EFFECT OF CINNARIZINE ON IgE ANTIBODY MEDIATED ALLERGIC REACTION IN MICE AND RATS

HIROICHI NAGAI, IKUHISA YAKUO, HIROAKI YAMADA, NAOKI INAGAKI, SHOICHI GOTO AND AKIHIDE KODA

Department of Pharmacology, Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi Gifu, Gifu, 502, Japan

(Received April 25, 1986)

The effect of cinnarizine on immunoglobulin E (IgE) antibody mediated allergic reactions in mice and rats was investigated. Nifedipine and tranilast were used as comparative drugs. 1) The dye leakage caused by IgE antibody mediated passive cutaneous anaphylaxis (PCA) in mouse ear was clearly inhibited by cinnarizine administered both orally and intraperitoneally. The inhibitory action of cinnarizine for PCA was as potent as nifedipine and superior to tranilast. 2) Cinnarizine clearly inhibited the increase in capillary permeability caused by histamine and calcium ionophore A 23187 (A 23187) but not by LTD₄ in mouse ear. Nifedipine inhibited the increases of capillary permeability caused by A 23187, histamine and LTD₄. Tranilast inhibited A 23187 induced vasculitis but not histamine or LTD₄-induced reaction. 3) Cinnarizine had no significant effect on the release of histamine caused by antigen or A 23187 from rat peritoneal mast cells. Nifedipine and tranilast inhibited the release of histamine caused by both antigen and A 23187 from rat peritoneal mast cells.

Keywords — cinnarizine; anti-allergic agent; calcium antagonist; IgE antibody

INTRODUCTION

Cinnarizine is one of the classical H₁ (histamine) receptor and a potent calcium (Ca) antagonist in cardiac and blood vessel smooth muscle.^{1–3)} Since histamine is well established as a mediator of immediate hypersensitivity reactions, antihistamines would therefore be expected to effectively block the effects of histamine during hypersensitivity reaction. In addition, the secretion of histamine from mast cells by antigen-antibody reaction is dependent on Ca and it has been suggested that the release is a consequence of the influx of Ca as the “trigger” for release. Furthermore, there are some reports to indicate that the efficacies of some anti-allergic agents (disodium cromoglycate, ketotifen and others) are closely related to the inhibition of Ca influx.^{4–6)} These evidences suggest that cinnarizine has a beneficial property to remedy immediate hypersensitivity. The study reported here was conducted to investigate the effect of cinnarizine on IgE antibody mediated allergic reactions in mice and rats.

MATERIALS AND METHODS

Drugs — Cinnarizine was kindly supplied by Eisai Pharmaceutical Co., Ltd., Tokyo, Japan. Nifedipine was purchased from Bayer Pharma-

ceuticals, A.G., Postsach, Federal Republic of Germany and tranilast was supplied by Kissei Pharmaceutical Co., Ltd., Matsumoto, Japan. Cinnarizine and nifedipine were suspended in 0.1% carboxy methyl cellulose saline solution. Tranilast was dissolved in 0.3% sodium bicarbonate immediately before use. Leukotriene (LT) D₄ was kindly supplied by Dr. S. Terao (Takeda Pharmaceutical Co., Ltd., Osaka, Japan).

Animals — Male DDY and female BALB/c mice weighing 18 to 22 g and male Wistar rats weighing 200 g were used. Animals were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan).

