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# The effect of montelukast in a model of gouty arthritis induced by sodium monourate crystals.

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**Key words:** Acute gouty arthritis, air pouch, sodium monourate, montelukast, leukotrienes.

**Abstract.** Non-steroidal anti-inflammatory drugs (NSAIDS) are the first line of therapy in acute gouty arthritis. NSAIDs inhibit the cyclooxygenase pathway, but not the lipooxygenase activity and can have many adverse effects and thus have a limited effect on the control of inflammation in this disease. In this work we studied the effect of montelukast on the cellular inflammatory infiltrate in a model of murine arthritis induced by sodium monourate crystals (SMU), using a subcutaneous air cavity (air pouch) in BALB/c mice. Seven groups of BALB/c mice (n = 4) were distributed into five experimental groups and two inflammatory control groups, a positive and a negative one. Previous to SMU exposure, the experimental groups received montelukast (1 and 0.01 mg/Kg/w) and/or indomethacine (2.5 mg/Kg/w), followed by administration of SMU in the air pouch. The total and differential counts of inflammatory cells were analyzed after 2, 6, 12 and 24 hours. Montelukast, significantly reduced the total number of cells ($p<0.05$), with a predominant impact on polymorphonuclear over mononuclear cells, especially after 12 hours of the medication. The montelukast/indometacine combination showed an additive effect. Our data show that montelukast has an anti-inflammatory effect in the model of gouty arthritis. Consequently, anti-leukotrienes could represent a new and effective therapy, either isolated or combined with conventional therapy of gouty arthritis.

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**Efecto de montelukast en un modelo de artritis gotosa inducido por cristales de monourato de sodio.**
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**Palabras clave:** Artritis gotosa aguda, bolsa de aire, monourato de sodio, Montelukast, leucotrienos.

**Resumen.** En artritis gotosa aguda las drogas antiinflamatorias no esteroideas son la primera línea terapéutica. Este tratamiento no es satisfactorio porque inhibe la ciclooxigenasa sin modificar la actividad de la lipooxigenasa, y puede acompañarse de numerosos efectos adversos. Investigamos el efecto de montelukast sobre el infiltrado celular inflamatorio en un modelo de artritis múrida inducida por cristales de monourato de sodio (MUS) en el modelo experimental de la bolsa de aire (*air pouch*). Siete grupos de ratones BALB/c (n = 4) fueron distribuidos en cinco grupos experimentales y dos grupos controles inflamatorios: positivo y negativo. Los grupos experimentales recibieron, montelukast (1 y 0,01 mg/Kg/p) y/o indometacina (2,5 mg/Kg/p) por vía oral, previo a la administración de MUS en la bolsa del aire. El conteo absoluto y diferencial de las células inflamatorias fue analizado después de 2, 6, 12 y 24 horas de tratamiento. El tratamiento con montelukast redujo significativamente el número total de células presentes en el infiltrado inflamatorio ($p < 0,05$), con un efecto mayor sobre polimorfonucleares que sobre las células mononucleares, y con un máximo efecto a las 12 horas después de la administración del medicamento. La combinación montelukast/indometacina mostró un efecto aditivo. Los resultados demuestran que montelukast tiene un efecto antiinflamatorio en el modelo de la artritis gotosa. Por lo tanto, los anti-leucotrienos podrían representar una nueva y eficaz terapia, aislada o en combinación con la terapéutica convencional, para la artritis gotosa.

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## INTRODUCTION

Gouty arthritis (GA) is a rheumatic condition characterized by hyperuricemia and sodium monourate crystal (SMU) deposits in one or some peripheral joints (1, 2). Once SMU crystals are deposited, joint tissues are colonized by neutrophils, monocytes/macrophages, platelets, and mast cells (3, 4). Recruitment and activation of neutrophils by SMU play a critical role in the pathogenesis and perpetuation of the acute gout (5).

