



Research article

Optimization of a histamine-induced allergic conjunctivitis model in Guinea pigs

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ABSTRACT

Allergic conjunctivitis is one of the most common immune diseases in the field of ophthalmology. The number of patients suffering from allergic conjunctivitis has been increasing, and there is still a strong need for development of therapeutic agents for this disease. In drug development, the utmost important point to improve the success probability is to accurately single out good compounds in the early stage of drug development. Therefore, drug efficacy evaluations in the nonclinical stage should be conducted with high reliability and accuracy. However, there are no literatures investigating the preparation and evaluation methods of animal models of conjunctivitis in details nor the standardized criteria.

In this study, we verified the reproducibility of an animal model in the previous report and made improvements in test methods focusing on a guinea pig model of histamine-induced allergic conjunctivitis. Furthermore, the drug efficacy evaluation was conducted using a commercially available antihistamine drug, levocabastine hydrochloride, to judge the suitability of the improved model.

As a result, the dose level of histamine needed to be increased to use the existing model for drug efficacy evaluation, but allergic-like symptoms were induced very easily and stably in this model. For observations of symptoms of conjunctivitis, we eliminated ambiguity of evaluation by adopting the Draize scale and ensured a higher objectivity on the evaluation method. The drug efficacy evaluation of levocabastine hydrochloride in the prepared model revealed that drug efficacy of the antihistamine drug was captured according to the standardized test method and highly-reproducible results were obtained.

1. Introduction

Allergic conjunctivitis is one of the most common immune diseases in the field of ophthalmology. It is sometimes considered to be less significant since it rarely affects vision. However, symptoms of allergic conjunctivitis including itching, redness, and swelling have a great impact on improving the individuals' quality-of-life (QOL) and efficiency, and the number of patients suffering from allergic conjunctivitis has been increasing over the world (Dupuis, Prokopich, Hynes, & Kim, 2020; Jalbert & Golebiowski, 2015). Accordingly, there is still a strong need for development of more economical and effective therapeutic agents for this disease.

In drug development, it is very important to use appropriate models for drug evaluation since patients can receive benefits of drug therapy only after an enormous amount of time and resources were devoted to its

development. New drug development usually begins with screening for candidate compounds and undergoes a number of nonclinical studies. Then, only those considered safe and effective for humans can proceed to clinical trials, which typically takes years to reach. In recent years, the need to improve the reproducibility of nonclinical studies serving as a base for drug development has been pointed out since if the results of nonclinical studies are not reproducible at the clinical trial stage, all the time and resources spent up to that point will be wasted (Howells, Sena, & Macleod, 2014; Richter, 2017). Besides, no matter how safe and useful a compound is, it will be dropped out in the early phase of development and never be available for the patient as a drug as long as an inappropriate evaluation method is chosen and resulted in erroneous evaluation. That is, a key point for improving the success probability of clinical development is how to accurately single out good compounds at the screening stage of drug development, and which is why high reliability

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and accuracy are required in evaluation of drug efficacy in the early stage of development. Furthermore, improving evaluation accuracy can contribute to controlling enormous drug development costs. It is considered to be of great significance not only in Japan, where the burden of medical expenses is a national issue, but also from the perspective of international drug development.

