Effects of Certain Antiallergic Drugs on Experimental Conjunctivitis in Guinea Pigs

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Effects of certain antiallergic drugs on experimental conjunctivitis were studied with guinea pigs. Chlorpheniramine, ketotifen and levocabastine were effective in inhibiting histamine- and antigen-induced conjunctivitis in guinea pigs. By contrast, amlexanox was only effective in inhibiting antigen-induced conjunctivitis. Topical application of antigen released 46.5 ± 3.8% of histamine from the conjunctiva in sensitized guinea pigs. Both levocabastine and amlexanox were effective in inhibiting conjunctival histamine release induced by antigen application. Lacrimal histamine contents were also increased after antigen challenge. The increase in the histamine content of tears was inhibited by pretreatment with levocabastine and amlexanox, but no significant effect was observed with chlorpheniramine and ketotifen. From these findings, it is concluded that certain antiallergic drugs, but not amlexanox, exhibited potent inhibition on experimental conjunctivitis in guinea pigs. In addition, it has been established that measurement of histamine in the conjunctiva and tears as well as observation of conjunctivitis syndromes are useful for evaluating the effectiveness of antiallergic drugs on various kinds of allergic conjunctivitis in clinical situations.

Key words allergic conjunctivitis; chlorpheniramine; ketotifen; histamine release; tear histamine

H₁-Blocking agents, chlorpheniramine and ketotifen, are widely used for the therapy of allergic conjunctivitis in clinical situations. Therefore, there is a considerable literature about the effectiveness of H₁-blockers in patients with allergic conjunctivitis, but little work has been published on the effects of antiallergic drugs in experimental allergic conjunctivitis. We have reported that topical application of histamine and antigen to the eyes induced severe and long-lasting conjunctivitis in guinea pigs. In addition, it has been found that histamine release from the conjunctiva occurs following antigen-antibody reaction in guinea pigs. On the other hand, it has been reported that the level of histamine in tears is increased in allergic conjunctival disease. However, we have very little information on whether histamine levels in tears in experimental animals are increased or not by antigen-antibody reaction.

The purpose of the present study was to evaluate the effect of certain antiallergic agents on experimental conjunctivitis induced by histamine and antigen. The determination of histamine in the conjunctiva and tears after antigen challenge in sensitized guinea pigs and the effect of antiallergic drugs on the histamine content of both were also elucidated.

MATERIALS AND METHODS

Animals Male guinea pigs (400—500 g, Nippon SLC) were used. The animals were housed in an air-conditioned room at 25 ± 1°C with 55 ± 5% humidity. The animals were given food and water ad libitum. Five to 7 animals were used in each group.

Drugs The drugs used were chlorpheniramine hydrochloride (Wako, Osaka), ketotifen fumarate (Sankyo, Tokyo), levocabastine hydrochloride (ophthalmic solution, Janssen-Kyowa, Tokyo) and amlexanox (Eli Lilly, ophthalmic solution, Senju, Osaka). Chlorpheniramine hydrochloride and ketotifen fumarate were dissolved in saline. All drugs used in the experiment were applied topically.

Histamine or Antigen-Induced Conjunctivitis Fifteen min after drug application, histamine solution (25 μl) was instilled in to the bilateral conjunctivae, and the degree of inflammation was determined by a scoring system. In most cases, the vehicle was instilled in to the right eye and the test drug was applied to the left. In the case of antigen-induced conjunctivitis, the animals were immunized with egg albumin (1 mg) as an antigen according to the method of Mota. Two weeks after immunization, 25 μl egg albumin solution (20 mg/ml) was applied to the eyes 15 min after drug administration. The antibody titer of the antisera was 1:256 as determined by 7-d homologous PCA in guinea pigs.

The Severity of Inflammation The scoring system used to classify the severity of conjunctivitis was as follows: 0 = no symptoms; 1 = slight hyperemia; 2 = severe hyperemia; 3 = severe hyperemia and slight edema; and 4 = severe edema.

