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The Effect of Piroxicam on Locomotor Activity in Rats With Adjuvant-Induced Arthritis

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ABSTRACT

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To evaluate the effectiveness of piroxicam, a clinically effective, long-acting, nonsteroidal antiinflammatory drug, in a potential animal model of chronic nociception, the locomotor activity of arthritic rats was monitored over long periods of time by computer. Rats injected with complete Freund's adjuvant (CFA) displayed locomotor deficits as measured by both horizontal and vertical activity. In experiment 1, to test the effectiveness of piroxicam therapy in severely arthritic animals, administration of piroxicam (1–10 mg/kg/day) was initiated 14 days after CFA injection and activity was recorded for 5 days. It was found that a single treatment significantly increased the mobility of the arthritic animals and the 4 additional days of treatment induced continued improvement in mobility and reduction in paw swelling. In experiment 2, to assess the effect of piroxicam on the development of the immobility associated with CFA-induced arthritis, piroxicam administration (0.3–3.3 mg/kg/day) was initiated prior to CFA injection and locomotor activity was monitored for 16 days. It was demonstrated that the activity of CFA-injected rats severely and progressively decreased and that piroxicam significantly increased the activity of the arthritic rats, while having no effect on the activity of the nonarthritic control animals. These studies demonstrate the efficacy of piroxicam in arthritic rats and, although further investigation is necessary, demonstrate that the measurement of locomotor activity in arthritic rodents may be an important tool for the identification and evaluation of antiinflammatory/analgetic agents.

Key words: adjuvant arthritis, locomotor activity, piroxicam

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INTRODUCTION

Piroxicam (Feldene®), an N-heterocyclic carboxamide of 1,2 benzothiazine 1,1 dioxide, is a new, long-acting, nonsteroidal antiinflammatory (NSAI) agent with analgesic and antipyretic properties [Brogden et al., 1981] and is structurally unrelated to previously available drugs. In vitro and in vivo, it is an inhibitor of prostaglandin synthesis by selective reversible inhibition of the cyclooxygenase step of arachidonic acid metabolism [Brodgen et al., 1981]. In a double-blind study in patients with rheumatoid arthritis, piroxicam decreased pain and inflammation and significantly improved such indirect measures as grip strength, walking time, and morning stiffness [Weintraub et al., 1977]. In osteoarthritic patients piroxicam produced significant improvement in pain, swelling, and general well-being within 2 weeks after initiation of therapy [Aderounmu et al., 1980].

In rats, piroxicam has been shown to produce potent, dose-dependent suppression of carrageenan-induced paw edema and significant suppression of both the primary and secondary lesions of complete Freund's adjuvant (CFA)-induced arthritis [Wiseman et al., 1976]. In addition, piroxicam significantly reduced the rise in synovial fluid pressure and the migration of polymorphonuclear leukocytes into the joint space of dogs with urate-induced synovitis [Wiseman et al., 1976].

Like other NSAIs, piroxicam produced a total suppression of the abdominal stretching response in the phenylbenzoquinone writhing test but was shown to be inactive in opiate-responsive analgesia tests such as the flinch-jump and tail-pinch procedures in rats and the hot-plate and tail-flick procedures in mice [Milne and Twomey, 1980]. In the Randall-Selitto test, piroxicam significantly elevated the pressure threshold in the edematous hind paws of rats injected with brewer's yeast, as measured by the amount of pressure at vocalization and/or struggle responses [Milne and Twomey, 1980].

In the present study, to evaluate the effectiveness of piroxicam in a chronic animal condition, the locomotor activity of CFA-induced arthritic rats was monitored continuously over a prolonged period of time. It was predicted that arthritic animals would exhibit significantly less activity than nonarthritic animals and that piroxicam would dose-dependently and significantly increase the activity of the arthritic animals.

