Effects of various pharmacologic agents on allergic inflammation of the eye

The roles of chemical mediators in ocular inflammation

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Effects of pharmacologic agents on experimental ocular inflammation induced by reverse passive Arthus reactions were investigated by a slit-lamp technique utilizing fluorescein-labeled rabbit serum albumin as an indicator. Cobra venom factor completely eliminated inflammatory responses, indicating that the complement system is a trigger for this type of ocular inflammation. Antihistamines mainly suppressed the early vascular response. Reserpine and indomethacin remarkably inhibited the increase of the permeability of the blood-aqueous barrier over the first 5 hr. Epinephrine and steroid hormone were also effective. Neither diethylcarbamazine nor isonicotinic acid showed effects on the permeability changes induced in this type of inflammation.

Key words: ocular inflammation, complement, histamine, serotonin, prostaglandin, slow reacting substance A, Arthus reaction, blood-aqueous barrier, fluorophotometry

Various chemical mediators have been demonstrated in inflamed tissues, and the mechanisms of their functions are being elucidated. Histamine is released from mast cells and appears to mediate an increase in vascular permeability during the initial phase of inflammatory processes. Recently, prostaglandins have been discovered to be possible mediators of increased vascular permeability, particularly during the late phase of inflammation. However, the roles of these mediators in inflammation in situ are not yet well known.

In a previous paper we reported a new method for the continuous and quantitative observation of permeability changes of the blood-aqueous barrier. The experiments based on this method showed that the permeability of the barrier changes in a biphasic pattern in the eyes inflamed by reverse passive Arthus reactions.

In the present paper we have attempted to ascertain the effects of antagonists to various chemical mediators on ocular inflammation using the same animal model.

Materials and methods

Animals. New Zealand Albino rabbits weighing 2.0 to 3.5 kg were employed. Before being used, the eyes were examined for the presence of preexisting disease.

Antiserum, antigen, and indicator. Bovine gamma globulin (BGG) (Miles Laboratories, Inc., Kankakee, Ill.) was dissolved in phosphate-buffered saline, pH 7.2, at a concentration of 20 mg/ml. Anti-BGG rabbit serum was obtained by repeated immunization of rabbits with BGG in complete Freund's adjuvant, and fluorescein-
Fig. 1. Changes in the permeability of blood-aqueous barrier during allergic inflammation of rabbit eyes and effects of pharmacologic agents on ocular inflammation. One eye received anti-BGG serum (open points) and the control eye on the opposite side was injected with normal serum (black points). The intravenous challenge of antigen was done at the zero time. Each point and error bar indicate, respectively, the mean value and standard error of each group of animals. Significance (p) values were calculated by Welch’s t-test. A, Nontreated nine animals. B, Four rabbits depleted of C3 by means of cobra venom factor: No. 1 (○—○), No. 2 (△—△), No. 3 (□—□), and No. 4 (▽—▽). C, Promethazine (○—○) in eight animals, Metiamide (△—△) in six animals. D, Reserpin (▽—▽) in four animals, and reserpin with niame (○—○) in five animals. E, Indomethacin (○—○) in five rabbits. F, Diethylcarbamazine (○—○) in five animals.

Labeled rabbit serum albumin (FITC-RSA) was prepared by conjugating a rabbit serum albumin fraction with FITC (Sigma Chemical Co., St. Louis, Mo.) as described in our previous paper. A normal rabbit serum was used as a control. These agents were sterilized by passage through a 0.22 μm Millipore filter and stored at -20°C without preservative.

Allergic inflammation. Ocular inflammation was induced according to the previous method. Eyes were sensitized by a single intravitreal injection of anti-BGG serum (0.1 ml), while the control eyes received the same volume of normal rabbit serum in the center of the vitreous. Just after resolution of the initial reaction to the trauma of injection, the intravenous challenge with BGG solution (50 mg) caused moderate inflammation in the eyes that received the antiserum. By contrast, the control eyes developed no signs of inflammation.

Measurement of FITC-RSA in the anterior chamber. One-half milliliter of FITC-RSA per kilogram of body weight was injected intravenously 30 min before the challenge. The intensity of fluorescence from materials leaking into the anterior chamber was continuously measured by a slit-lamp microphotometer (Hamamatsu T. V. Co., Hamamatsu, Japan).

Depletion of complement with cobra venom factor (CoVF). Forty units of CoVF (Cordis Laboratories, Miami, Fla.) per kilogram of body weight
Table I. Effects of pharmacologic agents on permeability changes during the late phase of ocular inflammation

<table>
<thead>
<tr>
<th>Agent</th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
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<tbody>
<tr>
<td>Untreated</td>
<td>17.9 ± 3.9</td>
<td>23.0 ± 5.0</td>
<td>27.3 ± 4.6</td>
</tr>
<tr>
<td>Promethazine</td>
<td>9.1 ± 2.1 (0.05)</td>
<td>13.5 ± 3.7 (NS)</td>
<td>22.9 ± 5.3 (NS)</td>
</tr>
<tr>
<td>Metiamide</td>
<td>5.3 ± 1.7 (0.01)</td>
<td>8.0 ± 2.8 (0.05)</td>
<td>11.5 ± 2.9 (0.01)</td>
</tr>
<tr>
<td>Reserpine</td>
<td>1.0 ± 2.5 (0.01)</td>
<td>3.6 ± 3.1 (0.05)</td>
<td>5.3 ± 2.2 (0.01)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>3.9 ± 1.7 (0.01)</td>
<td>4.4 ± 1.5 (0.01)</td>
<td>7.4 ± 1.6 (0.01)</td>
</tr>
</tbody>
</table>

