Potentiation of Indomethacin-Induced Anti-Inflammatory Response by Montelukast in Formalin-Induced Inflammation in Rats

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Abstract- The leukotrienes and prostaglandins are biologically active metabolites derived from arachidonic acid. The leukotrienes have a role in inflammatory diseases such as allergic rhinitis, inflammatory bowel disease and asthma. Montelukast, a cysteinyl leukotriene receptor antagonist, is claimed to be effective in asthma. The present study aimed to assess the role of cysteinyl leukotriene receptor antagonist on peripheral inflammation and whether montelukast treatment enhances the anti-inflammatory effect of indomethacin. Anti-inflammatory response was measured using a plethysmometer. Histopathologic examination for leukocyte accumulation was done. Montelukast (0.5–2mg/kg, i.p.) produced a significant anti-inflammatory effect in dose dependent manner against formalin-induced rat paw oedema at 1h but not in 3 and 5 h. When indomethacin (5 mg/kg, i.p) was co-administered with montelukast (1 mg/kg, i.p), the anti-inflammatory effects of indomethacin were significantly increased as compared to the *per se* effect at 3 and 5 hour after formalin challenge. In histopathology it has been found that combination therapy significantly decreased migration of leucocytes into the site of inflammation. These results show that montelukast has anti-inflammatory properties in peripheral tissue and markedly potentiates the anti-inflammatory activity of indomethacin at 3 and 5 h. It is expected that combination of montelukast with cyclooxygenase inhibitor would prove to be a novel approach to manage complex inflammatory conditions.

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Introduction

Inflammation is the body's response against invading pathogens, which is typically characterized by redness, swelling, pain, and heat. It can also be viewed as a complex system and related to homeostatic perturbations which initiated from within the body (for example, in autoimmune disease) or from the outside (for example, in infections). Virtually all acute and chronic diseases are either driven or modulated by inflammation. Several reports have provided evidence that inflammation is involved in the pathogenesis of many diseases, including aging (1), cancer (2), atherosclerosis (3), cardiovascular disease (4), arthritis (5), neurodegenerative disease (6), diabetes mellitus (7), obesity (8), and other life-threatening and debilitating diseases (9). There is an increased recognition that the role of inflammation is more and more important in a wide spectrum of diseases (10).

The arachidonic acid cascade is highly activated during inflammation, resulting in the formation of eicosanoids which are mediated either cyclooxygenase (COX) or 5-lipooxygenase enzymes (11). These complex inflammatory reactions involve the release of a wide variety of inflammatory mediators *i.e.* prostaglandins, thromboxanes and leukotrienes.

Non-steroidal anti-inflammatory drugs (NSAIDs) exert analgesic, antipyretic, and anti-inflammatory effects through the inhibition of COX-catalyzed biosynthesis of prostaglandines (12). Note that NSAIDs do not eliminate inflammation but rather act by reducing the production of selected inflammatory mediators. Although, COX inhibitors are effective in reducing oedema and erythema, effects mediated by COX

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products, they are not effective in reducing the effects mediated by 5-lipoxygenase products, such as inflammatory cell infiltration and bronchial asthma (13).

Recently, cysteinyl leukotriene receptor antagonists (i.e. montelukast, zafirlukast and pranlukast) are advocated for the treatment of asthma (14-16). Present study mainly aimed to investigate the role of intraperitoneal (i.p.) administration of montelukast in peripheral inflammation of formalin-induced rat paw oedema. Further it investigated whether montelukast treatment can modulate the anti-inflammatory effect of indomethacin in formalin-induced paw edema in rats.

Materials and Methods

Animals

Male Wistar rats (150–200 g) were used during the study. Animals were randomly assigned in six groups (n=6) and were housed in polycarbonate cages (6 in each) with bedding under standard laboratory conditions. They were maintained on rat chow (Pars Khurakdam Shushtar, Iran) and had free access to tap water.