In the development of therapeutic agents for allergic conjunctivitis, several drug efficacy evaluation methods are known. The first is a method of passively sensitizing mast cells in the conjunctiva using antisera against antigens (Mehta, Calonge, Levene, Bloch, & Allansmith, 1990; Stock, Dwyer, Jones, & Waters, 1985). In this method, mast cell degranulation is caused by reacting the antigen with IgG bound to mast cells. Degranulation of mast cells results in the release of chemical mediators such as histamine and prostaglandins, which causes conjunctivitis symptoms such as hyperemia, edema, and itching. The second is a method of actively sensitizing the antigen by treating it with an adjuvant (Kato et al., 2004; Shii, Oda, Shinomiya, Katsuta, & Nakamura, 2009). In this method, IgG is produced from B cells and binds to mast cells, which induces mast cell degranulation and various mediators cause allergy-like symptoms. However, the symptoms induced by these methods are influenced by the function and state of immune cells in each individual animal. Consequently, the data tends to vary from individual to individual, which often makes it difficult to interpret the data. Considering the time and effort required and the variability of data, these methods may be suitable for the mechanism analysis and evaluation of allergies, but are considered to be unsuitable for the evaluation of many compounds. The third is a method using histamine, a chemical mediator (Fukushima & Tomita, 2009; Kamei, Izushi, & Tasaka, 1991) which is greatly involved in allergic reactions. It is known that histamine is released from mast cells in vivo and causes vascular dilatation, increased vascular permeability, and itching via histamine receptors (Elieh Ali Komi, Rambasek, & Bielory, 2018; Ohbayashi, Manzouri, Morohoshi, Fukuda, & Ono, 2010). In an animal model for allergic conjunctivitis, pseudo-conjunctivitis symptoms can be caused by directly exposing the conjunctiva to histamine. This method is not significantly affected by immune response of the animals, and therefore, unlike the method of passive or active sensitization, it is considered that the variation owing to the individual differences can be mostly eliminated. Furthermore, since conjunctivitis symptoms are induced simply by the instillation of histamine onto the conjunctiva, the operating procedures are likely to be easily standardized in a facility with a certain level of technique, and standardization of the operating procedures directly leads to highly accurate and reproducible results. The fact that the model is not affected by individual animals or experimenters indicates that the evaluation of multiple candidate compounds can be carried out according to certain criteria. However, no studies have been examined from this perspective.

In this study, we investigated the usefulness of the existing model, the possibility of standardization of operating procedures, and obtainability of the reproducible results presuming that a guinea-pig model of histamine-induced conjunctivitis is useful for evaluation of the early stage of therapeutic drug development for conjunctivitis. We also made improvements in the test methods to prepare an animal model more suitable for drug development.

2. Materials and methods

This study was conducted in compliance with the Japanese “Law for the Humane Treatment and Management of Animals” and the “Guidance for Animal Care and Use” of Ina Research Inc. and in accordance with the protocol reviewed by the Institutional Animal Care and Use Committee (IACUC) of Ina Research Inc., a facility which is fully accredited by AAALAC International.

2.1. Animals and housing conditions

Slc:Hartley guinea pigs (males, 6–7 weeks) were purchased from

Japan SLC, Inc. (Shizuoka, Japan). Up to 3 animals/cage were housed in stainless steel wire mesh cages (61 W × 22D × 21H cm) with wood blocks for gnawing as enrichment. The temperature and humidity in the animal room were maintained between 21.0 °C and 25.0 °C and between 40.0% and 70.0%, respectively. Lighting was set on a 12-h light-dark cycle (fluorescent lighting from 7:00 to 19:00). The animals were allowed free access to the pelleted feed (LRC4, Oriental Yeast Co., Ltd.) and drinking water via an automatic watering system.

2.2. Verification of induction conditions for conjunctivitis

Histamine dihydrochloride (Fujifilm Wako Pure Chemical Corporation) was dissolved in phosphate-buffered saline (hereafter referred to as “PBS”, pH = 7.4) before use. In order to verify the optimal induction conditions for evaluation, the initial histamine concentration and dose level were set at 100 ng/μL with a dose volume of 25 μL/eye with reference to the previous report (Kamei et al., 1991). For further verification, 3 additional models were prepared at histamine concentrations of 1000 ng/μL (25 μL/eye), 2000 ng/μL (12.5 μL/eye), and 2000 ng/μL (25 μL/eye). For each concentration, the histamine solution was instilled once/eye into the right and left conjunctival sacs of each animal to induce conjunctivitis. The conditions of each animal model were evaluated according to the conjunctivitis scores, and the optimum histamine concentration and dose level were determined. The conjunctivitis scores were modified from past reports (Fukushima, Nabe, Mizutani, Nakata, & Kohno, 2003; Kamei et al., 1991; Kyei et al., 2015) and set to 6 levels of severity from 0 to 5 (0: no symptoms, 1: minimal, 2: mild, 3: moderate, 4: marked, and 5: severe).