In the first experiment, to examine the effect of CFA-induced arthritis on locomotor activity and to test the effectiveness of piroxicam therapy in severely arthritic animals, piroxicam administration was initiated 14 days after injection of the adjuvant, and activity was recorded for 5 days. In the second experiment, to assess the effect of piroxicam on the development of the immobility found to be associated with adjuvant-induced arthritis, piroxicam administration was initiated prior to injection of the adjuvant, and activity was monitored for 16 days. In addition to simple forward/backward locomotion, rearing behavior was also monitored. Since rearing entails standing on the two hind paws, it was predicted that this activity, like horizontal locomotion, would be severely and progressively decreased in arthritic animals and would provide an additional endpoint responsive to the antiinflammatory and analgetic effects of piroxicam.

METHODS

Adult male Wistar-Lewis rats (Charles River Laboratories, Wilmington, MA) were used throughout these studies. Animals weighed 250–270 g at the time of injection of the adjuvant and were housed seven per cage prior to placement in the locomotor activity boxes for the recording of data. Adjuvant arthritis was induced by a single subplantar injection of 1 mg *Mycobacterium butyricum* suspended in 0.1 ml mineral oil (complete Freund's adjuvant) into the right hand paw as previously described [Walz et al., 1971]. The resulting swelling responses were measured by mercury displacement. In both experiments piroxicam was suspended in deionized water and administered orally, by gavage, once daily between 1200 and 1600 hr. Control animals received water only.

Locomotor activity data were continuously monitored and recorded by a PDP 11/34 computer for 5 days in the first experiment and for 16 days in the second experiment. Locomotion was measured as the number of crossovers from one quadrant of a chamber to another, which cause displacement of the gimbaled grid floor and result in the closure of read switches located in the four corners of the floor. Computer software examines the status of switch closures every 100 msec. Rearing was measured as the number of times contact was made with a touch-plate 7 cm above the floor of the box. During both experiments animals were constantly confined to these chambers and data were recorded continuously except for brief periods when animals were removed for drug injection, paw measurement, or routine chamber maintenance.

Experiment 1

On day 1, 36 animals were injected with CFA and nine animals received no injection (nonarthritic control). All animals were kept in home cages until day 12, when they were placed in the locomotor activity boxes for the recording of data. Activity was then monitored continuously for a period of 40 hr to assess predrug differences between the arthritic and nonarthritic control animals. From these baseline data the arthritic animals were divided into four counterbalanced groups. Once a day on days 14–18 the arthritic animals received piroxicam (1.0, 3.3, and 10.0 mg/kg) or vehicle, and the nonarthritic control animals received the vehicle. In addition to the data obtained from behavioral monitoring of the animals, measurements of paw volume were made on both the injected and uninjected rear paws, prior to the placement in (on day 12) and after the removal of (on day 18) the animals from the activity boxes. A series of Student's t tests and three one-way analyses of variance were used to analyze the data.

Experiment 2

One day before injection of the adjuvant (day 1) animals were divided into five groups: nonarthritic control, arthritic control, and three piroxicam-treated (0.3, 1.0, or 3.3 mg/kg) arthritic groups. Since a plateau in the effectiveness of piroxicam had been reached with the 10-mg/kg dose in the first experiment, this dose was eliminated and a lower dose was added to test its effectiveness in the more "chronic" experiment. After treatment with either piroxicam or its vehicle, the animals were placed into the locomotor activity boxes for the recording of data to evaluate the effect of piroxicam on the locomotor activity of untreated animals. Although the number of behavioral chambers prohibited the inclusion of three additional nonarthritic groups receiving piroxicam chronically as control groups, it has been repeatedly demonstrated (Seymour et al., unpublished experiments) that piroxicam administration has no effect on the locomotor activity of normal animals. On day 0, the day of the adjuvant injection, all animals except the nonarthritic controls were injected with CFA, followed by piroxicam or vehicle, and were returned to the boxes. Daily piroxicam administration was continued in this manner for the next 15 days. The two-way analyses of variance with repeated measures and Newman-Keuls comparisons were used to analyze the data.

