Mechanism of steroid action in ocular inflammation: Inhibition of prostaglandin production. Nava Floman and U. Zor.

Prostaglandin E (PGE) concentration in the aqueous humor of an intact rabbit eye was less than 0.1 ng. per milliliter and increased to 19 ± 3 ng. per milliliter 60 minutes following paracentesis. The rise in PGE level was associated with clinical signs of ocular inflammation. Pretreatment with triamcinolone reduced both the accumulation of PGE in the aqueous humor and the inflammatory response following paracentesis. Intravitreal injection of E. coli endotoxin into rabbit eyes increased PGE level in the anterior chamber to 72 ± 17 ng. per milliliter and induced acute uveitis. Slices of iris and ciliary body (ICB) derived from either rabbit eyes with endotoxin-induced uveitis or normal eyes were incubated for 60 to 240 minutes and the rate of PGE release into the medium was measured by radioimmunoassay. After a 4 hour incubation, the PGE release from inflamed ICB was threefold higher than that of normal ICB. Incubation of inflamed ICB with hydrocortisone or Millicorten (100 ng per milliliter) for 4 hours reduced PGE accumulation in the medium by 50 and 81 per cent, respectively. Aldosterone had no effect on the rate of PGE release from inflamed ICB throughout the incubation period. Hydrocortisone or Millicorten also reduced PGE tissue content of inflamed ICB by about 74 per cent during the period of incubation. Indomethacin (100 µg per milliliter) abolished PGE accumulation. The suppressive action of hydrocortisone on PGE release into the incubation medium was prevented by the addition of arachidonic acid (2 µg per milliliter), a substrate for prostaglandin synthesis. By contrast, the inhibitory action of indomethacin was not affected by provision of arachidonic acid. We suggest that glucocorticosteroids reduce PGE accumulation by limiting the availability of the substrate for prostaglandin biosynthesis and thus suppress the inflammatory response.

Prostaglandins (PG’s) are regarded as mediators of the inflammatory process in many organs, including the eye. High levels of PG’s have been demonstrated in aqueous humor of human and laboratory animals in some forms of ocular inflammation. Administration of exogenous PG’s induces the characteristic signs of ocular inflammation. The involvement of PG’s in the inflammatory response has been emphasized by the recent discovery that the anti-inflammatory properties of aspirin-like drugs are related to their direct inhibitory action on the microsomal PG synthetase system.

In contrast to aspirin-like drugs, corticosteroids (CS) are devoid of a direct inhibitory action on PC synthetase activity in microsomal fractions. However, this does not exclude the possibility that the anti-inflammatory properties of CS are related to suppression of over-all PG synthesis and/or release from intact tissue. Indeed, several reports suggesting an inhibitory action of CS on PG synthesis and release were recently published.

The present investigation was undertaken to explore whether CS interfere with: (1) the in vivo prostaglandin E (PGE) release into the anterior chamber following paracentesis of rabbit eyes and (2) the in vitro PGE synthesis and release by slices of inflamed rabbit iris and ciliary body (ICB). Preliminary results have been presented.

Method and Materials.

In vivo studies. Adult albino rabbits weighing 2.5 to 3.5 kilograms were anesthetized with 50 mg. of Ketalar and 30 mg of Nembutal per kilogram of body weight, administered simultaneously intramuscularly. The right eye was treated with 0.1 ml. of triamcinolone acetonide (40 mg per milliliter) injected subconjunctivally. One hour later ocular inflammation was induced in both eyes by an anterior chamber paracentesis procedure previously described by Miller, Eakins, and Atwal. Sixty minutes following paracentesis, samples of aqueous humor (to be known as “secondary” aqueous) were withdrawn from both eyes with a 27 gauge needle. To facilitate the removal of fluid with a high protein content from the anterior chamber, the animals were heparinized.

Monocular uveitis in anesthetized rabbits was induced by intravitreal injection at the pars plana, of 10 µg of E. coli endotoxin in 0.05 ml. of saline with a 27 gauge needle. Then 50 µl of saline were injected in the same way into the contralateral eyes, which served as controls. Thirty-six hours after injection of endotoxin the animals were
Animal number

Fig. 1. In vivo PGE levels in the reformed secondary aqueous humor 60 minutes following paracentesis. Clear bars, eyes not pretreated with triamcinolone; striped bars, triamcinolone pretreated eyes. Each pair of bars represents one animal.

killed by intravenously injected air. The eyes were rapidly enucleated and the aqueous humor was withdrawn for the determination of PGE level.