2.3. Verification of modified animal model of conjunctivitis

Based on the results of the verification of induction conditions, the optimum histamine concentration and dose level were set at 2000 ng/μL (12.5 μL/eye). In order to determine the suitability of the modified model of conjunctivitis for efficacy evaluation in drug development, a drug efficacy evaluation was conducted using an existing drug. Levocabastine hydrochloride (hereafter referred to as “levocabastine”, Janssen Pharmaceutical K.K.) was used as a test article for efficacy evaluation, and PBS was used as a control article. Levocabastine is an H1 blocker eye drop that exhibits histamine H1 antagonism (Noble & McTavish, 1995). Since levocabastine is a commercial drug for which nonclinical efficacy studies in guinea pigs had been undergone for application, it was selected as a suitable test article to confirm the validity of the modified model. The evaluation items commonly used in animal models of conjunctivitis, i.e., scoring of allergic conjunctivitis, observations of scratching behaviors, and measurements of conjunctival vascular permeability (Kamei et al., 1991; Kato et al., 2004; Shii et al., 2009), were selected to confirm the inhibitory effect of levocabastine on allergic conjunctivitis.

2.3.1. Dosing method

Histamine and levocabastine or PBS were instilled into the right and left conjunctival sacs of each animal. Levocabastine or PBS was instilled 15 min before histamine instillation. The lower eyelid was gently pulled away from the eye and the dosing formulation was instilled into the conjunctival sacs using an autopipette at 12.5 μL/eye for histamine and 25 μL/eye for levocabastine or PBS. The lower eyelid was held for approximately 15 s after instillation. The upper and lower eyelids were closed gently for a moment and then slowly released.

2.3.2. Scoring of allergic conjunctivitis

In the verification experiment using the modified model, the iris and conjunctival parts of the Draize scale (Draize, 1959) were used for the conjunctivitis score evaluation (Table 1). The condition of the conjunctivae and iris was observed macroscopically and scored according to the Draize scale prior to (immediately after instillation of

Table 1

Scoring standards for allergic conjunctivitis (excerpted from Draize scale).

Iris
(A) Appearance, values
Normal 0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive) 1
No reaction to light, hemorrhage, gross destruction (any or all of these) 2
Conjunctivae
(A) Redness (refers to palpebral and bulbar conjunctivae)
Vessels normal 0
Vessels definitely injected above normal 1
More diffuse, deeper crimson red, individual vessels not easily discernible 2
Diffuse beefy red 3
(B) Chemosis
No swelling 0
Any swelling above normal 1
Obvious swelling with partial eversion of lids 2
Swelling with lids about half closed 3
Swelling with lids about half closed to completely closed 4
(C) Discharge
No discharge 0
Any amount different from normal 1
Discharge with moistening of the lids and hairs just adjacent to lids 2
Discharge with moistening of the lids and hairs, and considerable area around the eye 3

The iris and conjunctival parts of the Draize scale were used for the conjunctivitis score evaluation. Total evaluation scores of the iris and conjunctivae were calculated and used as allergic conjunctivitis scores for each eye.

levocabastine or PBS) and at 30, 60, and 90 min after instillation of histamine. Detailed observations were conducted using a slit lamp. Total evaluation scores of the iris and conjunctivae were calculated and used as allergic conjunctivitis scores for each eye.

2.3.3. Observations of scratching behaviors

Animals were transferred to observation cages at approximately 60 min prior to induction of allergic conjunctivitis for acclimation to the observation environment. After dosing with histamine outside the cages, the animals were promptly returned to their cages and behaviors were video-recorded for 30 min after induction. The number of eye scratching behaviors with the hindlimbs was counted from the recorded images. Continuous scratching behavior was counted as one scratch.