Inflammatory cells secrete proinflammatory mediators and chemotactic agents such as leukotrienes (LT), tumoral necrosis factor (TNF), complement factor C5a, and interleukin 8 (IL-8) (3, 4, 6). Leukotrienes may be very relevant in this pathology, since LTB4 is the most potent chemotactic agent for neutrophils both, *in vitro and in vivo* (7). Consequently transendothelial migration occurs and more neutrophils colonize synovial tissues (8, 9). Non steroidal antinflammatory drugs (NSAIDS) are the first line therapy for this disease. Colchicine and steroids, local or systemic,

are also used. This therapy is partially effective, but can induce adverse reactions and/or toxicity in aproximately 80% of patients. The mechanism of action involves inhibition of the cyclooxygenase enzyme, and blockade of the synthesis of prostaglandins (PG), but they have no effect in the lipooxygenase enzyme activity, responsible for the synthesis of LTs (10, 11), which are the most important inflammatory mediators in gouty arthritis (10). In inflammatory pathologies, such as bronchial asthma (12, 13) and allergic rhinitis (13, 14), in which LTs are known to play an essential role, different selective antagonists of cysteinyl LTs are shown to have a beneficial effect.

Since LTs are important inflammatory mediators in gouty arthritis (15 Rae) and drugs such as montelukast (Mk) act as a competitive inhibitor of LTs by joining selectively to LT receptors (16), especially to the LTD4 receptor, we sought to investigate the effect of montelukast in a model of experimental arthritis induced by SMU crystals deposits in the air pouch model in BALB/c mice (17).

## METHODS

### Animals

One hundred and five male BALB/c mice, 10 weeks old, on a regular diet, *ad libitum,* under strict conditions of hygiene and temperature (25-26°C) were chosen for the experimental protocol. Surgical procedures and animal treatments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (FONACIT).

### Experimental protocol

BALB/c mice were treated to produce an air pouch on their backs, by applying a subcutaneous injection of 5 mL of sterile air on day one, and 3 mL 3 days later (17). Thereafter, mice were distributed in five experimental groups (15 each) and two control groups (15 each). The experimental groups were designated as A, B, C, D, E, a positive control group for inflammation (F) and a negative control group (G). All the experimental groups received Mk and/or indomethacine (Indo), orally as follows: Mk 0.01 mg/Kg/w and 1 mg/Kg/w group A and group B respectively; Indo 2.5 mg/Kg/w group C; groups D and E received both drugs at same doses. Groups F and G received, buffer phosphate saline (PBS, Dako) orally, as control. One hour later, groups A-F were injected in the air pouch with 2 mL of SMU (5 mg/mL) prepared in PBS according to the Denko method (18). Group G received only PBS pH 7.2. Each animal group received an overdose of ether and was then sacrificed at 2, 6, 12 and 24 hours, to obtain samples for its analysis.

### Sample collection and processing

The air pouch was washed with 2 mL PBS pH 7.2, plus 20 units of sodium heparine (Lilly®). Samples from all groups were obtained at 2 (n = 3), 6 (n = 4), 12 (n = 4) and 24 (n = 4) hours, and centrifuged at 1.200 rpm, at 25°C, for 5 minutes. The pellet was resuspended in 1 mL of PBS. Total and differential cellular counts were done using hematoxiline/eosine in a Neubauer chamber, with an optical microscope (400X).

### Statistical analysis

Data are expressed as mean ± 2 SD (n = 4) and the Student's test was used to analyze the results. A P value of less than 0.05 was considered statistically significant.

## RESULTS

### Effect of Mk on the inflammatory exudate induced by SMU in the air pouch of experimental mice

The total cell number in the inflammatory exudates of SMU-treated mice progres-

sively increased, reaching a value of 1,264,583 ± 434,771 cell/mL after 2 hours and a peak of 6,071,250 ± 1,043,857 cell/mL at 12 hours, followed by a decline to 3,795,000 ± 1,724,824 cell/mL at 24 hours. In the negative inflammatory control (–) the total cell number reached a peak of 200 ± 50 cel/mL. At doses of 0.01 and 1 mg/Kg/w, Mk significantly decreased the total cell number in inflammatory exudates ($p < 0.05$). When comparing both doses of Mk, it was found that Mk 1mg/Kg/w had a significantly greater effect than 0.01 mg/Kg/w, but only at 24 hours (Fig. 1). This reduction reached 46.5% and 47.8% at 2 hours; 78% and 69% at 6 hours; 82.1% and 86.9% at 12 hours and 73.1% and 87.5% at 24 hours, respectively. At the dose of 2.5 mg/Kg/w, Indo significantly decreased the total cell count at all times tested ($p < 0.05$) (Fig. 2). The inhibitory effect was 61.6% at 2 hours, 86.4 % at 6 hours, 87.4% at 12 hours and 86.7% at 24 hours (data not shown).

