Inhibitory Effects of a New Antiallergic Agent, Azelastine, on Passive Cutaneous Anaphylaxis and Expulsion of *Nippostrongylus brasiliensis*

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Abstract: A new antiallergic agent, 4-(*p*-chlorobenzyl)-2-[*N*-methyl-perhydroazepinyl-(4)]-1-(2H)-phthalazinone hydrochloride (azelastine), was found to exert inhibitory effects on passive cutaneous anaphylaxis in the rat and on expulsion of *Nippostrongylus brasiliensis* from the rat intestine when this agent was administered intravenously at appropriate doses.

Key Words: Azelastine; Passive cutaneous anaphylaxis; Worm expulsion; *Nippostrongylus brasiliensis*

INTRODUCTION

A newly developed phthalazine derivative, 4-(*p*-chlorobenzyl)-2-[*N*-methyl-perhydroazepinyl-(4)-1-(2H)-phthalazinone hydrochloride (azelastine), blocks H_1 receptor activity related to histaminic actions, even though its chemical structure differs from conventional antihistaminics. This agent has been shown to inhibit in vitro release of histamine from the rat mesenteric tissues (Tasaka and Akagi, 1979).

The purpose of the present note is to show that this agent is effective in inhibiting passive cutaneous anaphylaxis (PCA) in rats that have been sensitized with mouse immunoglobulin E (IgE) antibodies. We also describe an inhibitory effect of the drug on the expulsion of an intestinal parasite, *Nippostrongylus brasiliensis*.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 250–300 g were obtained from Shizuoka Experimental Animal Farms and used for all experiments.

PCA

Methods of PCA have been described in detail elsewhere (Ovary et al., 1975). Briefly, serially diluted samples of mouse sera containing anti-hapten IgE antibody were injected into the freshly shaved skin of the rat in triplicate. Since the antibody was elicited by immunization of mice with

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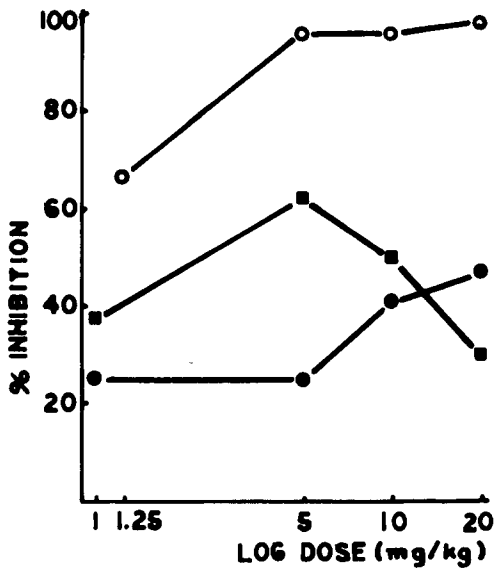

Figure 1 *Dose-dependent inhibition of rat PCA by oral or iv administration of azelastine and DSCG. Sprague-Dawley rats sensitized with mouse IgE antibody 24 hr previously were administered per os azelastine (●) or DSCG (■), or iv azelastine (○), 2 hr before elicitation of PCA. % Inhibition was calculated from the equation*

$$\frac{\text{Mean PCA titer in untreated control rats} - \text{Mean PCA titer in treated rats}}{\text{Mean PCA titer in untreated control rats}} \times 100$$

Each point represents mean % inhibition of PCA tested for two samples of mouse sera.

dinitrophenylated *N. brasiliensis* saline extract (DNP-Nb) as described previously (Kojima and Ovary, 1975), DNP-bovine serum albumin (DNP-BSA) dissolved in 0.5% Evans blue dye solution was used for challenge for PCA (Kojima et al., 1980) 24 hr after sensitization of the skin. The original PCA titers of the samples used ranged from 1:640 to 1:5,120 when PCA was carried out in untreated rats.

Parasite and Infection

A strain of *N. brasiliensis* has been maintained in our laboratory as reported (Kojima and Ovary, 1975). A group of 3–5 rats were inoculated subcutaneously with 1,000 or 2,000 third stage larvae of *N. brasiliensis*. Autopsy was carried out 2 days after the final administration of antiallergic agents; the whole length of the small intestine was opened longitudinally in a Petri dish and worm burdens were determined by counting parasites of the intestine and its contents under a dissecting microscope. Statistical analysis was carried out by Student's *t* test.

