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BRL 20459, a novel topically active non-steroidal anti-inflammatory drug

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BRL 20459 is a novel compound which displays anti-inflammatory activity when applied topically in the croton oil and cantharadin rat ear inflammation models. The compound does not inhibit uv-induced erythema in the guinea-pig or granuloma formation in the cotton pellet test in the rat. BRL 20459 does not inhibit prostaglandin synthesis nor does it interact with corticosteroid receptors in the thymus. In contrast to hydrocortisone, BRL 20459 did not cause thymus involution or reduce body weight gain in rats. BRL 20459 would seem to have a different mechanism of action to hydrocortisone, but this mechanism is as yet unknown.

Creams and ointments containing corticosteroids are widely used to treat skin inflammation associated with diseases such as allergic contact dermatitis, atopic eczema, primary irritant dermatitis and psoriasis (for review see Miller & Munro 1980). However, corticosteroids used over long periods can cause side effects including skin thinning (Kirby & Munro 1976; Dykes & Markes 1977), striae (Miller & Munro 1980) and suppression of pituitary–adrenal function (Carruthers et al 1975; Keczkes et al 1978) although the importance of this latter effect has been queried (Munro & Clift 1973; Sparkes 1976).

A compound with the broad spectrum of anti-inflammatory activity of corticosteroids, but without their side effects, would be useful especially in those chronic conditions requiring prolonged application of the drug. We describe studies on a structurally novel compound, 4α-β-methyl-6β-(3′-oxobutyl)-2,3,4,4a,5,6,7,8,-octahydro-2-naphthalenone (BRL 20459; I) (Goudie 1983).

METHODS

Croton oil-induced ear oedema

Female Wistar rats (Charles River, 170–200 g) were used and the method was modified from that of Tonelli et al (1965). Drugs were applied to the left ear either in the same solution as the croton oil or separately as indicated in the results. Croton oil alone was applied to the right ear. The animals were killed 6 h after applying croton oil and the ears excised at the hair line and weighed. In some experiments drugs were given orally suspended in 0·7% methyl cellulose 1 h before applying croton oil.

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Cantharadin-induced ear inflammation

The technique of Boris & Hurley (1977) was used except that tetrahydrofuran was used as the vehicle. The rat strain was as above, and animals were killed and ear weights determined 72 h after applying cantharadin.

Ultraviolet-induced erythema on the guinea-pig flank

The method was as described by Boyle et al (1982) except that drugs were applied in ethanol to two of the irradiated sites and ethanol alone to the other two. Erythema was scored on a 0 to 3 scale 2 h later by an observer unaware of the drug treatment. There was a maximum score of 6 per animal per treatment i.e. 54 per group of guinea-pigs.

Carrageenan induced-oedema in the rat paw

The method was as described by Boyle et al (1982) except that in some experiments the drug was injected with the carrageenan. In this latter procedure the compounds were initially dissolved in ethanol which was then diluted with 0·9% w/v NaCl (saline) to a final ethanol concentration of 12·5% v/v.

Cotton pellet-induced granuloma model

Compounds were dissolved in tetrahydrofuran and added to sterile cotton pellets. The solvent was allowed to evaporate overnight and the pellets implanted subcutaneously into male, Wistar rats under anaesthetic as previously described (Boyle et al 1982). Six days later the pellets were removed, dried at 80 °C overnight, and weighed.

[^{3}H]Dexamethasone binding

Thymus glands were removed from female Sprague Dawley rats (100–150 g) weighed and rinsed in ice-cold buffer (0·25 M sucrose, 1 mM $MgCl_2$, 10 mM Tris-HCl pH 7·6). The tissue was homogenized in a Silverson homogenizer in the above buffer at a ratio of 3 glands to 10 ml of buffer. The homogenate was centrifuged at 2000 rev min^{-1} in an MSE Mistral 4L centrifuge, the cytosol collected and diluted to the original volume.

Incubation tubes were prepared in triplicate containing 0·5 ml cytosol and 0·1 μCi [^{3}H]dexamethasone ([1(2)-^{3}H] TRK 417 batch 9, Amersham 25 Ci m mol^{-1}) to give a final concentration of 8×10^{-9} M. Non-radioactive competitors were added to incubation tubes to give final concentrations up to 1×10^{-4} M. The tubes were incubated at 4 °C for 2 h and 0·4 ml of a 100 mg ml^{-1} charcoal suspension in buffer was added. The tubes were immediately vortexed, centrifuged and the radioactive content of the supernatant determined in 10 ml of NE260 scintillation fluid (Nuclear Enterprises, Edinburgh).

Inhibition of prostaglandin synthesis

Bovine seminal vesicle prostaglandin synthesis was determined as described previously (Boyle et al 1982).

Materials

Croton oil, cantharadin, hydrocortisone and most general reagents were obtained from Sigma Chemical Company, London. (+)-Naproxen and indomethacin were generous gifts from Syntex Labs. Inc., Palo Alto, California, USA and Merck, Sharp and Dohme Limited, Hoddesdon, England respectively. Aspirin was purchased from BDH, UK. Carrageenan was prepared from a sample of Marine Colloids carrageenan by Mr Verrall, Beecham Pharmaceuticals, Brockham Park, UK.

