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Role of cysteinyl leukotrienes in nociceptive and inflammatory conditions in experimental animals

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Abstract

The leukotrienes are potent inflammatory mediators, which may have a role in inflammatory diseases such as allergic rhinitis, inflammatory bowl disease and asthma. Zafirlukast, a cysteinyl leukotriene receptor antagonist, is claimed to be effective in asthma. However, it is not known whether these leukotrienes are involved in nociceptive and peripheral inflammation. The present study aimed to assess the role of cysteinyl leukotrienes in nociceptive and inflammatory conditions in experimental animals. Central nociception was assessed with tail-flick and hot-plate methods and peripheral nociception was assessed by acetic acid-induced chemonociception in mice. Local administration (intraplantar) of carrageenan-induced hyperalgesia and inflammation, measured by paw withdrawal latency and paw volumes, respectively. Zafirlukast (2.5–20 mg/kg, p.o.) produced a significant and dose-dependent antinociceptive and antiinflammatory effect against acetic acid-induced chemonociception in mice and carrageenan-induced paw oedema in rats, respectively. Zafirlukast (2.5 and 5.0 mg/kg, p.o.) also attenuated the carrageenan-provoked hyperalgesia but did not alter the pain threshold in central nociception up to 20 mg/kg. Zafirlukast (5 and 10 mg/kg) significantly inhibited exudate formation and migration of polymorphonuclear leukocytes in carrageenan-induced pleurisy. Further, zafirlukast (5 mg/kg) also reduced myeloperoxidase activity in carrageenan-treated paw. When nimesulide (2 mg/kg, p.o.) was co-administered with zafirlukast, the antinociceptive, antihyperalgesic and antiinflammatory effects of nimesulide were significantly increased as compared to the per se effect. The results indicate that cysteinyl leukotrienes are involved in nociceptive/inflammatory conditions. It is expected that combination of cysteinyl leukotriene receptor antagonist with cyclooxygenase inhibitor would prove to be a novel approach to treat complex inflammatory conditions. © 2001 Published by Elsevier Science B.V.

Keywords: Cysteinyl leukotriene; Zafirlukast; Nociception; Cyclooxygenase inhibitor; Peripheral inflammation/hyperalgesia

1. Introduction

Inflammatory processes are the physiological response of the organism to different stimuli such as trauma, infections or immunological mechanisms. The arachidonic acid cascade is highly activated during inflammation, resulting in the formation of eicosanoids, and it is mediated by cyclooxygenase and 5-lipoxygenase enzymes (Heller et al., 1998). These complex inflammatory reactions involve the release of a wide variety of inflammatory mediators i.e. prostaglandins, thromboxanes and leukotrienes.

The pro-inflammatory activity of prostaglandins and thromboxanes has been well documented, and they have been shown to mediate nociception and oedema associated with inflammation. Beside prostaglandins, leukotrienes are also potent proinflammatory mediators involved in the pathophysiology of various inflammatory diseases such as asthma, psoriasis, rheumatoid arthritis and ulcerative colitis (O'Donnell, 1999; Harris et al., 1995). It is reported that leukotriene B_4 also produces hyperalgesia that is dependent on polymorphonuclear leukocytes (Levine and Goetzi, 1984; Hoheisel and Mense, 1994). Due to the contribution of eicosanoids to the pathogenesis of inflammation, and since the arachidonate metabolism appears to be a more complicated cascade of many reactions, both the cyclooxygenase and 5-lipoxygenase pathways are targets for the development of new anti-inflammatory agents.

Nonsteroidal anti-inflammatory drugs provide well-established anti-inflammatory therapy acting via inhibition of cyclooxygenase (Kulkarni et al., 2000), which prevents the formation of prostaglandins and thromboxanes. However, these compounds do not directly affect the 5-

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lipoxygenase pathway of the arachidonic acid cascade, allowing the production of pro-inflammatory hydroxy-eicosatetraenoic acid and leukotrienes. Although, cyclo-oxygenase inhibitors are effective in reducing oedema and erythema, effects mediated by cyclooxygenase products, they are not effective in reducing the effects mediated by 5-lipoxygenase products, such as inflammatory cell infiltration and bronchial asthma (Parker, 1987). Therefore, it is expected that the compounds having the additional property of blocking the action of 5-lipoxygenase enzyme mediated products, may be as more efficacious anti-inflammatory agents in comparison to pure cyclooxygenase inhibitor.