\*Difference of relative dye concentration between indicated times and 60 min after the antigen challenge. Significance levels (p) of difference from untreated by Student's t-test are in parentheses. NS = Not significant.

were injected intraperitoneally five times over a period of 48 hr just before the challenge, as described by Cochrane and associates. Serum complement activity was assayed according to the method of Mayer, and titers of C3 and C4 were measured with intermediate cells and guinea pig complement components according to the method of Nelson and associates.

Antagonists to chemical mediators. Promethazine hydrochloride (Shionogi & Co., Ltd., Osaka, Japan), a blocker of histamine H-1 receptors, and metiamide (SmithKline Corp., Philadelphia, Pa.), an H-2 blocker, were used as antihistamines. Diethycarbamazine (kindly supplied by Dr. Y. Iwasawa of Tnabe Seiyaku Co., Ltd., Tokyo), isonicotinic acid hydrazide (Wako Pure Chemical Industries, Ltd., Osaka), indomethacin (Sigma Chemical Co., St. Louis, Mo.), reserpine (Daiichi Seiyaku Co., Tokyo), nialamide (Sigma Chemical Co., St. Louis, Mo.), dexamethasone sodium phosphate (Banyu Pharmaceutical Co., Ltd., Tokyo), and epinephrine (Daiichi Seiyaku Co., Ltd., Tokyo) were also employed. Nialamide was dissolved in 2N HCl solution and then adjusted to pH 5.0 with 2N NaOH just before use, according to the method of Gershon and associates.

Results

The experiments were designed to determine the effects of various pharmacologic agents on permeability changes of the blood-aqueous barrier in allergic inflammation of the eye. This was accomplished with slit-lamp microphotometers using fluorescein-labeled rabbit serum albumin (FITC-RSA) as an indicator.

Permeability changes during the ocular inflammation. Nine animals were employed. One eye was sensitized by a single intravitreal injection of antiserum, while the control eye on the opposite side received normal serum. Just after resolution of initial reaction to the trauma of injection, 4 days after the ocular injection, a challenge consisting of an intravenous injection with antigen was performed, FITC-RSA having been injected into an auricular vein 30 min before the challenge. The leakage of the fluorescent substances into the anterior chamber was observed in the eyes which received antibody but was not detected in the control eyes at all.

The concentration of the dye in the circulating blood was measured 60 min after the stimulus. Dye concentrations at other time intervals were calculated as previously described. Ratios between the dye concentration observed in the anterior chamber and that in the circulating blood were also calculated.

Role of complement in the permeability responses. In order to elucidate the role of complement in allergic inflammation of the eye, permeability changes were examined during experimentally induced inflammation in animals depleted of complement by pretreatment with CoVF.

Four animals received 40 units/kg of CoVF for five doses. Since two of them (rabbits...
No. 1 and 2) were not sensitive to this drug, activities of serum complement were reduced to only 46% and 74% of pretreatment values, respectively. C3 activity was reduced to 45% in the rabbit No. 2. The other animals (Nos. 3 and 4) were more sensitive, and their C3 levels came down to 13% and 11% of the pretreatment values, respectively. By contrast, C4 values were not affected in any animals.

Relative dye concentration in the anterior chamber during the ocular inflammation is shown in Fig. 1, B. The immediate permeability response was completely suppressed in all animals. However, moderate leakage occurred in the late phase of inflammation in rabbits No. 1 and 2, corresponding to the delayed permeability response observed in the untreated animals. On the other hand, no permeability changes were detected in the other animals even during the late phase.

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*Antagonists to histamine.* Promethazine (8.5 mg/kg) was administered twice intravenously: once 5 min before and once 60 min after the stimulus. As shown in Fig. 1, C and Table I, the permeability response was inhibited significantly for the first hour, but the delayed response was not affected at all. Thirty minutes after the challenge, the relative concentration of FITC-RSA in the anterior chamber is 1.5% ± 0.5 (mean ± standard error) in promethazine-treated animals, in contrast to 7.7% ± 2.4 in untreated animals. However, dye leaked into the anterior chamber after 60 min. The increase of relative dye concentration in 2 hr, from 60 to 180 min after the antigen challenge, is 22.9% ± 5.3 in treated animals compared with 27.3% ± 4.6 in untreated animals.

An intramuscular injection with Metiamide (5 mg/kg) 30 min before the antigen challenge remarkably suppressed both the immediate and the delayed responses. The dye leakage occurred slightly during the early phase and moderately during the late phase.