Drugs

Montelukast and indomethacin was purchased from Sobhan Pharmaceutical Co., Tehran, Iran. Other reagents were provided by Merck.

Experimental conditions

The test compounds were dissolved in water and administered i.p. 30 min prior to noxious stimuli, and the control animals received corresponding amount of normal saline. Experiments were carried out at constant room temperature and humidity (22 ± 2 ºC; 60 ± 10% relative humidity).

Anti-inflammatory activity

Formalin-induced paw oedema: Acute oedema was induced in the right hind paw of rats by injecting 0.1 ml of solution of 2.5% formalin after 30 min of test drugs administration. Formalin was injected under the plantar region of right hind paw, and the volume was measured using a plethysmometer (UGO Basile, Italy) at 1, 3 and 5 hour after formalin challenge. Inflammation was expressed as the percentage change in paw volume (17). Percent inhibition of the edema was calculated as:

$$\% \text{ Inhibition of oedema} = \frac{V_2 - V_1}{V_1} \times 100$$

v_1: The volume of paw before injection of irritant

v_2: The volume of paw after injection of irritant

Histopathologic examination

Three samples of the formalin-treated paws in the third series were removed and fixed by immersion in 10% formaldehyde solution. After that, the fixed tissues were embedded in paraffin and cut into 3–4 μm sections. The slices were mounted on the glass slides and stained with hematoxylin and eosin for light microscopy analysis.

Statistical analysis

The difference in response to test drugs and controls was determined by one-way analysis of variance, followed by Tukey's test. $P<0.05$ was considered significant.

Results

Time-dependent increase in paw volume after formalin injection

Results are expressed as mean ± S.E.M. The subplanter injection of formalin in rats led to a time dependent gradual increase in paw volume (Figure 1).

Effects of montelukast and indomethacin on formalin-induced paw edema

Montelukast (0.5-2 mg/kg) dose-dependently and significantly decreased the formalin-induced increase in paw volume as compared to control rats. Indomethacin (5 mg/kg) also produced a significant anti- inflammatory effect as compared to control group ($P<0.05$). When indomethacin (5 mg/kg) co-administered with montelukast (1 mg/kg), the anti-inflammatory effect of indomethacin significantly increased as compared to per se effect (Figure 2).

Histopathologic examination

As shown in figure 3A, there was no sign of inflammation in the paw tissue of normal rats. The rats which had received formalin as inflammatory material revealed an acute inflammation in the dermis and epidermis with extensive extravasations, mainly polymorphonuclear (PMN) leucocytes (Figure 3B). There was also accumulation of PMN around capillaries in dermis. As observed in figure 3 (C and D), i.p. injection of montelukast (1 mg/kg) and indomethacin (5 mg/kg), reduced the tissue injuries induced by formalin in the paw skin. At the combination therapy (Figure 3E), not only the PMN infiltration was slight but also congestion was not observed. The paw tissues in this group were more likely a normal tissue than injured tissues.

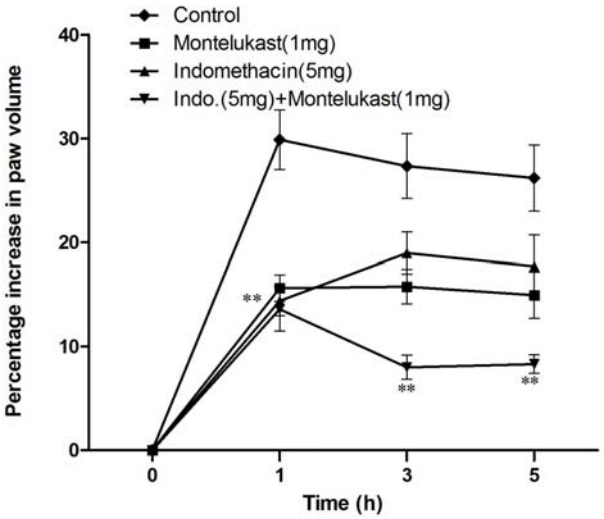

Figure 1. Time course of the alteration of the paw volume after subplanter injection of formalin in control animals, in rats treated with 1 mg/kg of montelukast, in rats treated with 5 mg/kg indomethacin and in rats treated with both montelukast and indomethacin. Data represent group means ± S.E.M. of six observations in each group. ***P*<0.01 compared with control.

