Prophylactic Effect of Highly Selective COX-2 Inhibition in Acute Monosodium Urate Crystal Induced Inflammation in the Rat Subcutaneous Air Pouch

SELIM NALBANT, LAN X. CHEN, MARIE S. SIECK, GILDA CLAYBURNE, and H. RALPH SCHUMACHER

ABSTRACT. ***Objective.*** To examine the ability of rofecoxib prophylaxis to blunt the effect of monosodium urate (MSU) crystal inflammation induced in the rat subcutaneous air pouch.

Methods. Eight rats were used in each of 4 groups. On day one, air was injected subcutaneously to create the pouches and gavage feedings were started with placebo or 2 different doses of rofecoxib. Six days later MSU crystals or saline were injected into the pouches. Twenty-four hours later, rats were examined, sacrificed, and pouch fluid studied.

Results. Rofecoxib 15 or 30 mg/kg given for 6 days before MSU crystal injection into rat air pouches significantly suppressed the inflammation following injection of 10 mg crystals ($p = 0.001$) and tended to suppress the milder inflammation induced by 5 mg MSU. Greater effects on phagocytosis were seen with 30 mg/kg rofecoxib. Tumor necrosis factor-α levels in pouch fluid measured by ELISA were not suppressed by the rofecoxib.

Conclusion. Prophylactic use of this cyclooxygenase 2 (COX-2) selective inhibitor in this pilot study suppressed acute MSU crystal induced inflammation. Effects on cytokines need further investigation. COX-2 inhibitors deserve consideration for prophylactic use in interim gout. (J Rheumatol 2005;32:1762–4)

Key Indexing Terms:
GOUT COXIB AIR-POUCH INFLAMMATION

Gout is the most common form of inflammatory joint disease in men over the age of 40[1]. Acute gout is an intense, extremely painful, inflammatory arthritis with relapsing inflammatory attacks resulting from formation of monosodium urate (MSU) crystals in the affected joint space. The prophylactic benefit of colchicine to decrease attacks of gout has been shown and colchicine is widely used in prevention of attacks[2]. Nonsteroidal antiinflammatory drugs (NSAID) are the most frequently prescribed for acute gout and they are also often used for prophylaxis but have not been systematically studied[3-5]. Cyclooxygenase 2 (COX-2) selective inhibitors (coxibs), such as celecoxib and rofecoxib (before withdrawal from the market), have been shown to be as effective as NSAID in the treatment of osteoarthritis, rheumatoid arthritis, and acute pain and another highly selective drug, etoricoxib, was as effective as indomethacin in acute gout and offered greater gastrointestinal safety[3].

Our study examined the ability of a coxib (rofecoxib) to act prophylactically to blunt the effect of MSU crystal induced inflammation in the rat subcutaneous air pouch model.

MATERIALS and METHODS

Crystal preparation. Synthetic and endotoxin free MSU crystals were prepared as described by McCarty and Faires[6]. They were washed, dried, and sterilized by autoclaving. Crystals were then suspended with sterile saline solution at a concentration of 1 mg/ml just prior to use.

Rat air-pouch model. Subcutaneous air pouches as previously described[7] were produced under ketamine anesthesia on the dorsum of Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) weighing 150-250 mg at time of experiment. Twenty-four milliliters sterile air was injected subcutaneously through a 0.25 μm microfilter into the backs of the animals to create a pseudosynovial cavity[8,9]. A second air injection was given on day 3, if needed, to keep the air pouch inflated.

Preparations of rofecoxib suspensions. Solutions of 0.5% methylcellulose in water were stirred while adding the appropriate amount of rofecoxib to make 2 doses (15 mg/kg and 30 mg/kg) of the drug. The suspensions were sonicated until homogeneous and stored at 2-8 ºC until use.

Experimental design. Rofecoxib or placebo was given by gavage in daily doses for 6 days before inflammation was induced by injecting 5 or 10 ml of MSU crystal suspension into 6-day-old rat air pouches. Study groups included (1) positive control group receiving MSU crystals only; (2) negative control group receiving sterile saline (no crystals or drugs); and (3) 2 treatment groups receiving MSU crystals and rofecoxib (either 15 mg/kg or 30 mg/kg). The last gavage of the drugs was given 30 min before the MSU injection. There were 8 rats in each group.