Antiserum — Anti-dinitrophenylated ascaris (anti-DNP-As) mouse homocytotropic antiserum was prepared in BALB/c mice by immunization with antigen and alum. The 48 h homologous passive cutaneous anaphylaxis (PCA) titer of antiserum estimated in mouse ear was 1:2⁸. Rat homocytotropic antibody against DNP-As was prepared according to the method of Tada and Okumura.⁷⁾

PCA and Vasculitis Caused by Histamine, LTD₄ or Calcium Ionophore A 23187 (A 23187) in Mouse Ear — PCA was carried out according to the method described previously.⁸⁾ Briefly, 10 μ l of 30-fold diluted anti-DNP-As mouse

serum was injected into the side of ears of DDY mice. After 48 h, PCA was elicited by an intravenous injection of 0.25 mg of dinitrophenylated bovine serum albumin dissolved in 0.5% Evans blue saline solution in a volume of 0.25 ml. Thirty min after the challenge, mice were sacrificed by cervical dislocation and both ears were removed to measure the amount of dye. To measure the extravasated dye, a pair of ears was dissolved with 0.7 ml of 1 N KOH solution in a stoppered tube at 37 °C overnight and 9.3 ml of 0.6 N H₃PO₄ solution and acetone (5 : 13) were added. After vigorous shaking, the precipitate was removed by filtration and amount of dye was measured colorimetrically at 620 nm. For calibration curves, a pair of ears was dissolved with 0.7 ml of 1 N KOH solution in the presence of 10 and 60 µg of Evans blue. A 23187-, histamine- and LTD₄-induced vasculitis were studied by the same method as mentioned in PCA. From the results of preliminary experiments, the concentrations of A 23187, histamine and LTD₄ were determined as 100, 100 and 50 ng, respectively.

Histamine Release from Rat Peritoneal Mast cells Caused by Antigen-IgE Antibody or A 23187

—The effect of drugs on histamine release from rat peritoneal mast cells were investigated by *ex vivo* experiments. Antigen-induced histamine release was conducted in rats which were passively sensitized with rat homocytotropic antibody against DNP-As. One ml of antiserum containing anti-DNP-As antibody was injected intraperitoneally (i.p.) 48 h prior to harvesting the peritoneal cells, and the drug was administered i.p. 1 h prior to harvesting. Harvested cells

were adjusted to 10⁴ mast cell/ml. Antigen (10⁻⁵ g/ml) was added to the cell suspension at 37 °C for 20 min. A 23187 induced histamine release was carried out by a similar method as antigen-induced histamine release. The amount of histamine was measured by a fluorescence method according to the method of May *et al.*⁹⁾

RESULTS

Effect on 48 h Homologous PCA in Mice

The effect of cinnarizine on homologous PCA in mice is indicated in Tables I and II. When administered both orally and intraperitoneally, cinnarizine (1–20 mg/kg) inhibited PCA as measured by dye leakage in mouse ear (Table I). The inhibitory potency of cinnarizine is similar to nifedipine but superior to tranilast (Table II).

Effect on A 23187-, Histamine- and LTD₄-Induced Vasculitis in Mouse Ear

The severity of vasculitis was expressed by the amount of extravasated dye. Time course for dye leakage and maximum amount of leaked dye in histamine- and LTD₄-induced responses were similar to those in PCA. The vasculitis caused by A 23187 was slightly more moderate than that by PCA as evidenced from the amount of extravasated dye. The dye leakage was initiated by the injection of 5 ng of A 23187 and reached a maximum by 100 ng of A 23187. Therefore, the experiments were performed using 100 ng of A 23187. Each of the examined drugs inhibited A 23187-induced vasculitis (Table III).

Tranilast showed the most potent inhibition whereas cinnarizine inhibited histamine-induced reaction but not LTD₄-induced vasculitis. Nife-

TABLE I. *Effect of Cinnarizine on 48 h Homologous Passive Cutaneous Anaphylaxis in Mice*

Route	Treatment	Dose (mg/kg)	N	Amount of dye ^{a)} (µg/a pair of ears)	Inhibition (%)
[A]	Control		10	13.0 ± 2.13	
	Cinnarizine	1	10	9.0 ± 2.20	30.8
		10	11	5.9 ± 1.31 ^{b)}	54.6
		20	11	3.0 ± 0.07 ^{b)}	76.9
[B]	Control		10	12.9 ± 1.76	
	Cinnarizine	1	10	6.9 ± 1.34 ^{b)}	46.5
		10	9	6.5 ± 1.08 ^{b)}	49.6
		20	10	6.1 ± 1.05 ^{b)}	52.7

Cinnarizine was administered [A] orally, 60 min and [B] i.p., 30 min prior to challenge. a) Each value represents the mean ± SE, b) p < 0.01.