The combination of Mk (0.01 mg/Kg/w) plus Indo (2.5 mg/Kg), and Mk (1 mg/Kg/w) plus Indo (2.5 mg/Kg) showed a stronger inhibitory effect at 12 hours when comparing with Mk at either dose alone (Fig. 3). When Mk 0.01mg/Kg/w and Indo (2.5 mg/Kg) were used in combination the reduction reached 92.7 % and 94.6% (Fig. 3).

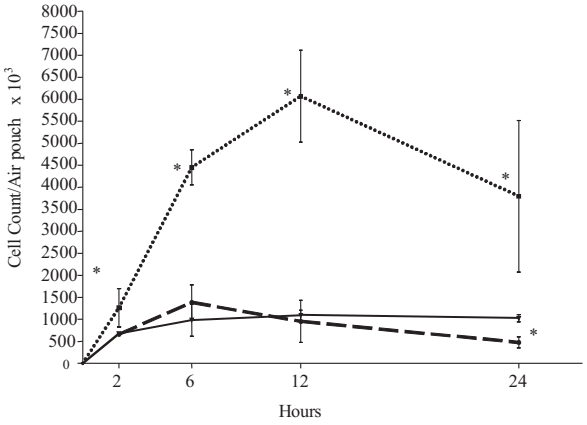


Fig. 1. Effect of montelukast on total cell count (mean ± 2D) of the inflammatory exudates induced by SMU crystals in the air pouch model in BALB/c mice. Dashed and solid lines indicate treatment with Mk 1 mg/Kg/w and 0.01 mg/Kg, respectively. Dotted line indicates without treatment. *$p < 0.05$.

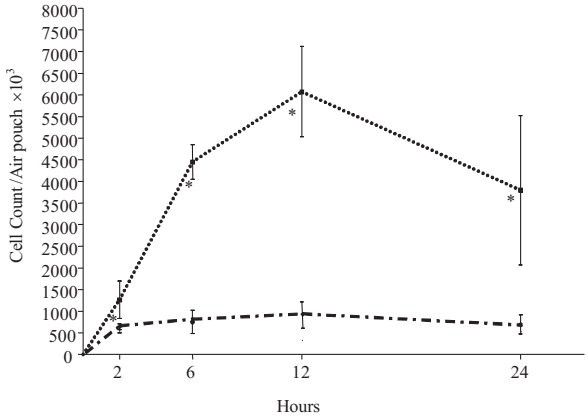


Fig. 2. Effect of indometacine on total cell count (mean ± 2D) of the inflammatory exudates induced by SMU crystals in the air pouch model in BALB/c mice. The pointed line indicates treatment with SMU, the dashed line indicates treatment with indometacine (2.5 mg/Kg). *$p < 0.05$.

**Effect of Mk and Indo on the differential cell count in the inflammatory exudate induced by SMU in the air pouch mice model**

Fig. 4 shows the differential cell count of inflammatory exudates in the experimental groups at 12 hours. SMU-treated air pouch mice showed a total number of 6,071,250 cell/mL, with a predominance of polymorphonuclear cells (73%). In PBS-treated mice the total number of cells was 200,000/mL, with a predominance of mononuclear cells (68 %). At the doses 0.01 and 1 mg/Kg Mk significantly decreased in the total number of cells 1083 (17%) and 957 (7%), respectively, with a greater effect over the percentage of