Recently, cysteinyl leukotriene receptor antagonists (i.e. zafirlukast, montelukast and pranlukast) are advocated for the treatment of asthma (Chung and Barnes, 1998; Vianna and Martin, 1998; Weisberg, 2000). Present study mainly aimed to investigate the role of cysteinyl leukotrienes in various nociceptive and inflammatory conditions in experimental animals. Since kinins, polymorphonuclear leukocytes, prostanoids and cytokines, all mediators, are involved in inflammation, we have sought to investigate if zafirlukast, a cysteinyl leukotriene receptor antagonist, has any anti-inflammatory effect in carrageenan-induced paw oedema. Effect of zafirlukast on cell migration and myeloperoxidase activity were also studied in carrageenan-induced pleurisy and carrageenan-induced paw oedema test, respectively. Further novel therapeutic approach, combinations of cysteinyl leukotriene receptor antagonist with cyclooxygenase inhibitor, was studied in animal models of nociception and inflammation.

2. Materials and methods

2.1. Animals

Albino Swiss mice (20–30 g) and Wistar rats (150–200 g) of either sex (Central Animal House, Panjab University, Chandigarh, India) were used. Animals were housed under standard laboratory conditions. They were maintained on rat chow (Ashirwad Industries, Chandigarh) and had free access to water.

2.2. Drugs

The following drugs were used: zafirlukast (Zeneca Pharmaceuticals, U.K.), nimesulide (Panacea Biotec, New Delhi), carrageenan lambda (type IV), *o*-phenylenediamine (Sigma, USA) and acetic acid (S.D. Fine Chemicals, India).

2.3. Experimental conditions

Unless otherwise stated, the conditions given below were employed in all experiments.

The test compounds were suspended in 0.5% carboxymethyl cellulose and administered per orally (p.o.) 30 min prior to noxious stimuli, and the control animals received corresponding amount of vehicle (0.5% carboxymethyl cellulose). Experiments were carried out at constant room temperature and humidity (28 ± 2.0 °C; $60 \pm 10\%$ relative humidity).

2.4. Analgesic study

2.4.1. Acetic acid-induced writhing assay

Acetic acid solution (1%, 10 ml/kg i.p.) was used to produce writhing in mice. The number of writhes (constriction of abdomen, turning of trunk (twist) and extension of hind legs) due to acetic acid was expressed as nociception response. Number of writhes per animal was counted during a 20-min session, beginning 3 min after the injection of acetic acid (Jain et al., 1999).

2.4.2. Tail-flick test (radiant heat-induced nociception)

The analgesic response was determined by measuring tail-flick latency to radiant heat. Baseline latency to tail withdrawal from the radiant heat source (3–5 s) was established. A cut-off time of 10 s was used to prevent any injury to the tail. Three trials were recorded for each animal to calculate mean basal latency. The increase in latency (nociception threshold) was recorded at 15, 30, 60, 120 and 180 min after drug administration (Jain et al., 1999).

2.4.3. Hot-plate test (thermal heat-induced nociception)

In this test, animals were individually placed on a hot plate maintained at a constant temperature (55 ± 0.3 °C).

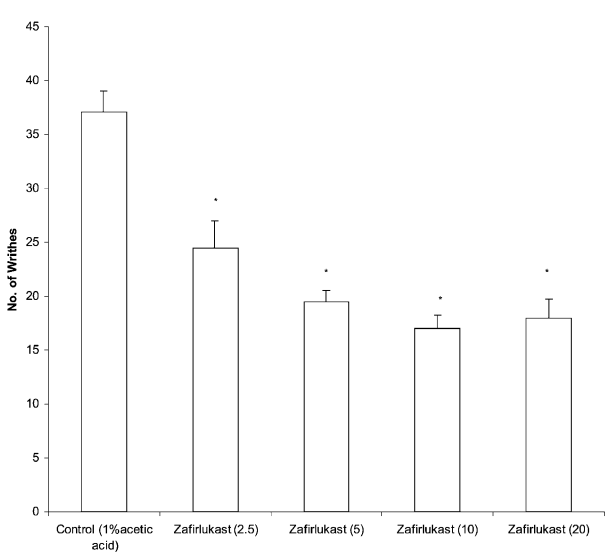

Fig. 1. Effect of zafirlukast (2.5, 5.0, 10.0 and 20.0 mg/kg, p.o.) against acetic acid-induced writhing in mice. Vertical lines show ±S.E.M (n = 6–8 per group). $^{*}P < 0.05$ compared with control.