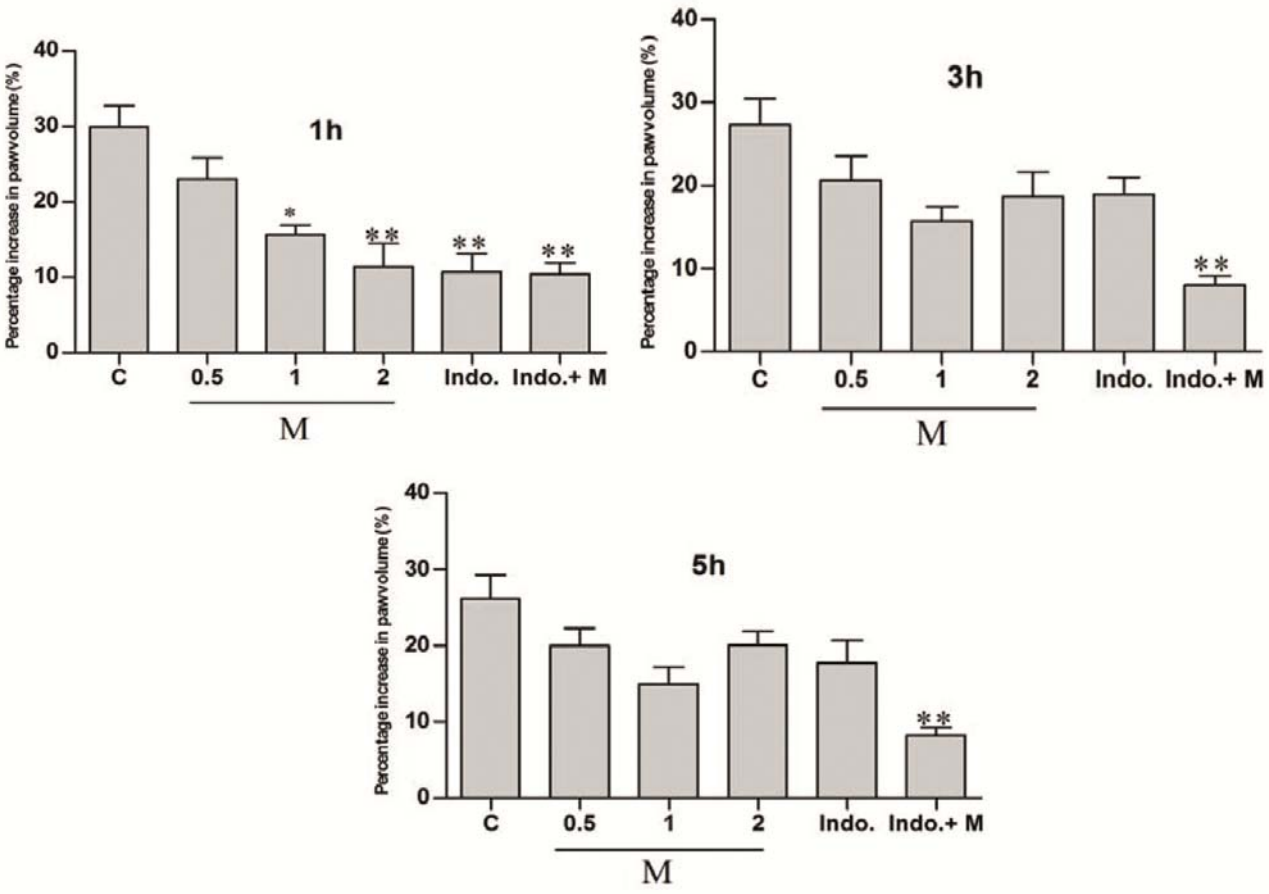

Figure 2. Anti-inflammatory effect of montelukast and its effect on indomethacin-induced anti-inflammatory response. Paw volume was measured 1, 3 and 5 h after formalin injection. Data represent group means ± S.E.M. of six observations in each group. **P*<0.05 and ***P*<0.01 compared with control, respectively. (M, montelukast; Indo., Indomethacin 5mg/kg; Indo.+M, Indomethacin 5mg/kg + montelukast 1mg/kg)

