# AHR-15010—A Novel Anti-arthritic Agent

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Abstract—AHR-15010 (3-(2-methoxyphenoxy)-1,2-propanediol bissulphamate ester) is a compound of novel structure that displays anti-arthritic activity in adjuvant arthritis in rats. When given orally from days 18 through day 50, (excluding weekends) after adjuvant injection, AHR-15010, at doses of 3·16 to 100 mg kg^{-1}, produced significant anti-inflammatory activity and reduced the severity of the hind paw joint lesions as monitored by X-ray analysis. AHR-15010, however, has no acute anti-inflammatory activity in the Evans Blue-carrageenan pleural effusion assay in rats, has no analgesic activity in mice, and has no activity in a classic, delayed-type, hypersensitivity assay in mice or in a cotton pellet granuloma test in rats. These data, in conjunction with biochemical data showing that AHR-15010 has no prostaglandin synthetase inhibiting activity, suggest that AHR-15010 is an anti-arthritic with a unique mechanism of action. AHR-15010 is a carbonic anhydrase inhibitor. Data are presented that suggest that AHR-15010 and acetazolamide, a prototype carbonic anhydrase inhibitor, may present novel approaches to the treatment of arthritis.

The arthritic diseases are characterized by the loss of the connective tissue structures of the joint. This joint degeneration is mediated by a variety of inflammatory cells and soluble factors released from those cells (Harris 1986). Recent work has also shown that bone resorption is an important clinical feature of the arthritic diseases (Alwan et al 1988; Sambrooke & Reeve 1988). This resorption can be induced by interleukin-1, bradykinin, or PGE_2 (Robinson et al 1975; Lerner et al 1987; Alwan et al 1988). Several drugs, including the NSAIDs and the gold salts, may exert some of their positive effects via inhibition of bone resorption (Katz & Gray 1986; Vargas et al 1987; Klaushofer et al 1988). In further support of this concept, Nolan et al (1989, unpublished data) shown that carbonic anhydrase inhibitors, which have been shown to inhibit bone resorption in-vitro and in-vivo (Minkin & Jennings 1972; Kenny 1985; Raisz et al 1988), display anti-arthritic activity in the rat.
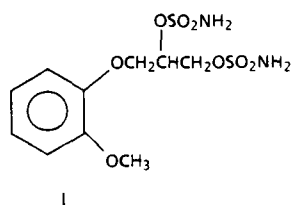
I. Structure of AHR-15010, 3-(2-methoxyphenoxy)-1,2-propanediol bissulphamate ester.

In our anti-arthritic programme, evaluation of anti-secretory and anti-arthritic compounds led to the identification of AHR-15010; 3-(2-methoxyphenoxy)-1,2-propanediol bissulphamate ester (I), as a unique agent for the treatment of arthritic diseases. This report describes the anti-arthritic activity of AHR-15010.

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Materials and Methods

Drugs and materials
AHR-15010 was synthesized in the Chemical Research Department at the A. H. Robins Co. (Richmond, VA). Indomethacin, acetazolamide, cyclophosphamide, triamcinolone acetonide, acetylcholine bromide, methylated bovine serum albumin (MBSA), and bovine carbonic anhydrase were from the Sigma Chemical Co. (St. Louis, MO). Cyclosporin (Sandimmune) was from Sandoz, Inc., (East Hanover, NJ), carrageenan (Viscarin) was from Marine colloids, Inc., (Springfield, NJ). *Mycobacterium butyricum* and Complete Freund's Adjuvant were from Difco Laboratories (Detroit, MI). Sheep vesicular glands were from the Wilson Food Corp. (Albert Lea, MN).