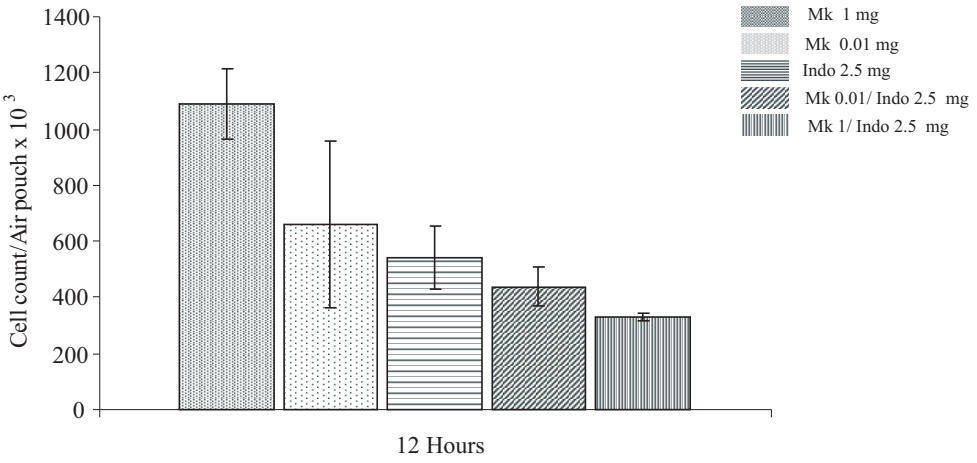


Fig. 3. Montelukast and/or Indometacine effect on the total cell count (mean + 2D) in the inflammatory exudate induced by MUS crystals at 12 hours. Histogram with fine points indicates treatment with Mk 1 mg/Kg/w; histogram with heavy points indicates treatment with Mk 0.01 mg/Kg/w; histogram with horizontal lines indicates treatment with Indometacine 2.5 mg/Kg/w; histogram with diagonals lines indicates treatment with Montelukast 0.01 + Indometacine 2.5 mg/Kg/w and histogram with vertical lines indicates treatment with Montelukast 1 + Indometacine 2.5 mg/Kg/w. *p< 0.05.

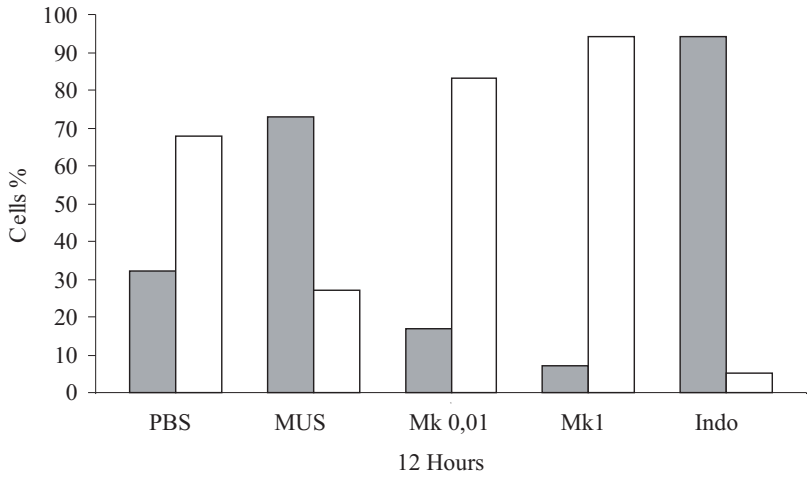


Fig. 4. Montelukast effect in the differential cell count (mean + 2D) in inflammatory exudate of gouty arthritis model at 12 hours. Values represent the percentage of polimorphonuclear cells (gray histogram) and mononuclear cells (white histogram).

polymorphonuclear cells. Indo also modified the total number of cells but the most important effect was seen on mononuclear cells 39,463 (5%).

## DISCUSSION

The current drug therapy for GA is limited and is frequently accompanied by common adverse reactions (19). NSAIDS, which are the first line of choice therapy for GA, have a partial effect on the characteristic inflammatory response of this disease, perhaps due to their inability to block the synthesis of LTs (19). As relatively innocuous antagonists of LTs receptors are now available and have been proven beneficial in diverse inflammatory conditions (12-14) we

sought to study the effect of Mk and compare it to that of Indo, the most commonly prescribed NSAID in gouty arthritis, using an experimental gouty arthritis model (air pouch) in BALB/c. SMU crystals can induce a rapid and intense inflammatory cellular infiltration, with a prominence of neutrophils in a subcutaneous cavity (air pouch), with a pattern similar to the inflammatory cell infiltrate seen in patients with acute gouty arthritis (20). Our results confirmed previous observations supporting the validity of this model (air pouch) to study this type of inflammation (7, 21).

