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Anti-Inflammatory and Safety Profile of DuP 697, a Novel Orally Effective Prostaglandin Synthesis Inhibitor

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ABSTRACT

DuP 697 (5-bromo-2[4-fluorophenyl]-3-[4-methylsulfonylphenyl]-thiophene) is a potent inhibitor of paw swelling in nonestablished and established adjuvant arthritis in rats (ED_{50} = 0.03 and 0.18 mg/kg/day, respectively). DuP 697 had no effect on phenylquinone writhing in rats (ED_{50} > 100 mg/kg), but was analgetic against inflammation-related pain in the Randall-Selitto assay (ED_{30} = 3.5 mg/kg) and was a very potent antipyretic agent (ED_{50} = 0.05 mg/kg). The drug was not ulcerogenic in rats at single doses up to 400 mg/kg. DuP 697 (5 mg/kg i.v.) did not alter renal blood flow or the renal vascular response to angiotensin II in furosemide-pretreated, volume-depleted rats. In contrast, indomethacin (5 mg/kg i.v.) decreased renal blood flow and potentiated the renal vascular response to angiotensin II in these animals. DuP 697 was a moderate inhibitor of bull seminal vesicle prostaglandin (PG) synthesis ($IC_{50} = 2.4 \times 10^{-5}$ M) and a potent inhibitor of rat brain PG synthesis ($IC_{50} = 4.5 \times 10^{-6}$ M) but was ineffective against rat kidney PG synthesis (IC_{50} 7.5×10^{-5} M). These differential effects of DuP 697 on PG synthesis by various tissues may account for its high potency as an anti-inflammatory and antipyretic agent and its minimal toxicity profile.

NSAID such as indomethacin and piroxicam are used as first line therapy in the treatment of rheumatoid arthritis. The NSAID share to various degrees the propensity for certain side effects. These include gastrointestinal irritation in many patients and nephrotoxicity in renal-compromised arthritics. Indeed, many NSAID classified as investigational new drugs in the 1980s share the gastrointestinal side effects of marketed drugs (Marsh *et al.*, 1986). These current therapeutic deficiencies provide the need to develop safer drugs; *e.g.*, agents which do not inhibit organ-protective PGs. DuP 697 (5-bromo-2-[4-fluorophenyl]-3-[4-methylsulfonylphenyl]-thiophene (fig. 1), is an anti-inflammatory drug with exceptional gastrointestinal safety in rats. In addition, DuP 697 may prove to be renal sparing inasmuch as it does not inhibit rat kidney PG synthesis or does it alter RBF in furosemide-pretreated, volume-depleted rats. In this paper, an overview of the *in vivo* anti-inflammatory and safety profile of the drug will be presented and compared to piroxicam which is given once per day (anticipated clinical schedule for DuP 697) and indomethacin and sulindac, NSAID reported to be nephrotoxic and renal sparing, respectively (Berg and Talseth, 1985). Most of the studies were performed in rats or their tissues to help define tissue selectivity for cyclooxygenase in one species. The mechanism of action of DuP 697 appears to be inhibition of PG synthesis in selected tissues including the brain. Results have been presented previously in abstract form (Ackerman *et al.*, 1986; Gans *et al.*, 1987).

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Methods

Drugs. Unless otherwise stated, drugs were prepared in 0.25% methylcellulose (Dow Chemical Co., Midland, MI; Type A15C) using distilled water and bead-milled for several hours to obtain a uniform suspension. Indomethacin, piroxicam, sulindac, furosemide and AII were obtained from Sigma Chemical Co. (St. Louis, MO) and DuP 697 was prepared by S. B. Haber. Ketamine was purchased from Parke Davis and Co. (Detroit, MI). Sodium 5-sec-butyl-5-ethyl-2-thiobarbiturate was obtained from Andrew Lockwood Associates. Sulindac sulfide was a gift from Merck & Company, Inc. (Rahway, NJ).

Animals. Sprague-Dawley rats and Lewis rats were obtained from Charles River Breeding Laboratories (Wilmington, MA). Munich Wistar rats were obtained from Harlan Labs (Madison, WI). Rats were maintained on a 12-hr light-dark cycle and allowed food and water *ad libitum* except where noted. These studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Statistics. Unless otherwise stated, data from *in vivo* experiments

ABBREVIATIONS: NSAID, nonsteroidal anti-inflammatory drugs; PG, prostaglandin; RBF, renal blood flow; AII, angiotensin II; PQW, phenylquinone writhing; %MPE, percentage of maximum possible effect; HEPES, 4-(2-hydroxyethyl)-1-piperaszineethanesulfonic acid; DMSO, dimethylsulfoxide; TD_{50}, toxic dose, 50%; IL-1, interleukin-1.

were evaluated using an analysis of variance followed by Duncan's Multiple Range test, with a P value of ≤ .05 considered statistically significant.

Adjuvant arthritis. Anti-inflammatory activity was evaluated according to the protocol of Pearson (1956), using male Lewis rats, 175 to 220 g, injected s.c. with 0.1 ml of adjuvant in the plantar area of the right hind paw. The adjuvant was prepared by bead-milling heat-killed, lyophilized *Mycobacterium butyricum* (Difco Laboratories, Detroit, MI) in light mineral oil (Fisher Scientific Co., Springfield, NJ) at a final concentration of 5 mg/ml. Twenty nonarthritic control rats were injected with mineral oil.

Established disease. Adjuvant-injected rats were held for 14 days to allow the development of polyarthritis. The volume of the noninjected hind paw of each rat was measured using an Ugo Basile mercury plethysmograph (Stoelting). Adjuvant-injected rats showing no evidence of arthritis were removed from the experiment and arthritic rats were distributed into groups of 10 having equal mean paw volumes with an approximately equal standard deviation. Nonarthritic (oil-injected) control rats were distributed into two groups of 10.

