Allergic Rhinitis Model with Brown Norway Rat and Evaluation of Antiallergic Drugs

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An animal disease model of allergic rhinitis was developed with Brown Norway (BN) rat, a high immunoglobulin E responder strain. BN rats were immunized with ovalbumin (OA) and made to suffer from allergic rhinitis. The severity of allergic rhinitis was assessed by determining the extent of the three kinds of markers, Evan’s blue, histamine and N-acetyl-β-d-glucosaminidase, released into the nasal perfusate following the OA challenge to the nasal cavity of OA-sensitized BN rats.

This experimental system was appreciated by antiallergic drugs; chlorpheniramine maleate inhibited the release of Evan’s blue and elevated that of histamine, but did not affect the N-acetyl-β-d-glucosaminidase level. Halopredone acetate inhibited the releases of all the three markers.

The estimation of the released markers using allergic rhinitis brought about in BN rats was found to be a useful experimental system for evaluating the effect of drugs on allergic rhinitis.

Keywords — allergic rhinitis model; Brown Norway rat; histamine; vascular permeability; antiallergic drug

Introduction

An animal disease model is often useful for evaluating the action of a drug and its therapeutical applicability. Allergic rhinitis is a type I allergy caused by an interaction between antigen and immunoglobulin E (Ig E) antibody on the surface of mucosal mast cells which are located on the epithelium of the nasal cavity. To evaluate the effects of antiallergic drugs on allergic rhinitis, the passive cutaneous anaphylaxis (PCA) reaction or the method using sensitized peritoneal mast cells is used preferably. In both methods, connective tissue mast cells are involved, but not mucosal mast cells. Since it is difficult to evaluate the preventive or alleviative action of antiallergic drugs by such experiments, it is necessary to develop a suitable assay model using allergic animals.

In the present study, Brown Norway (BN) rats, a high Ig E responder strain, were immunized with ovalbumin and made to suffer from allergic rhinitis. The severity of allergic rhinitis was assessed by determining the three kinds of markers, Evan’s blue, histamine and N-acetyl-β-d-glucosaminidase, released into the nasal perfusate. The effect of antiallergic drugs was also evaluated by the reduction of those released markers.

Materials and Methods

Materials — Ovalubmin (OA, grade V), Evan’s blue and chlorpheniramine maleate were purchased from Sigma Chemical Co. (MO, U.S.A.). Halopredone acetate was kindly given by Taiho Pharmaceutical Co. (Tokyo, Japan). Brown Norway (BN) rats and male Wistar rats were purchased from Sankyo Laboratories (Tokyo, Japan) and Shizuoka Agricultural Co. (Shizuoka, Japan), respectively.

Immunization — The BN rats (200—220 g) were immunized with a single intraperitoneal injection of 0.5 mg OA mixed with the adjuvant, 0.25 ml of aluminum hydroxide gel solution (30 mg/ml).

Passive Cutaneous Anaphylaxis Reaction — The Ig E titer in the serum was determined by PCA reaction according to the method of Mota. In brief, the doubling dilution of serum (50 μl) obtained from OA sensitized BN rats was injected intradermally into the shaved skin of male Wistar rats (220—240 g). After 72 h, OA (5 mg) in 1 ml of physiological saline containing 1% Evan’s blue was injected through the tail vein of the rats. After 30 min, the rats were sacrificed, the dorsal back skin peeled off, and the diameter of the blue circle on the underside of the skin was measured. The PCA titer was expressed as
the highest dilution giving a positive reaction of more than 5 mm in diameter.

To determine the class of antibody, a portion of the serum was treated at 56 °C for 2 h before the intradermal injection.

Antigen Challenge — OA-sensitized BN rats (18—20 d after immunization) were anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg). The trachea was cannulated with a polyethylene tube (PE-260, Cray Adams Co.) to keep an air way. A second cannula (PE-260) was inserted through the esophagus to the posterior part of the nasal cavity, and the nasopatinate was closed with an adhesive agent according to the method of Hussain et al.OA or OA with drugs in physiological saline (5 ml) were perfused from esophageal cannula through the nasal cavity at the rate of 1 ml/min (37 °C), and the perfusate passed out from nostrils was collected and was recirculated. Before antigen challenge, 1 ml of Evan's blue dissolved in physiological saline (1 %) was injected into the tail vein.

Assay of Allergic Markers — Periodically collected perfusate (1.0 ml) was centrifuged at 3000 rpm for 10 min, and Evan’s blue concentration in the supernatant was measured spectrophotometrically (610 nm). Histamine was determined spectrofluorometrically by the method of Shore et al. In brief, perchloric acid (10 N, 100 µl) was added to the perfusate (1 ml) obtained at 60 min and centrifuged. Following the extraction of histamine, it was reacted with o-phthalaldehyde in alkaline solution and fluorescence was measured (Ex. 360 nm, Em. 450 nm). N-Acetyl-b-D-glucosaminidase activity in the perfusate was measured using p-nitrophenyl-N-acetyl-b-D-glucosaminide as a substrate.