RESULTS

Experiment 1

Twelve days after the injection of CFA, the arthritic rats exhibited a pronounced decrease in crossovers and rearing. An independent t test between the number of crossovers observed 12 days after CFA injection indicated that the arthritic animals moved around their cages significantly less than the nonarthritic control animals. An additional t test indicated that the arthritic animals also reared significantly less than the nonarthritic control animals (Table 1). After dividing the arthritic animals into four counterbalanced subgroups, two analyses of variance indicated that there were no differences among these four groups in terms of

TABLE 1. The Effect of Adjuvant-Induced Arthritis on the Initial Exploratory Locomotor Activity of Rats 12 Days After Adjuvant Injection

	Normal control	Arthritic
Crossovers	642 ± 41	163 ± 13**
Rears	239 ± 14	20 ± 4 **

Normal control group, N = 9; arthritic group, N = 35. Mean (±SEM) number of crossovers and rears were obtained over a 40-hr period.
**P = < 0.001 as determined by two-tailed Student's t tests.

TABLE 2. The Effect of Piroxicam on the Normal Daily Activity of 14-day Arthritic Rats as Reflected by Mean (± SEM) Number of Crossovers and Rears Obtained Over the 24-hr Period Following the First Piroxicam Treatment

	Crossovers	Rears
Nonarthritic control	248 ± 37**	117 ± 41**
Arthritic control	69 ± 29	4 ± 6
Piroxicam (mg/kg)		
1	101 ± 42[a]	17 ± 8[a]
3.3	165 ± 42**	35 ± 17[a]
10[b]	169 ± 48**	37 ± 19*

N = 9 per group except the piroxicam 10 mg/kg group, where N = 8.
Statistical significance of differences from the arthritic control group as determined by Newman-Keuls comparisons: *P < 0.05; **P < 0.01.
[a]Not significant.
[b]One arthritic animal died.

crossovers ($F[3,31] = 0.31$, $P > .05$) or rearing ($F[3,31] = 0.85$, $P > .05$). Baseline activity, therefore, was shown to be equal for the four arthritic subgroups prior to the initiation of piroxicam administration.

The analysis of the data collected for a period of 24 hr after the first piroxicam injection showed that one piroxicam treatment significantly increased activity (both crossovers and rears) in the arthritic animals (Table 2). Two analyses of variance for the number of crossovers and rears on this day (day 14) indicated that significant differences occurred among experimental groups on both of these measures. A Newman-Keuls analysis indicated that all groups except the low-dose piroxicam (1.0 mg/kg) group moved significantly more than the arthritic control group. A similar analysis of rearing behavior indicated that only the nonarthritic control and the high-dose piroxicam (10.0 mg/kg) groups reared significantly more than the arthritic control group.

Administration of piroxicam for an additional 4 days resulted in further significant attentuation of the arthritis-induced locomotor deficits. The total number of crossovers and rears recorded over the next 4 days (days 15–18) of piroxicam administration formed the basis of the next analysis. Two analyses of variance indicated that significant differences occurred between groups on both measures (Table 3). A Newman-Keuls analysis of crossovers indicated that all groups except the low-dose piroxicam (1.0 mg/kg) group moved significantly more than the arthritic control group. An analysis of rearing yielded similar results.

As an additional demonstration of piroxicam efficacy, estimates of the average swelling of the rear paws of these animals, as measured by mercury displacement, indicated that

TABLE 3. The Effect of 5 days of Piroxicam Treatment on the Activity of Arthritic Rats on Days 15–18, as Reflected by Mean (± SEM) Number of Crossovers and Rears Emitted

	Crossovers	Rears
Nonarthritic control	1,000 ± 62**	411 ± 26**
Arthritic control	239 ± 37	7 ± 3
Piroxicam (mg/kg)		
1.0	373 ± 35[a]	37 ± 6[a]
3.3	526 ± 85*	69 ± 13*
10	649 ± 55**	68 ± 10*

Statistical significance of differences from the arthritic control group as determined by Newman-Keuls comparisons: *P < 0.05; **P < 0.01.
[a]Not significant.