In vitro studies. The iris and ciliary body (ICB) of the eyes with endotoxin-induced uveitis and control eyes were removed, cut into small slices, and incubated in a shaking bath for 30 minutes (in 1 ml. of KRB) at 37° C. The medium was then discarded and 1 ml. of KRB containing 0.5 per cent DMA and the drug (100 μg per milliliter as indicated) was added to the slices of ICB. In part of the experimental series arachidonic acid (2 μg per milliliter) was added to the incubation medium. Aliquots of the incubation medium were drawn at 60 to 240 minutes for PGE determination. At the end of 4 hours, the sliced ICB was blotted, weighed, and homogenized in 1 ml. of ice-cold 50 mM Tris-HCl buffer, pH 7.0, containing 0.02M EDTA. The homogenate was extracted once with two volumes of ether and the aqueous phase as well as the incubation media were then assayed for E-type PG content with a radioimmunoassay as previously described.10

Drugs. Ketalar (ketamine hydrochloride, Parke-Davis & Company, Detroit, Mich.), Nembutal (sodium pentobarbital, Abbott, Saint-Ring-sur-Avare), Kenalog (triamcinolone acetonide in aqueous suspension, Squibb & Sons, Ltd., Liverpool), E. coli endotoxin (lipopolysaccharide W., E. coli 0111:B4, Difco Laboratories, Detroit, Mich.) were made up in saline to a concentration of 0.2 mg per milliliter. Also used were Aldosterone, hydrocortisone acetate, (Iknapharm Lab., Ramat-Gan), indomethacin (Assia Chemical Lab., Tel-Aviv), arachidonic acid ([5,8,11,14-eicosatetraenoic acid], Sigma Chemical Co., St. Louis, Mo.), Millitocorten (dexamethasone tetrahydrophthalate, Ciba-Geigy), N,N-dimethylacetamide (Merck-Schuchhardt-München). PGE was generously made available by Dr. J. Pike of The Upjohn Company, Kalamazoo, Mich.

All drugs added to the incubation media were dissolved in N,N-dimethylacetamide (DMA) and brought to a final concentration of 100 μg per milliliter in Krebs-Ringer bicarbonate buffer (KRB), pH 7.4, containing glucose (1 mg. per milliliter) and 0.5 per cent DMA.

Results.

In vivo studies. Prostaglandin E was undetectable in aqueous humor of intact rabbit eye (less than 0.1 ng. per milliliter). Following paracentesis of the anterior chamber, the eye responded with hyperemia of conjunctiva and iris, miosis, and sometimes corneal edema. High levels of PGE (average of 19 ± 3 ng. per milliliter; Fig. 1) were detected in the re-formed secondary aqueous humor withdrawn 60 minutes after paracentesis. Administration of triamcinolone 60 minutes before paracentesis prevented PGE accumulation in the secondary aqueous humor to a value of 2 ± 0.5
Fig. 2. Rate of PGE release into the incubation media from normal and inflamed iris and ciliary body. Shown are mean values ± S.E.M. for 15 determinations. The broken line represents normal ICB.

ng. per milliliter (Fig. 1) and markedly reduced the clinical signs of ocular inflammation. Thirty-six hours following intravitreal injection of E. coli endotoxin, PGE concentration in the anterior chamber increased to 72 ± 17 ng. per milliliter and the eyes showed severe signs of uveitis.

In vitro studies. Prostaglandin E accumulated in the medium at a near linear rate during 4 hours of incubation of normal or inflamed ICB, but the rate of PGE accumulation was significantly higher when inflamed tissue was incubated (Fig. 2). The difference was twofold after 60 minutes and threefold after 240 minutes (Fig. 2). Incubation of inflamed ICB in KRB containing 0.5 per cent DMA did not affect PGE release. Addition of hydrocortisone or Millicorten (100 μg per milliliter) to the incubation media of the inflamed ICB reduced PGE accumulation in the medium after 4 hours of incubation by 50 and 81 per cent, respectively (Fig. 3). This inhibitory effect was first noted after 1 to 2 hours of incubation period. Aldosterone was ineffective in this respect (Fig. 3). Hydrocortisone or Millicorten, but not aldosterone, reduced PGE tissue content of inflamed ICB by 74 per cent during a 4 hour incubation period (data not shown). Indomethacin abolished PGE release into the incubation media (Fig. 3).

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Arachidonic acid (2 μg per milliliter) added to the medium abolished the inhibitory effect of hydrocortisone on PGE release from inflamed ICB, but did not prevent the inhibitory effect of indomethacin. Arachidonic acid on its own enhanced PGE release (Fig. 4).

Discussion. The present study was undertaken to examine whether steroidal anti-inflammatory drugs administered in vivo or in vitro interfere with PGE synthesis and release during ocular inflammation. Paracentesis and endotoxin-induced uveitis in rabbit eyes were used as models for ocular inflammation in the present study.

The participation of PC's in those models of ocular inflammation is well established and increased levels of PGE-like activity were demonstrated in the aqueous humor of those inflamed eyes. We, too, found that during ocular inflammation PGE concentration in the aqueous humor, as measured by radioimmunoassay, was remarkably higher than that found in the normal aqueous humor.

