National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Evaluation of analgesic, anti-inflammatory, and antipyretic activity of leukotriene receptor antagonist-montelukast: An experimental study

Atul Mallikarjun Kolhe, Anand Kale

Department of Pharmacology, SRTR Government Medical College, Ambajogai, Maharashtra, India

Correspondence to: Atul Mallikarjun Kolhe, E-mail: mallikarjun.kolhe@yahoo.in

Received: June 21, 2016; **Accepted:** July 11, 2016

ABSTRACT

Background: Various inflammatory mediators are involved in initiating and sustaining pain/inflammation cascade. Recently, leukotrienes (LTs) have been shown to be important mediators in pain and inflammation. **Aims and Objective:** An experimental evaluation of analgesic, anti-inflammatory, and antipyretic activity of LT receptor antagonist-montelukast. **Materials and Methods:** Wistar rats of either sex weighing 180-250 g and Swiss mice weighing 25-30 g were used. Analgesic activity of montelukast (20 mg/kg) was evaluated and compared with tramadol (10 mg/kg) and aspirin (300 mg/kg) using tail flick response method and acetic acid-induced writhing method. For evaluating anti-inflammatory activity, carrageenin-induced rat paw edema and formalin-induced arthritis models were used. The antipyretic activity was evaluated in Baker's yeast-induced pyrexia in rats. **Results:** Montelukast (20 mg/kg) had significant analgesic activity compared to control in acetic acid-induced writhing model but was ineffective in tail flick model. Montelukast showed significant anti-inflammatory activity in carrageenin-induced paw edema and formalin-induced arthritis models. Montelukast did not show antipyretic activity. **Conclusion:** Montelukast shows anti-inflammatory and analgesic activity which needs substantiation.

KEY WORDS: Montelukast; Tail Flick Method; Acetic Acid-induced Writhing

INTRODUCTION

The corticosteroids and non-steroidal anti-inflammatory drugs are used as anti-inflammatory and the later as analgesics and antipyretics also. These drugs offer a palliative relief and are associated with significant adverse effects. Hence, safer and effective drugs should be investigated.

Prostaglandins (PGs) are established mediators of inflammation, and their synthesis is inhibited by aspirin. PGs are generated during metabolism of arachidonic acid (AA) in cyclooxygenase pathway. However, AA is also metabolized via lipoxygenase (LOX) pathway, leading to the generation of leukotrienes (LTs).[1] These LTs can elicit most of the symptoms associated with inflammatory events including pain.

Recently, LTs have been shown to be important mediators in pain and inflammation. Aley and Levine (2003) reported the role of lipoxygenase metabolites in PG and epinephrine-mediated mechanical hyperalgesia.[2] Singh et al. (2004) reported that PGs and LTs have complementary effects in the development and persistence of inflammatory pain.[3]

The anti-inflammatory activity of LT receptor antagonists is established, but there are very few studies about their analgesic and antipyretic action. So, the present study has been proposed to evaluate analgesic, antipyretic, and anti-inflammatory

Access this article online

Website: www.njppp.com

Quick Response code

DOI: 10.5455/njppp.2016.6.0616811072016

activity of LT receptor antagonist-montelukast.

MATERIALS AND METHODS

Materials

The study was conducted after approval from the Institutional Animal Ethics Committee.

Experimental animals

Wistar rats weighing between 200 and 250 g and adult Swiss albino mice weighing between 20 and 25 g of either sex were used. Animals were procured from the central animal house of our institute. They were kept in polypropylene cages under controlled temperature 25°C ± 0.5°C.

Drugs and Chemicals

Aspirin and carboxymethylcellulose from Medley Pharmaceuticals, Mumbai, and montelukast from Cipla Pharmaceuticals Ltd, Mumbai, respectively, were gifted. Carrageenin (1% in 0.9% saline) and tramadol were obtained from Yarrow Chem Products Ltd. and Unijules Life Sciences Ltd., respectively.

Methods

Tail flick method in rats[4]

Animals were divided into following groups: (1) Control: Normal saline 2 ml/kg per oral (p.o), (2) Standard: Tramadol 10 mg/kg intraperitoneally (I.P), (3) Standard: Aspirin (300 mg/kg p.o), and (4) Test: Montelukast: (20 mg/kg p.o.).

