Effect of Anti-Inflammatory Agents on Acute Experimental Synovitis In Dogs. (31229)

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Laboratory procedures for screening and evaluating anti-inflammatory agents are usually done in rodents in tests such as ultraviolet erythema(1), granuloma pouch(2) or paw edema(3). A sensitive test in a non-rodent would be useful in determining the potential efficacy of such agents. Faires and McCarty (4) described a gouty synovitis (arthritis) in dogs and subsequently reported protection against this inflammatory response by prior intrasynovial administration of insoluble glucocorticoids(5). The present paper will describe the effects of oral administration of non-steroidal anti-inflammatory agents in attempting both to block and reverse urate synovitis in dogs.

Materials and methods. A colony of 17 trained, unanesthetized female hounds (18-25 kg) were used in all experiments. The dogs were kept under uniform conditions of feeding (Purina Lab Chow), temperature, humidity and artificial lighting. They were housed in individual cages and fed at 4:00 to 4:15 p.m. every afternoon; thus a dog receiving a drug on the morning of the test day had not been fed for 16 to 18 hours. Water was allowed *ad libitum* at all times. A microcrystalline urate suspension was prepared by reacting a hot sodium hydroxide solution with uric acid overnight. The suspension was washed, resuspended in saline, and finally sterilized (autoclaved) in rubber-stoppered 10 ml vials. The final pH was approximately 7.0 and the suspension contained 18-24 mg of urate per ml (dry weight)(4). The dogs were trained to lie quietly on their backs in a dog cradle under light restraint. The knee was shaved, painted with tincture of iodine, and a sterile 21-gauge needle inserted into the knee (stifle) joint. Slight aspiration produced a small amount of clear, viscous synovial fluid, indicating entry into the joint. The needle was left in place, a syringe containing the urate suspension attached, and volumes from 0.1 to 0.5 ml injected into the joint (approximately 2 to 10 mg of urate). Injections were made only into those knees from which synovial fluid could be aspirated. An interval of at least 14 days elapsed between each use of a dog. The knees were alternated; thus each animal was used twice a month but each knee only once a month. A scoring system was adopted in which inflammatory symptoms ranging from tenderness, limping, occasional 3-legged gait to complete 3-legged gait were scored from 1+ through 4+. Animals were kept in individual cages during the test but were removed and allowed to walk during the scoring sessions, which were usually every 30 minutes. Compounds were tested in 2 types of drug schedule. In the first, the dogs were pretreated orally with the test compound 45 to 60 minutes before the urate injection and were then observed and scored for maximum inflammatory effects during a 3-hour period after urate. In this procedure it was assumed that each dog would achieve a 4+ or maximum score and that any decrease in the score would indicate a protective effect of the compound. To check that a maximum inflammatory response occurred in this schedule, one control dog was always injected with urate but not given the test compound. In the second schedule, urate was given first and the dogs observed until maximum incapacitation (4+) occurred. The test compound was then given orally and the dogs observed and scored over a 3-hour period after compound. In this schedule each animal served as its own control, and a reversal of inflammatory symptoms rather than their prevention was assessed. With either schedule the maximum reduction of symptoms at any time during the 3-hour observation period was used to calculate per cent reduction. Orally administered compounds were given either by stomach tube as a fine aqueous suspension or in capsules.

Results. Fig. 1 shows the average 8-hour inflammatory response curve to 10 mg urate

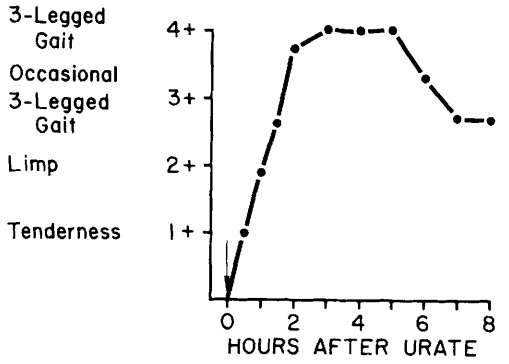

FIG. 1. Control response to intrasynovial injection of urate (10 mg) in unanesthetized dogs. Each point represents the mean response obtained in 32 experiments on 15 dogs over a 5-month period. Urate given at 0 hr (arrow).

in control animals. Severe symptoms were apparent within 2 hours after urate administration and lasted for approximately 4 hours, after which some recovery occurred. Complete clinical recovery was evident within 24 hours. This inflammatory response was reproducible from month to month with the same dog, but 2 of 17 dogs had to be removed from the test because of poor response. The inflammatory response to smaller doses of urate (2, 4 and 6 mg) was determined in a small series. Onset and intensity of the response were virtually indistinguishable from those after the 10 mg dose, but duration and reproducibility were somewhat decreased.

