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Some aspects of the pharmacology of ibufenac, a non-steroidal anti-inflammatory agent

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When given orally to animals ibufenac has a range of potencies 2 to 4 times that of aspirin. It also suppresses thurfyl nicotinate erythema in man. Like certain other analgesic–anti-inflammatory–antipyretic drugs, when administered intravenously it suppresses bradykinin-induced bronchoconstriction in the guinea-pig. It has no glucocorticoid activity. Ibufenac, a compound chemically unrelated to existing antirheumatic drugs, can thus be classified as a non-steroidal anti-inflammatory (antirheumatic) agent.

IBUFENAC (4-isobutylphenylacetic acid) is a non-steroidal anti-inflammatory agent which has proved to be of value in the treatment of rheumatoid arthritis and other rheumatic diseases (Chalmers, 1963; Thompson, Stephenson & Percy, 1964; Hart & Boardman, 1965; Mizushima, Kikutani & others, 1965). We describe here some aspects of its pharmacology with specific reference to its anti-inflammatory, analgesic and antipyretic effects.

Experimental

METHODS

In the animal experiments, male rats (Wistar, Boots) of about 150 g weight, female guinea-pigs (Tuck) of 500–800 g weight, and male mice (Horne) of 20–25 g weight were used. Unless otherwise stated, the animals were deprived of food overnight and ibufenac and the control drugs, either aspirin, phenylbutazone or hydrocortisone sodium succinate, were administered orally in graded doses in 10% mucilage of acacia. Control animals received the same volume of acacia mucilage.

ANTI-INFLAMMATORY ACTIVITY

Ultra-violet erythema in the guinea-pig. Anti-erythemic activity in the guinea-pig was assessed by the method described by Adams & Cobb (1958), the degree of erythema being assessed on a scale 0, 1, 2, 3 or 4.

Thurfyl nicotinate erythema in man. The effect of ibufenac on thurfyl nicotinate erythema in man was determined with a Lovibond reflecting tintometer by the technique of Adams & Cobb (1963). The test was made on two successive days in a group of six volunteers. On the first (control) day, the degree of erythema was assessed by comparing the difference in redness (ΔR_{40}) of the volar surface of the forearm immediately before (R_0), and 40 min after (R_{40}), the application of 5% thurfyl nicotinate cream. On the next day each subject took 960 mg of ibufenac immediately after breakfast and the R_0 value was measured 150 min later. The thurfyl

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nicotinate cream was then applied to an area of skin different from that used for the control experiment, and the R_{40} value determined 40 min later.

Formation of granulation tissue in the rat. The development of granulation tissue in the rat was examined by the technique of Bush & Alexander (1960), using carrageenan-impregnated cotton wool dental pellets. Four pellets were implanted subcutaneously into the ventral region of the rat, one near each axilla. The compounds were administered daily in divided doses at 9·00 and 16·00 hr for 7 days; at the end of this period the pellets were removed and dried to constant weight.

Adjuvant arthritis in the rat. This was produced by a single intradermal injection into the tail of 0·1 ml of a 6 mg/ml suspension of killed tubercle bacilli, derived from human strains PN, DT and C, in liquid paraffin. Assessment of the severity of the arthritis in the hind paws only was made 19 days after the injection of adjuvant. Each hind paw was given a score of 0 to 4, the maximal possible score for each rat therefore being 8. The compounds were administered by mouth in divided doses at 9·00 and 16·00 hr each day.

SUPPRESSION OF BRADYKININ-INDUCED BRONCHOCONSTRICTION IN THE GUINEA-PIG

The bronchoconstrictor response of guinea-pigs to intravenous bradykinin was investigated by a technique similar to that originally described by Collier & Shorley (1960). Aspirin and ibufenac, as the soluble sodium salts, were administered intravenously and a period of 5 min was allowed to elapse before the following dose of bradykinin. Tachyphylaxis to bradykinin is usual in the guinea-pig and in most experiments only one dose of a compound could be tested in each animal.

ANALGESIC ACTIVITY

This was assessed by determining the pain reaction threshold in rats to pressure applied to the yeast-inflamed paw, using the apparatus described by Randall & Selitto (1957). Compounds were administered 30 min before the injection of 0·1 ml of a 20% suspension of dried yeast into the plantar surface of the rat paw. Two hr after the injection the pain reaction threshold was assessed, two determinations being made on each foot.

