allopurinol has been found to possess significant analgesic activity as compared to control in doses of 100, 200, 400 mg/kg. The analgesic activity of allopurinol in doses of 100mg/kg was significantly more than control but not aspirin. With further increase in doses at doses of 200 mg/kg, the analgesic activity of allopurinol increases but the difference was not statistically significant. At doses of 200 mg/kg, the analgesic activity of allopurinol was comparable to aspirin.

At doses of 400 mg/kg the there was no statistically increase in the analgesic activity. The significant analgesic activity of allopurinol in tail flick method shows that it may act via the central mechanism of action.

In acetic acid induced writhing method, allopurinol has also shown the significant reduction in the number of writhes greatest reduction at the dose of 400 mg/kg. At the doses of 100 mg/kg allopurinol significantly reduce the number of writhes as compared to control. Further increase in doses there is an increase in the analgesic activity and % inhibition was 66% and 67% at the doses of 200 mg/kg and 400 mg/kg respectively. This indicates that allopurinol may also have some peripheral mechanism of action.

Our results are in accordance with Schmidt et al (2009)[6] who have shown that allopurinol induced anti-nociception may be related to increase in the accumulation of adenosine in the body. Adenosine and its analogues have been considered as important targets for new drug development. They have been shown to have analgesic properties at supraspinal, spinal as well as peripheral sites of action [10].

Sawynok et al (1998) [2] have shown that actions of adenosine are primarily mediated by A_1 receptors. Endogenous adenosine can be released in CNS and periphery and regulation of its levels by various pharmacological agents can alter pain processing through activation of adenosine A_1 receptors. Schmidt et al (2009) [6] found out that opioid pathway was not likely to be involved in the anti-nociception caused by allopurinol. They also demonstrated that allopurinol also

do not have any direct on A_1 receptors and that allopurinol act indirectly by increasing in the concentration of adenosine. Thus our study confirms the findings that allopurinol possesses the analgesic activity and could be useful for painful conditions but further more studies are required to elaborate the detail mechanism of action and toxicity at these doses before advocating this measure.

CONCLUSION

In our study, we found that. Allopurinol possess analgesic activity comparable with standard drug aspirin in tail flick as well as acetic acid induced method of analgesia and this drug should be further evaluated for this indication.

CONFLICT OF INTERESTS

Declared None

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