Topical indomethacin effect on neovascularisation of the cornea and on prostaglandin E₂ levels

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SUMMARY The effect of indomethacin 1% drops against a placebo (NaCl 0.9% solution) was tested in rabbits' corneas injured by a standardized chemical burn. The corneas treated with indomethacin 1% had less neovascularisation and a much smaller increase in the PGE₂ levels as compared with the control fellow eyes treated with a placebo. It appears that indomethacin 1% drops have a clear effect on the inflammatory response of the cornea and deserve a clinical trial in selected patients suffering from corneal inflammatory changes.

Prostaglandins are widely distributed in mammalian tissues.¹ Although the intimate mechanism of their synthesis and release are not well known, many tissues can be non-specifically stimulated to produce more prostaglandins than they contain.¹ Release of prostaglandins is associated with ocular inflammation,² but their exact role in inflammation has not been identified. The demonstration of prostaglandins in the tissues of chronic ocular inflammation is of interest.¹ A high content of leucocyte phospholipase provides the inflamed tissue with a possible source of prostaglandin E₂ (PGE₂), and it is not difficult to imagine clinical situations in which this mechanism could be a continuous source of the hormone.

In the present study we stimulated an inflammatory reaction in the cornea of rabbits by means of a standardised chemical burn, and used this model to investigate the effect of indomethacin 1%, a topical antiprostaglandin agent, on the corneal neovascular response to the injury and also on the levels of PGE₂ in the tissue.

Materials and methods

Twenty albino rabbits were used for this study. The experiments were done under the effect of topical anaesthesia with oxybuprocaine (Novesine) 0.25% in each eye. A standard epithelial and stromal lesion was performed with 10% sodium hydroxide. For this purpose a filter paper 6 mm in diameter was soaked with the solution of 10% sodium hydroxide and placed on the central cornea for 10 seconds. The corneal

burns resulting from this injury were seen to be of similar size and depth when examined with an operating microscope. The effect of aqueous suspension of 1% indomethacin drops (provided by Merck Sharp and Dohme) on the injured corneas was investigated in terms of its influence on corneal neovascularisation (11 rabbits) and also its effect on the levels of PGE₂ released (9 rabbits). This study was performed in a single-masked format: one eye was given a 1% indomethacin, 1 drop 3 times a day, while the fellow eye was given NaCl 0.9%, 1 drop 3 times a day. The drops were given by a laboratory technician, and the examiners were not informed about the medication given to each eye.

The eyes were evaluated in terms of corneal neovascularisation as follows: the ingrowth from the limbus was measured with the help of a caliper 7 days, 17 days, and 27 days after the alkali burn. Photographic records of the neovascular reaction were taken during these measurements. For the purpose of this study the vascularisation was graded from 1 to 4 as follows: grade 1, engorged limbal vessels; grade 2, less than 1.0 mm ingrowth of vessels into the cornea; grade 3, 1.25–3 mm; grade 4, more than 3.25 mm (Fig. 1).

Statistical evaluation was done using the SPSS software package. Kruskal-Wallis one-way analysis of variance was applied for the neovascularisation progress in the treated and untreated groups during the follow-up period, and the Mann-Whitney U-test served for the analysis of neovascularisation in the treated and the untreated eyes at any given time.

The PGE₂ levels in the injured corneas were studied as follows: in four rabbits the injured corneas were

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Fig. 1 Grading of corneal neovascularisation in corneas of rabbits. A=grade 1; B=grade 2; C=grade 3; D=grade 4.

removed after two days and in five rabbits six days after the corneal injury. The corneal tissue was homogenised with the help of polytrone P 10-00 on 1.0 ml of 50 mM TRIS HCL buffer, containing ethylenediamine tetra-acetic acid (EDTA) 0.2 M at pH 7.0. The homogenate was extracted twice with two volumes of ether, and both the aqueous phase and the incubation media were assayed for PGE₂ concentration using a radioimmunoassay.³ The statistical evaluation of the PGE₂ levels was done by the method of Wilcoxon matched-pairs signed ranks test.