Tail flick latency was measured by the method originally described by D' Armour and Smith in 1941, using analgesiometer. This test was performed before and at the end of 30, 60, 90, and 120 min after drug administration. % analgesia was expressed as:

$$\% \text{ analgesia} = \text{M.P.E} = \frac{\text{T.L.-B.L}}{\text{M.L.-B.L}} \times 100$$

Where, M.P.E. = Maximum possible effect, T.L. = Test latency,

M.L. = Maximum latency, B.L. = Basal latency or control latency

Acetic acid-induced writhing in mice[5]

Animals were divided into following groups: (1) Control: Normal saline (2 ml/kg p.o.). (2) Standard: Aspirin (300 mg/kg p.o.). (3) Test: Montelukast (20 mg/kg p.o.).

After 60 min of drug administration, 0.1 ml of 1% acetic acid solution was given to mice IP. The mice were placed individually into glass beakers and 5 min were allowed to elapse. The mice were then observed for 10 min, and the number of writhes was recorded. A writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

$$\% \text{ inhibition} = \frac{\text{Number of writhes in control group} - \text{Number of writhes in test group}}{\text{Number of writhes in control group}} \times 100$$

Carrageenin- induced hind paw edema in rats[6]

Animals were divided into following groups: (1) Control: Normal saline (2 ml/kg p.o.), (2) Standard: Aspirin (300 mg/kg p.o.), and (3) Test: Montelukast (20 mg/kg p.o.).

Paw edema was induced by an intradermal injection of 0.1 ml of carrageenin (1% in normal saline) into the plantar surface of the right hind paw.

Paw edema volume was determined using plethysmometer before and at 60, 120, and 180 min after carrageenin injection. All the drugs were administered 1 h before carrageenin.

Percentage inhibition of paw edema was calculated using following formula:

$$\% \text{ Inhibition at given time interval} = \frac{\text{Paw volume in control group} - \text{Paw volume in test group}}{\text{Paw volume in control group}} \times 100$$

Formalin-induced arthritis in rats[7]

Animals were divided into following groups: (1) Control: Normal saline (2 ml/kg p.o.), (2) Standard: Aspirin (300 mg/kg p.o.), and (3) Test: Montelukast (20 mg/kg p.o.).

Chronic phase of inflammation was induced by subcutaneous injection of

0.1 ml of 2% formalin under the plantar aponeurosis of the right hind paw on the 1st and 3rd day of the experiment.

The drug was given daily for 10 days. The linear cross section (LCS) immediately below the ankle joint of the right hind paw was measured daily with Vernier caliper. The difference in LCS on day 1 and day 10 was calculated.

Baker's yeast-induced pyrexia method[8]

Pyrexia was induced in rats by administering freeze-dried Baker's yeast as

20% suspension in 0.9% saline (1 g/kg s.c.) in the nape of neck. The temperature was measured by introducing 3 cm digital thermometer into the rectum. All the study groups were treated 4 h after injection of Baker's yeast. Rectal temperature was measured at 0, 3, 4, and 6 h.

Statistical analysis

Data were analyzed using graph pad prism software version 5.01. Comparison between different groups was done by one-way analysis of variance followed by Bonferroni post-test for comparison between multiple groups. The $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows that the basal latency (i.e. basal mean reaction time) was comparable in all the four groups in the tail flick model of analgesia in rats.

Montelukast has not shown significant analgesic activity ($P > 0.05$) as compared to control at all-time intervals.

The analgesic activity of aspirin and tramadol was significantly higher compared to control group at all-time intervals.

Table 2 shows the total number of writhes in acetic acid-induced writhing model in mice.

The total number of writhes was highest in the control group and lowest in the aspirin group. A number of writhes in montelukast group were significantly less ($P < 0.05$) as compared to the control group but was significantly more as compared to aspirin group. The percentage analgesia was more in the aspirin group (78.03%) than in montelukast group (62.12%).

Table 3 shows that all three groups, i.e. Control, aspirin, and montelukast show progressive paw volume increase at 1, 2, and 3 h.

At 1 h interval, montelukast and aspirin had significantly ($P < 0.05$) less increase in paw volume compared to control group.

At 2 h interval, montelukast and aspirin had significant ($P < 0.05$) less paw volume increase compared to control group.

At 3 h interval, paw volume increase was highest in the control group. Montelukast had significant less paw volume increase as compared to control group but significantly more than that of aspirin.

Table 4 shows the effect of different drugs on percentage inhibition of paw volume in carrageenin-induced paw edema in rats.