In-vivo methods
Rat adjuvant arthritis. Arthritis was induced in female Lewis Wistar rats by a method modified from that of Walz et al (1971). Fifty μL of a suspension of 1·5% *Mycobacterium butyricum* was injected into the subplantar surface of the right hind paw of all rats. Eighteen days later, the volume of both hind limbs was determined by mercury displacement, and the rats with significant uninjected hind paw oedema (paw volume > 2·4 mL) were assigned to treatment groups of 7 by balancing with regard to paw volume size. In the therapeutic dosing regimen, drugs were administered by gavage once daily to the rats beginning on day 18 after adjuvant injection and continuing through day 50 after adjuvant injection (excluding weekends). In the prophylactic experiments drugs were administered daily to groups of 10 rats beginning on the day of adjuvant injection and continuing for 50 days. Oedema was determined on day 29 and day 50 by paw volume difference (from day 18 paw volume in therapeutic experiments and from day 0 paw volume in prophylactic experiments). On day 50 the rats were killed by CO_2 inhalation and the uninjected hind limb cut off above the knee and X-rayed by standard procedures using a dental X-ray unit (Gendex, Model GX-770, Milwaukee, WI). The

X-rays were scored in a blinded manner by 2 investigators on an arbitrary 1 to 10 scale (1 = no damage; 10 = maximum damage).

Evans Blue-carrageenan pleural effusion in rats. Carrageenan pleurisy was carried out in male Sprague-Dawley rats essentially as outlined by Sancilio & Fishman (1973). The fasted rats were randomly assigned to treatment groups of 6 animals. One h after the oral administration of the compounds, the rats were anaesthetized with ether, and 5 mL of 0·075% Evans Blue, 0·5% carrageenan, in saline (at 37°C) injected intrapleurally. Five h later the rats were killed by CO_2 inhalation, and the pleural fluids were collected and measured.

Analgesia-acetylcholine-induced writhing in mice. Female ICR mice, 20–30 g, were used in a procedure modified from that of Collier et al (1968). Mice were dosed orally, by gavage, 20 min before an intraperitoneal challenge with acetylcholine bromide (6 mg kg^{-1}). Immediately following the challenge each mouse was placed under an inverted 1 L beaker and observed for 3 min for abdominal constriction. Analgesic activity was evidenced by the absence of this response.

Delayed type hypersensitivity (DTH) in mice. Mice were sensitized to methylated BSA (mBSA) by a subcutaneous injection of an emulsion (0·1 mL) made from equal volumes of Complete Freund's Adjuvant (CFA) and 5 mg mL^{-1} mBSA in saline. One week later the mice were challenged by injecting 25 μg of mBSA (in 50 μL saline) into the subplantar surface of 1 hind paw. Twenty-four h after this challenge, the mice were killed by cervical dislocation and the injected foot cut off at the ankle and weighed immediately. AHR-15010, vehicle (0·5% Tween 80), or reference drugs were administered for 3 consecutive days, either before sensitization or for 3 days before challenge.

Gastric toxicity in rats. Fasted male Sprague-Dawley rats, 180–240 g, were randomly allocated to groups of 7. Rats were treated orally with AHR-15010, indomethacin, or vehicle (0·5% Tween 80, 10 mL kg^{-1}) by gavage. Six h after dosing, the rats were killed by CO_2 inhalation; the stomachs were removed, opened, and scored on the following basis: 0 = no damage, 1 = few (1–3) erosions, < 3 mm in diameter, 2 = many (> 3) small erosions, 3 = few (1–3) large erosions, > 3 mm in diameter or 4–5 mm in length, 4 = many (> 3) large erosions.

Intestinal toxicity in rats. Fed male Sprague-Dawley rats, 180–240 g, were randomly allocated to treatment groups of 7. The rats were dosed daily by gavage with the drugs or vehicle for 11 consecutive days (excluding weekends). Twenty-four h after the last dose, the rats were killed by CO_2 inhalation and the intestines examined for lesions by using a scoring system as described by Sancilio et al (1977). The intestinal damage was assessed both on a quantal and on a quantitative basis as follows: 0 = no ulcers, 10 = ≤2 ulcers, 20 = 3–5 ulcers, 30 = 6–10 ulcers, 40 = > 10 ulcers, 50 = intestinal adhesions, 60 = death due to peritonitis.