TABLE 4. The Effect of Piroxicam Treatment on the Inflammatory Response of the Adjuvant-Injected (Primary Response) and the Uninjected (Secondary Response) Paws of Arthritic Rats

	Average swelling (mm Hg displaced)			
	Primary response		Secondary response	
	Initial	Final	Initial	Final
Nonarthritic control	25 ± 3	26 ± 3	25 ± 3	25 ± 4
Arthritic control	57 ± 12	57 ± 11	33 ± 5	38 ± 4
Piroxicam (mg/kg)				
1.0	56 ± 13	51 ± 9*	34 ± 4	30 ± 5*
3.3	47 ± 18	44 ± 8*	34 ± 6	29 ± 4*
10.0	58 ± 8	45 ± 6**	33 ± 3	29 ± 4**

Initial measurements were taken prior to piroxicam treatment (day 12) and final measurements were taken after 5 days of piroxicam treatment (day 18) and are expressed as mean SEM. Decreases in paw volume were evaluated by two-tailed dependent Student's t tests. *P = < 0.05; **P < 0.01.

piroxicam significantly reduced swelling in both the adjuvant-injected paws (primary response) and the opposite, uninjected paws (secondary response) (Table 4).

Experiment 2

Figures 1 and 2 demonstrate the time course of the CFA-induced locomotor disruption and the reduction of these deficits by piroxicam therapy. Horizontal movements (crossovers) gradually declined over time with the most pronounced disruption (less than 20% of control activity per day) occurring with the onset of the secondary response (days 11–14). During this period piroxicam significantly increased horizontal locomotor activity. By contrast, rearing activity (Fig. 2) appeared to be more sensitive to the effects of CFA, with almost complete absence of rearing coinciding with the injection of CFA (day 1). Following a transient recovery (days 2–4), a gradual decline in rearing ended in a complete absence of rearing on days 11–14. As depicted in Figure 2, piroxicam attentuated the disruptive effects of CFA on rearing throughout the time course examined.

Two analyses of variance (for crossovers and rears) with six repeated measures were performed on these data, using days −1, 0, 1, 2, 11, and 14 as the repeated measures. These specific days were chosen to analyze differences among groups occurring prior to, concurrent with, and immediately after injection of the adjuvant and at the time point where the most pronounced behavioral defects were observed (Figs. 1, 2). Newman-Keuls analyses for crossovers and rears indicated that prior to the injection of CFA on day −1, no differences were