RESULTS

Croton oil-induced ear oedema

Table 1 shows that BRL 20459, hydrocortisone and naproxen all inhibit croton oil-induced ear oedema in the rat when applied 20 min after the irritant. BRL 20459 produced a dose related inhibition with an ED50 of 2·8 mg/ear. Hydrocortisone reduced ear weight to below that of control possibly due to its vasoconstrictor action (McKenzie & Stoughton 1962). This procedure reduces the possibility of chemical interaction between the drug and the irritant, but very similar results were obtained when drug and irritant were co-administered. Hydrocortisone produced a marked reduction in oedema of the ear treated with irritant alone indicating significant systemic effects of the drug. BRL 20459 had no effect on the contralateral ear.

Table 1. Effect of BRL 20459, hydrocortisone and naproxen on croton oil-induced ear oedema in the rat. Croton oil was applied to both ears of rats that were killed 6 h later. Drugs were applied to the left ear 20 min after croton oil.

	Treated ear		Untreated ear	
Group	Wt (mg ± s.e.)	Inhib. %	Wt (mg ± s.e.)	Inhib. %
Normal control	112·2 ± 2·7		110·4 ± 2·5	
Croton oil control	140·7 ± 6·4		135·2 ± 6·2	
BRL 20459 3 mg/ear	110·4 ± 2·8***	106	132·9 ± 5·5	9
Naproxen 3 mg/ear	111·5 ± 4·8**	102	148·1 ± 6·9	−52
Hydrocortisone 1·5 mg/ear	92·0 ± 2·0***	171	106·0 ± 3·5***	118

** $P < 0·01$, *** $P < 0·001$.
Student's t test compared to croton oil control.
n = 10.

Oral dosing of hydrocortisone at 10 mg kg^{-1} 1 h before applying croton oil inhibited the induced oedema by 62%, whereas an oral dose of 50 mg kg^{-1} BRL 20459 had no effect.

Cantharadin-induced ear inflammation

Table 2 shows that BRL 20459 and hydrocortisone significantly reduced cantharadin-induced ear inflammation in the rat. Naproxen increased ear weight and although this was not significant in this test, non-steroidal anti-inflammatory agents have consistently stimulated swelling in this model. None of the drugs altered inflammation in the untreated ear.

Table 2. Effect of BRL 20459, hydrocortisone and naproxen on cantharadin-induced inflammation in the rat ear. Ear weights were determined 72 h after application of cantharadin.

	Treated ear		Untreated ear	
Group	Wt (mg)	Inhib. %	Wt (mg)	Inhib. %
Normal control	95·0		92·9	
Inflamed control	162·0		146·3	
BRL 20459 4 mg/ear	140·5*	32	134·2	22
Naproxen 4 mg/ear	184.8	−34	145·0	2
Hydrocortisone 2 mg/ear	133·1**	43	142·0	8

Mann Whitney 'U'.
n = 10.
** $P < 0·01$, * $P < 0·025$.

Ultraviolet-induced erythema on the guinea-pig flank

Aspirin, but not hydrocortisone or BRL 20459, inhibited uv-induced erythema in the guinea-pig when applied topically (Table 3). Earlier experi-

Table 3. Effect of aspirin, hydrocortisone and BRL 20459 on uv erythema in the guinea-pig. The experiment was carried out as described in the methods section. All drugs were applied at 5 mg/site in 100 µl ethanol and the inflammation scored at 2 h.

Compound	Total score vehicle control	Total score compound	Inhib. %
Aspirin	46	10	78****
Hydrocortisone	38	35	8
BRL 20459	41	35	15

Significantly different from ethanol treated side assessed by the Student's t test.
n = 9, **** $P < 0.001$.

ments have shown that the erythema can be inhibited by the topical application of indomethacin, 2 mg being a fully effective dose.

Carrageenan-induced paw oedema in the rat

BRL 20459 was inactive in this test at an oral dose of 38 mg kg^{-1}. When co-injected with carrageenan into the paw BRL 20459 (5 mg/paw) produced 0% inhibition compared with 16% ($P < 0.05$) for indomethacin (0·05 mg/paw) and 45% ($P < 0.01$) for hydrocortisone (0·2 mg/paw).

Cotton pellet-induced granuloma model

Table 4 shows that hydrocortisone inhibited granuloma formation around the drug treated pellet, with a slight, but not statistically significant, effect on the contralateral pellet. This effect was accompanied by a marked reduction in thymus weight and bodyweight gain. Naproxen reduced granuloma formation around the treated pellet without affecting the contralateral pellet, thymus weight or bodyweight gain. BRL 20459 was without signficant effect on any parameter.