At 3 h interval, aspirin and montelukast have shown significant anti-inflammatory activity as evident from the difference in paw volume.

Montelukast group and aspirin group had significantly ($P < 0.05$) less increase in paw volume compared to control group.

It was observed that percentage inhibition value of aspirin group at 3 h was 81.25% which was greater than that of montelukast group 58.31%, and this difference was statistically significant.

Table 5 shows the effect of different drugs on LCS below the ankle joint in formalin-induced arthritis in rats. The mean difference between the LCS just below the ankle on the 1st day and 10th day was calculated for each group. Lower the difference in LCS, higher is the anti-inflammatory action.

Table 5 shows that the least difference in the mean LCS was found in the aspirin group which was significantly ($P < 0.05$) lower than the control group and montelukast group.

The difference in LCS in montelukast group was also significantly less ($P < 0.05$) as compared to control group.

The percentage of the anti-inflammatory effect of montelukast was 70.98%, while that of aspirin was 87.32%.

Table 6 shows in the control group there was a consistent rise in rectal temperature up to 4 h following Baker's yeast injection.

Table 1: Effect of different drugs on nociception in tail flick model of analgesia in rats

Groups (n=6)	Basal latency (in seconds)	At 30 min (in seconds)	At 60 min (in seconds)	At 90 min (in seconds)	At 120 min (in seconds)
Control (normal saline 2 ml/kg p.o.)	3.458±0.0955	3.750±0.1258	3.700±0.1238	3.983±0.2104	3.600±0.1461
Aspirin (300 mg/kg p.o.)	3.562±0.1076	7.800±0.1713*#	7.133±0.1333*#	7.083±0.1424*#	6.800±0.2530*#
Tramadol (10 mg/kg i.p.)	3.463±0.0315	8.167±0.2275*#	9.133±0.3724*#@	8.900±0.0856*#@	7.983±0.1167*#@
Montelukast (20 mg/kg p.o.)	3.418±0.0682	3.900±0.2098	4.000±0.073	4.067±0.0667	3.767±0.0614

Values are mean±SEM. n=6 in each group. *P<0.001 as compared to control. #P<0.001 as compared to montelukast. @P<0.001 as compared to Aspirin. SEM: Standard error of mean

Table 2: Effect of different drugs in acetic acid-induced writhing models in mice

Groups (*n*=6 animals)	Number of writhes (In 10 min)	Percentage analgesia
Control (normal saline 2 ml/kg p.o.)	22.00±0.5774	-
Aspirin (300 mg/kg p.o.)	4.833±0.4014***$^{\#\#\#}$	78.03%
Montelukast (20 mg/kg/p.o.)	8.333±0.3333***	62.12%

Values are mean±SEM, *n*=6 in each group. ***P<0.001 as compared to control. $^{\#\#\#}P$<0.001 as compared to montelukast. SEM: Standard error of mean

Montelukast has not shown any significant reduction in rectal temperature at any hourly interval. The changes in the rectal temperature in montelukast group were comparable to control group ($P > 0.05$).

The reduction in the temperature in aspirin group was significantly greater compared to control group and montelukast group ($P < 0.05$) at 3, 4, and 6 h interval.

DISCUSSION

LTs are one of the important mediators of inflammation, and their antagonists are reported to have good therapeutic

Table 3: Effect of different drugs on paw volume in carrageenin-induced paw edema in rats

Groups (*n*=6)	Paw volume increase (in ml)		
	At 1 h	At 2 h	At 3 h
Control (normal saline 2 ml/kg p.o.)	0.7000±0.0856	1.333±0.0988	1.600±0.0730
Aspirin (300 mg/kg p.o.)	0.1000±0.0447***	0.2000±0.0516***	0.300±0.0447***$^{\#}$
Montelukast (20 mg/kg p.o.)	0.3000±0.0683*	0.4000±0.0894***	0.667±0.0421***

Values are mean±SEM, *n*=6 in each group. *P<0.01 as compared to control. ***P<0.001 as compared to control. $^{\#}P$<0.001 as compared to montelukast. SEM: Standard error of mean

Table 4: Effect of different drugs on percentage inhibition of paw volume in carrageenin-induced paw edema in rats