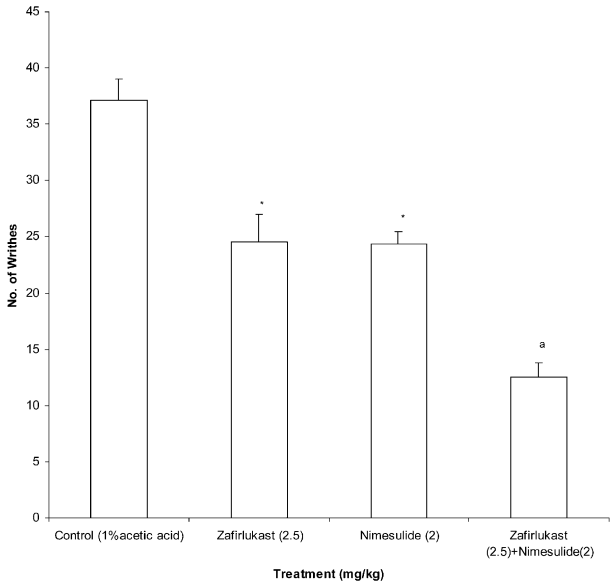

Fig. 2. Effect of zafirlukast (2.5 mg/kg, p.o.), nimesulide (2.0 mg/kg, p.o.) and its combination against acetic acid-induced writhing in mice. Vertical lines show ±S.E.M ($n = 6–8$ per group). $^{*}P < 0.05$ compared with control; $^{a}P < 0.05$ compared with nimesulide (2 mg/kg) and zafirlukast (2.5 mg/kg).

The latency to the first sign of paw licking or jump response to avoid heat nociception was taken as an index of nociceptive threshold with cut-off time 15 s. The nociceptive threshold was observed at 15, 30, 60, 120 and 180 min after the drug administration (Raghavendra et al., 2000).

2.4.4. Induction and assessment of carrageenan-induced inflammatory nociception (hyperalgesia)

Acute inflammation was induced in the right hind paw of rats by injecting 0.1 ml of freshly prepared solution of 1% carrageenan. The left paw received 0.1 ml of saline, which served as control. The response to inflammatory nociception was determined by measuring paw withdrawal latency of carrageenan injected paw when dipped in the water bath maintained at 47 ± 0.5 °C. Baseline latency to paw withdrawal from thermal source was established thrice, 5 min apart, and averaged. A cut-off time of 15 s was imposed to avoid any injury to the paw. The paw withdrawal latency for left and right paw were observed at 30, 60, 120, 180, 240 and 300 min after drug administration (Jain et al., 2001).

2.5. Anti-inflammatory activity

Carrageenan-induced paw oedema: Acute oedema was induced in the right hind paw of rats by injecting 0.1 ml of freshly prepared solution of 1% carrageenan after 30 min of test drugs administration. The left paw received 0.1 ml of saline, which served as control. Carrageenan was injected under the plantar region of right hind paw, and the volume was measured using a plethysmometer (UGO Basile, Italy) at 30, 60, 120, 180, 240 and 300 min after carrageenan challenge. Inflammation was expressed as the percentage change in paw volume (Jain et al., 1999).

2.6. Leukocyte migration and exudate formation in carrageenan-induced pleurisy in rats

Pleurisy was induced according to the method reported by Engelhardt et al. (1995), by intrapleural injection of 0.5 ml of a 2% carrageenan solution in 0.9% NaCl solution, under ether anaesthesia on the right side of the mediastine between the 4th and 5th rib. The test drugs were administered orally 2 h before induction of pleurisy. Twenty-four hours after administraion of carrageenan, the animals were sacrificed by an overdose of ether, the pleural exudate was collected and the pleural cavity washed with 2 ml of 0.9% saline (containing 5 IU heparin/ml). The exudate volume was recorded. Exudate cells were sedimented by centrifugation, resuspended in 0.15 ml of rat serum, stained on slide, and differential counts of at least 200 cells per preparation were determined.

2.7. Estimation of myeloperoxidase activity in carrageenan-treated paw in rats

Myeloperoxidase activity was determined by modified technique of Bird et al., (1988). Inflammatory reaction was

Table 1
Effect of zafirlukast (2.5–20 mg/kg, p.o.) on the tail-flick latency of mice (values are expressed as mean ± S.E.M.)