From the University of Pennsylvania School of Medicine and the Arthritis Center, VA Medical Center, Philadelphia, Pennsylvania, USA.

Supported by Merck Sharp and Dohme, Inc.

S. Nalbant, MD, Rheumatology Research Fellow, University of Pennsylvania School of Medicine; L.X. Chen, MD, Clinical Assistant Professor, University of Pennsylvania; M.S. Sieck, Laboratory Technician; G. Clayburne, Laboratory Technician; H.R. Schumacher, MD, Professor of Medicine, University of Pennsylvania, Chief of Rheumatology, VA Medical Center.

Address reprint requests to Dr. H.R. Schumacher, VA Medical Center, University & Woodland Aves., Philadelphia, PA 19104-4283, USA. E-mail: schumacr@mail.med.upenn.edu

Accepted for publication April 20, 2005.

Twenty-four hours after crystal injection, at the peak of acute inflammation (based on results from our preliminary studies, data not shown) rats were sacrificed (in a closed CO_2 box). Pouches were injected with 5 ml saline, and the lavage fluid was collected by aspiration for subsequent analyses.

Assessment of inflammation. White blood cell (WBC) counts of air pouch fluid were performed under regular light microscopy[10]. The percentage of phagocytic cells in air pouch fluid containing urate crystals (percentage of phagocytosis) was quantified by counting 100 phagocytic cells and recording those containing crystals. Tumor necrosis factor-α (TNF-α) levels in the air pouch fluid were measured only in the MSU 10 mg group by enzyme immunoassay using commercial assay kits (Mouse Biotrak Assay, Amersham Bioscience, Piscataway, NJ, USA).

Statistical analysis. Data are summarized in Table 1. Values are expressed as mean ± standard deviation (SD). Between group comparisons of variables were performed by one-way analysis of variance, and differences between the positive control and other groups were examined by using Dunnett's Multiple Comparison Test. P values less than 0.05 were considered significant.

RESULTS

As shown in Table 1, injection of MSU crystals into the preformed air pouch induced acute inflammation in this experimental model. Both leukocyte counts and TNF-α as inflammation markers were significantly increased in positive control animals compared to the negative control group.

Effects of treatment on pouch fluid WBC counts. Infiltrating WBC in air pouch lavage fluid were significantly reduced compared to untreated controls in animals treated with either 15 or 30 mg/kg of rofecoxib except for insignificant differences for mice injected with 15 mg/kg rofecoxib and 5 mg MSU crystals ($p > 0.05$). The difference between the 2 dosages of rofecoxib was not significant ($p > 0.05$) when comparing mice injected with either 5 or 10 mg of MSU crystals (Figure 1).

Effects on percentage of phagocytosis of pouch fluid. With the lower dosage (5 mg) of MSU crystals, the percentage of phagocytosis was significantly decreased at both dosages of rofecoxib ($p < 0.05$). The percentage of phagocytosis was also significantly more decreased at the higher dose of rofecoxib ($p < 0.05$). With the 10 mg dose of MSU crystals, phagocytosis trends were less clear.

Table 1. Effects of rofecoxib on markers of MSU crystal induced inflammation in air pouch fluids. Values are mean ± SD unless otherwise stated.

	MSU (10 mg)			MSU (5 mg)	
	WBC/mm^3	% Phagocytosis	TNF-α, ng/ml	WBC/mm^3	% Phagocytosis
Saline (negative control group)	50 ± 70.7	0	0.79 ± 0.05	43.7 ± 41.7	0
MSU alone (positive control group)	1412.5 ± 39.7	4.1 ± 1.9	1.19 ± 0.07	156.2 ± 252.7	7.3 ± 4.43
Rofecoxib 15 mg/kg + MSU	477.8 ± 3.8	10.1 ± 8.7	1.29 ± 0.15	111.1 ± 102.4	3.2 ± 2.8
Rofecoxib 30 mg/kg + MSU	444.5 ± 184.5	1.6 ± 1.4	1.53 ± 0.30	72.2 ± 68.2	1.4 ± 1.3