ANTIPYRETIC ACTIVITY

Pyrexia was induced in rats by means of a subcutaneous injection of 1 ml/100 g bodyweight of a 20% suspension of dried yeast. Sixteen hr later the rectal temperature of each rat was determined, animals with a temperature below 38·5° being discarded. The temperatures of the remainder were again recorded 1, 2 and 4 hr after drug administration.

GLUCOCORTICOID ACTIVITY

This was assessed by the increase of liver glycogen in the non-adrenalectomized mouse (Silber, 1959). Five hr after administration of the

compounds the mice were killed, the livers removed and the glycogen concentration estimated by means of the anthrone reaction (Carroll, Longley & Roe, 1956).

Results

ANTI-INFLAMMATORY ACTIVITY

Ultraviolet erythema in the guinea-pig. The collective results from a series of ultraviolet erythema experiments in the guinea-pig are shown in Table 1. An exact comparison of potency between ibufenac and aspirin is impossible since the dose-response slopes for the two compounds are obviously different. The results do suggest, however, that the potency of ibufenac is in the range 2 to 4 times that of aspirin. The shallower dose-response slope is not specific for ibufenac since we have found that other related phenylalkanoic acids have a similar shape.

TABLE 1. EFFECT OF IBUFENAC AND ASPIRIN ON THE DEVELOPMENT OF ULTRAVIOLET ERYTHEMA IN THE GUINEA-PIG. The degree of erythema in each animal was scored 0, 1, 2, 3 or 4.

Compound	Oral dose mg/kg	Mean erythema response	No. of animals
Aspirin	40	3·85	34
	80	1·27	33
	160	0·18	34
Ibufenac	10	3·53	34
	20	2·12	34
	40	0·97	34
	80	0·25	28
Control	—	4·00	30

TABLE 2. EFFECT OF AN ORAL DOSE OF 960 MG OF IBUFENAC ON THE ERYTHEMA PRODUCED IN MAN BY THE APPLICATION OF 5% THURFYL NICOTINATE CREAM TO THE VOLAR SURFACE OF THE FOREARM

	Tintometer readings (red units only)					
	Control: no drug			After 960 mg ibufenac		
Subject	R_0	R_{40}	ΔR_{40}	R_0	R_{40}	ΔR_{40}
A	4·3	6·2	1·9	4·2	5·1	0·9
B	4·3	5·5	1·2	4·8	5·4	0·6
C	4·2	5·7	1·5	4·1	4·9	0·8
D	4·2	5·6	1·4	4·0	5·2	1·2
E	5·4	6·4	1·0	5·4	5·6	0·2
F	4·2	5·8	1·6	4·3	5·4	1·1
Means	4·43†	5·87	1·43*	4·47†	5·27	0·80*
s.e.	0·20	0·15	0·13	0·22	0·10	0·15

* Significantly different $P < 0.01$.

† Not significantly different.

Thurfyl nicotinate erythema in man. The effect of 960 mg of ibufenac on the development of thurfyl nicotinate erythema in man is shown in Table 2: only the figures for red units are presented. Ibufenac did not affect the basic skin colour (R_0), but the degree of erythema (ΔR_{40}) was significantly reduced. This response is similar in degree to that obtained

on another occasion with 320 mg of aspirin. This qualitative similarity of the two compounds is of particular interest since 600 mg of phenylbutazone, 400 mg of oxyphenbutazone and 650 mg of sodium salicylate did not suppress the erythema (Adams & Cobb, 1963).

TABLE 3. ACTION OF IBUFENAC AND HYDROCORTISONE SODIUM SUCCINATE ON THE FORMATION OF GRANULATION TISSUE IN THE RAT

Compound	Oral dose mg/kg/day	No. of rats	% Body weight change	Mean granulation tissue per rat in mg ± s.e.	% Inhibition
Control	—	9	+13·6	23·4 ± 1·20	0
Hydrocortisone sodium succinate	16	8	+ 6·2	18·3 ± 1·11*	21·8
	32	8	+ 3·7	16·5 ± 0·81*	29·5
	64	8	0	11·1 ± 0·57*	52·6
Ibufenac	80	9	+14·8	20·2 ± 1·04	13·7
	160	9	+14·6	19·8 ± 0·79*	15·4
	320	9	+15·5	17·2 ± 0·37*	26·5

* Differs significantly from control $P \leqslant 0.05$.