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**Fig. 2.** Cumulative Amount of Evan's Blue Release into the Nasal Perfusate of Brown Norway Rat

OA (0.5 mg)-sensitized rats were challenged with different concentrations of OA (0.01 %(A), 0.1 %(B) or 1 %(C)) solution, in the nasal cavity. Values represent the mean of three experiments ± S.E.

- ○, non-sensitized rat; ○, OA-sensitized rat; △, 0.1 % BSA was challenged in the nasal cavity of OA-sensitized rat.

a) *p* < 0.05.
Fig. 3. Release of Histamine(A) and N-Acetyl-β-D-glucosaminidase (B) into the Nasal Perfusate

The nasal cavity of OA-sensitized rat was challenged with 0.1% of OA solution for 60 min, and the release of histamine and N-acetyl-β-D-glucosaminidase was determined. Values represent the mean ± S.E. Number in parenthesis indicates the number of experiment.

Results

Ig E Production

Figure 1 shows Ig E production in BN rats over a period of 30 d following immunization. The PCA titer gradually increased, attaining the maximum level of 1024 on day 15, and the titer of 512 was maintained for 15 d thereafter. No positive PCA reaction could be observed when the serum was heated.

Release of Allergic Markers into the Perfusate

Figure 2 shows the release of Evan’s blue following perfusion of the nasal cavity of sensitized or non-sensitized rats with challenged antigen (OA: 0.01, 0.1 and 1%). The release of Evan’s blue significantly increased in the sensitized rats after 30 min when they were challenged with 0.1 and 1.0% OA solutions.

Instead of OA solution, 0.1% bovine serum albumin (BSA) was perfused through the nasal cavity of OA-sensitized BN rats. As shown in Fig. 2 (B), the released extent of Evan’s blue was the same as that in non-sensitized rats. This fact indicates that vascular permeability is not accelerated by bovine serum albumin and differs from that of the immunized antigen.

Figure 3 shows the amounts of histamine(A)

Fig. 4. Effects of Chlorpheniramine Maleate and Halopredone Acetate on the Evan’s Blue Released into the Nasal Perfusate

Values represent the mean ± S.E. of 3—8 experiments. •, non-sensitized rat was challenged with 0.1% OA; ○, OA-sensitized rat was challenged with 0.1% OA; ▲, OA-sensitized rat was challenged with 0.1% OA and 4.4 mM chlorpheniramine maleate; △, OA-sensitized rat was challenged with 0.1% OA and 0.55 mM chlorpheniramine maleate; □, OA-sensitized rat was challenged with 0.1% OA and 23.2 μM halopredone acetate. a) p < 0.01.
Fig. 5. Effect of Chlorpheniramine Maleate and Haloprep-
done Acetate on the Release of Histamine to the Perfusate.
Values represent the mean ± S.E. Number in parenthe-
sis indicates the number of experiments. The nasal cavity
of OA-sensitized rat was challenged with following solu-
tions for 60 min.
A, non-sensitized rat was challenged with 0.1 % OA;
B, OA-sensitized rat was challenged with 0.1 % OA; C,
OA-sensitized rat was challenged with 0.1 % OA and 4.4
mM chlorpheniramine; D, OA-sensitized rat was
challenged with 0.1 % OA and 23.2 mM halopredone.

and N-acetyl-β-D-glucosaminidase(B) released
during 60 min of perfusion with 0.1 % OA. Both
markers released in sensitized rats were signifi-
cantly larger than those in non-sensitized rats.

**Effect of Antiallergic Drugs**
The effects of chlorpheniramine maleate and halopredone acetate on the release of allergic markers were investigated using the allergic rhi-
nitis model of BN rats. In the case of chlor-
pheniramine, the release of Evan’s blue
decreased with the increasing of the concentra-
tion, and a significant difference was observed
at 4.4 mM (Fig. 4). On the contrary, histamine
release was elevated (Fig. 5), and the release of
N-acetyl-β-D-glucosaminidase could not be influ-
enced by this drug (Fig. 6). In the case of
halopredone acetate, the release of both markers,
histamine and N-acetyl-β-D-glucosaminidase,
into the perfusate was inhibited (Fig. 5 and 6).

Fig. 6. Effect of Chlorpheniramine Maleate and Halopre-
done Acetate on the Release of N-Acetyl-β-D-
glucosaminidase to the Perfusate
Values represent the mean ± S.E. Number in parenthe-
sis indicates the number of experiments. The nasal cavity
of OA-sensitized rat was challenged with following solu-
tions for 60 min.
A, non-sensitized rat was challenged with 0.1 % OA;
B, OA-sensitized rat was challenged with 0.1 % OA; C,
OA-sensitized rat was challenged with 0.1 % OA and 4.4
mM chlorpheniramine; D, OA-sensitized rat was
challenged with 0.1 % OA and 23.2 mM chlorpheniramine.