Histamine Contents in the Conjunctiva and Tears Fifteen min after antigen application, the guinea pig conjunctiva was carefully excised, weighed and washed twice with saline. The tissues were homogenized with 0.4 N perchloric acid and placed in an ice-bath for 1 h. The percentage of histamine release was calculated as described previously. In the case of tears, 15 min after antigen application, 50 μl saline was applied to both eyes. These procedures were repeated 3 times and 200 μl sample was carefully collected from the both eyes. The sample and the same quantity of 0.4 N perchloric acid were mixed. After centrifugation at 10000 × g for 10 min at 4°C, the histamine content of the supernatant was determined by high performance liquid chromatography using a fluorometric detector (CCP & 8010 Series, Tosoh, Tokyo, Japan).

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Fig. 1. Sequential Changes in the Severity of Antigen- and Histamine-Induced Conjunctivitis in Guinea Pigs

Each point indicates mean ± S.E.M. (n = 5). Antigen-induced: ○, control; ●, 1 μg/μl; ▲, 5 μg/μl; ■, 10 μg/μl; △, 20 μg/μl. Histamine-induced: ○, control; ●, 1.5 ng/μl; ▲, 7.5 ng/μl; ■, 7.5 ng/μl.

Fig. 2. Effects of Certain Antiallergic Drugs on Antigen-Induced Conjunctivitis in Guinea Pigs

Each point indicates mean ± S.E.M. (n = 5). *, **, Significantly different from the control at p < 0.05 and p < 0.01, respectively by the Mann-Whitney U-test.

Statistical Analysis The values were expressed as mean ± S.E.M. The Mann-Whitney U-test and ANOVA with Dunnett’s test were used to calculate the statistical difference between the means of the test and control groups. ID₅₀ (95% confidence limit) values at 30 min after drug administration (the time at which a maximal effect was exhibited) was calculated according by the probit method.

RESULTS

Antigen- and Histamine-Induced Conjunctivitis Figure 1 shows the sequential changes in the severity of antigen- and histamine-induced conjunctivitis in guinea pigs. In both antigen- and histamine-induced conjunctivitis, dose-related inflammation was observed. To test the drug effect, 20 μg/μl antigen solution and 750 ng/μl histamine solution were used in antigen- and histamine-induced conjunctivitis, respectively.

Effect on Antigen-Induced Conjunctivitis in Guinea Pigs Results are shown in Fig. 2 and Table 1.

Table 1. ID₅₀ Values of Certain Antiallergic Drugs on Antigen- and Histamine-Induced Conjunctivitis in Guinea Pigs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>ID₅₀, ng/μl (95% confidence limits)</th>
<th>Antigen</th>
<th>Histamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpheniramine</td>
<td>18.4 (0.90—39.3) (3.71—36.5)</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>Ketotifen</td>
<td>4.12 (0.19—16.0) (0.03—11.4)</td>
<td>2.82</td>
<td></td>
</tr>
<tr>
<td>Levocabastine</td>
<td>4.14 (0.14—17.2) (3.93—12.1)</td>
<td>7.67</td>
<td></td>
</tr>
<tr>
<td>Amlexanox</td>
<td>2767 (1230—22400) &gt; 5000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chlorpheniramine suppressed antigen-induced conjunctivitis in a dose-dependent manner. A significant inhibition was observed after application of 50 and 100 ng/μl and the IC₅₀ value was 18.4 (0.90—39.3) ng/μl. Ketotifen and levocabastine were also effective in suppressing antigen-induced conjunctivitis. The potencies of two drugs were almost the same. The IC₅₀ values of ketotifen and
levocabastine were 4.12 (0.19—16.0) ng/μl and 4.14 (0.14—17.2) ng/μl, respectively. The effect of amlexanox was extremely weak, but a significant inhibition was noted at concentrations of 2500 and 5000 ng/μl. The IC₅₀ value of amlexanox was 2767 (1230—22400) ng/μl.

Effect on Histamine-Induced Conjunctivitis in Guinea Pigs Chlorpheniramine showed a dose-related effect on histamine-induced conjunctivitis, and a significant effect was observed at 10 and 100 ng/μl. The IC₅₀ of chlorpheniramine was 11.2 (3.71—36.5) ng/μl. Both ketotifen and levocabastine also caused an inhibition of histamine-induced conjunctivitis, and their IC₅₀ values were 2.82 (0.03—11.4) ng/μl and 7.67 (3.93—12.1) ng/μl, respectively. Amlexanox exhibited no inhibitory effect on histamine-induced conjunctivitis even at a concentration of 5000 ng/μl (Fig. 3 and Table 1).