Treatment (mg/kg)	*n*	Basal tail-flick latency (s)	Tail-flick latency (s) after treatment at (min)				
			15	30	60	120	180
Zafirlukast (2.5)	6	3.88 ± 0.18	3.83 ± 0.16	4.0 ± 0.36	4.16 ± 0.30	4.0 ± 0.25	4.0 ± 0.36
Zafirlukast (5.0)	6	3.71 ± 0.10	4.33 ± 0.42	4.0 ± 0.25	4.33 ± 0.33	4.0 ± 0.25	4.0 ± 0.25
Zafirlukast (10.0)	5	4.19 ± 0.22	4.80 ± 0.37	4.4 ± 0.24	4.8 ± 0.37	4.6 ± 0.40	4.6 ± 0.24
Zafirlukast (20.0)	5	4.46 ± 0.17	5.0 ± 0.51	4.6 ± 0.40	5.2 ± 0.37	5.0 ± 0.31	5.0 ± 0.31

Table 2
Effect of zafirlukast (2.5–20 mg/kg, p.o.) on hot-plate latency (paw licking response) in mice (values are expressed as mean ± S.E.M.)

Treatment (mg/kg)	*n*	Basal reaction time (paw licking) (s)	Reaction time (s) after treatment at (min)				
			15	30	60	120	180
Zafirlukast (2.5)	5	5.2 ± 0.58	5.6 ± 0.40	5.0 ± 0.31	5.0 ± 0.55	4.4 ± 0.24	5.0 ± 0.36
Zafirlukast (5.0)	5	6.2 ± 0.58	6.2 ± 0.58	5.89 ± 0.59	5.8 ± 0.58	5.8 ± 0.58	5.4 ± 0.24
Zafirlukast (10.0)	5	5.2 ± 0.74	5.2 ± 0.20	5.0 ± 0.31	5.6 ± 0.68	4.6 ± 0.24	5.0 ± 0.31
Zafirlukast (20.0)	5	5.0 ± 0.31	5.0 ± 0.31	4.6 ± 0.40	4.65 ± 0.40	4.80 ± 0.37	5.4 ± 0.24

induced in left paw by intraplantar injection (0.1 ml/paw) of carrageenan type IV (Sigma) in rat (right paw received 0.1 ml of saline). Test drugs were administered orally 30 min before carrageenan challenge. Three hours after administraion of carrageenan, animals were sacrificed, a fixed length of right and left paw were cut and homogenized in 5 ml of phosphate buffer (0.01 M). Homogenized tissue was centrifuged at 10,000 rpm. Supernatant collected was mixed with *o*-phenylenediamine (660 μg/ml in phosphate buffer) and 300 mM H_2O_2 was used to initiate the reaction. Absorbance was observed at 492 nm at an interval of 30 s for 5 min.

Change in optical density per minute was calculated and results were expressed as %myeloperoxidase activity, considering 100% myeloperoxidase activity in control group.

2.8. Statistical analysis

Results are expressed as mean ± S.E.M. The difference in response to test drugs and controls was determined by one-way analysis of variance, followed by Dunnett's *t*-test. $P < 0.05$ was considered significant.

3. Results

3.1. Antinociceptive activity

Zafirlukast (2.5–20 mg/kg) exerted a significant ($P < 0.05$) dose-dependent antinociceptive effect (reduction in