Formation of granulation tissue in the rat. The comparative effects of ibufenac and hydrocortisone sodium succinate on the development of granulation tissue in the rat are shown in Table 3. Although doses of 160 and 320 mg/kg of ibufenac produced a significant reduction in granulation tissue compared with controls this was quite modest and the slope was shallow, in marked contrast to that produced by hydrocortisone sodium succinate. These results are in agreement with the findings of Winder, Wax & Welford (1965), who also found a lower ceiling and a shallower dose-response curve for the non-steroidal anti-inflammatory compounds mefenamic acid, flufenamic acid, meclofenamic acid and phenylbutazone than for glucocorticoids. There was a typical depression of general body growth in those animals receiving hydrocortisone sodium succinate, which did not occur with ibufenac.

TABLE 4. EFFECT OF PHENYLBUTAZONE, ASPIRIN AND IBUFENAC ON THE DEVELOPMENT OF ADJUVANT ARTHRITIS IN THE RAT. The degree of arthritis in each hind paw was scored 0 to 4.

Compound	Oral dose mg/kg/day	No. of rats	Mean arthritis score on hind feet (max. 8)	% Inhibition of arthritis
Control	—	6	5·9	0
Phenylbutazone	12·5	6	3·3	44·1
	25	6	1·8	69·5
	50	6	1·5	74·6
	100	6	0·7	88·2
Aspirin	25	6	6·5	−10·2
	50	6	5·6	5·1
	100	5	6·2	−5·1
	200	5	2·6	56·0
Ibufenac	25	6	5·5	6·8
	50	6	5·8	1·7
	100	6	2·6	56·0
	200	6	2·4	59·4

Adjuvant arthritis in the rat. From Table 4 it will be seen that ibufenac was twice as active as aspirin but less effective than phenylbutazone in suppressing the development of adjuvant arthritis. Glenn, Bowman & others (1967) found ibufenac to be approximately twice as active as aspirin and half as active as phenylbutazone in reducing established arthritis in the rat.

SUPPRESSION OF BRADYKININ-INDUCED BRONCHOCONSTRICTION IN THE GUINEA-PIG

The effective dose of a compound was considered to be one which reduced by 50% or more the bronchoconstrictor effect previously produced by a similar dose of bradykinin. In a series of experiments the effective intravenous dose for aspirin was in the range 1 to 2 mg/kg and for ibufenac 2·5 to 5 mg/kg. The results for aspirin were similar to those originally obtained by Collier & Shorley (1960, 1963) and, compared with the potencies of other compounds recorded by these workers, ibufenac is less active than mefenamic and flufenamic acid, approximately equipotent with phenylbutazone, and more active than amidopyrine. The activity of ibufenac in this sytem supports the evidence of Collier & Shorley (1960) that a relation exists between the ability of certain analgesic/antipyretic compounds to suppress bradykinin-induced bronchoconstriction and ultra-violet erythema in guinea-pigs.

ANALGESIC ACTIVITY

The results of the analgesic studies are shown in Table 5. The log dose-response curves of the two drugs are linear and parallel and the potency of ibufenac compared with aspirin is 3·4 with 95% confidence limits of 2·8 to 4·1.

TABLE 5. EFFECT OF IBUFENAC AND ASPIRIN ON THE PAIN THRESHOLD OF THE INFLAMED FOOT OF THE RAT. 18 rats were used for each dose group.

Compound	Oral dose mg/kg	Mean pain threshold (mm/Hg)
Ibufenac	15	71·1
	30	104·2
	60	143·3
	120	166·4
Aspirin	30	54·8
	60	89·8
	120	97·1
	240	148·8
Control	—	40·4

ANTIPYRETIC ACTIVITY

The results of these tests depicted graphically in Fig. 1 indicate that ibufenac is an effective antipyretic compound having about four times the potency of aspirin.

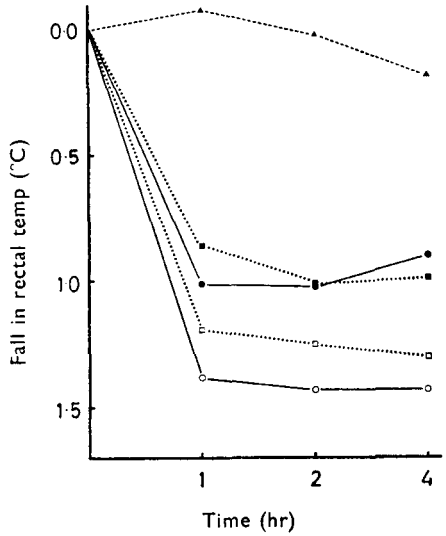

FIG. 1. Antipyretic effects of orally administered ibufenac and aspirin in the yeast-fevered rat. Each point represents the mean for 9 rats. ▲, Control; ■, aspirin 50 mg/kg; ●, ibufenac 12·5 mg/kg; □, aspirin 100 mg/kg; ○, ibufenac 25 mg/kg.

