Mouse Ear PCA as a Model for Evaluating Antianaphylactic Agents

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Abstract

Passive cutaneous anaphylaxis (PCA) reaction elicited in ears of male ddY mice was studied by means of assessing dye leakage. In the ear, PCA reaction was more sensitive and reproducible than that in the dorsal skin. The reaction was significantly suppressed by ketotifen, one of antianaphylactic agents.

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Passive cutaneous anaphylaxis (PCA) reaction [9] is one of the most useful methods for evaluating antianaphylactic agents [1–3]. In mice, PCA reaction was usually elicited in dorsal skin and the activity of antianaphylactic agents was evaluated by means of reduced dimension or diameter of PCA site [4]. In our preliminary experiment, however, the PCA reaction in dorsal skin of mice was little reproducible. Furthermore, dimension or diameter of PCA site was not always a good parameter for quantitative evaluation of PCA reaction.

In 1961, Feinberg [5] reported the suitability of mouse ear for PCA reaction. Recently, Iwata et al. [6] titrated mouse IgE antibody by PCA reaction in the mouse ear. In the present study, PCA reaction was elicited in mouse ear and the antianaphylactic activity of ketotifen was evaluated by means of the reduced amount of dye leakage.

Mouse homocytotropic antiserum was obtained from female BALB/c mice 10 days after immunization with ovalbumin (OA) and alum. PCA titer of the antiserum estimated in mouse ear was 1:28. 10 µl of the antiserum in appropriate dilution was injected into ears of male ddY mice weighing 25–30 g. 48 h after the sensitization, mice were challenged with 0.25 ml of 0.5% Evans blue dye solution containing 0.25 mg of OA intravenously. After 30 min, animals were sacrificed by cervical dislocation and the ears were removed.

The amount of dye leaked in the ear was measured according to the method described by Katayama et al. [7] with some modifications. In brief, a pair of ears obtained from each mouse were dissolved in 0.75 ml of 1 JV KOH solution at 37 °C overnight and 9.25 ml of a mixture of 0.6 JV H₃PO₄ solution and acetone (5:13) was added. After vigorous shaking, precipitates were filtered off and amount of dye was measured colorimetrically at 620 nm.

The relationship between dilution of antiserum for sensitization and amount of dye leaked in ears is shown in figure 1. The amount of extravasated dye caused by 10-fold diluted antiserum was

39.10 ± 0.16 µg and the reaction was reduced proportionally to the degree of dilution. A submaximal reaction was caused by 30-fold diluted antiserum.

The responsivity of the ear for PCA reaction was compared with dorsal skin by measuring the amount of extravasated dye and the dimension of the lesion. PCA reaction in dorsal skin was performed in a similar manner as that in ear. As shown in table I, the reaction in the ear was more sensitive and reproducible than that in dorsal skin when the reaction was measured with not only the amount of extravasated dye but also its dimension. These results agree with the previous report by Feinberg [5]. The effect of ketotifen (Sandoz), one of antianaphylactic agents and a potent antihistamine [8], on mouse ear PCA is shown in figure 2. The agent dissolved in saline was given to mice at doses of 0.1, 1 and 10 mg/kg intraperitoneally 30 min prior to antigenic challenge. As is evident, the PCA reaction in mouse ear was suppressed significantly in a dose-dependent fashion. As compared with rats, mice are easily handled because of their small size. High titer antiserum can be obtained in

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Table I. Comparison of the response of ear and dorsal skin in mouse PCA reaction¹

Site

Sensitization

Amount of dye², µg

Dimension³, mm²

mean ± SE

range

mean ± SE

range

Ear

10 9

2.15 ± 0.07 20.13 ± 2.13

1.90–2.53

8.22–30.83

63.40 ± 4.06

40.60–83.87

Dorsal skin

10 9

2.04 ± 0.16 4.74 ± 0.96

1.26–3.00 2.21–11.54

7.01 ± 3.82

0–34.90

1 Ear or dorsal skin were sensitized with 10 µl of 30-fold diluted antiserum. After 48 h, mice were challenged to elicit PCA reaction.

2 Each value represents the amount of dye obtained from a pair of ears or a pair of dorsal skin sites.

3 Each value represents the dimension obtained from a PCA site both in ear and in dorsal skin.

Fig. 1. Relationship between dilution of antiserum and dye leakage in mouse ear PCA. Each value represents the mean $\pm$ SE of 4 or 5 mice. Dotted area represents the spontaneous level. Fig. 2. Effect of ketotifen on mouse ear PCA. Ketotifen was given to mice i.p. 30 min prior to challenge. Each column represents the mean $\pm$ SE of 8 or 9 mice. Dotted area represents the spontaneous level. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, by Student's t test. mice. Furthermore, it is not necessary to clip hair when performing mouse ear PCA. All these results suggest that mouse ear PCA might be a useful method for evaluating antianaphylactic agents like rat homologous PCA in the back skin [1–3].

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30

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Γ 'i

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MM 10

-I f I f'I"

1 2 5

Concentration of antiserum, %

10

0.1 1

Control Ketotifen, mg/kg

N(3',4'-dimethoxycinnamoyl)anthranilic acid (N-5'). J. Allergy clin. Immunol. 57: 396–407(1976).

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