

We aim to clarify whether suplatast and azelastine (anti-allergic drugs) can shorten the half-life of immunoglobulin E (IgE) in the circulating blood. Thirty Wistar rats were divided into six groups. Distilled water or anti-allergic drugs were given orally for 6 days after the first sensitization. Two milligrams of monoclonal dinitrophenyl (DNP)-specific rat IgE was administered to the rats, which had been given suplatast or azelastine orally. The level of DNP-specific rat IgE in the serum was estimated by IgE-capture enzyme-linked immunosorbent assay, and the turnover of IgE was analyzed from its pharmacokinetic parameters. The elimination half-life of rat IgE was about 12 h irrespective of the sensitized state. The intercompartmental rate constants (K_{ct} and K_{tc}) in the suplatast-administered or azelastine-administered group were larger than those of the distilled water-administered group under non-sensitized conditions. These findings suggested that the anti-allergic drugs used in the present study facilitated the excretion of IgE from the circulation in rats.

Key words: Anti-allergic drugs, Turnover, IgE, Pharmacokinetics, Rat

Azelastine and suplatast shorten the distribution half-life of IgE in rats

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Introduction

Anti-allergic drugs have been used in the clinical field to control allergic reactions. Their clinical efficacy is based mainly on the inhibition of immunoglobulin E (IgE) production, the degranulation of target cells, and the migration of eosinophils, which were observed at the extravascular sites. The action of anti-allergic drugs is effective especially at the extravascular site of allergic reactions. It was predicted that IgE in the circulating blood played an important role to develop and continue the IgE-mediated inflammation at the extravascular site. Recently, we demonstrated that the short half-life of rat IgE in the circulation was attributable to the distribution of IgE from the intravascular to extravascular compartment.¹ Furthermore, it was expected that the alteration of IgE turnover in the circulation could lead to the modification of IgE-mediated inflammation at the extravascular site.

However, it has not yet been clarified whether anti-allergic drugs exert effects on the turnover of IgE in the circulation. In the present study, we tested whether the turnover of IgE was altered by the administration of anti-allergic drugs (suplatast and azelastine) in non-sensitized and sensitized rats by ovalbumin (OVA).

Materials and methods

Animal care and management

Wistar strain male rats weighing 250–300 g (Kyudo Co., Ltd., Kumamoto, Japan) were employed in the experiments. We followed the Standards Relating to the Care and Management of Experimental Animals (Notification No. 6, 27 March 1980, from the Prime Minister's Office, Tokyo, Japan) for the care and use of the animals, together with the guide for animal experiments issued by the University of the Ryukyus. All animal studies were reviewed and approved by the Animal Care Committee at the University of the Ryukyus.

Administration of anti-allergic drugs

Thirty Wistar rats were divided into six groups, each consisting of five rats. Distilled water or anti-allergic drugs were given orally for 6 days from the first sensitization in the sensitized animals. Sensitization of rats was performed as described previously.¹ Group A was given distilled water orally. Group B was given 100 mg/kg of suplatast orally. Group C was given 1 mg/kg of azelastine orally. Group D was sensitized with OVA and given distilled water orally. Group E was sensitized with OVA and given 100 mg/kg of

suplatast orally. Group F was sensitized with OVA and given 1 mg/kg of azelastine orally.

Administration of monoclonal dinitrophenyl-specific rat IgE and blood sampling

Two milligrams of monoclonal dinitrophenyl (DNP)-specific rat IgE was given intravenously to the sensitized rats at day 8 and to the normal rats via the jugular vein cannula. Blood samples (0.5 ml) were taken at 15 min, and 1, 3, 6, 12 and 24 h after the administration of IgE from the jugular vein cannula, and an identical volume of physiological saline was subsequently injected. The serum was kept at -20°C until determination of its IgE levels. The IgE-capture enzyme-linked immunosorbent assay for the estimation of rat IgE antibodies to DNP-*Ascaris suum* was performed as previously described.²

Drugs and reagents

Azelastine hydrochloride (Eisai, Tokyo, Japan) was dissolved in methanol and then further diluted with distilled water for oral administration. Azelastine is an anti-allergic drug that inhibits the leukotriene production, the release of histamine, and the migration and infiltration of granulocytes. Suplatast tosilate (Taiho Pharmaceutical Co., Tokyo, Japan) was dissolved in distilled water before use. Suplatast is another type of anti-allergic drug that inhibits the IgE production, the production of interleukin-4 and interleukin-5, and the infiltration of eosinophils. The 2,4-dinitrobenzene sulfonic acid sodium salt was purchased from Tokyo Kasei Inc., Ltd. (Tokyo, Japan). Killed *Bordetella pertussis* was purchased from Kaken Chemical Ltd. (Osaka, Japan). Albumin, chicken egg albumin (ovalbumin), and 2,2'-azino-di-[3-ethyl-benzthiazoline-6-sulfonic acid] were purchased from Sigma Chemical Co. (St Louis, MO, USA). Tween 20 and gelatin fine