Test compounds were administered p.o. once daily for 7 days (days 14–20). Nonarthritic and arthritic control rats received vehicle alone for 7 days. Noninjected hind paw volumes were measured 20 hr after the last dose (on day 21) and body weights were determined every second day.

Percentage of decrease from control noninjected mean paw volume was calculated with the following formula using group means:

$$\frac{\text{(Arthritic Vehicle Control)} - \text{(Arthritic Treatment)} \times 100}{\text{(Arthritic Vehicle Control)} - \text{(Nonarthritic Vehicle Control)}}$$

$$= \text{Percent Decrease from Control Mean Paw Volume (ml)}$$

The ED_{50} value was estimated using the mean percentage of decrease values plotted on semi-logarithmic paper.

Nonestablished disease. Animals were distributed randomly into groups of 10 and dosed p.o. by gavage once daily for 18 days beginning the day of adjuvant injection. On day 18, the volume of the noninjected hind paw of each rat was measured as described above. Percentage of decrease in paw volume and ED_{50} values were determined as described above. Leukocyte counts were determined using a Coulter counter and blood obtained *via* cardiac puncture.

PQW in rats. The PQW test for analgesic activity was modified from the methods of Siegmund *et al.* (1957) and Blumberg *et al.* (1965). Fasted male Sprague Dawley CD rats (65–95 g) were treated p.o. with randomized and coded doses of test compounds or vehicle-control, then challenged with i.p. injections of phenyl-*p*-benzoquinone (phenylquinone, 1.0 mg/kg) 5 min before specified observation times. Animals were observed 10 min for the characteristic abdominal constriction and stretching response beginning 5 min after phenylquinone injection. Separate groups of animals were used for each observation period. Analgesic activity was calculated as the percentage of animals failing to respond to the phenylquinone challenge dose. Greater than 95% of vehicle-control animals exhibited a writhing response.

Randall-Selitto assay. The drugs were also evaluated as analgesics in the rat inflamed yeast-paw test modified from the method of Randall and Selitto (1957) using an Ugo Basile analgesiometer (Stoelting). The pressure stylus was fitted at the tip with a 0.64 cm stainless-steel ball. The weighted slide was used with three 70 g weights and had a maximum travel of 25 cm. Fasted male Sprague-Dawley CD rats (60–95 g) were prescreened on both rear paws for preyeast threshold pain responses (vocalization or struggle) of less than 15 cm slide travel. The right rear paw was then inflamed by a subplantar injection (0.1 ml) of a 20% aqueous suspension of Fleischmann's active dry yeast. Coded drug doses were administered p.o. 2 hr after yeast injection; pain reaction thresholds were determined 0.5, 1, 2 and 4 hr later. ED_{30} values were calculated by regression analysis from %MPE transformed data, in which a 10.0 cm increase over the mean vehicle-control response was considered to constitute 100% maximum possible analgesic effect:

$$\%\ \text{MPE} = [(\text{Value-Control})/10.0] \times 100.$$

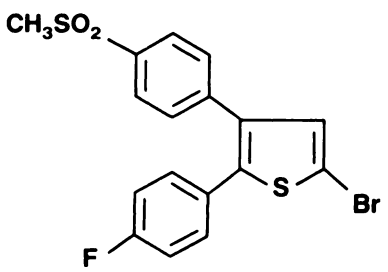

Fig. 1. DuP 697 (5-bromo-2-[4-fluorophenyl]-3-[4-methysulfonylphenyl]-thiophene.

Antipyretic activity. The antipyretic activity was determined using the method described by Smith and Hambourger (1935). Male Sprague-Dawley rats (130–175 g) were placed in polypropylene holding boxes with wire bar covers, five animals per box, and allowed to equilibrate in a temperature-controlled room during the early afternoon of day 1 of experiment. In late afternoon of day 1, rectal temperatures were taken using an Ellab thermometer (Type TE-3-S) with a thermocouple probe (model RM 4) inserted to a constant depth (3.5 cm). Rats were then injected s.c. at the nape of the neck with a freshly prepared 20% suspension of dried brewer's yeast (Schiff Bio-Food Products) in 0.9% saline. Normothermic control groups received only saline injection. Animals were then food-deprived overnight with water provided *ad libitum*. On day 2, 16 to 17 hr after yeast or saline injection, rectal temperatures were recorded, and coded graded p.o. doses of test compound or control vehicle were administered to febrile and normothermic animals. Rectal temperatures were recorded hourly after drug or vehicle administration. ED_{50} values were estimated using linear regression analysis after determining percentage of decrease in pyrexia at each dose level at each time interval studied.

Bovine seminal vesicle PG synthesis. The mechanism of anti-inflammatory activity of DuP 697 was evaluated using several cyclooxygenase assays. Inhibition of bovine seminal vesicle PG synthesis was measured according to White and Glassman (1974) as modified by Vigdahl and Tukey (1977). [^{14}C]Arachidonic acid was used as the substrate at a final concentration of 0.02 mM. Reaction conditions favored PGE_2 as the primary product, (>85% of oxidized products, Takeguchi *et al.*, 1971). Bovine seminal vesicle PG synthetase (Miles) was suspended at a concentration of 2 mg/ml in 0.25 M sucrose and 0.005 M potassium phosphate (pH 7.4). Inhibitors were bead-milled overnight and diluted in reaction buffer (0.2M Tris-Cl, pH 8.5) or dissolved in ethanol and brought to volume with reaction buffer. The amount of ethanol added to the reaction did not influence PG production. Drugs were incubated 2 min at 37°C with the enzyme and buffer before adding substrate to initiate the reaction which ran for 10 min at 37°C. The PG were separated from the unreacted arachidonic acid on silica gel columns using hexane-tetrahydrofuran-acetic acid (70:3:1) to remove the arachidonic acid followed by ethylacetate-ethanol (51:9) to remove the PG which were not separated one from the other. A compound that changed the ratio of PG products but did not alter the cyclooxygenase activity would not be detected in this system. Overall, > 95% of the radioactivity in the reaction was recovered. Based on thin-layer chromatographic studies, there was no evidence that any of the inhibitors tested changed the type of PG synthesized. A semilog plot of percentage of inhibition *vs.* final concentration of inhibitor was used to determine the IC_{50} by inspection.