**Discussion**
Mast cells are classified into the connective
tissue mast cell and the mucosal mast cell, and they
differ from each other in the degree of differen-
tiation, the morphology, and the sensitivity to
dye or chemicals, such as compound 48/80, poly-
myxin, bee venom 401 etc.\textsuperscript{15–17} Connective
tissue mast cells participate in the PCA reaction
and sensitized peritoneal mast cells are used in the
evaluation of antiallergic drugs \textit{in vitro}. However,
since the mucosal mast cells are asso-
ciated with the development of the allergic rhini-
titis, attention should be focused on the mucosal
mast cells \textit{in vivo}. Naclerio \textit{et al.}\textsuperscript{18} reported a
novel human \textit{in vivo} model for the evaluation of
topical antiallergic medication, but it involves
some risk to the patient with allergic rhinitis. The
evaluation of antiallergic drugs using an ex-
perimental animal model was developed with
Wistar rats, but the class of antibody that participated was unclear. Consequently, the establishment of an Ig E-dependent animal disease model of allergic rhinitis is significant for the estimation of the severity of allergic rhinitis and the evaluation of antiallergic drugs.

In type I allergy, degranulation of mast cells occurs following the interaction between challenged antigen and Ig E, bound to the surface of the mast cells by their Fc-receptors. However, Ig G is also thought to be responsible in part for the induction of allergic rhinitis in actively immunized rats. Thus, it is necessary to determine the class of the antibody produced by OA immunization. It was reported that the binding capacity of Ig E to Fc-receptors on the mast cells is abolished by pretreatment at 56 °C for 2 h. In this experiment, no PCA reaction could be observed by thermally treated serum of OA-sensitized BN rats (Fig. 1). The Ig E levels in BN rat (Fig. 1) exceeded those in Wistar rat whose highest Ig E level was 64 on day 15 following immunization (data not shown). These results are consistent with the report that the BN rats are a high Ig E responder strain. Taking the above findings into consideration, Ig E antibody may have strongly participated in sensitized BN rat rather than Ig G.

As for the degranulation in mast cells, it is well known that many kinds of chemical mediators such as histamine, serotonin, N-acetyl-β-D-glucosaminidase, prostaglandins etc. are released. It is considered that the typical symptoms of allergic rhinitis such as sneezing, rhinorrhea and rhinostegnosis are due to the action of these chemical mediators. To estimate the severity of allergic rhinitis or the regression from allergic rhinitis quantitatively, the release of the markers, histamine, Evan’s blue and N-acetyl-β-D-glucosaminidase, into the perfusate were determined. Because histamine is a typical mediator, our attention was initially directed to this compound. As histamine promotes the vascular permeability and Evan’s blue binds preferentially to the serum albumin, the release of this dye into the nasal perfusate is indicative of the action of histamine. Further, Schwartz et al. reported that a lysosomal enzyme such as N-acetyl-β-D-glucosaminidase is released at the time of degranulation of sensitized mast cells. Thus, the release of these three markers was used as the indicator of the severity or regression of allergic rhinitis. As shown in Fig. 2, a significant release of Evan’s blue was observed at and after 30 min following the antigen challenge; this probably means that such a length of time is necessary for penetration of OA through the mucus and its access to the mast cells. The release of N-acetyl-β-D-glucosaminidase and histamine was also increased significantly in accordance with the development of allergic rhinitis (Fig. 3). From the above results, an animal model of allergic rhinitis may be made antigen specific by perfusing OA through the nasal cavity of OA-sensitized BN rats.

Then, the effects of chlorpheniramine maleate, a well-known H₁-antagonist, and halopredone acetate, a glucocorticoid, on the release of the three kinds of allergy markers were examined using this experimental system. Chlorpheniramine binds to the H₁-receptors competitively with histamine, and inhibits the vascular permeability induced by histamine. Consequently, the release of Evan’s blue was inhibited by addition of 0.55 and 4.4 mM chlorpheniramine (Fig. 4). Chlorpheniramine can not depress the degranulation of mast cells, thus the release of histamine and N-acetyl-β-D-glucosaminidase from the drug treated rats is considered to be a same level to that of OA challenged rats. Mota and Dias da Silva, Read and Lagunoff reported that a high concentration of H₁-blockers promote the histamine release from mast cells. Consequently, the elevated histamine release may be a result from the membrane damage of mast cells by a high concentration of chlorpheniramine (Fig. 5).

It is considered that one pharmacological action of glucocorticoid is the stabilization of membrane, such as plasma and/or lysosomal membrane. Consequently, the decreased release of the three allergic markers by halopredone acetate reflects well the action of a glucocorticoid. But, Schleimer reported that steroids effectively inhibit the late-phase reaction of Ig E dependent responses which occur as a consequence of mast cell degranulation. Only a slight inhibition of histamine release by halopredone within
60 min (Fig. 5) may reflect the pharmacological properties of steroids.

The present results indicate that an animal disease model of allergic rhinitis was developed with BN rats. Pharmacological properties of two kinds of antiallergic drugs were appraised by this experimental system. Thus, this allergic rhinitis model with BN rats is useful for evaluating the effect of antiallergic drugs on allergic rhinitis through the determination of the release of the three markers into the perfusate.

References


26) G. W. Read and D. Lagunoff: Antagonism of the flin- common pathway of mast cell histamine secretion