2.3.4. Measurements of conjunctival vascular permeability

Evans blue was dissolved in saline to yield a 1 w/v% Evans blue solution (hereafter referred to as “Evans blue solution”). At 5 min after dosing with levocabastine or PBS, Evans blue solution was administered intravenously at a volume of 0.25 mL/100 g of body weight, and the histamine solution was administered 10 min later. At 30 min after dosing with the histamine solution, the animals were anesthetized by inhalation of isoflurane, JP (Mylan Seiyaku Ltd.) and euthanized by exsanguination from the posterior vena cava and abdominal aorta to remove the conjunctival tissues from both eyes. Wet weights of the removed conjunctival tissues were measured, and the tissues were cut finely and immersed in a 1 N KOH solution (0.5 mL, 37 °C) overnight (at least 18 h). An H₃PO₄-acetone solution (4.5 mL, 0.6 N H₃PO₄:acetone = 5:13) was added to the tissue solution and mixed well. This extracted solution was centrifuged (approx. 1800 ×g, room temperature, 20 min) to obtain the supernatant. Absorbance of the supernatant was measured at 620 nm. Extracted dye amounts per 1 g of conjunctival tissue were calculated based on the calibration curve generated in advance.

2.4. Statistical analysis

Statistical analysis was performed as follows in the verification experiment using the modified model:

For the conjunctivitis scores, the mean group values and standard

errors were calculated from all scores in the left and right eyes at each observation time point. Data were compared between the control and levocabastine groups using the Wilcoxon rank sum test. The mean group values and standard errors were calculated for the number of scratching behaviors and the amount of conjunctival vascular permeability. Data were analyzed for homogeneity of variance between the control and levocabastine groups using the F-test (5% level of significance). Aspin-Welch's *t*-test was conducted due to unequal variances.

Differences from the control group was evaluated at the two-tailed 5% level of significance and presented as $p < 0.05$ or $p < 0.01$ in the tables.

3. Results

3.1. Optimal conjunctivitis induction conditions

As a result of instillation of histamine at 100 ng/μL with a dose volume of 25 μL/eye, very slight edema or hyperemia (score 1, minimal) was observed in the bulbar conjunctiva from 30 to 60 min after instillation. No changes were observed in the eyelids and the score was clearly lower than that in the previous report, indicating that the dose level was insufficient for use in drug efficacy evaluation.

Accordingly, histamine concentrations and dose levels were increased to 1000 ng/μL (25 μL/eye), 2000 ng/μL (12.5 μL/eye), and 2000 ng/μL (25 μL/eye). As a result, no changes were observed at 1000 ng/μL (25 μL/eye), a dose 10 times the initial dose, whereas hyperemia and edema were clearly observed macroscopically at 2000 ng/μL (25 μL/eye), a dose 20 times the initial dose. At 2000 ng/μL (25 μL/eye), very strong edema and hyperemia were observed from the bulbar conjunctiva to the upper and lower eyelids 30 min after instillation, receiving the highest score of 5 (severe). At 2000 ng/μL with half the volume of 25 μL/eye, 12.5 μL/eye, the severity of hyperemia and edema slightly reduced as compared to 2000 ng/μL (25 μL/eye), and the score at 30 min after instillation was 3 (moderate) (Fig. 1a).

3.2. Verification of modified conjunctivitis model

3.2.1. Scoring of allergic conjunctivitis

Redness/congestion and chemosis/swelling of the conjunctivae, and discharge from the conjunctivae were observed in all animals in the control group after instillation of histamine, indicating that allergic conjunctivitis was induced by histamine (Fig. 1b).

The mean scores of allergic conjunctivitis according to the Draize scale prior to (immediately after instillation of levocabastine or PBS) and at 30, 60, and 90 min after instillation of histamine were 0.0, 4.0, 2.8, and 1.8 in the control group and 0.0, 0.6, 0.6, and 0.0 in the levocabastine group, respectively (Fig. 2).

In the levocabastine groups, the scores after histamine instillation were significantly lower than those in the control group at all observation time points, indicating that levocabastine showed statistically significant inhibitory effects on histamine-induced allergic conjunctivitis signs.