The administration of Mk significantly reduced the cellular infiltrate induced by SMU crystals in the air pouch during both, the early and late phases of inflammation. It also modified the differential cellular count by significantly decreasing the percentage of polymorphonuclear cells. Our results may suggest that Mk mainly affects the influx of neutrophils, having only a partial effect on mononuclear cells. Doses as low as 0.01 mg/Kg/w of Mk were shown as effective as 1 mg/Kg/w, except at 24 hours. The inhibitory effect of Indo alone in the overall cell count was seen earlier and was slightly superior than that of Mk. Interestingly, unlike the effect of Mk, Indo showed a predominat effect on mononuclear rather than polymorphonuclear cells, that may be explained by the preferential blockade of the cyclooxygenase pathway that has a lesser effect on the chemotaxis of polymorphonuclear cells. When combined at optimal doses, both drugs showed an clear additive effect. Our results confirm that mediators derived from the cyclooxygenase and the lipooxygenase pathways participate in the inflammatory response of gouty arthritis.

Previous investigations (22) showed that LTB4 is the strongest LT triggering inflammation induced by SMU, by having a significant effect on the chemotaxis and activation of neutrophils (4, 23). In addition, LTB4 is known to induce the synthesis of pro-inflammatory cytokines such as IL-8, IL-1 y TNF-α (23-25). Mk is a selective antagonist of the LTD4 receptor, a pathway shown to play minor role gouty arthritis (26, 27), Therefore, it is possible that LTD4 may have a heretofore unknown role in acute gouty arthritis. Alternatively, Mk may act inhibiting the synthesis and/or the effect of LTB4 as recently suggested by the demonstration that antagonist of LT receptors are alosteric inhibitors of the 5-lipooxygenase enzyme (28).

In fact, in the study by Ramires et al. (28), and similar to findings reported in a zimosan rat model model of rheumatoid arthritis (29), it was shown that blocking the activity of LTs plays an important role in decreasing the inflammatory response induced by SMU crystals and zimosan. Our results suggest that antagonists of LT receptors, alone or in combination with NSAIDS, can be a potentially beneficial therapy in the control of acute GA.

## REFERENCES


1. **Cohen M, Emmerson B.** Crystal-related arthropaties gout. In: Klippel J.H; Dieppie P and Arnett. (ed). Rheumatology. 2nd Ed. London. St Louis, Mosby.1998. pp. 14. 1-14.14.
2. **Emmerson B.** The management of gout. Curr Op Immunol 1996; 206:334-345.
3. **Pascual E.** Hiperuricemia and gout. Curr Op Rheumatol 1994; 6: 454-458.
4. **Naotaka S, Keiko S, Shiota N, Shimoura K, Hideki H.** Pathophysiological role of mast cells in collagen-induced arthritis: Study with a cysteinyl leukotriene receptor antagonist, montelukast. Eur J Pharmacol. 2006; 548:158-166.
5. **Gaudry M, Roberge C, de Médicis R, Lussier A, Poubelle P, Naccache P.** Crystal-iniduced neutrophil activation. III. Inflammatory microcrystals induce a distinct pattern of tyrosine phosphorylation in hu-