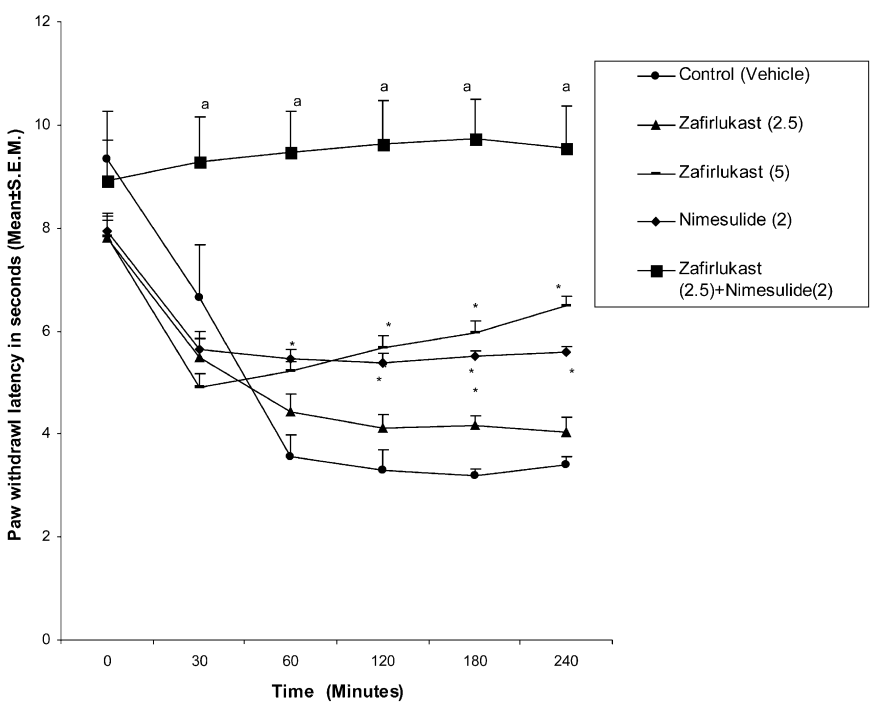

Fig. 3. Effect of zafirlukast (2.5 and 5.0 mg/kg), nimesulide (2.0 mg/kg) and its combination against carrageenan-induced hyperalgesia (inflammatory pain) in rats. Vertical lines show ±S.E.M ($n = 6–8$ per group). * $P < 0.05$ compared with control; [a] $P < 0.05$ compared with zafirlukast (2.5 mg/kg) and nimesulide (2 mg/kg).

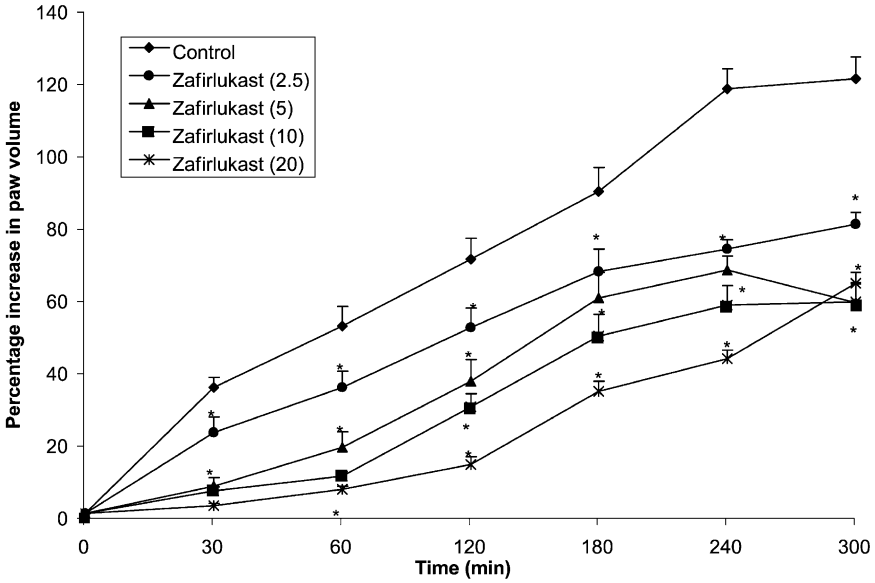

Fig. 4. Effect of various doses of zafirlukast (2.5, 5.0, 10.0 and 20.0 mg/kg) against carrageenan-induced paw oedema in rats. Vertical lines show ±S.E.M ($n = 6–8$ per group). * $P < 0.05$ compared with control.

number of writhes) against acetic acid-induced chemonociception in mice (Fig. 1). Zafirlukast showed peak antinociceptive effect after 30 min of its administration and the effect persisted till 120 min in writhing assay (data not given). Nimesulide (2 mg/kg), a reference drug, also significantly increased nociception threshold. When nimesulide (2.0 mg/kg) was co-administered with zafirlukast (2.5 mg/kg), the antinociceptive effect of nimesulide significantly increased as compared to per se effect (Fig. 2). Zafirlukast (2.5–20 mg/kg) did not show any increase in nociception threshold against tail-flick and hot-plate assays (Tables 1 and 2).