Treatment with Azelastine and Other Antihistaminics

Azelastine, clemastine fumarate, and DSCG were kindly provided by Eisai Co, Ltd. (Tokyo, Japan). These agents were dissolved in physiological saline and administered by gastric intubation at a dose of 1 to 20 mg/kg in a volume of 0.5 ml. For an intravenous (iv) injection, the

Abbreviations. azelastine: 4-(*p*-chlorobenzyl)-2-[*N*-methyl-perhydroazepinyl-(4)]-1-(2H)-phthalazinone hydrochloride; BSA: bovine serum albumin; DNP: dinitrophenyl; DSCG: disodium cromoglycate; iv: intravenous(ly); Nb: *Nippostrongylus brasiliensis* saline extract; PCA: passive cutaneous anaphylaxis; Ig: immunoglobulin.

drugs were dissolved in 1.5 ml of saline. Oral or iv administration was carried out 2 hr before elicitation of PCA. In order to examine an inhibitory effect on worm expulsion, various doses of azelastine or clemastine were administered iv for 3 consecutive days starting from day 7 postinfection.

RESULTS

An inhibitory effect of PCA was clearly demonstrated when azelastine was administered iv at a dose of 5 mg/kg or more (Fig. 1). A single oral dose of 5 mg/kg of DSCG resulted in 62 ± 8.1 (mean ± SE)% inhibition of PCA, while only 25 ± 7.0% inhibition occurred by oral administration of the same dose of azelastine. However, when the dose of azelastine was increased up to 20 mg/kg, its inhibitory effect became more than that of the same dose of DSCG but did not exceed the maximum level obtained by oral administration of 5 mg/kg of DSCG (Fig. 1).

Since a strong inhibition of PCA was observed by iv administration of azelastine, rats infected with *N. brasiliensis* were treated with iv injections of various doses of this agent for 3 consecutive days. On day 11 postinfection, worm burdens were determined by dissecting the entire length of the small intestine. Results of two experiments indicated that worm expulsion was inhibited by azelastine at a dose of 5 mg/kg/day or more (Table 1). Clemastine was not effective in inhibiting worm expulsion at this dose.

DISCUSSION

The present study demonstrated that the iv administration of azelastine is quite effective in inhibiting PCA and worm expulsion. It is known that rat mast cells have receptors for mouse IgE but not for IgG, resulting in elicitation of PCA as early as 2 hr after skin sensitization (Ovary et al., 1975). Since azelastine was administered 2 hr before challenge for PCA, which has a 24 hr sensitization period, it is likely that this agent inhibits release of histamine from mast cells. This is

Table 1 *Effects of azelastine and clemastine fumarate on expulsion of* Nippostrongylus brasiliensis *from the rat intestine*[a]

Group No.	Compound	Dose (mg/kg/day)	No. of worms recovered (mean ± SE)[b]	p[c]
Exp. 1				
1	control	—	125.8 ± 109.9	—
2	azelastine	1.0	224.0 ± 274.8	ns[d]
3	azelastine	2.5	280.0 ± 64.5	ns
4	azelastine	5.0	451.3 ± 86.1	<0.01
5	azelastine	20.0	462.3 ± 64.9	<0.01
6	clemastine	5.0	271.7 ± 64.9	ns
Exp. 2				
1	control	—	146.7 ± 110.0	—
2	azelastine	5.0	281.0 ± 150.2	<0.025
3	azelastine	20.0	851.7 ± 187.0	<0.01
4	clemastine	5.0	308.0 ± 213.0	ns

[a] Rats were inoculated subcutaneously with 1,000 (Exp. 1) or 2,000 (Exp. 2) third stage larvae of *N. brasiliensis* on day 0. Various daily doses of azelastine or clemastine fumarate were administered intravenously from day 7 to day 9. Autopsy was carried out on day 11.

[b] Number of worms recovered from the intestine of three to five rats.

[c] *p* value versus control determined by Student's *t* test.

[d] ns: not significant.

supported by in vitro experiments in which azelastine has been found to inhibit histamine release from the rat mesenteric tissues (Tasaka and Akagi, 1979). Another possibility is that azelastine binds to H_1 receptors antagonistically with histamine, thus inhibiting the increase of permeability of the vessels.

A significant inhibition of PCA was also demonstrated by an oral administration of DSCG at a dose of 5 mg/kg (Fig. 1). This is in accord with the results obtained by Orr et al. (1970), but not with those by Spicer et al. (1975) and Hurtado et al. (1978). The discrepancy on the inhibitory effect of oral administration of DSCG was not well-understood in the present study.

The expulsion of intestinal parasites has been noted to be a complex phenomenon of immunological and nonimmunological reactions of the host (Wakelin, 1978). Besides parasite-specific humoral and cellular immune responses, nonlymphoid effector cells, such as mast cells or basophils, may be involved in the expulsion, probably via a direct (Jones and Ogilvie, 1971) or an indirect (Barth et al., 1966) effect of amines (histamine and 5-hydroxytryptamine) on worms. Indeed, by using W/W^v mice, a strain that is genetically defective in mast cells (Kitamura et al., 1978), we have recently shown that mast cells are essentially involved in expulsion of *N. brasiliensis* (Kojima et al., accepted for publication). Results of the present study, in which inhibition of worm expulsion was demonstrated by iv injections of azelastine, are consistent with this observation. This experimental model may be useful for evaluation of antiallergic agents.

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