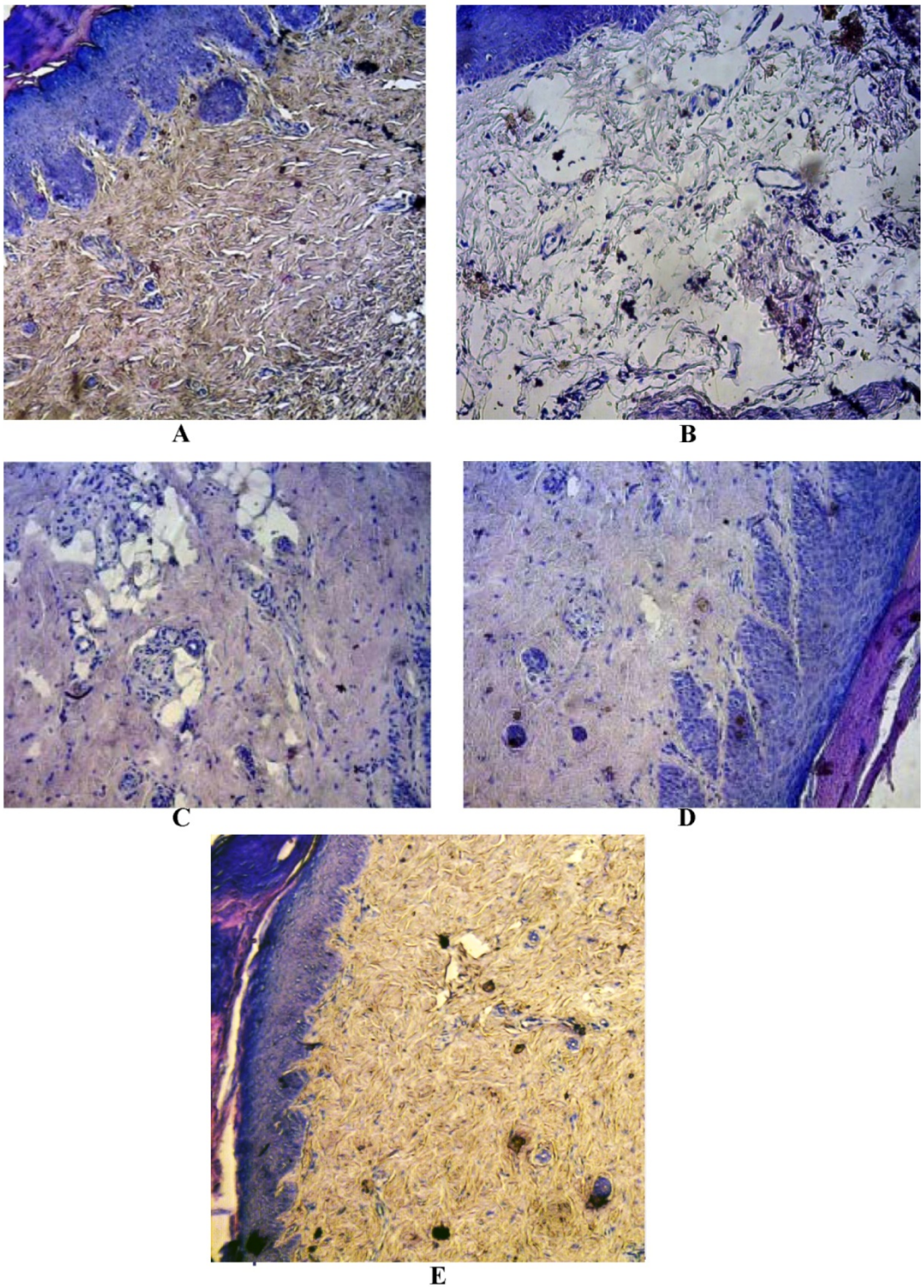

Figure 3. Histopathologic examination of paw skin of rats treated with montelukast and indomethacin, 5 h after subplantar injection of formalin. A: Normal rats show the normal appearance of epidermis and dermis without any lesion. B: formalin-injected paw skin in control group (vehicle-treated). Vasodilatation with edema, and migration of leukocytes were observed. C: formalin-injected paw skin of rats treated with indomethacin (5mg/kg, i.p.). The edema of was noticeable only in some parts. D: formalin-injected paw skin of rats treated with montelukast (1 mg/kg, i.p.). E: formalin -injected paw skin of rats treated with indomethacin (5mg/kg, i.p.) and with montelukast (1 mg/kg, i.p.). The appearance of tissues was more similar to the normal tissues. The injuries induced by formalin and the PMN infiltration reduced, and the congestion of capillaries was rare. Sections were stained with hematoxylin and eosin, magnification ×40.

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 (18).

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 (19). It is reported that leukotriene B4-induced hyperalgesia is totally dependent on the mobilization of polymorphonuclear leukocytes at the site of inflammation (20) and unconstrained by the cyclooxygenation of arachidonic acid.

The results of present study showed that montelukast, a cysteinyl leukotriene receptor antagonist, inhibited the inflammatory response in rats. Although the precise site and mechanism of the anti-inflammatory effect were not addressed in the present investigation, it would seem likely that montelukast blocks the cysteinyl leukotrienes receptors as a consequence of inhibition of the effect of formed leukotrienes due to inflammatory stimuli. The results of the present study clearly indicate that montelukast effectively potentiates the