Rat kidney PG synthesis. Because the anti-inflammatory, antipyretic and analgesic activities were demonstrated in rats, cyclooxygenase assays were developed using rat tissues to further explore the effect of DuP 697 on cyclooxygenase. The preparation of medullary and cortical kidney microsomes was based on the method of Hassid and Dunn (1980). The primary metabolite of exogenous arachidonate in a rat kidney microsomal fraction appeared to be PGE_2. Inhibition of microsomal PGE production was measured by radioimmunoassay (Ad-

vanced Magnetics, [^{3}H]PGE_2 kit) following silica gel chromatography as described by Vigdahl and Tukey (1977) to isolate a PG fraction. PGE represents PGE_1 and PGE_2 which are indistinguishable by this radioimmunoassay analysis (cross-reactivity: 100, 6.4, 4.9 and 2.7% per ng for PGE_1, $PGF_{2\alpha}$, PGB_2 and 6-keto $PGF_{1\alpha}$, respectively).

The enzymatic reaction was buffered with 0.2 M HEPES (pH 7.5) containing 0.09 mM EDTA, 0.62 mM epinephrine and 2.1 mM reduced glutathione. Inhibitors were prepared on the day of the test in 5% DMSO and 95% 0.2 mM HEPES (pH 7.5). Five percent of DMSO did not influence PGE production. Enzyme (162 μg of medullary microsome protein or 117 μg of cortical microsome protein), buffer and inhibitor were incubated 5 min at 37°C before the addition of arachidonate. Medullary PGE production was complete by 1 min and cortical PGE production was complete by 2 min. The 100-μL reaction was terminated by a 7-min heat inactivation of the enzyme, which did not alter PGE_2 immunoreactivity. Addition of 0.025 mM arachidonate increased medullary PGE production 4-fold above background levels and 2-fold above PGE levels from endogenous arachidonate present in the microsomal preparation. Cortical PGE production was elevated 3-fold above background levels in the presence of 0.012 mM arachidonate. IC_{50} values for net PGE production were determined by inspection of a semilog plot of percentage of inhibition *vs.* final inhibitory concentration.

Rat brain PG synthesis. Sprague-Dawley rats were decapitated and the brains were removed minus the cerebellum and olfactory lobe and homogenized in 3 ml/g of wet weight tissue, of 0.1 M sodium phosphate and 0.1 mM dithiothreitol, pH 7.5 (isolation buffer) in a Polytron 3 × 10 sec at the high setting. This homogenate was centrifuged for 10 min at 10,000 × *g*, 4°C, and supernatant removed and centrifuged for 1 hr at 21,000 × *g*, 4°C. The pellet was washed, resuspended in isolation buffer and centrifuged again for 1 hr, 21,000 × *g*, 4°C. The second pellet was resuspended in isolation buffer plus 0.25 M sucrose in one-eighth the original volume of isolation buffer. This microsomal preparation was frozen under argon and stored at −70°C.

The concentration of microsomal preparation used gave 600 to 1300 pg of net PGE production. Compounds were assayed for enzyme inhibition by preincubation with the enzyme for 10 min in an ice bath. Assay buffer was 0.1 M Tris·HCl, pH 8.0; all compounds, the enzyme and substrate were dissolved in this assay buffer. A cofactor solution was added to give a final concentration of 2.55 mM glutathione, 0.105 mM EDTA and 0.75 mM epinephrine, and the reaction tubes were incubated in a water bath at 37°C for 2 min. The substrate, 1.5 mM arachidonic acid, was then added, and the reaction proceeded for 2 min at 37°C in a total volume of 0.1 ml. The enzyme was then inactivated in a boiling water bath for 7 min. Tubes were then frozen on dry ice. PG were separated from the arachidonic acid on silica gel columns. The PG-containing fraction was dried under nitrogen gas and resuspended in Advanced Magnetics bovine gamma globulin assay buffer. Radioimmunoassay of PGE was performed as described above. Drug samples were compared to controls, and the percentage of inhibition was calculated as follows:

$$[(\text{net control}) - (\text{net sample})]/[\text{net control}] \times 100.$$

Results were plotted on semilog graph paper and IC_{50} values determined by inspection.

Gastrointestinal lesions. The gastrointestinal safety of DuP 697 was evaluated relative to indomethacin and piroxicam. Male Sprague-Dawley rats weighing approximately 165 g were given single p.o. doses of drug or vehicle. Eighteen to 24 hr after dosing, animals were anesthetized with 1.0% sodium pentobarbital i.p. in 0.9% saline. Each rat was then injected i.v. with 1.0 ml of pontamine sky blue 6BX dye (15%, 0.9% saline) and euthanatized. The small intestine and stomach were removed, opened, rinsed with tap water and examined for the presence of mucosal lesions using a binocular dissecting microscope (10×) in coded, randomized order. Leakage of the protein-bound blue dye from damaged blood vessels aided visualization of mucosal changes. The occurrence of one lesion, regardless of its severity (erosion or hemmorrhage), was considered a positive result. The median dose for production of gastrointestinal lesions (TD_{50}) was calculated by the moving-average method of Thompson (1947).

Margin of gastrointestinal safety was defined as the ratio of the gastrointestinal TD_{50} to the established adjuvant arthritis ED_{50}, with a higher ratio indicating greater safety.

Renal function. As an *in vivo* correlate of potential nephrotoxicity of DuP 697, the drug was tested in volume-depleted rats in which the renin-angiotensin system is activated. These experiments were performed in male, Munich Wistar rats (six/group) with group mean weights of 230 to 240 g. The rats were maintained on a low sodium diet (0.4% sodium chloride by weight) and were given a 30 mg/kg i.p. injection of furosemide 12 hr before the experiment to salt-deplete the animals and activate the renin-angiotensin system.

