Eosinophil Chemotaxis and Anterior Uveitis From Topical Dimaprit and Nordimaprit

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Topical application of the H₂-histamine receptor agonist, dimaprit (S-[4-N,N-dimethylaminopropyl]isothiourea), produced eosinophil chemotaxis into the anterior segment of rabbit eyes only when an 
H₂-antagonist was co-administered. Nordimaprit (S-[4-N,N-dimethylaminethyl]isothiourea), a structural homologue of dimaprit that lacked activity at histamine receptors, produced eosinophil chemotaxis whether or not an H₂-antagonist was co-administered. Onset of eosinophil chemotaxis began after 2 or more days of treatment, and was accompanied by corneal edema, opacification, and ocular inflammation. There was no concurrent eosinophilia in the peripheral blood or in the conjunctiva. The response occurred in pigmented and albino rabbit eyes, and was facilitated by prior co-administration of proparacaine eye drops. Another dimaprit homologue without activity at histamine receptors, homodimaprit (S-[4-N,N-dimethylaminobutyl]isothiourea), did not produce eosinophil chemotaxis when applied topically, nor did the H₂-agonists impromidine, histamine, or 4-methylhistamine, whether co-administered with an H₂-antagonist or not. It was concluded that dimaprit and nordimaprit produced a selective eosinophil chemotaxis unrelated to H₁ and H₂-histamine receptor activity. However, the H₂-agonist activity of dimaprit appeared to inhibit this response unless neutralized by an H₂-antagonist. Topical application of dimaprit with an H₂-antagonist or nordimaprit alone may allow large numbers of non-degranulated eosinophils, free of other cell types, to be harvested from the aqueous humor. Invest Ophthalmol Vis Sci 27:1504–1511, 1986

Histamine is both an H₁- and H₂-receptor agonist. Drugs that are more specific for each of these receptors have been synthesized. For example, dimaprit,1 impromidine,2 and 4-methylhistamine3 are primarily H₂-agonists, while cimetidine4 and tiotidine5 are primarily H₂-antagonists. In addition, chemical homologues of these drugs have been made that lack significant agonist or antagonist activity at histamine receptors, e.g., nordimaprit and homodimaprit (Fig. 1). Use of these various agents has allowed identification of H₁- and H₂-receptors at a variety of sites. For example, H₂-receptors have been identified in the uterus, heart, and on the ocular surface.7,8

The chemotactic effect of histamine on eosinophils is a subject of controversy. Clark et al9 and Turnbull10 believed that histamine was selectively chemotactic for eosinophils. However, Litt11 reported that histamine-induced eosinophil movement was a non-specific effect caused by its acidity. Wadee et al12 also found that histamine produced random eosinophil migration (chemokinesis) and reduced true chemotaxis. Jones and Kay13 reported that an initial exposure to histamine was chemotactic, but that repeated histamine exposure prevented this effect. This apparent self-deactivation might have been the result of histaminase release by eosinophils.14 The limited evidence in the literature supports the view that eosinophil movement, be it chemotaxis or chemokinesis, is the result of stimulation of H₂-receptors.11,12 Clark et al15 found that, with regard to migration stimuli, eosinophils behaved as a heterogeneous population. They speculated that this might be due to variations in the relative frequencies of H₁- and H₂-receptors, or to the existence of a third type of histamine receptor, i.e., an H₃-receptor.

During preliminary studies on the intraocular pressure effects from the topical application of a combination of an H₂-antagonist, cimetidine, and an H₂-agonist, dimaprit, an acute inflammatory response was noted. The anterior segment of rabbit eyes developed a selective eosinophil infiltration. It was accompanied by miosis, corneal edema, and a variable amount of iris depigmentation. The present paper describes the investigations of this response.