Histological preparation of 12 corneas (six rabbits) were done 30 days after the corneal injury. The tissue was stained with haematoxylin and eosin and examined under a light microscope.

Results

Neovascularisation in corneas treated with an aqueous suspension of indomethacin 1% or with a placebo. Fig. 2 shows that the degree of corneal neovascularisation

Fig. 2 Neovascularisation in corneas treated with an aqueous suspension of indomethacin 1% or with a placebo. ▨ Corneal neovascularisation in control eyes. □ Corneal neovascularisation in indomethacin 1% treated eyes. ● Corneas treated with NaCl 0.9%. ○ Corneas treated with indomethacin 1%.

Fig. 3A Light microscopic preparation of one rabbit with injured corneas. There is a mild corneal scarring and infiltration of white blood cells. This cornea was treated for 30 days with indomethacin 1%.

Fig. 3B More severe corneal scarring with infiltration of white blood cells. This cornea was treated with a placebo. (HE, $\times 38$).

tion in both groups increased gradually during the follow-up period. However, the rate of neovascularisation was more advanced in the eyes that received NaCl 0.9% ($p < 0.001$) than in the eyes treated with 1% indomethacin ($p < 0.05$). Furthermore at any given time after the injury the eye receiving the placebo had a more advanced degree of neovascularisation. The statistical difference in the degree of neovascularisation between the treated group and the control was not significant seven days ($p < 0.073$) after the injury. However, 17 days ($p < 0.01$) and 27 days ($p < 0.025$) after the injury it became statistically significant that the treated group had less neovascularisation. Twenty-seven days after the injury only in two treated eyes there was more neovascularisation than in the fellow untreated eyes.

In all the injured corneas a chronic white cell infiltration consisting of lymphocytes, macrophages, plasma cells, and eosinophils was seen. This infiltration was more severe in the non-treated corneas (Fig. 3).

PGE₂ levels in corneas treated with aqueous suspension of indomethacin 1% or with a placebo. Fig. 4 presents the PGE₂ levels extracted from both

the treated and the untreated corneas two and six days after the injury. The PGE₂ levels in both groups two days after the injury were by far more elevated ($p < 0.01$ in both groups) than the corresponding groups after six days. However, at any given time there were larger PGE₂ levels in the untreated group of corneas than in the treated one ($p < 0.009$ two days, $p < 0.02$ 6 days).

Discussion

In most experimental models corneal vascularisation is seen to be associated with an inflammatory reaction.⁴⁻⁶ The white cell infiltration that accompanies the inflammation could play a direct or indirect role in the increased release and synthesis of PGE₂ in the tissue.¹ Furthermore there is experimental evidence that PGE₂ can induce corneal neovascularisation.⁷ Anti-inflammatory substances such as indomethacin can inhibit prostaglandin synthesis,^{8,9} and the lesser neovascular response to inflammation seen in our model could well be related to this mechanism. We found that topical indomethacin 1%

Fig. 4 PGE_2 levels in a group of injured corneas treated with aqueous suspension of indomethacin 1% or with a placebo. ▨ PGE_2 levels in control eyes. □ PGE_2 levels in indomethacin 1% treated eyes.

results in less neovascularisation of the injured cornea, a lower degree of corneal infiltration with inflammatory cells, and also a lower concentration of PGE_2 levels as compared with placebo treated injured corneas.

In previous studies topical indomethacin reduced the deleterious effect of thermal injury and also of prolonged use of contact lenses.^{10,11} However, when the drug was injected below Tenon's space it had no effect on corneal neovascularisation.¹² We have no clear explanation for the discrepancy except that a different route of indomethacin administration was used.

Indomethacin 1% drops have been used effectively in clinical ophthalmology to reduce the inflammatory response of the iris produced by cataract surgery¹³ and has also been reported to have some effect on the prevention of cystoid macular oedema after this operation.^{14,15} To the best of our knowledge this drug has not yet been tested in terms of its clinical effects in chronic corneal inflammation in man. This study may

indicate that it can be worthwhile to try topical indomethacin in patients with chronic corneal inflammation to test its possible anti-inflammatory potential.

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