man neutrophils. Lab Clin Invest 1993; 4:1649-1655.
6. **Terkeltaub R, Zachariae C, Santoro D, Martin J, Peveri P, Matsushima K**. Monocyte -derived neuthrophil chemotactic factor interleukin-8 is a potential mediator of crystal-induced inflammation. Arthritis Rheum 1991; 34:894-903.
7. **Crooks S, Stockley R.** Leukotriene B4. Int J Biochem Cell Biol 1998; 2:173-178.
8. **Hoosiau F.** Cytokines in rheumatoid arthritis. Clin Rheumatol 1995; 14:10-13.
9. **Ryckman C, McColl S, Vandal K, Médicis R, Lussier A, Poubelle P, Tessier PA.** Role of S100A8 and S100A9 in Neutrophil recruitment in response to monosodium urate monohydrate in the air-pouch model of acute gouty arthritis. Arthritis Rheum 2003; 48:2310-2320.
10. **Ferrari A, Van Linthoudt D, Morrone L, Branigan P, Schumacher H, Baker D.** Nonsteroidal anti-inflammatory drugs and prostaglandins: Their interactions and effects on the particulate-induced inflammatory process implicated in joint implant-loosening and on monosodium urate crystal-induced inflammation. Am J Ther 1996; 3:189-194.
11. **Serhan C, Lundberg U, Weissmann G, Samuelsson B**. Formulation of leukotrienes and hydroxy acids by human neutrophils exposed to monosodium urate. Prostaglandins 1984; 17:563-581.
12. **Bisgaard, H.** Leukotriene modifiers in pediatric asthma management. Pediatrics 2001; 10:381-389.
13. **Nayak A.** A review of montelukast in the treatment of asthma and allergic rhinitis. Expert Opin Pharmacother 2004; 5:679-686.
14. **Banasiak N, Meadows-Oliver M.** Leukotrienes: their role in the treatment of asthma and seasonal allergic rhinitis. Pediatr Nurs 2005; 31:35-38.
15. **Rae S, Davidson EM, Smith MJ.** Leukotriene B4, an inflammatory mediator in gout. Lancet 1982; 2:1122-1124.
16. **Jones T, Labell M, Belley E, Champion L, Charette L, Evans J.** Phahmacology of Montelukast sodium (Singulair), a potent a selective leukotriene D4 receptor antagonist. Can J Physiol Pharmacol 1995; 73: 191-201.
17. **Edwards J, Sedgwick A, Willoughby A.** The formation of a structure with the features of synovial lining by subcutaneous injection of air: An in vivo tissue culture system. J Pathol 1980; 134:147-156.
18. **Denko CW, Whitehouse MW.** Experimental inflammation induced by naturally occurring microcrystalline calcium salts. J Rheumatol 1976; 54-62.
19. **Fam A.** Treating acute gouty arthritis with selective COX 2 inhibitors. Biomedical J 2002; 325:980-981.
20. **Schiltz C, Liote F, Prudhommeaux F, Meunier A, Champy R, Callebert J. Bardin T.** Monosodium rate monohydrate crystal induced inflammation in vivo: quantitative histomorphometric analysis of cellular events: Arthritis Rheum 2002; 46: 1143-1150.
21. **Rull M, Clayburne G, Sieck M, Shumacher H**. Intra-articular corticosteroid preparations: different characteristics and their effect during inflammation induced by monosodium urate crystals in the rat subcutaneous Air pouch. Rheumatology 2003; 9:1093-1100.
22. **Sally E**. Leukotriene Inhibitors. In Rich R, Fleisher T, Shearer W, Kotzin B and Schroeder Jr H (Eds): Clinical and Practice Immunology. England. Mosby Ed. 2001:108.1-108.7.
23. **Di Giovine FS, Malawista SE, Nuki G, Duffi GW.** Interleukin 1 (IL-1) as a mediator of Crystal Arthritis: stimulation of T cell and synovial mitogenesis by urate Crystal -induced IL-1. J Immunol 1987; 138: 3213-3218.
24. **Punzi L, Calo L, Plebani M.** Clinical significance of cytokine determination in synovial fluid. Crit Rev Clin Lab Sci 2002, 39:63-88.
25. **Edgar J, Payan D**. Inmunopathogenic roles of leukotrienes in human disease. J Clin Immunol 1984; 4:79-84.
26. **Brain S, Williams T**. Leukotrienes and inflammation. Pharmacol Ther. 1990; 46(1): 57-66.
27. **Thomsen M, Ahnfelt-Ronne I.** Leukotrienes: A review of the significance in man

and the possibilities for therapeutic intervention. Ugeskr Laerger. 1991; 153(3): 173-176.

28. **Ramires R, Caiaffa M, Tursi A, Haeggstrom J, Macchia L.** Novel inhibitory effect on 5-lipoxygenase activity by the anti-asthma drug Montelukast. Biochem Biophys Res Commun. 2004; 324: 815-821.
29. **Castro F, Martins M, da Silva J, Carneiro V, Márquez M, de Albuquerque R, de Queiroz F.** Blockade of leukotriene B4 prevents articular incapacitation in rat zimosan-induced arthritis. Eur J Pharmacol. 2004; 497: 81-86.