Materials and Methods

Dutch Belted rabbits, i.e., pigmented rabbits, were used, except for one experiment with albino rabbits.
Animals were maintained in compliance with the ARVO Resolution on the Use of Animals in Research. Cimetidine, dimaprit hydrochloride, nordimaprit dihydrochloride, 4-methylhistamine dihydrochloride, histamine dihydrochloride, homodimaprit, and thiourea were 0.21 M unless so stated. Their pH's were 5.2, except for thiourea, which was 7.3. Solutions were made up every 2-3 days. Impromidine was supplied as a 3 mM, pH 4.5 solution by the manufacturer; it was used as such and also at dilutions of 0.3, 0.6, 1.2, and 1.8 mM. Tiotidine solubility was limited, and a 0.01 M, pH 5.6 solution was used, as it was close to saturation. A proparacaine HCl, 0.5% commercial preparation provided local anesthesia and was used at times to promote corneal penetration of the other drugs. When proparacaine was administered, it was always the first eye drop given. When both an H₂-antagonist and an H₂-agonist were administered, the former was given before the latter. Drugs were administered as 50 μl eye drops or injected subconjunctivally or intravitreally in 50 μl volumes. Eye drops were given by holding the lids away from the globe and opposed for several seconds to prevent overflow. Topical applications of different drugs were separated by at least 5 min.

The eosinophil uveitis was evaluated by observing for miosis, iris depigmentation, and corneal clouding. Histologically, the response to all drug treatments was evaluated using light microscopy with hematoxylin-eosin stains and Luna eosinophil granule stain. 16 Differential white cell counts of Giemsa stained peripheral blood smears were performed before and at the conclusion of all drug treatments. Rabbits were killed in a CO₂ gas tank or by intravenous pentobarbital.

Intraocular Pressure

Five rabbits had their intraocular pressures measured 4 times a day, while the right eye received: a) days 1-7, dimaprit HCl, 0.1 M (2.5%), twice a day; b) days 7-14, dimaprit HCl, 0.21 M (5%), twice a day; and c) days 14-25, cimetidine and dimaprit HCl, both 0.21 M and both twice a day. The left eyes received NaCl eye drops of matching pH and tonicity. Intraocular pressures were measured using proparacaine topical anesthesia and a pneumotonometer.

Eosinophil Chemotaxis

Eye drops were applied three times a day for 12 or more consecutive days in the various combinations shown in Tables 1 and 2. One eye would receive a drug or drug combination without proparacaine, and the contralateral eye would receive the same drug or drug combination with proparacaine. Due to the systemic toxicity of impromidine, only one eye of each rabbit was treated when this drug was used. If uveitis occurred, the eye drops were continued for an additional 48 hr to intensify the reaction, and then the rabbit was killed. Each of three albino rabbits received proparacaine + cimetidine + dimaprit to the right eye and proparacaine + dimaprit to the left eye, three times a day until 48 hr after at least one eye developed an inflammatory response. The rabbit was then killed.

Table 1. Topical drugs producing anterior segment eosinophil chemotaxis

<table>
<thead>
<tr>
<th>H₂-antagonist activity</th>
<th>Number Eyes Treated</th>
<th>Number Eyes Responding</th>
<th>Response Onset (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proparacaine + cimetidine</td>
<td>5</td>
<td>5</td>
<td>2-3</td>
</tr>
<tr>
<td>Proparacaine + cimetidine + nordimaprit</td>
<td>5</td>
<td>5</td>
<td>2-3</td>
</tr>
<tr>
<td>H₂-antagonist and H₂-agonist activities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tiotidine + dimaprit</td>
<td>4</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Proparacaine + cimetidine + dimaprit</td>
<td>6</td>
<td>6</td>
<td>3-11</td>
</tr>
<tr>
<td>Proparacaine + tiotidine + dimaprit</td>
<td>4</td>
<td>4</td>
<td>3-4</td>
</tr>
<tr>
<td>No H₂ activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nordimaprit</td>
<td>5</td>
<td>5</td>
<td>5-10</td>
</tr>
<tr>
<td>Proparacaine + nordimaprit</td>
<td>5</td>
<td>5</td>
<td>2-3</td>
</tr>
</tbody>
</table>
Time Course Study

Ten rabbits were treated bilaterally with proparacaine + cimetidine + dimaprit eye drops three times a day. Two rabbits each were killed after 1, 2, and 4 days of treatment. The four remaining rabbits were treated until eosinophil uveitis occurred in at least one eye, and then the eye drops were discontinued. Four and eight days later, two rabbits each were killed. The eyes of all rabbits were removed for histological examination.