3.2.2. Observations of scratching behaviors

The mean group values for the number of eye scratching behavior were 16.6 and 1.0 in the control and levocabastine groups, respectively (Table 2). A statistically significant decrease was noted in the levocabastine group as compared to the control group, indicating an inhibitory effect of levocabastine on the eye scratching behavior.

3.2.3. Measurements of conjunctival vascular permeability

The mean group values for extracted dye amounts in the conjunctival tissue were 68.1 and 18.3 μg/g in the control and levocabastine groups, respectively (Table 3). A statistically significant decrease was noted in the levocabastine group as compared to the control group, indicating an inhibitory effect of levocabastine on the enhancement of vascular

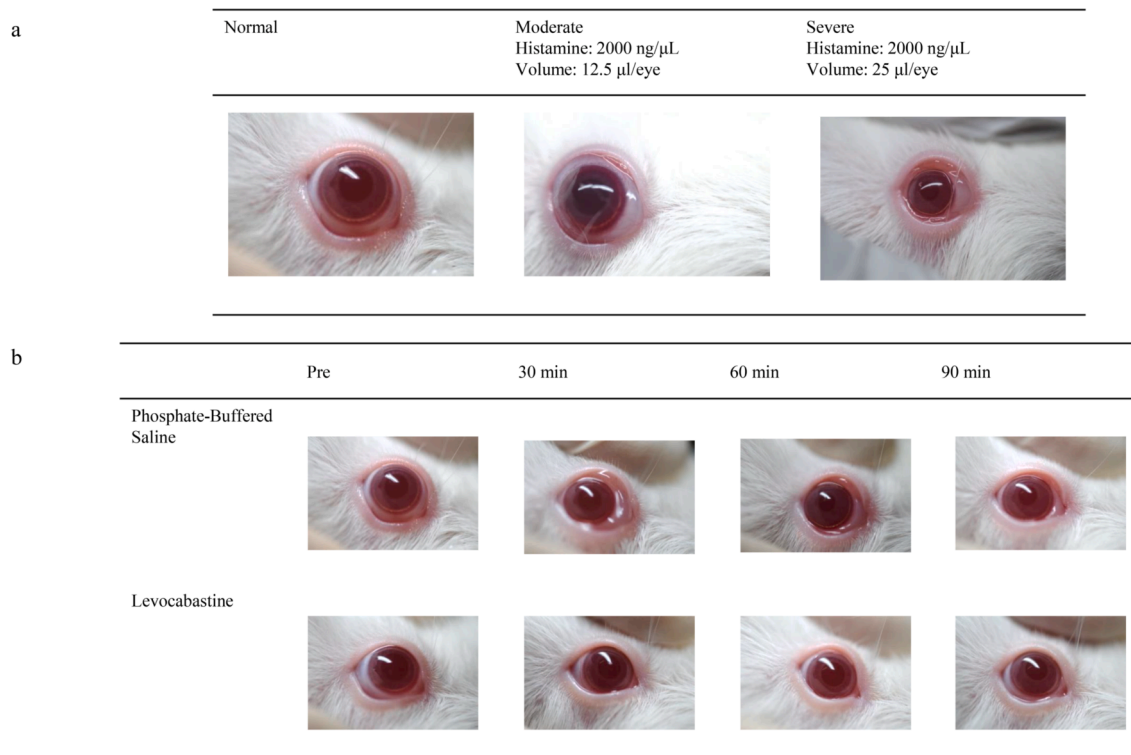


Fig. 1. Conjunctivitis-like symptoms in guinea pigs caused by histamine instillation.

A representative example of guinea pig hyperemia and edema caused by histamine instillation. a: Differences in conjunctivitis symptoms at different histamine dose levels (2000 ng/ μ L (25 μ L/eye) vs 2000 ng/ μ L (12.5 μ L/eye)). b: Hyperemia and edema in the control group vs the levocabastine group prior to and at 30, 60, and 90 min after instillation of histamine at histamine dose level of 2000 ng/ μ L (12.5 μ L/eye).