TABLE II. *Effect of Cinnarizine, Nifedipine and Tranilast on 48 h Homologous Passive Cutaneous Anaphylaxis in Mice*

Treatment	Dose (mg/kg)	N	Amount of dye (μg / a pair of ears)	Inhibition (%)
Control		10	12.0 ± 0.10	
Cinnarizine	10	9	6.7 ± 0.37^b	44.2
	20	9	6.8 ± 0.58^b	43.7
Nifedipine	10	10	7.3 ± 1.13^b	39.2
	20	9	6.8 ± 0.59^b	43.3
Tranilast	20	8	13.7 ± 1.22	—
	50	8	9.7 ± 0.58^a	19.2
	100	8	5.5 ± 1.10^b	54.2

Each drug was administered i.p. 30 min prior to challenge with antigen. a) $p < 0.05$, b) $p < 0.01$.

TABLE III. *Effect of Cinnarizine, Nifedipine and Tranilast on Calcium Ionophore A 23187 [A]-, Histamine [B]- and Leukotriene D_4 [C]-Induced Vasculitis in Mice Ear*

Inducer	Treatment	Dose (mg/kg)	N	Amount of dye (μg / a pair of ears)	Inhibition (%)
[A]	Control		8	8.1 ± 0.82	
	Cinnarizine	10	9	7.0 ± 0.99	13.6
		20	9	5.7 ± 0.69^a	29.6
	Nifedipine	10	10	5.2 ± 0.92^a	35.8
		20	7	5.0 ± 1.18^a	38.3
	Tranilast	100	8	3.6 ± 0.59^b	55.6
[B]	Control		10	11.1 ± 1.70	
	Cinnarizine	1	10	10.4 ± 0.94	6.3
		10	10	7.4 ± 1.56^a	33.3
		20	9	5.0 ± 0.70^b	55.5
	Nifedipine	10	7	10.0 ± 2.10	9.9
		20	7	6.7 ± 1.53^a	39.6
	Tranilast	50	8	10.5 ± 1.63	5.4
		100	10	10.1 ± 1.47	9.0
[C]	Control		10	8.5 ± 1.11	
	Cinnarizine	1	10	7.5 ± 1.63	11.8
		10	10	8.0 ± 0.67	5.9
		20	10	7.5 ± 0.97	11.8
	Nifedipine	10	7	6.8 ± 1.06	20.0
		20	9	5.9 ± 0.70^a	30.6
	Tranilast	100	9	4.0 ± 0.53^b	52.9

Calcium ionophore A 23187 (10^{-5} g/ml), histamine (10^{-5} g/ml) or LTD₄ (10^{-6} g/ml) in a volume of 10 μl was injected into mouse ear 30 min before sacrifice. a) $p < 0.05$, b) $p < 0.01$.

dipine inhibited the increase of extravasated dye caused by both histamine and LTD₄ and tranilast inhibited LTD₄-induced reaction.

Effect on Histamine Release from Rat Peritoneal Mast Cells Caused by Antigen and IgE Antibody or A 23187

Cinnarizine had no significant effect on antigen- or A 23187-induced histamine release (Table IV).

Contrary to cinnarizine, nifedipine and tranilast inhibited the release of histamine by both inducers.