anti-inflammatory effects of indomethacin. The mechanism by which montelukast potentiated indomethacin anti-inflammatory effects is still unknown. However, according to histopathological results it might be due to effects of leukotrienes on the mobilization of leukocytes at the site of inflammation.

Nickerson-Nutter and Medvedeff reported the efficacy of leukotriene synthesis inhibitor along with cyclooxygenase inhibitors in animal model of rheumatoid arthritis (21). Levine *et al*. reported the effects of leukotrienes on the mobilization of polymorphonuclear leukocytes at the site of inflammation (20). Yamauchi *et al*. reported the efficacy of Pranlukast in reduction of the eosinophil ratio in the induced sputum (22).

As already known, NSAIDs are associated with several adverse reactions, primarily gastrointestinal toxicity such as hemorrhagia and ulcerations (23). Among others, the administration of high doses of NSAIDs is also established as a risk factor in NSAID-related gastrointestinal complications (24). Accordingly, one might expect to eliminate this risk factor by a strategy, which provides an effective treatment with a dose of NSAIDs as low as possible. Indeed, montelukast treatment did enhance anti-inflammatory activity of 5 mg/kg indomethacin. Our data proposes that montelukast has the ability to reduce the anti-inflammatory doses of NSAIDs since montelukast effectively potentiates indomethacin-induced anti-inflammatory responses.

In summary, the result revealed that montelukast, a cys-leukotriene-1 receptor antagonist, has peripheral anti-inflammatory effect and markedly potentiates the anti-inflammatory activity of indomethacin. The possible mechanism by which montelukast exerts its potentiation effect on indomethacin actions may include its inhibitory effects on the mobilization of leukocytes at the site of inflammation.