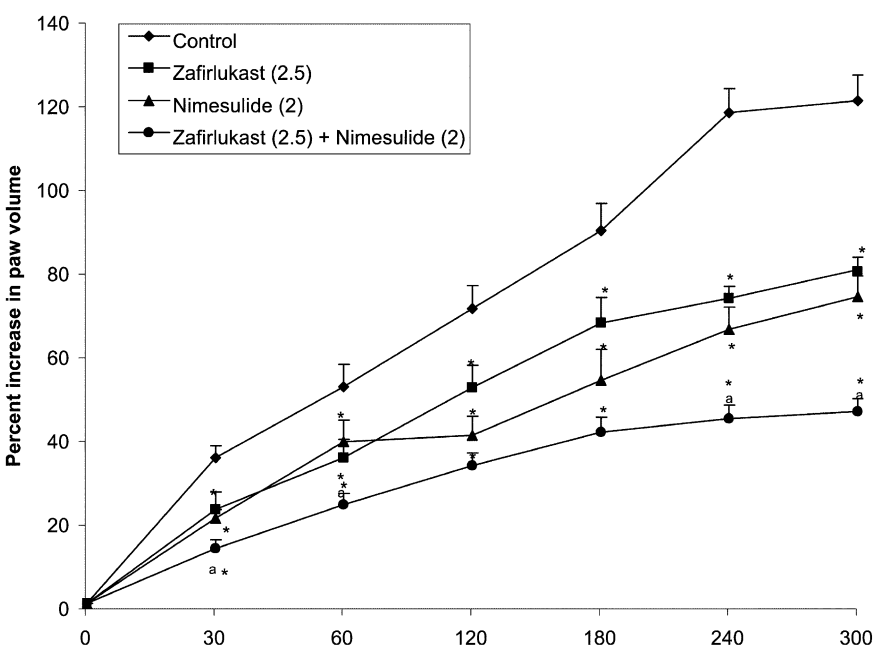

Fig. 5. Effect of zafirlukast (2.5 mg/kg), nimesulide (2 mg/kg) and its combination against carrageenan-induced paw oedema in rats Vertical lines show ±S.E.M. ($n = 6–8$ per group). * $P < 0.05$ compared with control. [a] $P < 0.05$ compared with nimesulide (2 mg/kg) and zafirlukast (2.5 mg/kg).

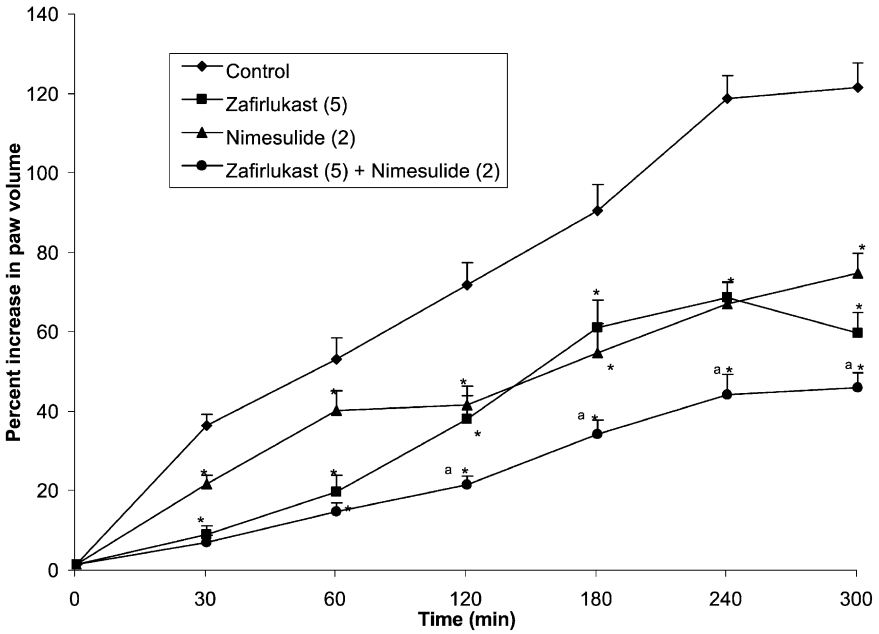

Fig. 6. Effect of zafirlukast (5.0 mg/kg), nimesulide (2 mg/kg) and its combination against carrageenan-induced paw oedema in rats. Vertical lines show ±S.E.M. ($n = 6–8$ per group). $^{*}P < 0.05$ compared with control. $^{a}P < 0.05$ compared with nimesulide (2 mg/kg) and zafirlukast (5 mg/kg).

3.2. Effect of zafirlukast on inflammatory nociception in rats

Carrageenan-treated paws showed decrease in paw withdrawal latency in comparison to saline-treated paws. Zafirlukast (5.0 mg/kg) significantly reversed the carrageenan-induced inflammatory nociception (hyperalgesia). Nimesulide (2 mg/kg) also reversed the hyperalgesic effect. In combination, zafirlukast (2.5 mg/kg) significantly potentiated the anti-hyperalgesic effect of nimesulide (2.0 mg/kg) (Fig. 3).