On the day of the experiment, the rats were anesthetized with ketamine (30 mg/kg i.m.) and sodium 5-sec-butyl-5-ethyl-2-thiobarbiturate (50 mg/kg i.p.). Cannulas were placed in the jugular vein for infusions and in the femoral artery for measurement of arterial pressure. A 1.5-mm flow probe was placed on the renal artery of the left kidney for measurement of RBF using an electromagnetic flowmeter (Carolina Instruments, King, NC). The rats received a maintenance i.v. infusion of 0.9% sodium chloride at 1 ml/hr throughout the experiment.

After surgery, a 30-min equilibration period was allowed. Then RBF was measured and the renal vasoconstrictor response to a bolus 100 and 200 ng/kg i.v. injection of AII was recorded during a 10-min control period. The rats then received a 1 mg/kg i.v. injection of either indomethacin, sulindac or DuP 697. After a 30-min equilibration period, RBF and the renal responses to AII were again measured. The rats then received an additional 2-mg/kg dose of the drugs and 30 min later the experimental measurements were repeated. The rats were then given an additional 2-mg/kg dose of indomethacin, sulindac or DuP 697 and RBF and the renal vasoconstrictor responses to AII studied after a 30- min equilibration period. Indomethacin and sulindac were dissolved in 0.1 M sodium carbonate and DuP 697 was dissolved in ethanol at a concentration of 5 mg/ml. Thus, the volume of vehicle injected was small (50–100 μl) and had no effect on RBF or the renal vasoconstrictor response to AII.

Results

Adjuvant arthritis

Established disease. DuP 697 significantly decreased paw volume in the established adjuvant arthritis model with an ED_{50} value of 0.18 mg/kg/day. In comparative studies, piroxicam had an ED_{50} of 0.27 mg/kg/day (table 1) and indomethacin had an ED_{50} of 0.27 mg/kg/day.

Nonestablished disease. DuP 697 was a potent inhibitor of paw swelling in the nonestablished adjuvant arthritis model with an ED_{50} for inhibition of noninjected paw volume of 0.03 mg/kg/day on day 18. By comparison, DuP 697 was more potent than indomethacin which had an average extrapolated ED_{50} of 0.05 mg/kg/day (table 2).

Neither drug statistically significantly reduced circulating white blood cell counts. Arthritic rats had a circulating white blood cell count of 17 ± 0.6 × 10^3 cells/mm^3 whereas rats treated with 3.2 mg/kg of DuP 697 had 19 ± 1.3 × 10^6 cells/mm^3. DuP 697 and indomethacin also produced dose-related increases in body weight gain (table 2).

PQW. DuP 697 failed to inhibit PQW in rats (ED_{50} > 100 mg/kg). In contrast, indomethacin and piroxicam were equipotent in the rat PQW test with ED_{50} values of 2.9 and 3.5 mg/kg, respectively.

Randall-Selitto. DuP 697 produced significant analgetic effects in the yeast-inflamed paw test with an ED_{30} = 3.5 mg/kg at 4 hr after injection (table 3). DuP 697 had a slower onset

TABLE 1

Effect of DuP 697 in established adjuvant arthritis in rats

Rats were sensitized in the hind footpad with *M. butyricum*, and dosed p.o. on days 14 to 20 after adjuvant. Noninjected paw volumes and body weight changes were measured on day 21.

Drug	Dose	Mean Body Wt. Gain	Paw Volume	Decrease in Paw Volume	ED_{50}
	mg/kg/day p.o.	*g*	*ml, mean ± S.E.*	*% control*	*mg/kg*
Vehicle	Nonarthritic	+95	1.20 ± 0.02		
Vehicle	Arthritic	−3	2.50 ± 0.09		
DuP 697	0.1	+7	2.20 ± 0.10	23	
$n = 2$[a]	0.3	+17	1.77 ± 0.04***	56	
	1.0	+21	1.58 ± 0.04***	71	
	3.0	+27	1.40 ± 0.05***	85	0.18
Vehicle	Nonarthritic	+121	1.44 ± 0.03		
Vehicle	Arthritic	+18	2.58 ± 0.09		
Indomethacin					
$n = 5$[a]	0.1	+23	2.20 ± 0.02**	33	
	0.3	+31	1.92 ± 0.05**	58	
	1.0	+37	1.87 ± 0.08***	68	0.27
Vehicle	Nonarthritic	+94	1.27 ± 0.02		
Vehicle	Arthritic	−2	2.52 ± 0.09		
Piroxicam	0.03	−2	2.51 ± 0.09	0	
$n = 1$[a]	0.1	−2	2.30 ± 0.08	4	
	0.3	+8	1.87 ± 0.06***	52	0.27
	0.9	+18	1.67 ± 0.04***	69	

[a] Number of assays (10–20 rats/group/assay).
** $P \leq .01$; *** $P \leq .001$ compared to arthritic control, Duncan's Multiple Range test.

TABLE 2

Effect of DuP 697 and indomethacin in nonestablished adjuvant-induced arthritis in rats

Rats were sensitized in the hind footpad with *M. butyricum*. Animals were dosed p.o. on days 1 to 18 after adjuvant injection. Paw volume was measured on days 14 and 18 and blood drawn on day 18 for circulating white cell counts.

