sheets. The cells retain their epithelial morphology and synthesize cAMP in response to β-adrenergic but not serotonergic stimulation.

Key words: epithelium, cornea, rabbit, cell culture, β-adrenergic response, growth factors, cholera toxin, cAMP, serotonergic response

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References

A Comparison of the Ocular Anti-inflammatory Activity of Steroidal and Nonsteroidal Compounds in the Rat

P. Dharrocherjee, R. N. Williams, and K. E. Eakins

The anti-inflammatory activities of steroidal and nonsteroidal compounds have been evaluated in the rat model of ocular inflammation induced by subcutaneous injection of lipopolysaccharides. Dexamethasone sodium phosphate, BW755C, flurbiprofen, indomethacin, and benoxaprofen were administered orally or topically for 24 or 48 hrs. Oral administration of dexamethasone, BW755C, and flurbiprofen inhibited iris-vasodilatation and leukocyte accumulation in the anterior chamber in a dose-dependent manner. Indomethacin and benoxaprofen were active only at high doses. Topical administration of these compounds inhibited the inflammatory responses in a similar manner. The inhibitory effect on leukocyte accumulation by these compounds was greater than their effect on vasodilatation. BW755C, a phenyl pyrazoline derivative, which is an inhibitor of both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism was the most active nonsteroidal compound and had an anti-inflammatory profile similar to dexamethasone. The results of this study also indicate that the model of rat ocular inflammation induced by subcutaneous injection of endotoxin can be used satisfactorily for comparative evaluation of anti-inflammatory agents. Invest Ophthalmol Vis Sci 24:1143–1146, 1983

The anti-inflammatory drugs such as aspirin and indomethacin, inhibit the enzyme, cyclo-oxygenase, which converts arachidonic acid into prostaglandins. Unlike the aspirin-like drugs, the anti-inflammatory corticosteroids reduce the formation of both cyclooxygenase and lipoxygenase products of arachidonate metabolism by preventing the release of the precursor acid, arachidonic acid. This additional inhibition of leukotriene formation might be responsible for the wider profile of anti-inflammatory activity exhibited by the corticosteroids.

Although effective clinically as anti-inflammatory agents, corticosteroids have a large number of undesirable side-effects when used either systemically or topically. A compound having the ability of a corticosteroid to prevent the formation of the products of
both pathways of arachidonic acid metabolism but free of their undesirable side-effects, would be valuable as a topical ocular anti-inflammatory drug. The nonsteroidal anti-inflammatory compound BW755C (3-amino-1-(3 trifluoromethylphenyl)-2-pyrazoline) may be representative of such a class of drugs, since it is equi-active in inhibiting cyclo-oxygenase and lipoxygenase activity, and possesses a wider profile of anti-inflammatory activity than the aspirin-like drugs.4

In the present study, we have compared the anti-inflammatory activity of the aspirin-like drugs, flurbiprofen, indomethacin and benoxaprofen, with a corticosteroid, dexamethasone, and BW755C, following both oral and topical administration using the newly described rat model of ocular inflammation.5,6

**Materials and Methods.** Ocular inflammation in the rat was induced by injecting Shigella endotoxin into the foot pad as described by Bhattacherjee, Williams, and Eakins.6 Immediately after the injection, groups of 8–10 rats were treated with the anti-inflammatory compounds either orally three times a day for 24 hrs or topically three times a day either for 24 or 48 hrs. A constant volume of 10 μl and 500 μl was used respectively for topical and oral administration. At each dose level, a control group treated with vehicle alone was always included.

Animals were then examined under a slit lamp 24 and 48 hrs after endotoxin injection and commencement of the treatments. Dilatation of iris blood vessels was assessed, and the severity was scored (0 for normal, 1 as mild, 2 moderate, and 3 as severe). At the end of the experimental period, animals were killed with sodium pentobarbitone, the anterior chamber punctured, and the aqueous humor collected in a disposable graduated capillary tube and mixed with 0.1 ml heparinized saline. Leukocytes were counted in a single blind manner in a Neubauer Chamber. To compare the activity of various compounds and to analyse the data statistically, each value of the vasodilation score and leukocyte count was converted to the percent of the mean of the control. The mean of these values were then compared statistically with that of the control using unpaired t-test for groups consisting of unequal number of observations.

**Preparation of drug solutions.** Dexamethasone Sodium phosphate and BW755C were dissolved in saline immediately prior to administration. Indomethacin, flurbiprofen and benoxaprofen were dissolved in saline with the aid of equimolar concentration of sodium carbonate yielding clear solutions with pH between 7.0 and 7.5.