Diuresis in rats. Fasted male Sprague-Dawley rats were dosed with the vehicle, 0·5% Tween 80, at 40 mL kg^{-1}, and the urine was collected for 6 h. The rats were ranked according to urine output (mL/100 g/6 h) and were balanced into treatment groups of 6. The rats were treated 2 to 5 days later with AHR-15010, acetazolamide, or vehicle; urine output was determined as above.

Statistics. In all animal experiments involving several treatment groups, Dunnett's t-test was used to determine significance ($P < 0.05$). For quantal data, differences between a control and treated group were analysed by using Fisher's Exact Test. Potency was determined by regression analysis (Bliss & White 1967).

In-vitro methods

Prostaglandin synthetase inhibition. Inhibition of prostaglandin synthetase was determined as outlined by Graff & Anderson (1989). Briefly, AHR-15010, indomethacin, or vehicle (ethanol) was added to the sample chamber of a Yellow Springs Instrument that contained 3 mL of 0·1 M Tris-HCl, pH 8·0; 1 mM phenol; 2μM haematin; and 10 μL of solubilized sheep vesicular gland microsomes (equilibrated to 30°C). The reaction was initiated by the addition of 30 μL of 10 mM ammonium arachidonate. The rate of oxygen consumption was determined polarographically with a Yellow-Springs Instrument Model 53 oxygen monitor. IC50 values were determined by regression analysis.

Carbonic anhydrase inhibition. Approximately 10 units of bovine erythrocyte carbonic anhydrase was incubated with various amounts of AHR-15010 or acetazolamide in DMSO (30 μL) in 6 mL of barbitone buffer, pH 8·3 (at 5 C). The reaction was initiated by the addition of substrate (4 mL of CO_2-saturated H_2O, 5 C). The rate of enzyme-catalysed change in pH was followed, and the IC50 values were determined from the concentration–% inhibition curves.

Results

Anti-arthritic activity

When given in a therapeutic dosing regimen, AHR-15010 produced dose-dependent inhibition of the oedema (days 29 and 50) and of the X-ray changes of the arthritic limbs of the rats (Table 1). Acetazolamide, a carbonic anhydrase inhibitor, and indomethacin, a typical prostaglandin synthetase

Table 1. Activity of AHR-15010 in rats with adjuvant arthritis.

| Drug | Dose (mg kg^{-1}) | Oedema | | X-ray score[a] | Number of trials[b] |
		day 29	day 50		
Vehicle	—	+0·20	−0·05	8·79	12
Indomethacin	3·16	−0·76[c]	−0·76[c]	4·00[c]	19
Acetazolamide	10·00	−0·34[c]	−0·50[c]	6·35[c]	3
AHR-15010	1·00	−0·15	−0·45[c]	7·01[c]	5
	3·16	−0·40[c]	−0·55[c]	6·20[c]	4
	10·00	−0·50[c]	−0·67[c]	5·47[c]	5
	31·60	−0·60[c]	−0·79[c]	5·78[c]	4
	100·00	−0·77[c]	−0·94[c]	5·82[c]	1

[a] Max score = 10; normal = 1. [b] There are 7 rats/group in each trial. [c] $P < 0.05$, Dunnett's t-test.

inhibiting NSAID (non-steroidal anti-inflammatory drug), were also active (Table 1). When given prophylactically (from the day of adjuvant injection through day 50), AHR-15010 did not attenuate the acute inflammatory response (day 15 or day 18 oedema), but significantly inhibited oedema formation from day 29 onward (Fig. 1), as was shown in the therapeutic dosing experiments. Indomethacin and triamcinolone acetonide produced significant anti-inflammatory activity at all times tested (Fig. 1). In either dosing regimen, the profile of activity of AHR-15010 was similar to that of the carbonic anhydrase inhibitor, acetazolamide (Table 1 and Fig. 1).