Subconjunctival and Intravitreal Drug Injection

Five rabbits received daily subconjunctival injections for 14 consecutive days. After applying one eye drop of proparacaine to each eye, a temporal subconjunctival injection of dimaprit was administered bilaterally, and a nasal subconjunctival injection of cimetidine was administered to the right eye. On the fifteenth day, the animals were killed, and their eyes and blood smears were examined microscopically.

Intravitreal drugs were injected once in both eyes of eight rabbits anesthetized with pentobarbital. Both eyes received the same drugs using 25-gauge needles inserted through the pars plana: rabbit 1, cimetidine; rabbits 2 and 3, dimaprit HCl; rabbit 4, impromidine; rabbit 5, tiotidine; rabbit 6, dimaprit + cimetidine in the same 50 μl injection; and rabbits 7 and 8, dimaprit and cimetidine in two separate, but simultaneous, 50 μl injections to each eye.

In Vitro Depigmentation

Iris-ciliary bodies were removed from rabbits immediately after death. Each tissue was divided into four approximately equal-sized parts. One of the four parts was incubated in each of the following for 3 hr at 37°C: dimaprit HCl, 5%; cimetidine, 5%; dimaprit, 2.5%-cimetidine, 2.5%; or saline, 1.2%. Tissues were then observed grossly and by light microscopy for disruption of the pigment-containing cells.

Results

In the preliminary studies, dimaprit HCl, 2.5% and 5.0%, had no effect on intraocular pressure in pigmented rabbits. The combination of dimaprit HCl 5% and cimetidine 5.3% also had no effect on intraocular pressure, but did produce miosis followed within 24 hr by a variable amount of iris depigmentation and corneal clouding (Fig. 2). Light microscopy of hematoxylin- and eosin-stained tissue slides revealed edema and marked eosinophil infiltration of the cornea, iris, and ciliary body (Fig. 3). The aqueous humor was filled with eosinophils (Fig. 4). Luna-stained tissue slides confirmed that the cells were eosinophils (Fig. 5). The conjunctiva, although edematous and hyperemic, was not infiltrated with eosinophils. There was marked loss of iris stromal pigment. The iris pigment epithelium was affected, but less so. The ciliary bodies were less depigmented than the irides, but also demonstrated some loss of pigment epithelium as well as disruption of the non-pigmented epithelium. This in vivo pigment cell disruption could not be reproduced in vitro by incubating the iris and ciliary body for 3 hr in solutions of dimaprit, cimetidine, or a cimetidine-dimaprit combination.

Pigmented rabbits were given eye drops of H2-agonists and antagonists, alone or in combination (Tables 1, 2). Dimaprit alone or with proparacaine did not produce a response. Nor did other H2-agonists and antagonists, alone or in various combinations. Only when an H2-agonist (cimetidine or tiotidine) was administered with the H2-agonist dimaprit was eosinophil...
Fig. 2. Eosinophil anterior uveitis. A, Miosis, iris depigmentation, and corneal clouding produced by proparacaine + cimetidine + dimaprit eye drops three times a day. B, Contralateral eye which received proparacaine + cimetidine. Photographs were obtained 3 days after discontinuing the drops to allow sufficient corneal clearing for visualization of the iris of the responding eye.

Proparacaine produced. Prior administration or proparacaine appeared to facilitate the development of eosinophil uveitis from an H2-antagonist + dimaprit combination, e.g., only one of four eyes developed eosinophil uveitis when proparacaine was not used, while nine of nine eyes developed eosinophil uveitis when proparacaine was used.