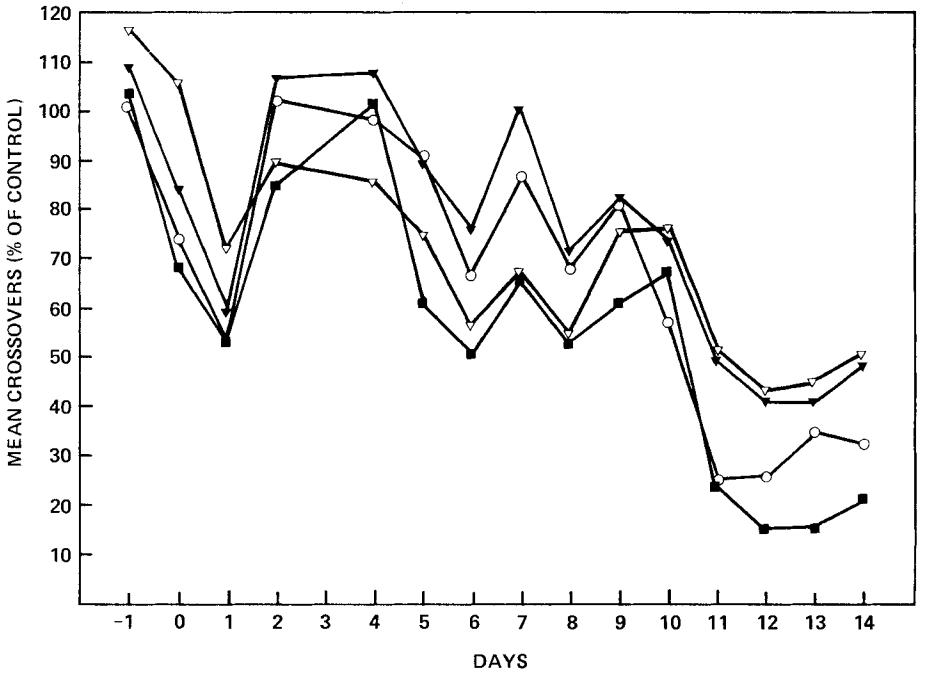

Fig. 1. The effect of piroxicam therapy on the mean locomotor activity of adjuvant-arthritic rats over 16 days (■, arthritic control; ○, arthritic/piroxicam 0.3 mg/kg; ▲, arthritic/piroxicam 1.0 mg/kg; △, arthritic/piroxicam 3.3 mg/kg) expressed as percent of the nonarthritic control group. See Table 5 for summary of significant differences. Data for day 3 are missing because of computer failure on that day.

found among groups in terms of crossovers or rearing, an indication that piroxicam had no effect on the locomotor activity of normal rats. Additional analyses for the remaining days indicated that both the nonarthritic animals and arthritic animals that received piroxicam therapy (3.3 and 1.0 mg/kg) consistently exhibited more activity than the arthritic control animals (Table 5).

DISCUSSION

The results of experiment 1 demonstrated that a single piroxicam treatment significantly increased the mobility of the arthritic animals and that 4 days of additional piroxicam therapy induced continued improvement in mobility and amount of swelling.

The results of experiment 2 demonstrated that the activity of arthritic rats was severely and progressively decreased and that piroxicam therapy induced significant improvement in these symptoms, enabling arthritic rats to behave more like the control rats. The development and severity of the locomotor deficits induced by CFA were significantly attenuated by both the early and chronic administration of piroxicam, although the level of activity exhibited by the nonarthritic control group was not attained even with the highest dose of piroxicam used.

These results appear to share the temporal patterns of results reported by other investigators. Colpaert et al. [1982] reported that the nociception associated with adjuvant-arthritis had its onset on days 10 and 11, and its peak on days 18–21. In experiment 2, beginning on day 10, both rearing and crossovers became severely decreased, but this decrease was dose-dependently attentuated by piroxicam. Al-Haboubi and Zeitlin [1982] reported that CFA

TABLE 5. Summary of Newman-Keuls Comparisons of All Treatment Groups With the Arthritic Control Group as Depicted in Figures 1 and 2 on Days −1, 0, 1, 1, 11, and 14.

		Crossovers			Rears		
Day	Group	Mean	SEM	P	Mean	SEM	P
−1	Arthritic control	269	22	—	109	13	—
	No differences						
0	Arthritic control	97	11	—	42	10	—
	Nonarthritic control	140	11	<0.01	78	10	<0.01
	Arthritic/piroxicam 3.3[a]	147	12	<0.01	80	12	<0.01
1	Arthritic control	56	6	—	11	2	—
	Nonarthritic control	104	8	<0.01	84	14	<0.01
	Nonarthritic/piroxicam 3.3	—		—	39	9	<0.01
2	Arthritic control	88	9	—	18	2	—
	Nonarthritic control				63	9	<0.01
	Arthritic/piroxicam 3.3				50	6	<0.05
	Arthritic/piroxicam 1.0				49	7	<0.05
11	Arthritic control	35	9	—	3	1	—
	Control	144	19	<0.01	83	14	<0.01
	Piroxicam 3.3	74	10	<0.05	37	10	<0.01
	Piroxicam 1.0	71	5	<0.05	—	—	—
14	Arthritic control	26	6	—	3	1	—
	Control	163	18	<0.01	99	12	<0.01
	Piroxicam 3.3	61	11	<0.05	38	11	<0.01
	Piroxicam 1.0	57	7	<0.05	—	—	—

Only those groups that were significantly different are shown. For example on day 1 only the healthy animals exhibited significantly more crossovers than the arthritic controls. By contrast, on day 14 all but the lowest dose piroxicam group (0.3 mg/kg) exhibited significantly more crossovers than the arthritic controls.