Acute activity—effect on Evans Blue-carrageenan pleural effusion and on acetylcholine-induced writhing in mice. When given at doses of 1 to 31·6 mg kg^{-1} p.o., to rats 1 h before carrageenan challenge, AHR-15010 had no significant, acute anti-inflammatory activity (Table 2). The reference NSAID, indomethacin, produced significant activity at 3·16 mg kg^{-1}. AHR-15010 also had no activity in a model of analgesia, the acetylcholine-induced writhing test in mice. At doses as high as 316·2 mg kg^{-1}, AHR-15010 did not inhibit the writhing response; the reference, indomethacin, caused a 90% blockade of the response at 2 mg kg^{-1} (Table 3).

Effect on DTH reaction in mice
AHR-15010 given for 3 consecutive days at 10 mg kg^{-1} before sensitization or for 3 days before challenge had no significant effect on the DTH response in mice. The immuno-modulatory drugs, cyclophosphamide and cyclosporin, and the steroid, triamcinolone acetonide, depending upon the
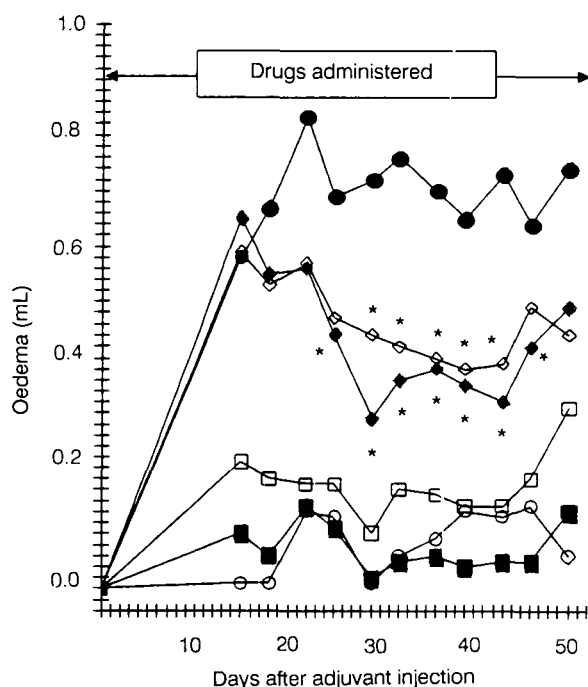

Fig. 1. Inhibition of adjuvant arthritis in rats—prophylactic dosing: ● arthritic control; ○ negative control; ◆ AHR-15010, 10 mg kg^{-1}; ◇ acetazolamide, 31·6 mg kg^{-1}; □ indomethacin, 3·16 mg kg^{-1}; and ■ triamcinolone acetonide, 1 mg kg^{-1}. * $P < 0.05$, Dunnett's t-test. All data points for the negative control, indomethacin and triamcinolone acetonide are significantly less than control ($P < 0.05$).

Table 2. Effect of AHR-15010 on the Evans Blue-carrageenan pleural effusion in rats.

Compound	Dose (mg kg^{-1} p.o.)	% Change in oedema from control
Control	—	—
Indomethacin	3·16	−31[a]
AHR-15010	1	+5
	3·16	−6
	10	−8
	31·6	−8

[a] $P < 0.05$, Dunnett's t-test.

Table 3. Acetylcholine-induced writhing in mice. Effect of AHR-15010. (20 min pretreatment time.)

Compound	Dose (mg kg^{-1} p.o.)	% Block of writhing response
Control	—	0
Indomethacin	2·0	90[a]
AHR-15010	10	0
	31·6	20
	100	0
	316·2	10

[a] $P < 0.05$, Fisher's Exact test.

schedule of treatment, significantly enhanced or suppressed the reaction (Table 4).

Gastric and intestinal toxicity
AHR-15010, when given to fasted rats in doses in vast excess of those required for anti-arthritic activity, caused minimal gastric lesions (Table 5). AHR-15010 was approximately 0·077 times as potent as indomethacin in producing gastric lesions. When given for 11 consecutive days, AHR-15010, unlike indomethacin, produced no intestinal lesions even at the highest dose tested, 316·2 mg kg^{-1} (Table 6).