Time course studies demonstrated that the marked eosinophil infiltration of the anterior segment was not present until the miosis occurred that heralded the onset of the intraocular inflammation. At the time of the onset of miosis, the inflammatory cells were approximately half eosinophils and half neutrophils. Shortly thereafter, the response intensified and became almost purely eosinophilic. When treatment was discontinued after eliciting the full response, the eosinophils disappeared over the next few days. By the fourth day, few could be identified; grossly, the corneal clouding had nearly resolved, and the iris remained depigmented. At no time did the peripheral blood smears demonstrate eosinophilia. Differential cell counts for eosinophils remained at 2-3% before, during, and after treatment.

Albino rabbits responded in a similar manner, except that iris depigmentation was not possible. The right eyes, treated with proparacaine + cimetidine + dimaprit, developed eosinophil anterior uveitis in 3-8 days, while the left eyes, treated with proparacaine + dimaprit, did not. There was no eosinophilia of the peripheral blood.

Topical administration of the two dimaprit homologues without H2-receptor activity produced differing results. Nordimaprit produced an intense eosinophil uveitis (Table 1), whether administered alone, with proparacaine, or with an H2-antagonist. However, homodimaprit was without effect (Table 2). The rapidity of the onset of the response to nordimaprit was facilitated by the prior administration of proparacaine or an H2-antagonist. Nordimaprit, like dimaprit, produced severe corneal opacification. There were several differences in the reactions from these two drugs. First, the eosinophil-filled aqueous humor produced by the topical H2-antagonist + dimaprit treatments did not contain fibrinous material, while all combinations of topical nordimaprit produced a marked fibrinoid response (Fig. 6). Second, nordimaprit did not cause iris depigmentation by gross inspection. However, on microscopic examination, no intact iris stromal melanocytes could be identified in nordimaprit-treated eyes, although the iris pigment epithelial melanocytes were intact. Third, nordimaprit produced either a transient miosis, lasting less than 24 hr, followed by a marked mydriasis, or directly produced a marked mydriasis.

Subconjunctival dimaprit, with or without cimetidine, failed to produce a marked eosinophil infiltration. Beginning on the third to fifth day of treatment, there was a localized area of iris depigmentation superiorly in both eyes of all five rabbits. However, this did not progress, nor did corneal opacification or miosis occur. Histologic examination revealed some mild fibrin deposition in the anterior chambers with an occasional eosinophil present.

The single intravitreal injections of the H2-antagonists, cimetidine and tiotidine, alone or in combination with the H2-agonist, dimaprit, failed to produce an inflammatory reaction of any type. Eyes were examined histologically 4 days after drug administration, except for rabbits 3 and 8 which were examined 8 days after
Fig. 3. Microscopic appearance of the edema and eosinophil infiltration produced by proparacaine + cimetidine + dimaprit eye drops in the cornea (top, left) and iris (top, right). (Hematoxylin-eosin, X160).

Fig. 4. Microscopic appearance of eosinophil accumulation in aqueous humor in response to topical proparacaine + cimetidine + dimaprit. (second row, left) Adjacent to cornea, (second row, right) adjacent to iris. (Hematoxylin-eosin, X80).

Fig. 5. Microscopic appearance of eosinophils in aqueous humor in response to topical proparacaine + cimetidine + dimaprit. The cells are intact and do not appear to have degranulated. (third row, left) Hematoxylin-eosin stain. (third row, right) Luna stain, which gives eosinophil granules a rust brown appearance. (X400).

Fig. 6. (bottom) Microscopic appearance of the fibrinoid material and eosinophils in the aqueous humor in response to topical nordimaprit. (Hematoxylin-eosin, X160).
treatment. When the H2-agonist, dimaprit, was injected alone, it produced a marked posterior uveitis, vitritis, and retinitis in three of four eyes within 48 hr. This response consisted primarily of neutrophil cells with a relative eosinophilia of approximately 15–30%.