Drug	Dose	Body Wt. Gain	Paw Volume	Inhibition in Paw Volume	ED_{50}
	mg/kg/day p.o.	*g*	*ml, mean ± S.E.*	*%*	*mg/kg*
Vehicle, Nonarthritic control		89	1.04 ± 0.01		
Vehicle, Arthritic Control		−10	2.36 ± 0.06		
DuP 697	0.0125	−6	1.83 ± 0.10***	40	
	0.05	6	1.66 ± 0.13***	47	
	0.2	21	1.46 ± 0.04***	68	
	0.8	38	1.21 ± 0.03***	87	
	3.2	41	1.24 ± 0.03***	85	0.03
Indomethacin	0.1	8	1.60 ± 0.06***	58	
	0.3	21	1.47 ± 0.05***	67	
	0.9	26	1.32 ± 0.05***	79	0.05

*** $P \leq .001$ as compared to arthritic control, Duncan's Multiple Range test.

TABLE 3

Anti-inflammatory/analgetic activity of DuP 697 and other NSAID in the rat yeast-inflamed paw (Randall-Selitto) test

Compound	Route	ED_{30}	Peak-Effect Time (range)	Maximum Analgesic Effect (Dose)[a]
		mg/kg		
DuP 697	p.o.	3.5	(4+ hr)	54% (33–100 mg/kg)
Indomethacin	p.o.	1.0	(0.5–4 hr)	69% (3 mg/kg)
Piroxicam	p.o.	3.2	(0.5–4 hr)	67% (11–33 mg/kg)

[a] % MPE based on a 10 cm increase above base line (100% analgesic effect).

of action but was equipotent with piroxicam (ED_{30} = 3.2 mg/kg at 0.5 to 4 hr after injection). The magnitude of analgesic activity for DuP 697 (54 %MPE, 100 mg/kg, 4 hr) was equivalent to maximal responses produced by piroxicam (67 %MPE, 33 mg/kg, 1 hr). These data suggest that DuP 697 has significant anti-inflammatory/analgesic activity in this acute pain model. DuP 697 and reference NSAID were ineffective in raising the pain threshold on the noninflamed paw.

Antipyretic activity. DuP 697 demonstrated a potent dose-related antipyretic effect in rats. The p.o. activity of DuP 697 *vs.* yeast-induced pyresis (ED_{50} = 0.05 mg/kg, 5 hr) (fig. 2) appeared more potent than indomethacin or piroxicam. The onset, duration and time of peak antipyretic effect of DuP 697 were similar to those seen with indomethacin. DuP 697 had no effect on body temperature of normothermic animals at a dose of 18 mg/kg.

Bovine seminal vesicle PG synthesis. DuP 697 inhibited bovine seminal vesicle PG biosynthesis in a concentration-related manner. The individual IC_{50} values for each of three separate experiments were 1.75, 3.1 and 2.0×10^{-5} M; the mean for these experiments was 2.4×10^{-5} M. The Michaelis-Menten apparent K_m for arachidonic acid in this system was 0.03 mM (data not shown) which agrees well with the literature (McDonald-Gibson *et al.*, 1973).

The effectiveness of DuP 697 was compared to that of indomethacin ($IC_{50} = 1.3 \times 10^{-6}$ M, $n = 3$), piroxicam ($IC_{50} = 2.6 \times 10^{-4}$ M, $n = 3$) and sulindac sulfide ($IC_{50} = 3.3 \times 10^{-6}$ M, $n = 3$), the active form of the prodrug sulindac (fig. 3). DuP 697

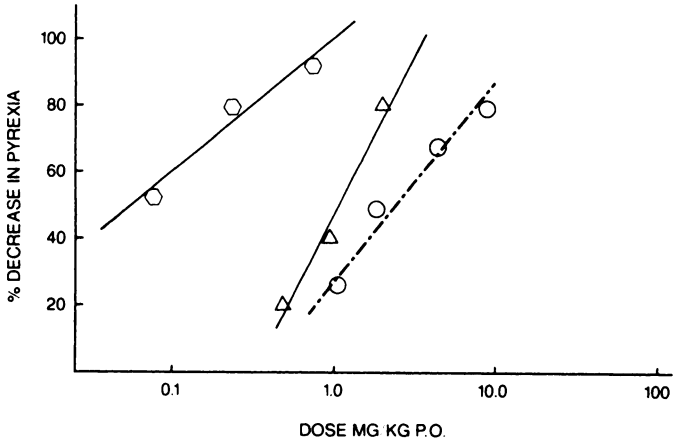

Fig. 2. Antipyretic effect of DuP 697 (○), indomethacin (△) and piroxicam (○) at the time of peak antipyretic effect in febrile rats. Symbols represent mean of five rats/group. Peak effect times were 5 hr for DuP 697, 4 hr for indomethacin and 2 hr for piroxicam.

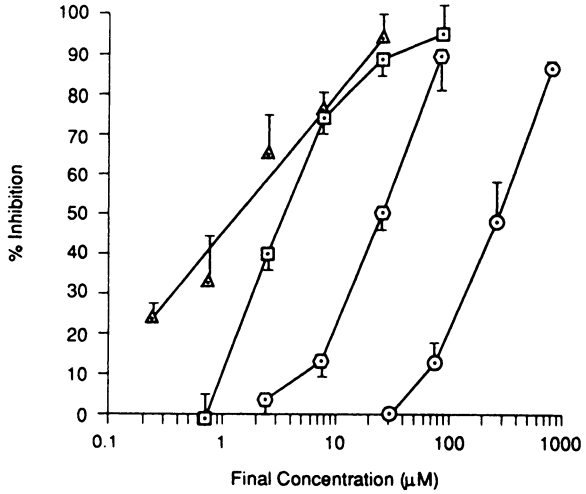

Fig. 3. Inhibition of bovine seminal vesicle PG synthesis by DuP 697 (○), indomethacin (△), sulindac sulfide (□) and piroxicam (○). Mean ± S.D. for three experiments.

was about one-tenth as potent as indomethacin and sulindac sulfide and more potent than piroxicam in this *in vitro* assay.

Rat kidney PG synthesis. The mean IC_{50} values for DuP 697 to inhibit medullary and cortical PGE production (fig. 4, A and B) were $> 7.5 \times 10^{-5}$ M ($n = 3$) and 6.2×10^{-5} M ($n = 3$), respectively. When compared to indomethacin and sulindac sulfide with IC_{50} values of 2.5×10^{-6} M ($n = 3$) and 1.7×10^{-6} M ($n = 3$), respectively, DuP 697 appeared at least 10-fold less potent at inhibiting medullary PGE production. Similarly, DuP 697 was less potent than indomethacin, sulindac sulfide and piroxicam at inhibiting cortical PGE production.