3.3. Anti-inflammatory activity

Carrageenan (1% w/v) produced paw oedema in control group, indicating inflammatory response. Zafirlukast (2.5–20 mg/g) dose-dependently and significantly decreased the carrageenan-induced increase in paw volume as compared to control rats (Fig. 4). Zafirlukast exhibited a better profile of anti-inflammatory effect in early phase as compared to the late phase of inflammation. Nimesulide (2 mg/kg), a reference drug, also produced a significant anti-inflammatory effect as compared to control group ($P < 0.05$). Zafirlukast exhibited a better profile of anti-inflammatory effect in early phase as compared to the late phase of inflammation. When nimesulide (2 mg/kg) co-administered with zafirlukast (2.5 and 5.0 mg/kg), the

Table 3
Effect of zafirlukast on exudate volume and number of cells in the exudate 24 h after induction of pleurisy

Treatment (mg/kg)	Exudate volume (ml)	PMNs $\times 10^6$	Monocytes $\times 10^6$
Control (2% carrageenan)	4.18 ± 0.19	65.75 ± 3.96	19.5 ± 3.77
Zafirlukast (5 mg/kg)	3.21 ± 0.18[a]	45.25 ± 3.19[a]	10.5 ± 0.95[a]
Zafirlukast (10 mg/kg)	2.08 ± 0.15[a]	34.50 ± 2.75[a]	9.75 ± 1.37[a]

[a] $P < 0.05$ compared with control (2% carrageenan).

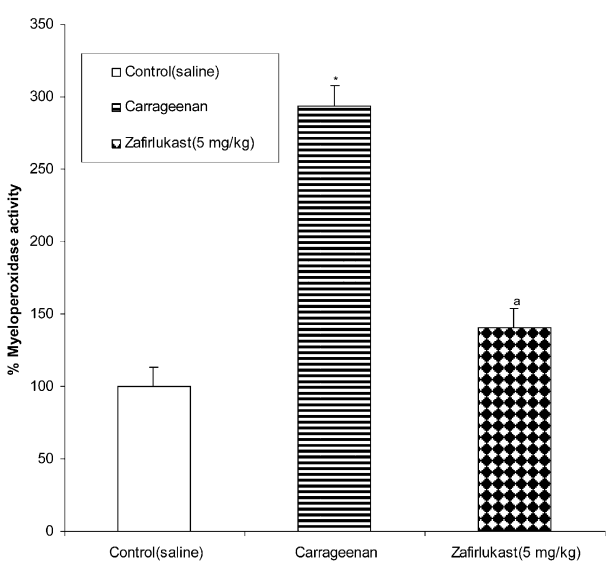

Fig. 7. Effect of zafirlukast (5 mg/kg, p.o.) on myeloperoxidase activity in carrageenan-induced paw edema in rats. $^{*}P < 0.05$ compared with control (basal MPO level considered 100%), $^{a}P < 0.05$ compared with MPO activity in carrageenan-treated paw.

anti-inflammatory effect of nimesulide significantly increased as compared to per se effect (Figs. 5 and 6).

3.4. Effect of zafirlukast on leukocyte migration and myeloperoxidase activity

Table 3 shows the volume of exudate and the number of cells in the exudate 24 h after induction of pleurisy. Zafirlukast (5 and 10 mg/kg) produced inhibition of exudate formation and migration of polymorphonuclear leukocytes and monocytes. Myeloperoxidase enzyme in carrageenan-treated paw was found to be three times higher than that of saline-treated paw. The increased myeloperoxidase level was significantly reduced on zafirlukast (5 mg/kg) treatment (Fig. 7).

4. Discussion

Many factors contributed to the complex course of inflammatory reactions. Microbiological, immunological and toxic agents can initiate the inflammatory response by activating a variety of humoral and cellular mediators.

Arachidonic acid is the mother substance of the pro-inflammatory eicosanoids, is released from the membrane phospholipids in the course of inflammatory activation, and it is metabolized to prostaglandins and leukotrienes by cyclooxygenase and lipoxygenase pathways, respectively (Rabasseda, 2000).