Impromidine, 3 mM was lethal within 48 hr when applied topically or injected intravitreally. Preceding each impromidine eye drop (an H2-agonist) with a cimetidine eye drop (an H2-antagonist) did not prevent or delay this lethality. The eyes were not inflamed, either by gross or histological examination. In the 12 hr preceding death, the rabbits appeared normal, but exhibited labored breathing. Autopsy revealed bilateral massive pulmonary edema and marked brain edema with herniation of the cerebellum. Lower concentrations of impromidine, 0.3–1.8 mM, were not as toxic, and were successfully given for a 2-week period as eye drops (Table 2) with and without cimetidine. These eyes did not exhibit ocular inflammation or eosinophil infiltration by gross inspection or on histologic examination.

Discussion

The eosinophil chemotactic effect of topical dimaprit appeared to be augmented by two factors. First was the presence of an H2-antagonist which, presumably, neutralized dimaprit’s H2-agonist activity. For example, dimaprit and proparacaine + dimaprit did not elicit a response, while tiotidine + dimaprit, proparacaine + cimetidine + dimaprit, and proparacaine + tiotidine + dimaprit produced a marked, and essentially pure, eosinophil inflammation. Second was the use of topical proparacaine which, presumably, enhanced corneal penetration of the H2-antagonist + dimaprit. Thus, (1) cimetidine + dimaprit was ineffective, while proparacaine + cimetidine + dimaprit produced a marked response, (2) only one of four eyes responded to tiotidine + dimaprit while all four eyes responded to proparacaine + tiotidine + dimaprit, and (3) the eye receiving proparacaine always responded before the contralateral eye that did not receive proparacaine. This last was true for nordimaprit as well as H2-antagonist + dimaprit combinations.

Based on the effectiveness of proparacaine, it was assumed that subconjunctival and intravitreal injections, by bypassing the corneal epithelium barrier, would produce eosinophil chemotaxis more rapidly, and with less effort than using the same drugs as eye drops. That this did not occur was unexpected. Two weeks of daily subconjunctival injections of dimaprit or cimetidine + dimaprit, each preceded by a topical drop of proparacaine anesthesia, failed to produce a significant response in any of the eyes treated. Intravitreal cimetidine + dimaprit was also without effect. Intravitreal dimaprit alone did produce a marked response in three of four eyes, but the many eosinophils were outnumbered by even larger accumulations of neutrophils. The reasons for these differences between topical and injected dimaprit were not clear. The conjunctiva did not accumulate eosinophils during topical or subconjunctival drug application. Thus, the eosinophil response differed from a naturally occurring immediate hypersensitivity reaction, and suggested that corneal or intraocular drug metabolism played a role.

Dimaprit was S-[4-N,N-dimethylaminopropyl]-isothiourea (Fig. 1). Nordimaprit had one less carbon atom and lacked activity at histamine receptors. Yet, nordimaprit produced a marked eosinophil chemotaxis under all conditions of topical administration; i.e., alone, with H2-antagonists, and with proparacaine. Dimaprit’s structural similarity to nordimaprit appeared to be the cause of its chemotactic effect, because other H2-agonists, e.g., impromidine, 4-methylhistamine, and histamine, whether given alone, with an H2-antagonist, or with proparacaine, were ineffective. Homodimaprit (S-[4-N,N-dimethylaminobutyl]isothiourea) and thiourea were also ineffective. The mechanism by which dimaprit and nordimaprit exerted their actions was unknown. However, both of these drugs have been shown to affect another white cell, the lymphocyte, in a manner suggesting that histamine receptors were not involved. Dimaprit profoundly inhibited mitogen-induced lymphocyte activation,17–19 and its action was not diminished by H2-receptor antagonists. Further, nordimaprit was even more effective than dimaprit. Perhaps, in the present studies, dimaprit and nordimaprit produced eosinophil chemotaxis indirectly. N-methyl-p-methoxyphenethylamine formaldehyde condensation product caused rabbit conjunctival mast cells to degranulate and produced a localized eosinophilia.20 While many mast cells are found in the conjunctiva, a smaller number is found intraocular.21,22 Mast cells contain an eosinophil chemotactic factor. If dimaprit or nordimaprit caused intraocular mast cells to degranulate, an eosinophil migration could result.