powder were obtained from Nacalai Tesque (Kyoto, Japan), and hydrogen peroxide from Santoku Chemical Ind. (Miyagi, Japan). Amino-hexanoyl-biotin-*N*-hydroxysuccinimide, peroxidase-conjugated streptavidin and mouse anti-rat myeloma IgE (MARE-1) was obtained from Zymed Laboratories (San Francisco, CA, USA). Unless otherwise stated, all other chemicals were of reagent grade.

Analysis

Calculation of kinetic parameters was performed as previously described.¹ Statistical analysis was performed by the unpaired Student's *t*-test for between-group comparisons. Data are expressed as mean \pm standard deviation (SD). When $p < 0.05$, the means were considered to be significantly different.

Results and discussion

The level of monoclonal DNP-specific rat IgE in the serum underwent a rapid decrease between 15 min and 3 h, and then declined slowly between 6 and 24 h following intravenous (i.v.) administration. The level of monoclonal DNP-specific rat IgE in the suplatast-administered or azelastine-administered group were lower than those of the distilled water-administered group under non-sensitized conditions at 1 h after administration (groups B and C versus group A, $p < 0.05$) (Fig. 1). The IgE levels at approximately 15 min–3 h and 6–24 h, respectively, displayed broadly linear decreases on the semi-logarithmic graphs. The turnover of monoclonal DNP-specific rat IgE was therefore analyzed employing a two-compartment model for the pharmacokinetics.

The pharmacokinetic parameters of monoclonal DNP-specific rat IgE are summarized in Table 1. The coefficient α in the suplatast-administered or azelas-

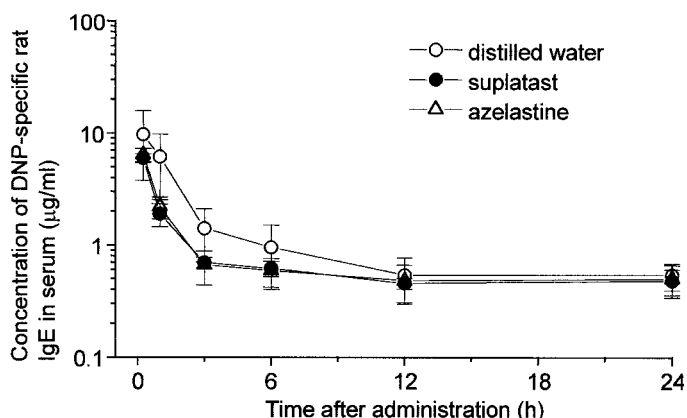


FIG. 1. Serum concentration-time curves for monoclonal DNP-specific rat IgE in non-sensitized rats following i.v. administration. Two milligrams of monoclonal DNP-specific rat IgE were administered to rats via the jugular vein. \circ , Distilled water-administered groups (group A); \bullet , suplatast-administered groups (group B); \triangle , azelastine-administered groups (group C). Data are expressed as mean \pm SD ($n = 5$).

Table 1. Pharmacokinetic parameters for monoclonal dinitrophenyl (DNP)-specific rat immunoglobulin E (IgE) administered to non-sensitized and sensitized rats

Parameter	Distilled water		Suplatast (100 mg/kg)		Azelastine (1 mg/ml)	
	Group A	Group D	Group B	Group E	Group C	Group F
α (h^{-1})	2.1 ± 0.85	1.8 ± 0.26	7.3 ± 2.8**	2.9 ± 1.3	6.4 ± 2.8*	2.9 ± 1.2
β (h^{-1})	0.076 ± 0.053	0.063 ± 0.020	0.065 ± 0.047	0.062 ± 0.043	0.055 ± 0.039	0.046 ± 0.010
K_{el} (h^{-1})	0.49 ± 0.22	0.52 ± 0.28	0.53 ± 0.31	0.40 ± 0.37	0.58 ± 0.50	0.36 ± 0.13
K_{tc} (h^{-1})	0.27 ± 0.05	0.26 ± 0.14	0.90 ± 0.51*	0.42 ± 0.31	0.92 ± 0.63*	0.38 ± 0.15
K_{ct} (h^{-1})	1.45 ± 0.85	1.63 ± 0.82	5.88 ± 2.17**	2.16 ± 0.86	4.97 ± 2.24*	2.81 ± 1.20
V_1 (ml/kg)	567 ± 285	754 ± 692	1068 ± 357*	953 ± 487	993 ± 225*	706 ± 222
V_2 (ml/kg)	3470 ± 2750	3381 ± 899	7313 ± 1768*	6534 ± 5526	6789 ± 3409	5237 ± 1452*
$t_{1/2\alpha}$ (h)	0.35 ± 0.12	0.40 ± 0.06	0.11 ± 0.04**	0.27 ± 0.09*	0.12 ± 0.04**	0.23 ± 0.08*
$t_{1/2\beta}$ (h)	13.1 ± 5.7	12.0 ± 3.9	16.0 ± 10.8	18.4 ± 15.1	22.2 ± 19.9	15.5 ± 3.01