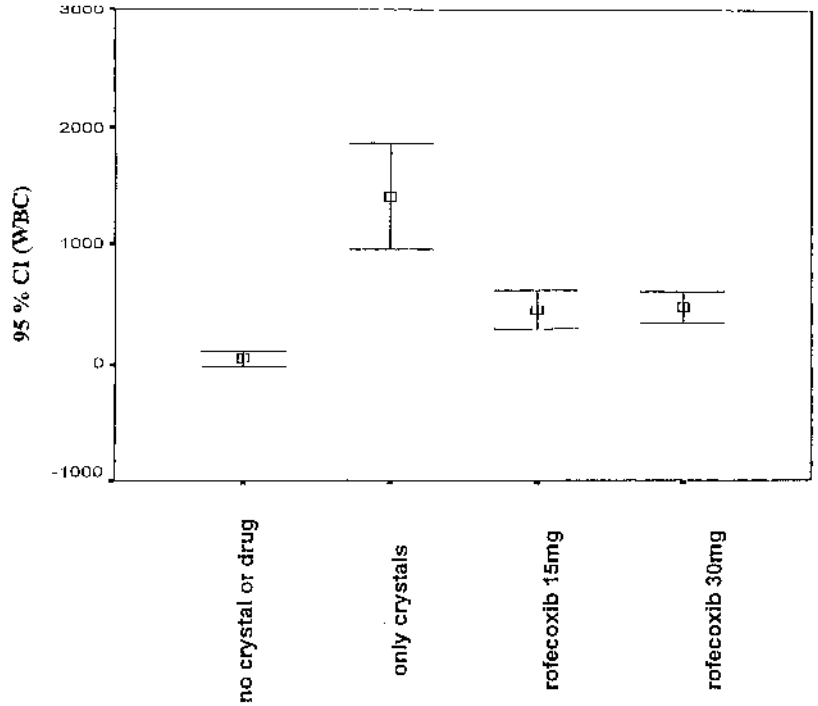

Figure 1. Leukocyte counts in air pouch fluids at 10 mg dosage of MSU crystals. WBC were significantly reduced by rofecoxib. Numbers of infiltrating WBC in the air pouch lavage fluid were significantly lower after treatment with both 15 and 30 mg/kg rofecoxib compared to MSU alone ($p < 0.05$). There was no significant difference between the 2 dosages of rofecoxib ($p > 0.05$).

Effect on air pouch fluid TNF-α levels. TNF-α concentrations in air pouch fluid were only assayed in the 10 mg MSU crystal group. In this group of rats, the increased TNF-α level caused by MSU injection was not altered by rofecoxib treatment. Although not statistically significant, rofecoxib at both dosages tended to increase TNF-α slightly (Table 1, Figure 2).

DISCUSSION

It has been shown that infiltration of joints by neutrophils plays a significant role in the development of gouty arthritis[11]. Therefore, a drug that protects patients from gout flares should have the capacity to suppress neutrophil infiltration. It has been shown clinically that at least one coxib can suppress inflammation during gout attacks in humans[3]. However, possible prophylactic effects of coxibs against gout attacks has not been studied. Rofecoxib prophylaxis at both doses (15 mg/kg and 30 mg/kg) significantly suppressed inflammation induced by MSU crystals in our rat model. The effect was seen more clearly in neutrophil numbers than in the percentage of phagocytosis. Coxibs may not directly affect phagocytosis but may suppress inflammatory response to MSU crystals on the basis of neutrophil migration and infiltration[12]. The rofecoxib doses we used were high but comparable to doses/kg used in published studies in rats. Testing with lower doses would be of value.

Because TNF-α is one of the major cytokines in joints in chronic arthritis, the effect of rofecoxib on TNF-α was also studied. Rofecoxib did not suppress TNF-α levels; indeed, they even tended to increase. This result is consistent with a study by Pinheiro and coworkers who showed that rofecoxib and celecoxib exerted a stimulatory effect on the production of TNF-α[13]. As seen with other NSAID, this is hypothesized to be due to inhibition of prostaglandins, which normally inhibit the production of TNF-α[7].