*Antiserotonin.* Reserpine induces the release of serotonin from basophils and platelets, thus depleting them. An intramuscular injection of reserpine (1 mg/kg) into four rabbits 1 day before the stimulus strongly suppressed the usual permeability increase accompanying the ocular inflammation during the first 3 hr.

Forty milligrams per kilogram of nialamide was injected intramuscularly with the simultaneous administration of reserpine (1 mg/kg) in five animals. As shown in Fig. 1, D, nialamide did not block the suppressive action of reserpine on permeability responses.

*Inhibitors of slow reactive substance A.* The diethyldiethylcarbazine (16 mg/kg) intravenously injected 30 min before the stimulus did not show any inhibitory effect on the ocular inflammation in three rabbits. Therefore, larger amounts of the drug (40 mg/kg) were administered in another two animals, but the drug, even in these large amounts, did not affect the permeability responses of the eyes (Fig. 1, F).

Isonicotinic acid hydrazide (20 to 50 mg/kg), given intravenously 30 min before the challenge, also failed to produce anti-inflammatory effects in the eyes of five animals.

*Inhibitor of prostaglandin biosynthesis.* The oral administration of indomethacin by rubber catheter (8 mg/kg) was performed in five animals 1 hr before the antigen challenge, also failed to produce anti-inflammatory effects in the eyes of five animals.

*Isonicotinic acid hydrazide.* The diethylcarbazine (16 mg/kg) intravenously injected 30 min before the antigen challenge, also failed to produce anti-inflammatory effects in the eyes of five animals.

*Other agents.* Epinephrine (48 μg/kg in total amounts) was injected intravenously at 1 hr intervals, beginning immediately before the stimulus, for a period of 4 hr. Dexamethasone sodium phosphate (2 mg/kg) was administered intramuscularly 30 min before the antigen challenge. Both drugs completely blocked vascular leakage throughout the 5 hr observation period.

**Discussion**

Cobra venom factor completely suppressed ocular inflammation at a dose which depleted almost 90% of C3 activity. It has been mentioned that the complement system plays an important role as an initiator of acute inflam-
mation by various types of injuries. The activation of complement is responsible for the anaphylatoxin activities of C3a and C5a, which mediate the release of vasoactive amines from mast cells, basophils, and platelets. In our study the complement system also appeared to play a role as a trigger of the ocular inflammation. However, in rabbits partly depleted of complement, the permeability response occurred only in the late phase.

Histamine is one of the best-known vasoactive amines and is a mediator of increased vascular permeability in inflammation. In our experiments the immediate permeability response was inhibited partly by promethazine hydrochloride, a blocker of the histamine H-1 receptor, and more remarkably by metiamide, an H-2 blocker, which also moderately suppressed the delayed response. The results suggest that histamine might be important for the initial increase of permeability of the blood-aqueous barrier in ocular inflammation and that this mediator might also influence the late phase. This sequential action of histamine has been observed in other types of inflammation. In addition, the experiments suggest that H-1 receptor antagonists have little effect on the vascular responses of inflammation in contrast to the strong effects of an H-2 receptor blocker.

The suppression of the ocular inflammatory responses by the histamine antagonists may be partly due to their action in blocking chemical mediators other than histamine. Antihistamines are not specific in their action on histamine. Promethazine has been shown to cause a suppression of serotonin and other mediators. On the other hand, our experiments demonstrated the strong inhibitory effects of reserpine on the permeability response over the entire phase of ocular inflammation, suggesting that serotonin, as well as histamine, may be a major mediator of increased permeability in allergic inflammation. In acute inflammations of the skin, histamine and serotonin have been demonstrated to be responsible for the immediate vascular response.

Although diethylcarbazine and isonicotinic acid hydrazide inhibit the release of SRS-A in rats subjected to antigen-antibody interactions, these drugs had no effect on the vascular responses seen in allergic inflammation of rabbit eyes, suggesting that SRS-A may not be a potent mediator for this type of inflammatory response among rabbits.

Prostaglandins (PGs) are present in normal ocular tissues and are demonstrated in the inflamed anterior chamber. Ocular inflammation caused by various injuries can be suppressed with indomethacin, indicating that PGs are involved in ocular inflammation. In our experiments indomethacin strongly suppressed both the immediate and delayed phases of the vascular responses, suggesting that both phases may be mediated by PGs. PGs cause increased permeability not only because of their action on the release of histamine but also because of their direct action on the vascular system. As shown in our data, histamine is responsible for vascular responses during the early phase of ocular inflammation but plays little part in the late phase. Therefore, PGs are most probably involved in the immediate vascular response through the release of histamine and in the delayed response by direct action on blood vessels.

In summary, we suggest that the complement system plays an important role as a trigger for the release of chemical mediators in the ocular inflammation produced by reverse passive Arthus reactions. Histamine and serotonin may be major mediators of vascular responses, particularly during the early phase. The release of these mediators may be caused by activated complement from mast cells, platelets, and leukocytes. The activation of the complement system is also associated with the release of PGs, which in turn have direct effects on the vascular system and regulate the release of vasoactive amines and SRS-A. PGs appear to be responsible for both the immediate and delayed inflammatory responses.

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