Two milligrams of monoclonal DNP-specific rat IgE were administered to rats via the jugular vein. Groups A, B, and C are data from non-sensitized rats; groups D, E, and F are from the sensitized rats by ovalbumin (see text). Data are expressed as mean ± SD ($n = 5$). * $p < 0.05$, ** $p < 0.01$ (versus distilled water-administered group). See text for definitions of parameters.

tine-administered group was larger than that of the distilled water-administered group in the non-sensitized state (group B versus group A, $p < 0.01$; group C versus group A, $p < 0.05$). The values for the distribution half-life ($t_{1/2\alpha}$) in the suplatast-administered or azelastine-administered group were smaller than those of the distilled water-administered group irrespective of the sensitized state (groups B and C versus group A, $p < 0.01$; groups E and F versus group D, $p < 0.05$). The intercompartmental rate constants (K_{ct} and K_{tc}) in the suplatast-administered or azelastine-administered group were larger than those of the distilled water-administered group in the non-sensitized state (K_{tc} , groups B and C versus group A, $p < 0.05$; K_{ct} , group B versus group A, $p < 0.01$; group C versus group A, $p < 0.05$). The value of the distribution volume of the central compartment (V_1) in the suplatast-administered or azelastine-administered group was larger than that of the distilled water-administered group under non-sensitized conditions ($p < 0.05$). The value of the distribution volume of the tissue compartment (V_2) in the suplatast-administered or azelastine-administered group was larger than that of the distilled water-administered group under non-sensitized conditions ($p < 0.05$). The elimination half-life ($t_{1/2\beta}$) of monoclonal DNP-specific rat IgE was about 12 h following i.v. administration in rats irrespective of the sensitized state. The values for the elimination half-life ($t_{1/2\beta}$) in the anti-allergic drug-administered groups did not differ from those of the distilled water-administered group, irrespective of the sensitized state.

The half-life of rat IgE in the circulating blood was reported by Tada *et al.*³ to be about 12 h. In agreement, the elimination half-life ($t_{1/2\beta}$) of monoclonal DNP-specific rat IgE was found to be about 12 h following i.v. administration in rats irrespective of the sensitized state.¹

In the present study, we demonstrated that the levels of monoclonal DNP-specific rat IgE underwent a rapid decrease in the suplatast-administered or azelastine-administered group in comparison with those of the distilled water-administered group under non-sensitized conditions at 15 min–3 h following administration of IgE. Pharmacokinetic analysis revealed that the values of the distribution half-life ($t_{1/2\alpha}$) in the suplatast-administered or azelastine-administered group were smaller than those of the distilled water-administered group irrespective of the sensitized state, suggesting that the anti-allergic drugs used can facilitate the excretion of IgE from the circulation. The intercompartmental rate constants (K_{ct} and K_{tc}) in the suplatast-administered or azelastine-administered group were larger than those of the distilled water-administered group in the non-sensitized state. The values of the distribution volume of the tissue compartment (V_2) in the suplatast-administered or azelastine-administered group were larger than those of the distilled water-administered group under sensitized or non-sensitized conditions. However, it remains to be clarified in which tissue the distribution of IgE was increased.

Suplatast is known to inhibit the production of IgE, the induction and degranulation of mast cells, and the migration of eosinophils.^{4,5} In addition, azelastine is known to inhibit the release of chemical mediators from mast cells and to decrease vascular permeability.⁶ On the contrary, Tada *et al.*³ suggested in their report that a significant part of the injected IgE might have been fixed to tissue mast cells with a resultant rapid decrease in IgE level in the circulation as compared with that of immunoglobulin G. Therefore, suplatast and azelastine might alter the turnover of IgE in the circulation in part due to action on the target cells in the tissue and any direct effects on the endothelial cells.

We conclude from the present study that the clinical efficacy of the anti-allergic drugs may be based not only on their well-known effects, but also on a facilitation of the excretion of IgE from the circulation. We propose the new concept that allergic reactions can be controlled through alternations in the turnover of IgE elicited by anti-allergic drugs.

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