GLUCOCORTICOID ACTIVITY

From Table 6 it will be seen that, whilst 2 mg/kg of hydrocortisone sodium succinate produced a significant deposition of glycogen in the liver, 200 mg/kg of ibufenac had no effect.

TABLE 6. INFLUENCE OF IBUFENAC AND HYDROCORTISONE SODIUM SUCCINATE ON GLYCOGEN DEPOSITION IN GROUPS OF 10 MICE

Compound	Oral dose mg/kg	Mean liver glycogen mg/10 g mouse ± s.e.
Control	—	4·5 ± 0·31
Hydrocortisone sodium succinate ..	2	9·6 ± 1·30†
	4	11·2 ± 1·56†
	8	24·8 ± 4·39†
Ibufenac	50	4·0 ± 0·91*
	100	4·0 ± 1·06*
	200	3·2 ± 0·79*

* Not significantly different from control $P > 0.05$.

† Significantly different from control $P < 0.05$.

Discussion

A distinction is usually made between the acute inflammatory response of tissues to a transient stimulus, and the chronic inflammation which occurs when a stimulus is persistent. Ultraviolet erythema and thurfyl nicotinate erythema are typical of acute inflammation, whilst cotton-wool pellet granuloma and adjuvant arthritis are examples of different types of chronic inflammation.

Although ultraviolet irradiation in the guinea-pig and the application of thurfyl nicotinate in man are capable of producing oedema as well as erythema, our techniques are planned so as to measure only erythema (Adams & Cobb, 1963). Ibufenac is similar to many other non-steroidal anti-inflammatory drugs in its ability to inhibit ultraviolet erythema, but in thurfyl nicotinate erythema its action is more specific since, although aspirin is effective in very low doses, sodium salicylate, phenylbutazone and oxyphenbutazone are all inactive (Adams & Cobb, 1963).

In the granulation tissue experiments the proliferative component of inflammation predominates; whilst ibufenac, like a number of other potent non-steroidal anti-inflammatory agents, is slightly suppressive (Winder, Wax & Welford, 1965), it is markedly less effective than hydrocortisone.

Adjuvant arthritis in the rat is a complex chronic inflammatory condition of immunological origin, characterized by a persistent erythema and oedema of the paws, in some respects resembling active rheumatoid arthritis. The effect of ibufenac in this condition is probably a measure of its ability to reduce increased capillary permeability.

The overall pharmacological results indicate that ibufenac possesses anti-inflammatory, analgesic and antipyretic properties, its oral potency in animals being two to four times that of aspirin. In rheumatoid arthritis and allied conditions the compound is about twice as active as aspirin (Hart & Boardman, 1965). Thus some of the pharmacological effects of ibufenac appear to be similar to those of aspirin, but the difference between the dose-response curves of the two compounds in ultraviolet erythema indicates possible differences in their modes of action in this type of inflammation.

The failure of ibufenac to influence glycogen deposition in the mouse, or to simulate glucocorticoids in respect of both whole body growth and the formation of granulation tissue in the cotton pellet test, indicate that the compound is devoid of glucocorticoid activity.

On the basis of its pharmacological and clinical activities ibufenac can be regarded as a member of that non-steroidal group of anti-inflammatory–antirheumatic compounds which includes phenylbutazone, oxyphenbutazone, indomethacin, aspirin and the fenamic acids. This similarity of ibufenac to the other aforementioned compounds has been confirmed at the biochemical level in a variety of *in vitro* systems. It uncouples oxidative phosphorylation in rat liver mitochondria, whilst in cartilage it inhibits the incorporation of inorganic phosphate into organic phosphates, and the biosynthesis of mucopolysaccharide sulphates (Whitehouse, 1964). Skidmore & Whitehouse (1966a,b) have shown that ibufenac and other acidic anti-inflammatory drugs inhibit histamine formation by competing with pyridoxal phosphate for the coenzyme binding site, believed to be a lysyl ϵ-amino-group, on mammalian histidine decarboxylase. Mizushima & Suzuki (1965) have demonstrated that ibufenac as well as phenylbutazone, flufenamic acid and indomethacin stabilizes serum albumin (FV) against heat coagulation, whilst Gerber, Cohen & Guistra (1967) showed that ibufenac and other anti-inflammatory compounds

accelerate the disulphide interchange between serum sulphydryl groups and an aromatic disulphide.

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