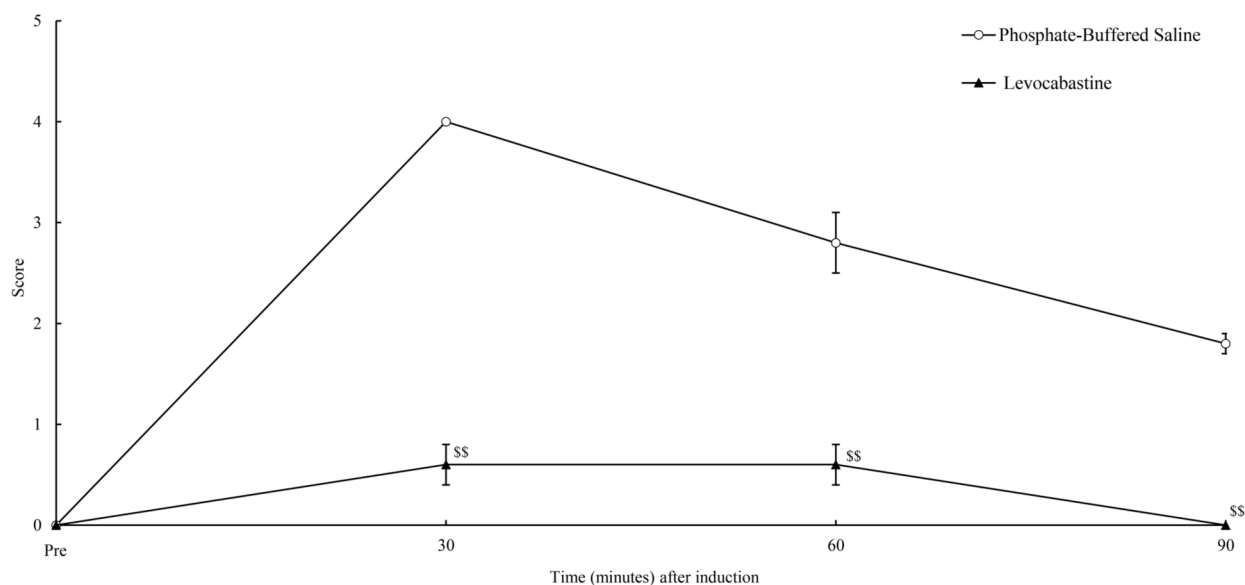


Fig. 2. Scoring of allergic conjunctivitis.

The condition of the conjunctivae and iris was observed macroscopically and scored according to the Draize scale prior to and at 30, 60, and 90 min after instillation of histamine.

Each value represents Mean $\pm$ S.E. ($N = 10$).

^{\$\$}: $P < 0.01$, significant difference between the control and levocabastine groups (Wilcoxon's rank-sum test).

permeability.

4. Discussion and conclusion

As a result of verification of the reproducibility of the previously

reported histamine model (Kamei et al., 1991), only slight edema or hyperemia was observed in the bulbar conjunctiva, and no changes were observed in the eyelids after instillation of histamine at 100 ng/ μ L with a dose volume of 25 μ L/eye, the same condition where the conjunctivitis symptoms were clearly observed in the previous report. The results of

Table 2

Observations of scratching behavior.

Group	Number of eye scratching behavior		
Phosphate-Buffered Saline	16.6 ± 3.5		–5
Levocabastine	1 ± 0.6	#	–5

After dosing with histamine, behaviors were video-recorded for 30 min after induction. The number of eye scratching behaviors with the hindlimbs was counted from the recorded images. Continuous scratching behavior was counted as one scratch.

Each value represents Mean ± S.E. (*N* = 5).

#: *P* < 0.05, significant difference between the control and levocabastine groups (Aspin-Welch's *t*-test).

Table 3

Measurements of conjunctival vascular permeability.

Group	Extracted dye amounts (μg/g conjunctival tissues)		
Phosphate-Buffered Saline	68.1 ± 10		–5
Levocabastine	18.3 ± 1.4	##	–5

The conjunctival tissues were removed 30 min after instillation of histamine, and the Evans blue pigment was extracted. The absorbance of the extracted solution was measured, and the amount of dye per 1 g of conjunctival tissue was calculated.