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until recently it was not clear whether the anti-inflammatory properties of corticosteroids (CS) are related to PG's. In isolated microsomal preparations, CS at a dose level of 100 µg per milliliter do not inhibit PG synthetase activity; whereas aspirin-like drugs were active in this respect. We studied the mode of action of CS on PG synthesis and release in intact tissue both in vivo and in vitro during ocular inflammation. Triamcinolone administered subconjunctivally decreased markedly PGE accumulation in the anterior chamber following paracentesis and abolished the clinical signs of inflammation. These results are in accordance with the observation of Eakins and associates, who observed that two patients suffering from anterior uveitis, and receiving local steroid therapy, had undetectable levels of PGE-like materials as compared to high levels of PGE found in untreated patients suffering from the same disease. Similarly, our investigation indicated that hydrocortisone and Millipent, incubated with slices of inflamed ICB, reduced significantly PGE release into the medium (Fig. 2). PGE tissue content of inflamed ICB was reduced as well when incubated with those steroids.

These data confirm previous suggestions that corticosteroids somehow interfere with the over-all production of PGE. Since free arachidonic acid, the substrate of PG synthesis, is a rate-limiting factor in PGE production, the corticosteroids may limit the availability of arachidonic acid and thus cause reduction in PC production and release. Indeed, addition of arachidonic acid prevented the inhibitory action of hydrocortisone on PGE accumulation in the medium, whereas it had no effect when PGE synthesis was inhibited by indomethacin (Fig. 4). Similar results were obtained with inflamed synovia.

Studies by Hong and Levine with transformed mouse fibroblasts showed that hydrocortisone inhibits arachidonic acid release, induced by added serum, but does not inhibit production of PG's from exogenously supplied arachidonic acid. Furthermore, Bhattacherjee and Eakins found that arachidonate administered topically to the eye caused increase of aqueous PGE levels and inflammatory signs. Pretreatment with dexamethasone was ineffective in these respects, whereas indomethacin was effective. These observations suggest causal relationships between arachidonic acid and CS and are in accordance with those of Gryglewski and associates, who found that inhibition by corticosteroids of the release of PGE-like substances from rabbit mesenteric vascular preparation and sensitized guinea pig lung could be fully reversed by arachidonic acid. The possibility that corticosteroids affect PGE levels in inflamed ICB by enhancing PGE catabolism is unlikely, since these steroids have been found not to affect PG degradation.

Relatively high concentration of steroids (100 µg per milliliter) used in the in vitro study exceeded the therapeutic plasma level in man. However, in the in vivo studies, we employed steroid dosages in accordance with accepted clinical procedure. Prevention by exogenous arachidonic acid of the inhibitory effect of hydrocortisone on PGE release excludes the possibility that the steroid action was due to a toxic effect. In addition, aldosterone, a steroid devoid of anti-inflammatory properties at a comparable dose level, did not reduce PGE release from inflamed ICB in vitro. Stabilization of lysosomal membranes by corticosteroids is well known, and may interfere with the release of lysosomal phospholipases and thus...
preclude the contact between these enzymes and membrane phospholipids with a consequent reduction in the availability of arachidonic acid for PG synthesis and release. This hypothesis is supported by the evidence that only pharmacological doses of corticosteroids which stabilized lysosomes also inhibited PGE release.

In addition, corticosteroids may reduce the availability of arachidonic acid by several alternative mechanisms, such as (1) inhibition of the release of cell membrane phospholipase, (2) inhibition of phospholipase activity, and (3) interference with the transport of the newly formed arachidonic acid to the microsomes.

Since CS and aspirin-like drugs act on separate loci of PG biosynthesis, it is suggested that combined treatment with both types of drugs, in submaximal doses, may prove beneficial in ocular inflammation.

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REFERENCES

Pathogenesis of corneal damage from Pseudomonas exotoxin A. BARBARA H. IGLEWSKI, ROBERT P. BURNS, AND ILENE K. GIPSON.

Pseudomonas aeruginosa exotoxin A was injected into rabbit corneas. Death of epithelial, endothelial, and stromal cells resulted, and necrosis of the cornea followed. Control eyes with exotoxin neutralized by specific antitoxin showed minimal damage. A dose-response pattern was evident. Antitoxin neutralization of pseudomonas exotoxin A in corneal ulcers may have possible therapeutic implications.

Pseudomonas aeruginosa, an opportunistic pathogen, is a common cause of severe corneal infections which progress rapidly and are very destructive. While endotoxin-induced damage from bacterial cell walls is the usual mechanism of gram-negative bacterial injury, a number of studies have implicated extracellular pseudomonal proteases as the substances responsible for corneal damage. Recently Liu produced from Pseudomonas aeruginosa a heat-labile protein exotoxin (exotoxin A) which is lethal for many experimental animals and cultured mammalian cells. Exotoxin A has the same enzymatic activity as diphtheria toxin fragment A. Both toxins catalyze the transfer of the adenosine diphosphate ribose (ADP-R) portion of nicotinamide adenine dinucleotide (NAD) to mammalian elongation factor 2 (EF-2), thereby inhibiting cellular protein synthesis. This preliminary report de-