TABLE IV. *Effect of Cinnarizine, Nifedipine and Tranilast on Antigen [A]- or Calcium Ionophore A 23187 [B]-Induced Histamine Release from Rat Peritoneal Mast Cells*

Inducer	Treatment	Dose (mg/kg)	N	Amount of histamine (ng/ml)	Inhibition (%)
[A]	Control		5	1160 ± 82.4	
	Cinnarizine	10	5	1351 ± 127.3	—
		20	5	1272 ± 198.2	—
	Nifedipine	10	5	958 ± 26.4	17.4
		20	5	376 ± 80.1 ^{a)}	67.2
	Tranilast	50	5	1048 ± 39.0	9.7
		100	5	636 ± 36.8 ^{a)}	45.2
[B]	Control		9	2217 ± 33.8	
	Cinnarizine	10	10	2303 ± 133.6	—
		20	10	2168 ± 52.0	2.2
	Nifedipine	10	13	1736 ± 31.6 ^{a)}	23.5
		20	12	1517 ± 54.0 ^{a)}	34.2
	Tranilast	50	10	1929 ± 19.7 ^{a)}	13.0
		100	12	1920 ± 42.2 ^{a)}	13.4

Each drug was administered 1 h prior to harvesting the cells. a) $p < 0.01$.

DISCUSSION

The results presented show the efficacy of cinnarizine on IgE antibody mediated allergic reaction and its possible mechanism in mice and rats. In other experiments, we demonstrated the efficacy of cinnarizine in guinea pig IgE antibody mediated allergic reaction.¹⁰⁾ The efficacy of cinnarizine on IgE antibody mediated allergic reaction is further demonstrated in clinical investigations.¹¹⁻¹³⁾ However, there is limited basic data or references concerning the efficacy and mechanism of cinnarizine on IgE antibody mediated allergic reaction. The present data support the efficacy of cinnarizine on IgE antibody mediated vasculitis in mice. In addition, the data suggest the possible anti-allergic mechanism of cinnarizine. Cinnarizine inhibited the increase of capillary permeability caused by both A 23187 and histamine as well as antigen and IgE antibody mediated reaction. But cinnarizine had no effect on the release of histamine by antigen-IgE antibody and A 23187. These results are different from both nifedipine and tranilast. Nifedipine inhibited the increase of capillary permeability and histamine release caused by immunological and chemical stimulations. On the other hand, tranilast inhibited both vasculitis and histamine release caused by antigen-IgE antibody and A 23187. This suggests that the inhibitory action of cinnarizine on the allergic increase of capillary

permeability is mainly due to its antagonistic action to histamine. Moreover, the anti-allergic action of tranilast may interfere with the release of histamine and nifedipine may inhibit both reactions. The mechanism of IgE antibody mediated vasculitis can be divided into two stages. The first stage is the mediator release from mast cells subsequent to antigen antibody reaction. The second stage is tissue reaction (increase of capillary permeability) caused by released mediators. Calcium plays a role in both instance.¹⁴⁾ Whereas there are many reports¹⁻³⁾ to support the calcium antagonist action of cinnarizine in smooth muscle preparations, cinnarizine did not affect the release of histamine from mast cells. This evidence is very interesting since it suggests the existence of different types of calcium channels on mast cell from smooth muscle. Similar results were reported by Middleton *et al.* and others.¹⁵⁻¹⁷⁾ But calcium channels in mast cell membrane have not yet been characterized. More detailed studies on mast cell calcium channels are necessary. From the nifedipine results, the release of histamine was inhibited by the drug. Since it is well known that nifedipine shows clear calcium antagonist action in smooth muscle,¹⁸⁻²⁰⁾ its action on mast cells may result from action on different types of calcium channels than cinnarizine. Therefore, some characteristics of calcium channel which are affected

by the above two calcium antagonists may be different. From this viewpoint, cinnarizine and nifedipine are important tools to investigate the characteristics of calcium channels in mast cell.

In conclusion, cinnarizine shows a potent anti-allergic action in mice IgE antibody mediated reaction. Its action is mainly due to anti-histaminic action and not to inhibition of allergic mediator release.