[^{3}H]Dexamethasone binding to thymus binding sites

BRL 20459 was compared with dexamethasone in one experiment and with dexamethasone and hydrocortisone in a second experiment using a concentration of 8×10^{-9} M [^{3}H]dexamethasone throughout. Dexamethasone at concentrations of 1×10^{-7} and 5×10^{-7} M displaced 60 and 50% of the binding, hydrocortisone at 5×10^{-7} M displaced 40% of the binding and BRL 20459 at 1×10^{-4} and 5×10^{-5} M displaced 26% and increased binding by 15% in the two tests.

Inhibition of prostaglandin synthesis

BRL 20459 did not inhibit bovine seminal vesicle prostaglandin synthesis at doses up to 200 µg ml^{-1}. This compares with 50% inhibition at 1·5 µg ml^{-1} for naproxen.

DISCUSSION

The croton oil-induced rat ear inflammation model is an acute, oedematous reaction which reaches a peak at 6 h and declines by 24 h (Tonelli et al 1965; Iizuka et al 1981). We have shown here that both steroidal and non-steroidal anti-inflammatory agents inhibit this reaction to croton oil. In contrast, cantharadin-induced inflammation of the rat ear is reported not to reach its peak response until day 7 and to be inhibited by steroidal anti-inflammatory agents, but not by non-steroidal anti-inflammatory agents, antihistamines or vasoconstrictors (Boris & Hurley 1977). A similar difference in drug responsiveness has been reported when these two irritants are used on mouse ears (Swingle et al 1981). The guinea-pig uv erythema model has been reported to be specific for inhibitors of prostaglandin synthesis (Peters et al 1977) and we have shown herein that aspirin was an effective inhibitor of this response whereas hydrocortisone was inactive. BRL 20459 paralleled hydrocortisone in being active in the croton oil and cantharadin models in the rat, but was inactive in the uv erythema model in the guinea-pig. The latter result correlates with the lack of effect of BRL 20459 on prostaglandin synthesis and this may also explain the lack of effect on carrageenan paw oedema.

Table 4. BRL 20459, naproxen and hydrocortisone on cotton pellet-induced granuloma formation.

Compound	Dose (right pellet only)	Left pellet Granuloma (mg ± s.e.)	Left pellet Inhib. %	Right pellet Granuloma (mg ± s.e.)	Right pellet Inhib. %	Thymus wt (mg)	Body wt gain (g)
None		47·2 ± 5·8	—	59·5 ± 7·1	—	462·5	25·7
BRL 20459	20 mg	56·4 ± 4·1	−19	45·4 ± 7·9	24	488·8	27·8
Naproxen	5 mg	49·0 ± 6·7	−4	36·7 ± 5·1	38***	461·4	27·4
Hydrocortisone	5 mg	33·5 ± 3·6	29	25·3 ± 2·9	57****	253·4****	18·4***

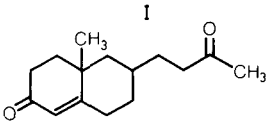
n = 8, *** $P < 0.01$, **** $P < 0.001$.
Student's t-test compared to control group.

Hydrocortisone inhibited croton oil-induced ear oedema of the opposite ear to that treated with the drug. This may have been due to absorption of the drug through the skin and distribution about the body, but could also have been due to oral absorption via paw licking after grooming. Experiments have shown that hydrocortisone could inhibit ear oedema in the croton oil model after oral absorption. In contrast, BRL 20459 had no effect on the contralateral ear and was ineffective orally and so the effect on the treated ear is a local effect. It is absorption of steroids through the skin which results in suppression of the pituitary–adrenal system in clinical use (Carruthers et al 1975).

BRL 20459 differed from both steroidal and non-steroidal anti-inflammatory agents in its inability to reduce granuloma formation in the rat cotton pellet test even when the pellet was impregnated with the compound before implantation. This experiment also showed the marked effect of corticosteroids on thymus weight and body weight gain. In contrast BRL 20459 was free from any toxic effects on the thymus weight or body weight gain.

Structurally BRL 20459 is a seco-steroid (I). However, this structural similarity was not sufficient to enable BRL 20459 to displace [^{3}H]dexamethasone from receptors in the rat thymus.

I



Lorenzetti (1979) has shown that the activity of corticosteroids in the croton oil model correlates well with their activity in human vasoconstrictor assays which have been claimed to be good predictors of clinical utility (McKenzie & Stoughton 1962). Boris & Hurley (1977) claimed that the relative activity of corticosteroids in the rat cantharadin test correlated with their clinical activity. BRL 20459 is active in both of these models and this spectrum of activity could be useful in treating dermatitis, however the potency of this particular compound is not sufficient to justify commercial development.

The mechanism of action of BRL 20459 is unknown. Its lack of interaction with corticosteroid receptors in the thymus and the lack of effect on granuloma formation in the cotton pellet test distinguish the compound from corticosteroids. BRL 20459 does not inhibit prostaglandin synthesis and inhibitors of prostaglandin synthesis together with vasoconstrictor agents, antihistamines and 5-HT antagonists are all reportedly inactive in the cantharadin test (Boris & Hurley 1977) whereas BRL 20459 is active. Thus BRL 20459 is unlikely to be working by any of these mechanisms so its mechanism of action remains to be elucidated.

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