Rat brain PG synthesis. In four experiments, DuP 697 inhibited rat brain microsomal PG synthesis with an average IC_{50} of 4.5×10^{-6} M (fig. 5). Indomethacin and sulindac sulfide were equipotent to DuP 697 with an IC_{50} of 3.8×10^{-6} M ($n = 3$) and 6.0×10^{-6} M ($n = 3$), respectively. Piroxicam was less potent than DuP 697, and inhibited PGE production by rat brain with an IC_{50} value of 1.4×10^{-5} M ($n = 3$).

Gastrointestinal lesions. Administration of DuP 697, at 25, 100, 200 or 400 mg/kg p.o., did not induce small intestinal lesions in rats, giving a TD_{50} of greater than 400 mg/kg (table 4). Piroxicam, by contrast, gave a small intestinal lesion TD_{50} of 3.4 mg/kg.

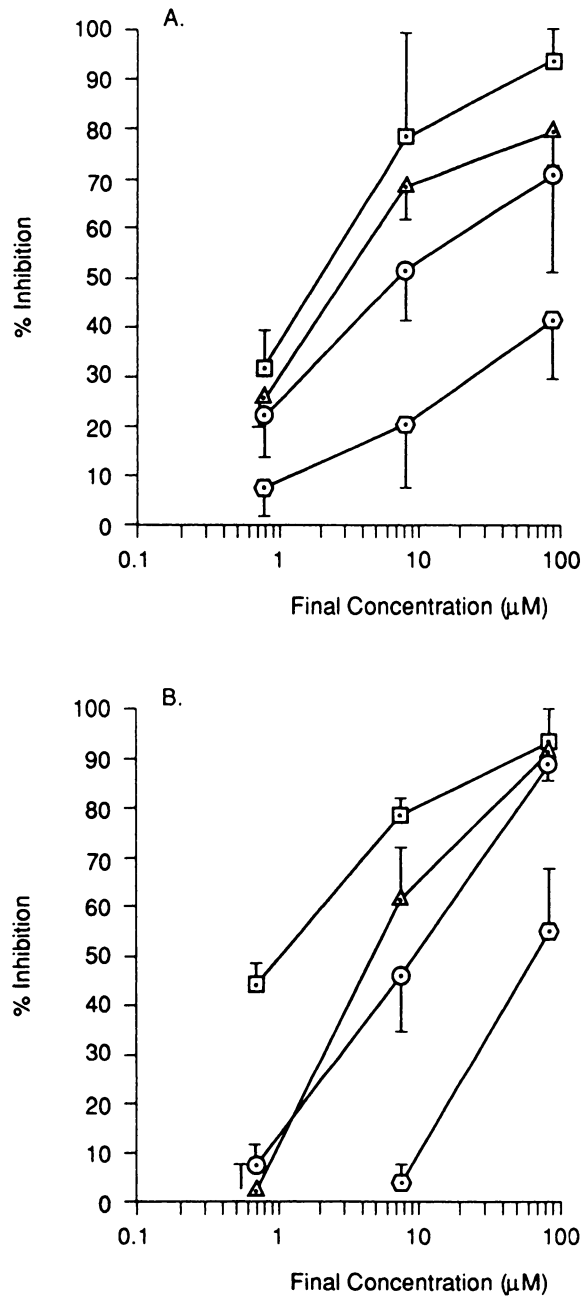

Fig. 4. A, inhibition of rat kidney medullary PGE production by DuP 697 (○), indomethacin (△) sulindac sulfide (□) and piroxicam (○). B, inhibition of rat kidney cortical PGE production. Mean ± S.D. for three experiments.

An apparent margin of gastrointestinal safety in the rat can be calculated by dividing the gastrointestinal TD_{50} by the established adjuvant arthritis ED_{50}. DuP 697 was found to have an exceptional margin of gastrointestinal safety in Sprague-Dawley rats of greater than 2000. This value can be compared to that obtained with piroxicam, with an apparent margin of gastrointestinal safety of 13.

Renal function. DuP 697 was tested in volume-depleted rats to determine if its lack of potent, *in vitro* renal cyclooxygenase inhibition would be evident *in vivo* in a model in which cyclooxygenase products are necessary to maintain RBF (McGiff and Itskovitz, 1973). DuP 697 at a cumulative dose of

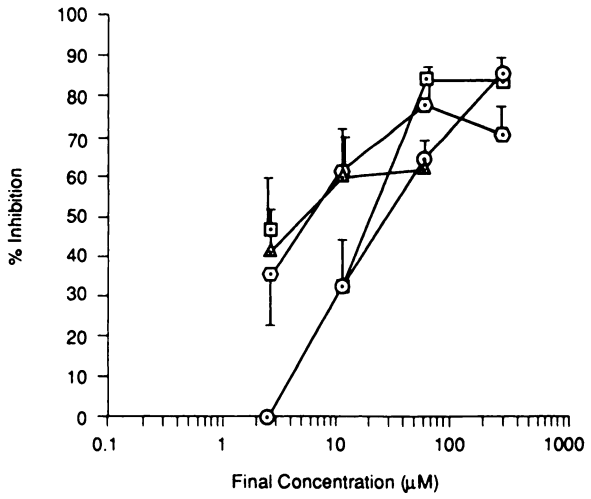

Fig. 5. Inhibition of rat brain PGE production by DuP 697 (○), indomethacin (△), sulindac sulfide (□) and piroxicam (○). Mean ± S.E. for three to four experiments.