Groups (*n*=6)	Difference in volume at 3 h (in ml)	% Inhibition at 3 h
Control (normal saline 2 ml/kg/p.o.)	1.600±0.07303	-
Aspirin (300 mg/kg p.o.)	0.300±0.0447*$^{\#}$	81.25
Montelukast (20 mg/kg p.o.)	0.667±0.0421*	58.31

Values are mean±SEM, *n*=6 in each group. *P<0.001 as compared to control. $^{\#}P$<0.001 as compared to montelukast. SEM: Standard error of mean

Table 5: Effect of different drugs on LCS below the ankle joint in formalin-induced arthritis in rats

Group (*n*=6)	Mean day 1 LCS (mm)	Mean day 10 LCS (mm)	Mean difference in LCS (mm)	% anti-inflammatory effect
Control (normal saline 2 ml/kg p.o.)	4.450±0.0619	7.917±0.072	3.550±0.1285	
Aspirin (300 mg/kg p.o.)	4.500±0.0894	4.950±0.1204	0.4500±0.0482*$^{\#}$	87.32
Montelukast (20 mg/kg p.o.)	4.267±0.0421	5.333±0.0614	1.033±0.021*	70.98

Values are Mean±SEM, *n*=6 in each group. *P<0.001 as compared to control. $^{\#}P$<0.001 as compared to Montelukast. LCS: Linear cross section. SEM: Standard error of mean

Table 6: Effect of different drugs on rectal temperature in Baker's Yeast-induced pyrexia model in rats

Group(*n*=6)	Rectal temperature in degree celsius at time (h)				
	-4 h	0 h	3 h	4 h	6 h
Control (normal saline 2 ml/kg p.o.)	37.22±0.0477	38.53±0.1229	38.58±0.1327	38.88±0.0703	38.60±0.0577
Aspirin (300 mg/kg p.o.)	37.35±0.0619	38.33±0.1333	37.42±0.0307*$^{\#}$	37.53±0.0333*$^{\#}$	37.48±0.0307*$^{\#}$
Montelukast (20 mg/kg p.o.)	37.25±0.0428	38.38±0.1447	38.47±0.1022	38.58±0.0980	38.47±0.843

Values are Mean±SEM, *n*=6 in each group. *P<0.001 as compared to control. $^{\#}P$<0.001 as compared to Montelukast. SEM: Standard error of mean

potential in many inflammatory clinical conditions. Bronchial asthma is one such chronic persistent inflammatory condition mediated by a wide variety of inflammatory cells and mediators such as LTs. Cysteinyl LT receptor antagonists such as Zafirlukast and montelukast are advocated for the treatment of bronchial asthma. It is reported that LT B4-induced hyperalgesia is mediated through mobilization of polymorphonuclear leukocytes at the site of inflammation.[2]

In our study, montelukast exhibited a significant anti-inflammatory effect in both acute and chronic models of inflammation, i.e. carrageenin-induced paw edema and formalin-induced arthritis method, respectively. Montelukast blocks the cysteinyl LT receptors and inhibits the effect of formed LTs due to inflammatory stimuli.

The acetic acid-induced writhing method is used to evaluate peripherally acting analgesics. In our study, in acetic acid-induced writhing model of analgesia, the analgesic action of montelukast was significantly more compared to control group but was significantly less as compared to aspirin group. The percentage analgesia in aspirin group was 78.72%, whereas in montelukast group it was 62.92%. Thus, both aspirin and montelukast had analgesic action, but aspirin had better analgesic efficacy compared to montelukast aspirin acts by different mechanisms of action, it inhibits cyclo-oxygenase-II and thereby PG synthesis. PGs sensitize afferent nerve endings to mechanical and chemical pain stimuli. Since these two drugs are effective in acetic acid-induced writhing model, hence their analgesic action may be peripheral.

The tail flick method is used to evaluate centrally acting analgesics. In this method, in our study, montelukast did not show significant analgesic activity as compared to control at all-time intervals. It may be possible that acute thermal stimuli fail to release the LTs centrally. A study was carried out by Singh et al., in 2005, which showed that Zileuton, 5-LOX inhibitor and cysteinyl LT receptor antagonists, montelukast, Zafirlukast inhibited the number of writhes in acetic acid-induced writhing method but did not show analgesic activity in tail flick and hot plate methods.[9] A study conducted by Jain et al. (2001) showed Zafirlukast, a cysteinyl LT receptor antagonist, inhibited the nociceptive and inflammatory response in mice and rats. Zafirlukast dose-dependently and significantly increased the nociception threshold in acetic acid-induced writhing method but failed to exert antinociceptive effect in tail flick and hot plate methods.[10] Muthuraman et al. (2012) showed that montelukast was found to be beneficial in ischemia-reperfusion associated vasculitic neuropathic pain due to its LT receptor antagonistic action.[11]

Thus, the analgesic action of montelukast seen in our study corroborates with the findings of other studies reported in the literature.