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References

1. Finch CE. Developmental origins of aging in brain and blood vessels: an overview. Neurobiol Aging 2005;26:281–91.
2. Caruso C, Lio D, Cavallone L, Franceschi C. Aging, longevity, inflammation, and cancer. Ann N Y Acad Sci. 2004;1028:1-13.
3. Paoletti R, Gotto AMJ, Hajjar DP. Inflammation in atherosclerosis and implications for therapy. Circulation 2004;109: III20–III6.
4. Rus H, Niculescu FI. Inflammation, aspirin, and the risk of cardiovascular disease. N Engl J Med 1997;337(6):423–4.
5. Firestein GS. Inhibiting inflammation in rheumatoid arthritis. N Engl J Med 2006;354(1):80–2.
6. Klegeris A, McGeer EG, McGeer PL. Therapeutic approaches to inflammation in neurodegenerative disease. Curr Opin Neurol 2007;20(3):351–7.
7. Libby P, Plutzky J. nflammation in diabetes mellitus: role of peroxisome proliferator-activated receptor-alpha and peroxisome proliferator-activated receptor-gamma agonists. Am J Cardiol 2007;99(4A):27B-40B.

8. Yan SF, Ramasamy R, Schmidt AM. Mechanisms of disease: advanced glycation end-products and their receptor in inflammation and diabetes complications. Nat Clin Pract Endocrinol Metab 2008;4(5):285-93.
9. Tsirpanlis G. Inflammation in atherosclerosis and other conditions: a response to danger. Kidney Blood Press Res 2005;28(4):211-7.
10. Nathan C. Points of control in inflammation. Nature 2002;420(6917):846–52.
11. Heller A, Koch T, Schmeck J, Van Ackern K. Lipid mediators in inflammatory disorders. Drugs 1998;55(4):487–96.
12. Nakadate T, Yamamoto S, Iseki H, Sonoda S, Takemura S, Ura A, Hosoda Y, Kato R. Inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion by nordihydroguaiaretic acid, a lipoxygenase inhibitor, and p-bromophenacylbromide, a phospholipase A2 inhibitor Gann 1982(73):841–3.
13. Parker CW. Lipid mediators produced through the lipooxygenase pathway. Annu Rev Immunol 1987;5:65-84.
14. Chung KF, Barnes PJ. Zafirlukast (Accolate). Drugs Today 1998;34(4):375–88.
15. Vianna EO, Martin RJ. Recent innovations in asthma therapy. Drugs Today 1998;34(4):341–51.
16. Weisberg SC. Pharmacotherapy of asthma in children with special reference to leukotriene receptor antagonists Pediatr Pulmonol 2000;29(1):46–61.
17. Jain NK, Singh A, Kulkarni SK. Analgesic, anti-inflammatory and ulcerogenic activity of a zinc–naproxen complex in mice and rats. Pharm Pharmacol Commun 1999;5:599-602.
18. Radhofer-Welte S, Rabasseda X. Lornoxicam, a new potent NSAID with an improved tolerability profile Drugs Today 2000;36(1):55–76.
19. Chung KF. Leukotriene receptor antagonists and biosynthesis inhibitors: potential breakthrough in asthma therapy. Eur Respir J 1995;8(7):1203–13.
20. Levine JD, Lau W, Kwiat G, Goetzl EJ. Leukotriene B4 produces hyperalgesia that is dependent on polymorphonuclear leukocytes. Science 1984;225(4663):743–5.
21. Nickerson-Nutter CL, Medvedeff ED. The effect of leukotriene synthesis inhibitors in models of acute and chronic inflammation. Arthritis Rheum 1996;39(3):515–21.
22 Yamauchi K, Tanifuji Y, Pan LH, Yoshida T, Sakurai S, Goto S, Kuroda S, Kobayashi H, Inoue H. Effects of pranlukast, a leukotriene receptor antagonist, on airway inflammation in mild asthmatics. J Asthma 2001;38(1):51-7.
23. Feldman M, McMahon AT. Do cyclooxygenase-2 inhibitors provide benefits similar to those of traditional nonsteroidal antiinflammatory drugs, with less gastrointestinal toxicity? Ann Intern Med 2000;132(2):134–43.
24. Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med 1999;340(24):1888–99.