5 mg/kg i.v. did not alter the renal vasculature response to AII (fig. 6A) nor did it reduce RBF (fig. 6B) in anesthetized, volume-depleted rats. In contrast, indomethacin significantly increased the renal vasoconstrictor response to AII at 3 and 5 mg/kg i.v. to 194 and 245% of control, respectively. Indomethacin also significantly reduced RBF at 3 and 5 mg/kg i.v. Sulindac did not alter the renal vascular response to AII, but it did significantly increase RBF by approximately 1 ml/min over the control value. The increase in RBF was the same at 3 and 5 mg/kg and was not significantly different from that of DuP 697. The volume-depleted rats treated with indomethacin did not produce enough urine to allow a comparison with DuP 697 and sulindac on the basis of sodium and water excretion. Neither indomethacin nor sulindac significantly altered the arterial blood pressure in the anesthetized, volume-depleted rats. DuP 697 slightly increased arterial blood pressure by about 15 to 20 mm Hg. The increase was significant ($P < .05$) at 1 mg/kg and remained essentially the same at the higher doses. In anesthetized dogs, DuP 697 did not have any effect on heart rate or blood pressure at doses up to 2.7 mg/kg (i.v.) nor did it alter the vasomotor response to a series of standard challenge agents including AII (1 μg/kg) (J. Stump, personal communication).

Discussion

Virtually all NSAID act in part by inhibiting the cyclooxygenase catalyzed step in PG biosynthesis. PG contribute to many aspects of the inflammatory response including hyperalgesia and vasodilation (Collier, 1980), and increased levels of these mediators are found at inflammatory sites. Reduction in PG concentration caused by NSAID contributes to their pharmacologic activity (Vane, 1987). However, in some tissues PG play a beneficial role and inhibition of their production contributes to the gastrointestinal and renal side effects of these drugs. An agent which inhibits specific tissue PG might provide anti-inflammatory activity without the gastrointestinal and renal side effects associated with universal PG inhibition. In the present studies, DuP 697 displayed relatively selective inhibition of PG synthesis *in vitro* with potent activity against rat brain PG synthesis, moderate activity against bovine seminal vesicle PG synthesis and virtually no activity against rat kidney PG synthesis. In contrast, indomethacin and sulindac sulfide had virtually the same IC_{50} value in each of the PG synthesis assays. The low potency of piroxicam against the bovine enzyme agrees with a report by Wiseman (1978).

The majority of NSAID used clinically in the therapy of rheumatoid arthritis cause gastrointestinal side effects (Rainsford, 1984). Although the PG inhibitory activity of many NSAID correlates with their capacity to produce gastric lesions, salicylate is an exception (Whittle *et al.*, 1980). In the present experiments, DuP 697 did not produce either intestinal or gastric ulcers in rats at single doses up to 400 mg/kg. This apparent gastrointestinal safety of DuP 697 may be due in part to its distinct chemical structure as a nonacidic thiophene (Shen *et al.*, 1981). The relatively weak inhibitory activity of DuP 697 against bovine seminal vesicle PG synthesis *in vitro* may explain its lack of ulceration potential in the rat. Experiments have not yet been completed to evaluate the effect of DuP 697 on PG synthesis in rat gastrointestinal tissue. Treatment of dogs with DuP 697 at single doses of 200 mg/kg or at 3 or 10 mg/kg/day for 2 weeks produced no ante-mortem or gross post-mortem findings which could be attributed to treatment. DuP 697 may thus prove to have a minimal propensity to induce gastrointestinal side-effects in humans.

Although PG may not be critical to normal renal function, most NSAID that inhibit PG synthesis can lead to the subsequent development of renal damage in humans (Nanra, 1980;

TABLE 4

Incidence of small intestinal and gastric lesions produced by DuP 697 and piroxicam

Rats were dosed p.o. with compound 18 to 24 hr before injection of pontamine sky blue dye to visualize mucosal lesions in the small intestine or stomach.

Compound	Dose	n	Intestinal		Gastric	
			No. With Lesions	TD_{50}	No. With Lesions	TD_{50}
	mg/kg p.o.			mg/kg		mg/kg
DuP 697	0	5	0		0	
	25	5	0		0	
	50	5	0		0	
	100	5	0		0	
	200	5	1		0	
	400	5	1	>400	0	>400
Piroxicam	0	5	0		0	
	1.0	5	0		0	
	2.0	5	1		0	
	4.0	5	3		0	
	8.0	5	5	3.4	0	>8.0
Indomethacin	8.0	5	5	<8.0	5	<8.0