There is evidence that LTs generated locally act to sensitize peripheral nociceptors to noxious stimuli and subsequently release other mediators in spinal cord resulting in hyperalgesia. Intraplantar injection of LTB_4 receptor agonist (LTB_4) and 8R-15(S)-dihydroxy-eicosa-5-cis-9,11,13-trans-tetrenoic acid (8R-15-diHETE) which are metabolites derived from 5 to 15 lipoxygenase pathways, respectively evoke profound hyperalgesic response.[12] Cysteinyl LT receptor antagonist-montelukast has demonstrated analgesic activity in acetic acid-induced writhing method. Hence, it may owe its analgesic action to antagonism of some of the LTs like LTB_4.

The absence of antipyretic effect of montelukast in yeast-induced pyrexia method suggests that it may not inhibit the synthesis of interleukins.

The anti-inflammatory action of montelukast is more established. However, further studies are needed to substantiate its analgesic activity.

CONCLUSION

Montelukast shows anti-inflammatory and analgesic activity which needs substantiation.

ACKNOWLEDGMENTS

We are thankful to Dr. D.B. Jadhav, in-charge, Animal House, for his support and help during the study period.

REFERENCES

1. Funk CD. Prostaglandins and leukotrienes: Advances in eicosanoid biology. Science. 2001;294(5548):1871-5.
2. Aley O, Levine JD. Contribution of 5- and 12-lipoxygenase products to mechanical hyperalgesia induced by prostaglandin E(2) and epinephrine in the rat. Exp Brain Res. 2003;148(4):482-7.
3. Singh VP, Patil CS, Kulkarni SK. Effect of zileuton in radicular pain induced by herniated nucleus pulposus in rats. Inflammopharmacology. 2004;12(2):189-95.
4. Parmar NS, Prakash S. Evaluation of analgesics, anti-inflammatory and anti-pyretic activity. In: Parmar NS, editor. Screening Methods in Pharmacology. New Delhi, India: Narosa Publishing House; 2006. p. 232-3.
5. Parmar NS, Prakash S. Evaluation of analgesics, anti-inflammatory and anti-pyretic activity. In: Parmar NS, editor. Screening Methods in Pharmacology. New Delhi, India: Narosa Publishing House; 2006. p. 225-6.
6. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med. 1962;111:544-7.
7. Brownlee G. Effect of deoxycortone and ascorbic acid on formaldehyde-induced arthritis in normal and adrenalectomized rats. Lancet. 1950;1(6596):157-9.
8. Deewan S, Kumar S, Kumar VL. Antipyretic effect of latex of

Calotropis procera. Indian J Pharmacol. 2000;32:252.
9. Singh VP, Patil CS, Kulkarni SK. Differential effect of zileuton, a 5-lipoxygenase inhibitor, against nociceptive paradigms in mice and rats. Pharmacol Biochem Behaviour. 2005;81:433-9.
10. Jain NK, Kulkarni SK, Singh A. Role of cysteinyl leukotrienes in nociceptive and inflammatory conditions in experimental animals. Eur J Pharmacol. 2001;423(1):85-92.
11. Muthuraman A, Ramesh M, Sood S. Ameliorative potential of montelukast on ischemia-reperfusion injury induced vasculitic neuropathic pain in rat. Life Sci. 2012;90(19-20):755-62.
12. Martin HA, Basbaum AI, Kwiat GC, Goetzl EJ, Levine JD. Leukotriene and prostaglandin sensitization of cutaneous high-threshold C- and A-delta mechanonociceptors in the hairy skin of rat hindlimbs. Neuroscience. 1987;22:651-9.

How to cite this article: Kolhe AM, Kale A. Evaluation of analgesic, anti-inflammatory, and antipyretic activity of leukotriene receptor antagonist-montelukast: An experimental study. Natl J Physiol Pharm Pharmacol 2017;7(1):32-37.

Source of Support: Nil, **Conflict of Interest:** None declared.