Over the recent years, it has become widely accepted that asthma is a chronic persistent inflammatory condition regulated by a wide variety of inflammatory cells and mediators such as leukotrienes. It has been shown that there is an increased level of cysteinyl leukotrienes in biological fluids from patient with chronic asthma and experimentally induced acute bronchospasm (Rola-Pleszcynski et al., 1993; Chung, 1995). It is reported that leukotriene B4-induced hyperalgesia is totally dependent on the mobilization of polymorphonuclear leukocytes at the site of inflammation (Levine and Goetzi, 1984) and unconstrained by the cyclooxygenation of arachidonic acid. Moreover, the inflammatory reactions (carrageenan oedema) used in this study is supported by several endogenous mediators such as kinins, polymorphonuclear leukocytes, prostanoids, nitric oxide, neuropeptides and cytokines (Damas et al., 1990; Damas and Remacle-Volon, 1992; Ialenti et al., 1992).

The results presented here show that zafirlukast, a cysteinyl leukotriene receptor antagonist, inhibited the nociceptive and inflammatory response in mice and rats, respectively. Although the precise site and mechanism of the antinociceptive and anti-inflammatory effect were not addressed in the present investigation, it would seem likely that zafirlukast blocks the cysteinyl leukotrienes receptors as a consequence of inhibition of the effect of formed leukotrienes due to inflammatory stimuli. Zafirlukast dose-dependently and significantly increased the nociception threshold in acetic acid-induced chemonociception. This study suggests that the acetic acid causes tissue injury and activates the release of lipid mediators like cysteinyl leukotrienes. However, higher doses of zafirlukast failed to exert antinociceptive effect in acute model of thermal nociception (tail-flick and hot-plate assay).Tail-flick and hot-plate nociceptive assays involve central (spinal and supra spinal) mechanisms (Kanaan et al., 1996), and it may be possible that acute thermal stimuli fail to release the leukotrienes.

The antinociceptive effect of nimesulide, a preferential cyclooxygenase-2 inhibitor, was also modulated by zafirlukast. This confirms the involvement of both cyclooxygenase and 5-lipoxygenase pathways in acetic acid-induced nociception.

Zafirlukast significantly and dose-dependently reduced the carrageenan-evoked increase in paw volume and inflammatory nociception (hyperalgesia) in rats.

Zafirlukast (5 and 10 mg/kg) also significantly inhibited exudate formation and migration of poymorphonuclear leukocytes in carrageenan-induced pleurisy test. Further, zafirlukast (5 mg/kg) reduced myeloperoxidase activity in carrageenan-induced inflammatory reaction in the rat paw. The hyperalgesic/inflammatory effects of carrageenan appear to be a function of an influx of polymorphonuclear leukocytes, besides other inflammatory mediators. It is consistent with the previous finding that leukotrienes are the potent polymorphonuclear leukocytes chemotactic and activating factor of the lipoxygenase pathway.

The anti-inflammatory effect of zafirlukast was significantly better than nimesulide in early phase of inflammation, suggesting the involvement of leukotrienes in early phase. When zafirlukast (2.5 and 5.0 mg/kg) was combined with nimesulide (2 mg/kg), the anti-inflammatory effect was superior at both phases of inflammation. The blockade of cyclooxygenase-2 enzyme and leukotriene receptors at the site of inflammation by nimesulide and zafirlukast, respectively, showed additive anti-inflammatory activity. The synergistic anti-hyperalgesic activity of nimesulide–zafirlukast combination reflects the complete antagonism of polymorphonuclear leukocyte-dependent release of leukotrienes and blockade of cyclooxygenase pathway in carrageenan-induced paw oedema.

Nickerson-Nutter and Medvedeff (1996) reported the efficacy of leukotriene synthesis inhibitor along with cyclooxygenase inhibitors in animal model of rheumatoid arthritis. Recently, Sheftell et al. (2000) also reported the role of leukotrienes in migraine associated headache; it furher supports the involvement of leukotrienes in nociceptive conditions.

More recently, various approaches have been made to develop dual inhibitors of cyclooxygenase and 5-lipoxygenase (i.e. darbufelone mesilate, flobufen, ML-3000, tebufelone and tepoxalin) (Unangst et al., 1994; Dyer and

Connor, 1997; Martin et al., 1999) to completely block the inflammatory mediators.

In summary, our results reveal that cysteinyl leukotrienes are involved in nociceptive/inflammatory conditions. The inhibitory effect of zafirlukast against carrageenan-induced poymorphonuclear leukocytes migration and increased myeloperoxidase activity suggests involvement of cysteinyl leukotrienes in inflammatory reaction. Furthermore, our finding may suggest a new therapeutic approach with co-administration of cysteinyl leukotrienes antagonist and cyclooxygenase inhibitor in the management of complex inflammatory conditions.

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