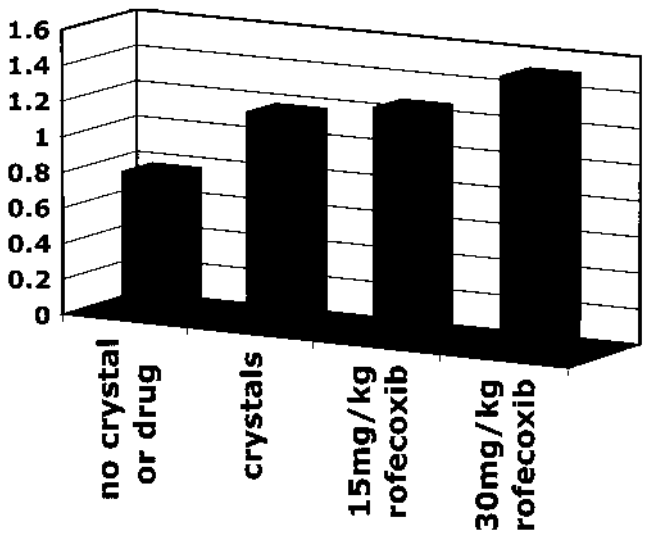

Figure 2. Levels of TNF-α in air pouch fluids. Rofecoxib at both dosages tended to increase TNF-α although neither level was significantly higher versus crystals alone.

Our results show that prophylactic inhibition of COX-2 suppressed some aspects of MSU crystal induced inflammation. These findings encourage us to further consider the possible use of these types of agents for prophylaxis in clinical practice.

ACKNOWLEDGMENT

We thank Merck Sharp and Dohme Inc. for supplying the rofecoxib for this study.

REFERENCES

1. Turner RE, Frank MJ, Van Ausdal D, Bollet AJ. Some aspects of the epidemiology of gout. Sex and race incidence. Arch Intern Med 1960;106:400-6.
2. Li-Yu J, Clayburne G, Sieck M, et al. Treatment of chronic gout. Can we determine when urate stores are depleted enough to prevent attacks of gout? J Rheumatol 2001;28:577-80.
3. Schumacher HR, Boice JA, Daikh DI, et al. Randomised double blind trial of etoricoxib and indometacin in treatment of acute gouty arthritis. BMJ 2002;324:1488-93.
4. Altman RD, Honig S, Levin JM, Lightfoot RW. Ketoprofen versus indomethacin in patients with acute gouty arthritis: a multicenter, double blind comparative study. J Rheumatol 1988;15:1422-6.
5. Lomen PL, Turner LF, Lamborn KR, Winblad MA, Sack RL, Brinn EL. Flurbiprofen in the treatment of acute gout: a comparison with indomethacin. Am J Med 1986;80:134-9.
6. Kohn NN, Hughes RE, McCarty DJ Jr, Faires JS. The significance of calcium phosphate crystals in the synovial fluid of arthritic patients: the "pseudogout syndrome". II. Identification of crystals. Ann Intern Med 1962;56:738-45.
7. Ferrari AJL, van Linthoudt D, Morrone L, Branigan P, Schumacher HR, Baker DG. 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;3:189-94.
8. Edwards JCW, Sedgwick AD, Willooghbg DA. The formation of a structure with the features of synovial lining by subcutaneous injection air: an in vivo tissue culture system. J Pathol 1981;134:147-56.
9. Nagase M, Baker DG, Schumacher HR. Prolonged inflammatory reactions induced by artificial ceramics in the rat air pouch model. J Rheumatol 1988;15:1334-8.
10. Leukocyte count. In: Schumacher HR, Reginato AJ, editors. Atlas of synovial fluid analysis and crystal identification. Philadelphia: Lea and Febiger; 1991: 27-31.
11. Pouliot M, James MJ, McColl SR, Naccache PH, Cleland LG. Monosodium urate microcrystals induce cyclooxygenase-2 in human monocytes. Blood 1998;91:1769-76.
12. Martin AR, Villegas I, La Casa C, Alarcon de la Lastra C. The cyclo-oxygenase-2 inhibitor, rofecoxib, attenuates mucosal damage due to colitis induced by trinitrobenzene sulphonic acid in rats. Eur J Pharmacol 2003;481:281-91.
13. Pinheiro RM, Calixto JB. Effect of the selective COX-2 inhibitors, celecoxib and rofecoxib, in rat acute models of inflammation. Inflamm Res 2002;51:603-10.