[a]Dosages are in milligrams per kilogram.

injection induced a biphasic swelling response. The mean volume of CFA-injected paws increased continuously, becoming 80 ± 26% larger than the noninjected paws 6 hr after injection. This response persisted for 1 to 2 days, decreased by day 5, and then increased again by day 14. The contralateral noninjected paws showed little increase in size until day 10, and then increased by 42% by day 14. Again, the presently reported activity data appear to validate these observations. Therefore, in addition to the demonstrated efficacy of piroxicam therapy in CFA arthritic rats, the present experiments also demonstrated the usefulness of the model itself. The observed decreases in the locomotor activity of these arthritic animals appeared to be the result of nociception resulting from movement. In both experiments, rearing activity was more profoundly affected than was horizontal locomotor activity, which reflected a severe decrease concurrent with the development of the secondary response. NSAIs such as piroxicam are efficacious antiinflammatory/analgetic agents, and the present experiments demonstrated that as inflammation was decreased, the activity of the arthritic animals increased.

Bottcher et al. [1981] also recently reported that acute paw inflammation induced by carrageenan injection significantly reduced the locomotor activity of rats when measured for brief periods 3 and 5 hr after carrageenan injection. In contrast to the carrageenan-induced effects, CFA-induced arthritis in rats is a condition in which the nociceptive stimulus persists for an extended period of time and may represent an animal model of chronic pain. Although additional investigation of the model using other NSAI, steroidal, and opiate drugs is necessary, the measurement of locomotor activity in arthritic animals appears to be an extremely useful method for the identification and evaluation of antiinflammatory/analgetic agents.

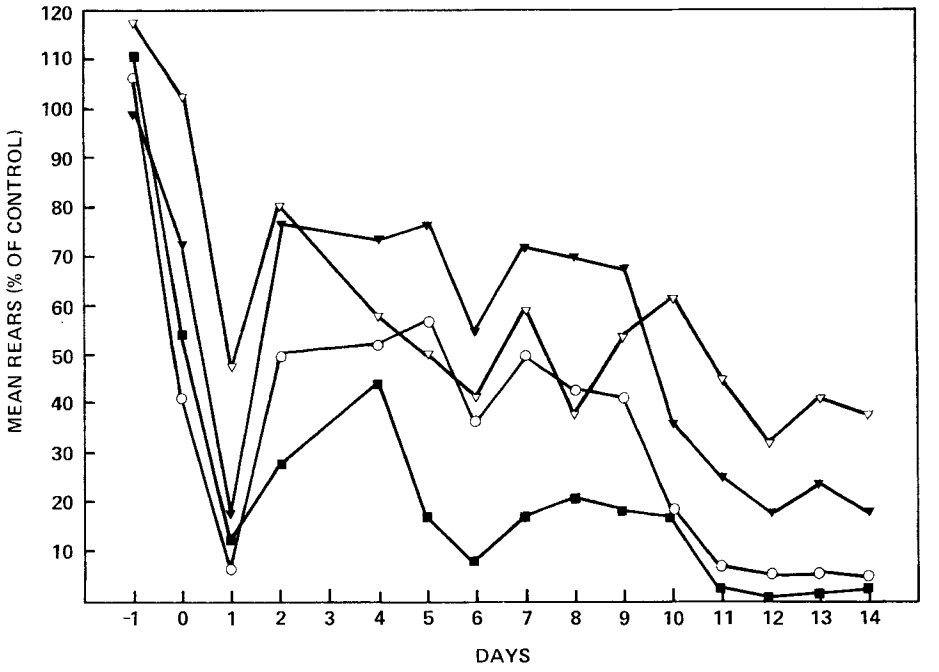

Fig. 2. The effect of piroxicam therapy on the mean rearing activity of adjuvant-arthritic rats over 16 days (■, arthritic control; ○, arthritic/piroxicam 0.3 mg/kg; ▲, arthritic/piroxicam 1.0 mg/kg; △, arthritic/piroxicam 3.3 mg/kg) expressed as percent of the nonarthritic control group. Data for day 3 are missing because of computer failure on that day.

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