The inflammatory responses evoked by topical nordimaprit and H2-antagonist + dimaprit combinations were severe. The anterior and posterior chambers had the histologic appearance of an eosinophil abscess, and eosinophils infiltrated the surrounding structures. Both albino and pigmented rabbits responded similarly. In the past, rabbit neutrophils have been confused with eosinophils, because the former have a cytoplasmic granule that stains red. Hence, these neutrophils have been termed "pseudo-eosinophils."23 However, with this in mind, it was relatively easy to distinguish the larger eosinophils and their larger red granules. In addition, the identity of the eosinophils was confirmed.
using Luna stain, which is specific for eosinophil granules.

While topical H$_2$-antagonist + dimaprit combinations and nordimaprit produced similar inflammatory responses with regard to cell type and corneal opacification, there were several differences. One was that miosis was a conspicuous component of the H$_2$-antagonist + dimaprit reactions, while nordimaprit-treated rabbits developed a marked mydriasis. The second difference was that H$_2$-antagonists + dimaprit caused more disruption of iris pigment epithelium cells than did nordimaprit. This pigment cell disruption did not appear to be a direct result of drug-pigment cell interaction, because incubating the iris and ciliary body with dimaprit or cimetidine + dimaprit did not produce it. Third, nordimaprit elicited a marked fibrinoid response in the aqueous humor. The reasons for these differences remain to be elucidated. Perhaps topical H$_2$-antagonists + dimaprit released substances from eosinophils that nordimaprit did not. For example, eosinophils can release anti-inflammatory agents, such as histaminase and a substance that inhibits histamine release. Eosinophil granules also contain a basic polypeptide that stimulates histamine release from basophils and enhances inflammation. This basic polypeptide comprises more than 50% of the granule protein in guinea pigs, and has a high arginine content. Dimaprit bears a closer structural relationship to arginine than does nordimaprit. Histamine released from basophils not only could elicit an inflammatory reaction itself, but it could react with H$_2$-receptor sites on eosinophils to cause them to release toxic superoxide anions as well. The resultant inflammation could produce the miosis and pigment-cell disruption found following the application of H$_2$-antagonist + dimaprit combinations. Impromidine 3 mM was uniformly lethal within 48 hr. Prior administration of the H$_2$-antagonist cimetidine was of no prophylactic value. Rabbits appeared to suffer cardiovascular collapse with marked pulmonary and brain edema. Such a picture was consistent with so-called histamine shock.

In the past, studies of eosinophil chemotaxis and degranulation have been hampered by the difficulty in obtaining large numbers of intact, i.e., non-degranulated and non-fragmented, cells. One source has been heparinized blood, which must be washed, centrifuged, and passed through density gradients to achieve relatively small and variably pure yields, i.e., 64–98% of the cells are eosinophils. A second source has been peritoneal cavity washings from animals that have received horse serum intra-peritoneal injections for 4–6 weeks. These eosinophils also require centrifugation for purification. Topical proparacaine + nordimaprit or proparacaine + tiotidine + dimaprit consistently and selectively drew large numbers of apparently intact eosinophils into the aqueous humor of rabbit eyes in 2–4 days. These combinations may offer a new, and possibly superior, method for obtaining eosinophils.

In summary, topical nordimaprit and dimaprit produced eosinophil chemotaxis into the eye. The eosinophils in the anterior and posterior chambers appeared intact, i.e., they had not degranulated. The common structural characteristics of dimaprit and nordimaprit may lead to new insights into the mechanism of eosinophil chemotaxis. In addition, these drugs may allow large numbers of intact eosinophils to be harvested from the aqueous humor for related studies.

**Key words:** eosinophil, histamine, chemotaxis, dimaprit, nordimaprit

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**References**