Effect on Histamine Release from the Conjunctiva The effects of antiallergic drugs on antigen-induced histamine release from the conjunctiva is shown in Fig. 4. Topical application of antigen released 46.5 ± 3.8% (n = 5) of histamine from the conjunctiva in sensitized guinea pigs. Both levocabastine (100 ng/μl) and amlexanox (2500 ng/μl) caused a significant inhibition of histamine release from guinea pig conjunctiva induced by antigen-antibody reaction. No marked effect was observed with chlorpheniramine (100 ng/μl) and ketotifen (100 ng/μl).

Effect on the Histamine Content of Tears The histamine content of tears was increased almost 5 times (post: 8.6 ± 0.8 ng/ml) after instillation of the antigen compared with that before antigen challenge (pre: 1.7 ± 0.4 ng/ml). Both chlorpheniramine (100 ng/μl) and ketotifen (100 ng/μl) had no effect on an increase in the histamine content of tears induced by antigen challenge. However, levocabastine (100 ng/μl) and amlexanox (2500 ng/μl) produced a significant decrease in the histamine content compared with the control group (post).

DISCUSSION

In the present study, it was found that chlorpheniramine, ketotifen and levocabastine were effective in inhibiting both histamine- and antigen-induced conjunctivitis in guinea pigs. Previously, we proposed that in the conjunctiva, histamine plays a dominant role in the process.
Fig. 5. Effects of Certain Antiallergic Drugs on an Increase in the Histamine Content of Tears in Guinea Pigs

Each point indicates mean ± S.E.M. (n = 5–7). * Significantly different from the control at p < 0.05 by ANOVA with Dunnett's test.

leading to allergic inflammation. However, as shown in Table 1, some differences were noted in the inhibition induced by certain antiallergic drugs on histamine-or antigen-induced conjunctivitis; i.e. chlorpheniramine and ketotifen exhibited a slightly more potent inhibition of conjunctivitis induced by histamine than that induced by antigen. These differences can be accounted for by the fact that some putative chemical mediators, such as platelet activating factor, leukotriene C₄ or substance P also participate in the antigen-antibody reaction.

On the other hand, ketotifen exhibited a more potent inhibition than chlorpheniramine and levocabastine on histamine-induced conjunctivitis. These findings are supported by the fact that ketotifen has a more potent antihistaminic activity than chlorpheniramine and levocabastine in the guinea pig ileum. As shown in Fig. 5, levocabastine was effective in inhibiting histamine release from guinea pig conjunctiva. On the other hand, chlorpheniramine and ketotifen had no effect. It has been reported that levocabastine causes an inhibition of histamine release from guinea pig lung fragments at concentrations of 10 and 100 μM. Therefore, it seems likely that the potent effect of levocabastine on allergic conjunctivitis may be due to inhibition of histamine release. As shown in Table 1, amlexanox was effective in only antigen-induced conjunctivitis. Amlexanox has also been reported to inhibit histamine release from rat peritoneal mast cells. In addition, amlexanox has been found to suppress leukotriene generation by inhibiting lipooxygenase.

In the present study, it was also found that the histamine content of tears increased after antigen challenge. In non-sensitized animals, the histamine content of tears was 1.7 ± 0.4 ng/ml, and it was increased 5 times (8.6 ± 0.8 ng/ml) by antigen challenge. The histamine content of tears was reported to be 2.2 ± 1.6 ng/ml in healthy subjects, 4.5 ± 2.3 ng/ml in patients with pollen conjunctivitis, 4.3 ± 3.9 ng/ml in those with chronic allergic conjunctivitis and 18.8 ± 16.6 ng/ml in those with vernal conjunctivitis. These findings were very similar to that seen in the present experimental conjunctival models. We have proposed that the main chemical mediator of allergic conjunctivitis induced by the antigen-antibody reaction in guinea pigs may be histamine. The present findings also confirm our proposal. Akagi et al. reported that mast cells are present in the conjunctival tissue of guinea pigs.

In conclusion, certain antiallergic drugs, but not amlexanox, exhibit potent inhibition of experimental conjunctivitis in guinea pigs. In addition, the measurement of the histamine concentrations the conjunctiva and tears as well as observation of conjunctivitis syndromes are useful for evaluating the effectiveness of antiallergic drugs on various kinds of allergic conjunctivitis in clinical situations.

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