Each value represents Mean ± S.E. (*N* = 5).

##: *P* < 0.01, significant difference between the control and levocabastine groups (Aspin-Welch's *t*-test).

the previous report were not reproducible, and the symptoms were too weak to be used for drug efficacy evaluation. Therefore, we examined whether conjunctivitis symptoms suitable for drug efficacy evaluation could be induced by increasing the dose levels of histamine. When histamine concentrations (dose volumes) were set at 1000 ng/μL (25 μL/eye), 2000 ng/μL (12.5 μL/eye), and 2000 ng/μL (25 μL/eye), no changes were observed at 1000 ng/μL (25 μL/eye), a dose 10 times the previously reported dose level, and hyperemia and edema were clearly observed macroscopically at 2000 ng/μL, a dose 20 times the previously reported dose level. At 2000 ng/μL, edema and hyperemia were observed more severely at a dose volume of 25 μL/eye, and the severity of hyperemia and edema slightly reduced in half the volume of 25 μL/eye, 12.5 μL/eye. In case of drug efficacy evaluation, it seems difficult to capture the changes that should be evaluated if the symptoms in the model are too severe. Therefore, we judged that the histamine concentration of 2000 ng/μL with a dose volume of 12.5 μL/eye, at which moderate symptoms appeared, is appropriate model conditions. The reason why the results in the previous report could not reproduced was considered to be differences in materials and/or experimental environments. Even though the same types of materials are used, it is commonly experienced in researches in the field of life science that the reproducibility of an experiment cannot be obtained due to small differences such as differences in lots for reagents, breeding environments, and suppliers of the animals (Collins & Tabak, 2014). In the case of the model that was the basis of this study, it may have been possible to obtain the same results if the environment, materials, and methods were exactly the same as the original. However, these information are rarely described in detail in papers, and it is impossible to match all the conditions completely when trying to reproduce them in different facilities. The key requirements expected of animal models used in the pharmaceutical field are that they should be “quite easy to perform”, “widely accessible”, and “reproducible” (Moeller, Ask, Warburton, Gauldie, & Kolb, 2008). “Reproducible” here means that the same result can be obtained even in different facilities if the same procedure is followed using almost the same material, even if it is not exactly the same as the original. Such a reproducible method is expected to yield the same results, even if there are slight changes in materials and methods. Reproducibility is essential for models used in the field of pharmaceuticals, which are developed

based on more objective evidence. Accordingly, when introducing a new model, it is necessary to conduct preliminary experiments, even on a small scale, to confirm that the method is reproducible, and then to modify the method to be reproducible if necessary.

As a second step, we confirmed using an existing drug (levocabastine) whether the animal model prepared under the conditions of histamine concentration of 2000 ng/μL with a dose volume 12.5 μL/eye can actually be evaluable for drug efficacy and verified whether the operation procedures can be standardized. As the indicators for drug efficacy evaluation, a commonly used scoring system for allergic conjunctivitis, observations of scratching behaviors, and measurements of conjunctival vascular permeability were selected. After instillation of histamine, redness/congestion and chemosis/swelling of the conjunctivae and discharge from the conjunctivae followed by eye scratching behavior were observed. Furthermore, vascular permeability was increased, which allowed to evaluate the drug efficacy by measuring the amount of pigment leakage in the conjunctiva. This model did not require individual immune responses and the data obtained were relatively comparable between animals. All symptoms were suppressed by levocabastine, the histamine H1 receptor antagonist. In other words, evaluation of the drug efficacy was feasible in the prepared model in the same way as the previous report (Kamei et al., 1991).