Carbonic anhydrase inhibition and diuretic activity
When tested in-vitro against carbonic anhydrase from bovine erythrocytes, AHR-15010 inhibited the enzyme with an IC50 of 0·13 μM (n = 20). Acetazolamide, the reference

Table 4. Effect of AHR-15010 and reference compounds on a DTH-reaction in mice.

Treatment	Dose (mg kg^{-1} p.o.)	Paw wt (mg ± s.d.)
Drugs administered 3 consecutive days before sensitization		
Control	—	175·8 ± 9·5
AHR-15010	10	164·1 ± 17·8
Cyclosporin	50	165·3 ± 11·9
Cyclophosphamide	50	215·1 ± 29·2[a]
Triamcinolone acetonide	1	165·3 ± 11·7
Naive Mice	—	149·0 ± 8·6[a]
Drugs administered 3 consecutive days before challenge		
Control	—	231·4 ± 46·7
AHR-15010	10	214·0 ± 47·7
Cyclosporin	50	173·0 ± 14·3[a]
Cyclophosphamide	50	161·4 ± 7·6[a]
Triamcinolone acetonide	1	187·2 ± 23·4[a]
Naive Mice	—	160·5 ± 18·7[a]

[a] $P < 0.05$, Dunnett's t-test.

Table 5. Gastric toxicity: AHR-15010 vs indomethacin. (acute study—1 dose).

Compound	Dose (mg kg^{-1} p.o.)	Quantal score	Mean ± s.d.	Potency
Control	10 mL kg^{-1}	0/7	0	
Indomethacin	6·0	4/7	0·71 ± 0·76	1·0
	12·0	5/7	2·14 ± 1·86	
AHR-15010	10	0/7	0	~0·077[a]
	31·6	0/7	0	
	100	6/7	1·14 ± 0·90	
	316·2	4/7	0·71 ± 0·76	

[a] Approximation due to lack of parallelism of regression lines.

Table 6. Intestinal toxicity: AHR-15010 vs indomethacin. (chronic study—11 days of dosing).

Compound	Dose (mg kg^{-1} p.o.)	Quantal score	Mean score ± s.d.	Potency
Control	10 mL kg^{-1}	0/7	0	
Indomethacin	1·5	0/7	0	
	3·0	2/7	2·86 ± 4·88	1·0
	6·0	5/7	35·7 ± 24·4	
AHR-15010	3·16	0/7	0	
	10·0	0/7	0	
	31·6	0/7	0	
	100·0	0/7	0	
	316·2	0/7	0	

carbonic anhydrase inhibitor, is about twice as potent, its IC50 being 0·070 μM (n = 110). When given to rats for four consecutive days at 31·6 mg kg^{-1} per day, AHR-15010 and the reference carbonic anhydrase inhibitor (acetazolamide) produced the expected diuretic effects after the first dose, but were substantially less active on subsequent days of treatment (Fig. 2).

Effect on prostaglandin synthetase activity in-vitro. AHR-15010 did not inhibit prostaglandin synthetase activity in-vitro at the highest dose tested, 500 μM (data not shown). Indomethacin has an IC50 of 0·23 μM in this test.

Discussion

The current therapy of the arthritic diseases usually employs NSAIDs, steroids, immunomodulators, or disease-modifying drugs. The long-term results of treatment with these drugs, however, may not limit disease progress as much as desired (Pincus et al 1984; Scott et al 1987); and there are now suggestions of initially treating these patients more aggressively (Wilske & Healey 1989) to limit subsequent joint destruction. All of these drug classes, however, have side effects and the search for novel anti-arthritic drugs continues.