Fig. 2 presents the dose-response curves of

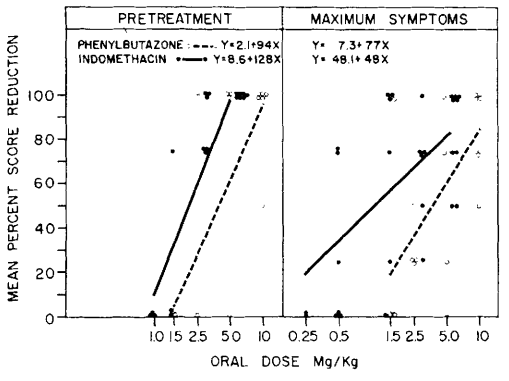

FIG. 2. Effect of phenylbutazone and indomethacin on synovitis induced by intrasynovially injected urate (10 mg) in unanesthetized dogs. Compounds were given 45 to 60 min before urate (pretreatment) or at time of maximum inflammatory effect (maximum symptoms). Each point represents results from one animal.

phenylbutazone and indomethacin when tested against 10 mg of intrasynovial urate. The doses in the pretreatment series were: phenylbutazone, 1.5, 2.5, and 10 mg/kg; indomethacin, 1, 1.5, 3, and 6 mg/kg. The doses at maximum symptoms were: phenylbutazone, 1.5, 2.5, 5, and 10 mg/kg; indomethacin, 0.25, 0.5, 1.5, 3, and 6 mg/kg. At the largest doses in the pretreatment series, indomethacin completely suppressed the inflammatory response in every experiment while phenylbutazone was similarly effective in 6 out of 7 dogs. The largest doses in the maximum symptoms schedule resulted in a mean suppression of only 84% with an onset of action of approximately 90 minutes for either compound. Greater variability of response for either compound and a more shallow dose-response curve for indomethacin occurred when compounds were administered at maximum symptoms. With either schedule the anti-inflammatory effect (mg for mg) of indomethacin was approximately twice that of phenylbutazone. To determine if decreasing urate would increase the sensitivity of the assay, doses of phenylbutazone and indomethacin which were approximately 50% effective by pretreatment against 10 mg of urate were tested against 4 mg in the same pretreatment schedule (Table I). Approximately similar protective results were obtained with 4 mg as with 10, indicating that within the range of urate tested the sensitivity of the test remains relatively constant.

TABLE I. Activity of Phenylbutazone and Indomethacin in Synovitis Induced by 4 mg Urate. Doses were estimated ED_{50} values from studies using 10 mg urate.

Compound	No. dogs	Oral dose (mg/kg)	Reduction in symptoms (mean %)	Range (%)
Phenylbutazone	9	5	64	(25-90)
Indomethacin	10	3	53	(25-75)

Compounds given 60 min prior to urate.

Table II shows the effects of some additional compounds on synovitis induced by 10 mg of urate. Acetylsalicylic acid at higher doses, even with an added antacid, caused

TABLE II. Effect of Compounds on Synovitis Induced by Intrasynovially Injected Urate (10 mg) in Dogs.

Compound given by pretreatment (minus 60 min)					Compound given at maximum symptoms		
Compound	Route	No. dogs	Dose (mg/kg)	Reduction in symptoms (mean %)	No. dogs	Dose (mg/kg)	Reduction in symptoms (mean %)
Acetylsalicylic acid*	Oral	4	45	6	4	45	6
		4	90†	0	4	90	0
		2	150‡	25	—	—	—
Codeine-PO_4	Sub. cut.	3	3	0	2	3	0
	(base)	3	5	0	3	5	0
Promazine HCl	Oral	2	5 §	0	3	7.5	0
		3	7.5‖	0	—	—	—
Chlorpromazine HCl	Oral	3	5‖	0	—	—	—
	i.m.	1	5	0	1	5	0
Hydrocortisone sodium succinate	i.v.	2	100	0	—	—	—
		1	300	0	—	—	—
CI-583	Oral	3	10	75	—	—	—
		3	15	75			

* Administered with 10 ml aluminum and magnesium hydroxide gel (Aludrox). † Emesis in one dog. ‡ Emesis in 2 dogs. § Salivation, pupil dilatation. ‖ Salivation, pupil dilatation, emesis in one dog.