Oxyphenbutazone being insoluble in water was solubilised in polysorbate mono-oleate and then diluted with phosphate buffer, pH 7.5 to a final concentration of 5% polysorbate mono-oleate.

**Results.** The anti-inflammatory effects of the test compounds following oral administration on the responses of the eye to endotoxin are summarized in Figure 1. Dexamethasone effectively inhibited both the vasodilatation and leukocyte accumulation in the aqueous humor. Each column expressed as the percent of control values is the mean of 16–20 eyes. The figures within the columns are the doses and the bars represent ±SEM. *P < 0.05.

**Fig. 1.** The inhibitory activity of the anti-inflammatory agents administered orally three times in 24 hrs on the iris-vasodilatation and leukocyte accumulation in the aqueous humor. Each column expressed as the percent of control values is the mean of 16–20 eyes. The figures within the columns are the doses and the bars represent ±SEM. *P < 0.05.
on the vascular and leukocyte responses in the first 24 hrs of treatment, were significantly more effective at the end of the 48 hrs. BW755 was found to inhibit both the vascular and the cellular component of the inflammatory response whereas flurbiprofen, even at 48 hrs, was without effect on the vascular response, except at one dose. Interestingly, flurbiprofen produced a biphasic dose-response curve on leukocyte accumulation, low doses (up to 2.5 μg) inhibiting and higher doses (5 and 10 μg) potentiating the appearance of cells in the anterior chamber. The maximal inhibition of the cellular response achieved with flurbiprofen was only 50%.

Neither indomethacin nor benoxaprofen exhibited significant anti-inflammatory activity after treatment for 48 hours. Oxyphenbutazone, which was only tested for 48 hrs, was effective in reducing leukocyte accumulation at relatively large doses (25 μg/eye). It should be noted, however, that the oxyphenbutazone data are not strictly comparable with those of the other compounds since the vehicle used to solubilise the former was different from that used for the other compounds (saline only) and may have enhanced the bioavailability of oxyphenbutazone.

Discussion. The results of the present study show that this model of ocular inflammation is suitable for the evaluation of anti-inflammatory activity of both steroids and nonsteroidal compounds administered either orally or topically. Compounds like dexamethasone, with proven clinical efficacy, suppressed the ocular responses of the rat to endotoxin in a well-defined, dose-dependent manner following both oral and topical administration.

The nonsteroidal compounds, administered orally, had variable effects on the inflammatory responses,
the effect being more pronounced on leukocyte accumulation than on vasodilation. Of these compounds, flurbiprofen and BW755C significantly inhibited both the leukocyte and vascular responses. On the other hand inhibition of leukocyte accumulation but not of vasodilation was seen only at high doses of indomethacin and benoxaprofen. There is general agreement in the literature that following oral administration, drugs such as indomethacin and the salicylates will inhibit cell migration at high doses.4,7,8 Flurbiprofen and benoxaprofen also inhibit cell migration at doses which are well above those required to inhibit prostaglandin biosynthesis.4

It is not clear why BW755C, flurbiprofen, and oxyphenbutazone were without effect after 24 hrs; possibly their rate of absorption following topical administration is slow. The reversal of the dose response curve for flurbiprofen following topical administration of either 5 or 10 μg/eye would seem paradoxical but might be explained if the higher doses of flurbiprofen were irritating to the ocular tissues.

Indomethacin was without effect on either parameter of inflammation after 48 hrs. It is of interest that topical administration of indomethacin was found to enhance significantly the number of polymorphonuclear leukocytes in the aqueous humour of the rabbit with immunogenic uveitis while betamethasone and dexamethasone, inhibited white cell entry into the anterior chamber.9 No sign of enhanced leukocyte accumulation was found in the present study following indomethacin treatment. These differences might result from the different species involved, however, oral administration of low doses of indomethacin have been found to enhance the accumulation of leukocytes in inflammatory exudates, at doses which significantly reduce prostaglandin production, prior to inhibiting cell migration at higher doses.4

It is now well established that prostaglandins and the lipoxygenase products of arachidonic acid are involved in the ocular responses to trauma and immunogenic or non-immunogenic stimuli.10-14 The mediators involved in endotoxin-induced ocular inflammation have yet to be identified. However, the fact that BW755C, which inhibits both the cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism, reduced vasodilation and leukocyte accumulation, suggests that both prostaglandins and hydroxy products are involved in this model of ocular inflammation.

Key words: rat, ocular, inflammation, evaluation, anti-inflammatory agents

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References