The results reported here indicate that AHR-15010 is a novel-acting anti-arthritic agent. AHR-15010 is anti-arthritic in the adjuvant rat (Table 1, Fig. 1), but lacks all the acute activities (anti-inflammatory activity in Evans Blue-carrageenan pleural effusion assay, analgesic activity in acetylcholine-writhing, Tables 2, 3) of typical prostaglandin synthetase inhibiting NSAIDs. AHR-15010 is not a prostaglandin synthetase inhibitor and has no gastric or intestinal liabilities (Tables 4, 5, 6). AHR-15010, given prophylacti-
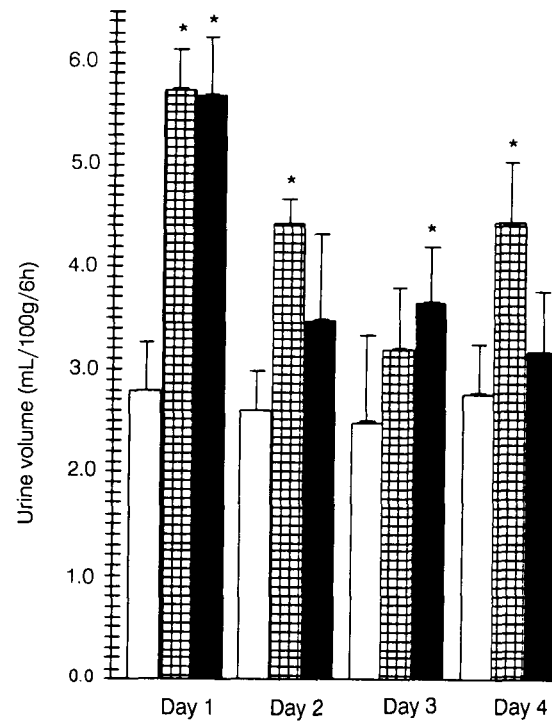

FIG. 2. Diuretic effects of AHR-15010 and acetazolamide in rats when given for four consecutive days: □ vehicle; ■ AHR-15010, 31·6 mg kg^{-1} and ▦ acetazolamide, 31·6 mg kg^{-1}. * $P < 0.05$, Dunnett's t-test.

cally (from day of adjuvant injection), does not inhibit the formation of the 'arthritic state' (day 15 oedema, Fig. 1) as do indomethacin, cyclosporin, cyclophosphamide, and triamcinolone acetonide (data not shown for cyclosporin or cyclophosphamide). These results, combined with the lack of an effect on a DTH reaction (Table 4), indicate that AHR-15010 has a unique pharmacological profile for an anti-arthritic compound.

The mechanism of the anti-arthritic action of AHR-15010 is unknown. However, AHR-15010 is a carbonic anhydrase inhibitor (Table 6) and acetazolamide, the prototype carbonic anhydrase inhibitor, is also anti-arthritic. Nolan et al (unpublished data) have shown that carbonic anhydrase inhibitors are anti-arthritic in the rat and that the activity is not due to their diuretic activity. The results here extend those observations. AHR-15010 has acute diuretic activity that lessens upon chronic usage (Fig. 2). The chronic anti-arthritic activity is not the result of this diuretic activity. In support of this concept, noncarbonic anhydrase inhibiting diuretics (such as frusemide and hydrochlorthiazide) are not anti-arthritic (data not shown). Carbonic anhydrase is involved in bone resorption (Hall & Kenny 1985), and carbonic anydrase inhibitors inhibit bone resorption in-vivo (Kenny et al 1979; Kenny 1985) and in organ cultures of bone (Minkin & Jennings 1972; Raisz et al 1988). Bone resorption is an important chemical component of rheumatoid arthritis (Sambrooke & Reeve 1988) and bone resorbing activity is found in rheumatoid synovia (Robinson et al 1975) and synovial fluid (Alwan et al 1988). It is interesting to speculate that AHR-15010 may work via inhibition of bone resorption. The early, oedematous changes in the arthritic rat may

be regulated, in part, by the prostaglandins. Since AHR-15010 has no effect on this process, it does not attenuate this process as do the classical NSAIDs. However, bone resorption may play a key role in the chronic phase of the disease. Since AHR-15010 and acetazolamide attenuate the disease when administered therapeutically, these drugs may be working via inhibition of bone resorption.

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