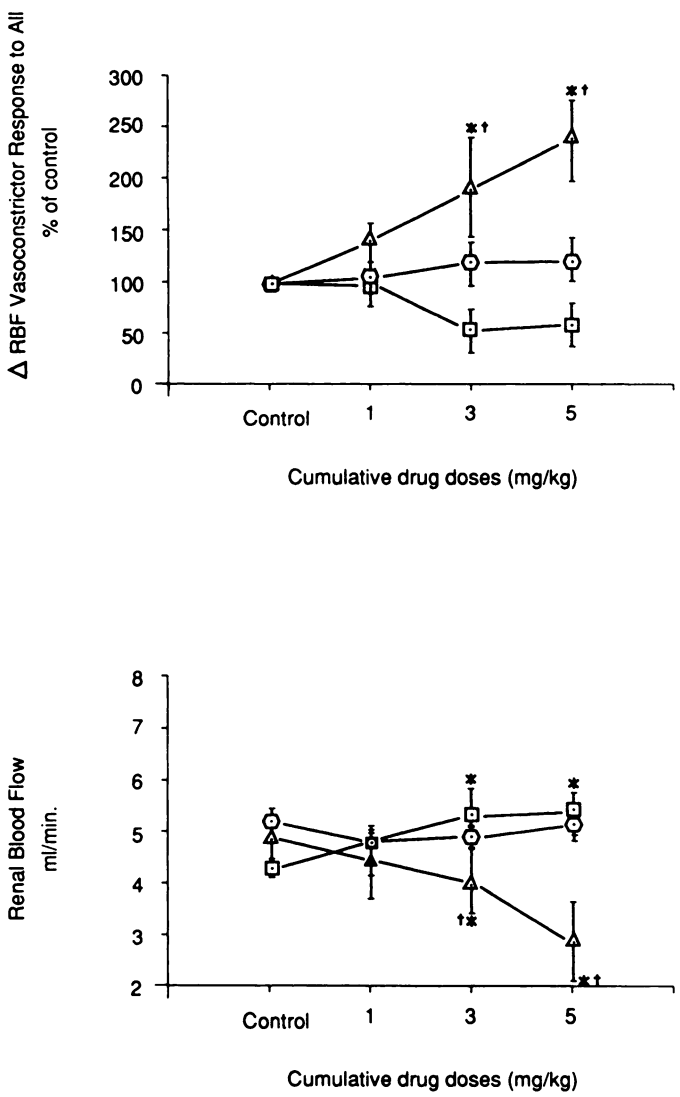

Fig. 6. Effects of DuP 697 (○), indomethacin (△) and sulindac (□) in furosemide-pretreated, volume-depleted rats. A, upper panel: effect on rat renal vasoconstrictor response to 200 ng/kg of AII. B, lower panel: effect on RBF. Mean of six rats/group ± S.E. *P < .05 from control. †P < .05 from corresponding values in DuP 697 and sulindac groups.

Dunn and Zambraski, 1980). One possible mechanism involved in ischemic tissue damage is inhibition of the vasodilators PGE_2 and prostacyclin contributing to reduced glomerular filtration and sodium and water excretion. We, therefore, evaluated the effect of DuP 697 on rat renal PG synthesis *in vitro*, comparing it to indomethacin, sulindac sulfide and piroxicam. Mean IC_{50} values for inhibition of rat medullary and cortical PGE production by DuP 697 were $> 7.5 \times 10^{-5}$ and 6.2×10^{-5} M, respectively, 10-fold less potent than indomethacin and sulindac sulfide. The critical site of renal PG production is unclear, because both the cortex and medulla possess synthetic capacity for these mediators. However, indomethacin and sulindac sulfide were equipotent at inhibiting PG production in both tissues.

Volume-depleted animals have been used to model the human high-renin states in which cyclooxygenase inhibitors, including indomethacin, have been shown to reduce RBF (Clive and Stoff, 1984, Blasingham and Nasjletti, 1980). In contrast, cyclooxygenase inhibitors have no effect in salt-replete animals. We chose to evaluate indomethacin, sulindac and DuP 697 in volume-depleted rats to be species consistent with the pharmacological profile. Under these experimental conditions, the renin-angiotensin system is activated and RBF is maintained by local production of vasodilatory PG to oppose the vasoconstrictor effects of AII. Indomethacin and sulindac were nephrotoxic and renal-sparing, respectively, in this model. Indomethacin dramatically reduced RBF and potentiated the vasoconstrictor response to AII. In contrast, sulindac increased RBF and had no effect on the renal vascular response to AII. Sulindac sulfide, the active form of the prodrug sulindac, is as potent a cyclooxygenase inhibitor as indomethacin, but its renal-sparing properties are attributed to the presence of predominantly the prodrug form in the kidney (Sedor *et al.*, 1984; Berg and Talseth, 1985). The slight elevation in RBF caused by sulindac may be an effect of the prodrug unrelated to the cyclooxygenase system. DuP 697 also had a renal-sparing profile in these studies. It did not potentiate the renal vasoconstrictor effect of AII, nor did it reduce RBF at doses where indomethacin had these effects. Because DuP 697 had anti-inflammatory potency similar to indomethacin and piroxicam in adjuvant arthritis, these results suggest a wider safety margin for DuP 697 than indomethacin with respect to renal safety in elderly renal compromised patients (Clive and Stoff, 1984; Dunn, 1984). These results also support the *in vitro* data suggesting tissue selectivity in cyclooxygenase inhibition by DuP 697.

Febrile responses are caused by the actions of IL-1 within the hypothalamus through the production and release of PGE_2 (Dinarello, 1984). DuP 697 was a potent inhibitor of lipopolysaccharide-induced PGE release from human peripheral blood monocytes; DuP 697 did not inhibit concomitant IL-1 production (Gans *et al.*, 1987). Thus, the antipyretic activity of DuP 697 may be related to its ability to inhibit PGE release induced by IL-1. In brewer's yeast-induced pyresis in rats, DuP 697 demonstrated a potent antipyretic response with a time course similar to that observed with indomethacin. DuP 697 exhibited an antipyretic profile characteristic of drugs with specific antipyretic activity as opposed to generalized hypothermic activity in that it reduced the febrile response to brewer's yeast to normothermic levels but was without effects on the temperature of normothermic rats. The *in vitro* ability of DuP 697 to inhibit rat brain PG synthesis may also be related to its *in vivo* antipyretic activity. As suggested by Milton and Wendlandt (1970), antipyretic activity may occur through inhibition of PG synthesis in the hypothalamus.

PGE was shown by several investigators to sensitize peripheral nerve endings and produce hyperalgesia (Ferreira and Vane, 1974; Ferreira, 1983; Willis and Cornelsen, 1973). DuP 697 was effective as an anti-inflammatory analgesic in the inflamed paw in the Randall-Selitto assay, but it did not increase the pain threshold of the normal paw and it lacked activity in the rat PQW assay. These results suggest that DuP 697 is an effective analgesic in which PGE is clearly involved in the inflammatory pain response, but it lacks analgesic activity in a noninflammatory pain model. The clinical significance of this observation is unknown.

In summary, DuP 697 is a new nonacidic nonsteroidal agent with anti-inflammatory, antipyretic and analgesic activity in inflammation-induced pain. In addition, DuP 697 is virtually devoid of gastrointestinal toxicity and does not alter RBF or AII responsiveness in volume-depleted rats. These properties

suggest a drug with the potential for both gastrointestinal and renal safety in arthritic patients.

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