emesis within 1 hour and was ineffective in either schedule. Codeine was analgesic, as evidenced by a decreased response to ear pinching, but was inactive in this test. Promazine and chlorpromazine caused central nervous system depression but were also inactive. Soluble hydrocortisone was inactive when the dogs were pretreated intravenously. CI-583 [*N*-2,6-dichloro-*m*-tolyl anthranilic acid], a potent anti-inflammatory compound described by Winder *et al*(6), was active in some preliminary studies. Full strength dimethyl sulfoxide and a counterirritant preparation (containing methyl salicylate, capsicum, chloroform, camphor and alcohol) were each applied topically to the knee joints of 3 dogs at minus 60 minutes and at 0, 1, 2, and 3 hours after urate; both failed to influence the clinical course of the arthritic syndrome.

Discussion. The inflammatory reaction to intrasynovial injection of urate crystals is prevented in dogs by intrasynovial pretreatment with various glucocorticoid preparations (5) and in humans by oral and intravenous administration of colchicine, hydrocortisone and phenylbutazone(7). Utilizing the same method in dogs we have shown that the inflammatory reaction can either be prevented or reversed by the nonsteroidal anti-inflammatory agents phenylbutazone and indomethacin(8). Both compounds were active by either treatment schedule, but when administered at maximum symptoms there was considerable variation in the results, particularly at the intermediate doses. When doses that were completely effective by the pretreatment schedule were given at maximum symptoms, complete suppression of the inflammatory response was not achieved. This method is thus similar to other anti-inflammatory tests, greater sensitivity and reproducibility being obtained when a compound is given before rather than after the experimental inflammation. Dose-response curves obtained with these 2 compounds indicate that both drugs are active at doses similar to those used in various human arthritic conditions(9) but that indomethacin is approximately twice as potent as phenylbutazone.

The 4-hour assay was able to detect activity in 3 structurally different anti-inflammatory, antirheumatic compounds but not in codeine or phenothiazine derivatives. In this test, acetylsalicylic acid appeared inactive; however, the dose was limited by the side effects encountered. Intravenous hydrocortisone failed to modify the arthritic response, in contrast to the long lasting protective ef-

fects reported for intrasynovially administered insoluble glucocorticoids(5). Two counterirritants applied topically to the arthritic knee before, during and after urate were inactive; but in preliminary tests the anthranilic acid derivative CI-583(6) prevented the inflammatory response. Within the doses of urate studied (4 and 10 mg), decreasing the dose of urate did not affect the response to either phenylbutazone or indomethacin. The results indicate that this procedure may be a useful laboratory test for oral screening and evaluation of nonsteroidal anti-inflammatory, antiarthritic agents.

Summary. Oral administration of the nonsteroidal anti-inflammatory agents phenylbutazone and indomethacin prevented or reversed a urate-induced synovitis in unanesthetized dogs. Acetylsalicylic acid, codeine, promazine, chlorpromazine, topical counterirritants and intravenous hydrocortisone were inactive. This model of gouty arthritis may be useful in detecting and evaluating orally active nonsteroidal anti-inflammatory agents.

Appreciation is expressed to Merck Sharp & Dohme for indomethacin; to Geigy Pharmaceuticals for phenylbutazone; and to Parke, Davis & Co. for CI-583.

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The Autofluorescent Cells of the Rat Thymus Under Certain Experimental Conditions.* (31230)

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In a previous work of fluorescence microscopy(1), we reported the presence of peculiar "autofluorescent cells" at the cortico-medullary junction of the young adult rat thymus (Fig. 1). The cytoplasm was filled with granules most of which were small and yellow autofluorescent. These granules which were not detectable at birth, and first became evident between days 15 and 20 and increased in number until about the sixth month(1,2). The granules which stained variably with the techniques of Sudan black(1,3) and P.A. Schiff(1,2), were thought to contain lipids(1, 3).

Because it was thought that the autofluorescent cells were absent in the thymus prior to its involution, it has been proposed that these cells are an expression of altered thymic metabolism accompanying thymic involution(3). It was later suggested that the origin of these cells might be related to lympholysis as their granules could represent indigestible residues from lysis of lymphocytes by macrophages(4). We previously suggested that these cells are involved in immunity or growth(1,5).

The first purpose of this work, to determine if the lipoid granules of the autofluorescent cells have an endogenous origin, was investi-

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