REFERENCES

- 1) T. Godfraid and N. Morel: An upper limit to the number of calcium channels in smooth muscle as estimated with cinnarizine, *Eur. J. Pharmacol.*, **41**, 245–246 (1977).
- 2) T. Godfraid and A. Kaba: Blockade or removal of the contraction induced by calcium and adrenaline in depolarized arterial smooth muscle, *Br. J. Pharmac.*, **36**, 549–560 (1969).
- 3) F. Awouters, C. J. Niemagers and P. A. Janssen: Cinnarizine and flunarizine, potent inhibitors of anaphylactic shock in guinea pigs, *Arch. Int. Pharmacodyn. Ther.*, **217**, 38–43 (1975).
- 4) D.A. Lowe, and B.P. Richardson: Effect of cycloheptadine, ketotifen and sodium nitroprusside on mechanical activity and calcium uptake in guinea pig taenia coli *in vitro*, *Respiration*, **39**, 44–46 (1980).
- 5) H. J. Johnson and M. K. Bach: Prevention of calcium ionophore-induced release of histamine in rat mast cells by disodium cromoglycate, *J. Immunol.*, **114**, 514–516 (1975).
- 6) A. Ujiie, M. Kojima, J. Naito, M. Nakazawa and A. Koda: Effect of N-5' on histamine release from rat peritoneal exudate cells induced by calcium ionophore and ATP, *Jpn. J. Pharmacol.*, **34**, 9–14 (1984).
- 7) T. Tada and K. Okumura: Regulation of homocytotropic antibody formation in the rat. I. Feed-back regulation by passively administered antibody, *J. Immunol.*, **106**, 1002–1011 (1971).
- 8) N. Inagaki, S. Goto, H. Nagai and A. Koda: Pharmacological characterization of mouse ear PCA, *Int. Archs. Allergy Appl. Immunol.*, **78**, 113–117 (1985).
- 9) C.D. May, M. Lyman, R. Alberto and J. Cheng: Procedure for immunochemical study of histamine release from leucocytes with small volume of blood, *J. Allergy*, **46**, 12–20 (1970).
- 10) H. Nagai, H. Yamada, I. Yakuo and A. Koda: Effect of cinnarizine on IgE mediated experimental allergic reactions in guinea pigs, in preparation.
- 11) B. Zolov: Clinical evaluation of cinnarizine in various allergic disorders, *Ann. Allergy*, **19**, 1290–1294 (1961).
- 12) P. E. Siegler, T. Bodi, M. A. Gershenfeld, A. D. Brown, A. D. Ducanens and J. H. Nodine: Human bioassay of antihistaminic agent, *Ann. Allergy*, **20**, 37–44 (1962).
- 13) M. B. Emanuel, J. A. Chamlerlain, S. Witing and B. G. Bigden: Cinnarizine in the treatment of chronic asthma, *Br. J. Clin. Pharmac.*, **7**, 189–198 (1979).
- 14) E. B. Weiss and L. Babero: Calcium homeostasis in asthma, *Chest*, **5**, 834–535 (1983).
- 15) E. J. Middleton: Airway smooth muscle, asthma and calcium ions, *J. Allergy Clin. Immunol.*, **73**, 643–650 (1984).
- 16) D. O. Williams, P. J. Barnes, H. P. Vickers and M. Rudolf: Effect of nifedipine on bronchomotor tone and histamine reactivity in asthma, *Br. Med. J.*, **283**, 343 (1981).
- 17) E. R. Macfadden: Calcium channel blocking agents and asthma, *Int. Med.*, **95**, 232–233 (1981).
- 18) A. Fleckenstein: A specific pharmacology of calcium in myocardium, cardiac pacemakers and vascular smooth muscle, *Ann. Rev. Pharmacol. Tox.*, **17**, 49–66 (1977).
- 19) R. A. Janis and A. Scriabine: Site of action of calcium channel inhibitors, *Biochem. Pharmacol.*, **32**, 3499–3507 (1983).
- 20) A. Schwartz and D. J. Triggle: Cellular action of calcium channel blocking drugs, *Ann. Rev. Med.*, **35**, 325–340 (1984).