Regarding standardization of operating procedures, since observations of eye scratching behaviors and measurements of conjunctival vascular permeability can be expressed quantitatively, we considered that a numerical evaluation, which is not affected by the subjectivity of evaluators, is feasible by standardize the operating procedures. The scoring system of allergic conjunctivitis, on the other hand, generally consists of severity grades, but the criteria of severity are often different and ambiguous between papers. For example, some evaluate conjunctival hyperemia and edema on one scale as a series of changes without distinguishing them (Kamei et al., 1991), some score hyperemia and edema separately on each scale (Shii et al., 2010), and others evaluate them by summing up the scores for each symptom (Nakano et al., 2009). In some reports, the intermediate symptoms of integer scores is defined as 0.5 (Fukushima et al., 2003). The levels of severity grades are also not standardized, with some having 4 levels and others having 5. Furthermore, most of the scores are labeled in simple terms such as “slight” and “severe”, but the definition of symptoms for the same severity label are different from paper to paper. Under such circumstances, only the experimenter perceives the severity of actual symptoms in a model, and it is understandable that the results from the same subject may be completely different between experimenters. Even if the experimenters constantly discuss the criteria and reach a consensus, the results often differ between experimenters. Ambiguous criteria also leave room for arbitrary data manipulation, which is not desirable from the perspective of data reproducibility. In order to solve such problems, it is necessary to clarify the judgment criteria and establish criteria independent from the senses of observers. Then, we presumed that the conjunctivitis scores could be standardized by adopting the Draize scale as the standard that meets the above conditions. Draize scale is commonly used in drug safety studies to assess the acute toxicity (irritability) of the eyes (Kowalski, Romanowski, Yates, & Mah, 2016; Lather, Sharma, & Khatkar, 2020). In this scale, the severity grades of symptoms are set for the cornea, iris, and conjunctiva, but only the criteria for iris and conjunctiva, where changes may be caused by histamine, were excerpted for evaluation in this study. In most of the conventional score evaluations, the criteria are not set for each part, the presence or absence of symptoms are judged by the subjectivity of observers, and the symptoms are classified into 4 or 5 levels of severity. Unlike the conventional scoring system, the scoring criteria in the Draize scale are set for each part based on the types of signs/symptoms and their changes, reducing an opportunity of subjective judgment and increasing the objectivity. As you can see from the fact that there are many drugs that have been evaluated for safety using the Draize scale are now on the market, since this standard has already been adopted in many experimental facilities involved in

drug development and the experimental procedures have been standardized, the Draize scale seems to be easily applicable to drug efficacy evaluation. However, since the Draize scale is the criteria for the toxicity evaluation of compounds, some evaluation criteria are unsuitable for drug efficacy evaluation. For instance, changes in the iris are evaluated in 3 levels of severity from 0 to 2, and the highest score is defined as bleeding and significant tissue destruction; however, this is not the case with the drug efficacy model. It may be necessary to observe slightly weaker reactions in detail in order to capture changes due to drug efficacy. In such cases it may be necessary to consider using a different criterion like the McDonald-Shadduck scoring system, which is commonly used and standardized in drug safety studies similar to the Draize scale (McDonald & Shadduck, 1977). This system allows more detailed observation of each part than the Draize scale, and since the scores are subdivided, it may be more suitable for capturing changes in drug efficacy evaluation.

We have focused on allergic conjunctivitis, the most familiar eye disease, and investigated animal models for the development of therapeutic agents for it. Specifically, we presumed that an animal model of histamine-induced allergic conjunctivitis would be useful as a screening model in the early stages of drug development. Then, we investigated whether the existing model was appropriate as a drug efficacy evaluation method in drug development, and further verified the possibility of standardization of operating procedures with highly reproducible results. As a result, the dose level of histamine needed to be increased in order to use the existing model for drug efficacy evaluation, but allergic-like symptoms were induced very easily and stably in this model. Furthermore, this study revealed that standardization of operating procedures is feasible and highly reproducible results can be obtained by devising the evaluation method. In drug development, it is of the utmost important to deliver good medicines to patients as soon as possible. For that purpose, improvement of the success probability is required in the clinical development, and it is essential to ensure the reproducibility of experiments in the nonclinical stage. We consider that verification like this study will be useful to carry out reproducible experiments established with objective judgments and quantitative expressions whenever possible.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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