Azelastine hydrochloride suppresses histamine-enhanced permeability of rat IgE across rat endothelial cells

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Falcon Cell culture insert®を使用して、ラット内皮細胞のIgE透過性に対する抗アレルギー剤の効果を検討した。内皮細胞を通じて通過するIgEの透過係数は、クリアランス速度を基本として算出した。ヒスタミン（1×10⁻⁶M）に24時間曝露した内皮細胞のIgE透過係数は、ヒスタミン非存在下に比して著しく上昇した。ヒスタミンとazelastine hydrochloride（1×10⁻⁶M）を同時に曝露したIgEの透過係数は、ヒスタミンのみの曝露に比較して著しく減少した（p<0.05）。ヒスタミンによって亢進したIgE透過性は、suplatast tosilateとketotifen fumarateでは抑制されなかった。しかしながら、azelastine hydrochlorideは内皮細胞のヒスタミンによるIgE透過性亢進を抑制する効果を示した。これらの結果から、IgEの血管透過性制御作用を強化した抗アレルギー剤は、アレルギー反応の制御という観点からは有効な薬剤である。

The IgE which exists in the tissue is produced in the tissue and/or distributed from the intravascular to extravascular portion. It was inferred therefore that the magnitude of the allergic reaction in the tissue may be accorded to the degree of IgE passage through the vascular wall. Concerning the regulatory mechanisms of IgE passage from the circulation to the tissue, one possibility is that the IgE passage to the tissue could be related to the interactions of IgE with the IgE-receptor bearing cells and/or to their regulatory effects on the barrier function of the endothelial cells. The endothelial cells might play an important role in the regulatory mechanisms of IgE passage from the circulating blood to the tissue. IgE passage through the endothelial cells could increase under pathophysiological conditions such as the release of histamine from mast cells and allergic reaction in the tissue. Our previous study indicated that permeability of rat IgE was enhanced by histamine on rat aortic endothelial cell.

Antiallergic drugs have been used in the clinical field to control allergic reactions. Their clinical efficacy is based mainly on inhibition, of the production of IgE, degranulation of target cells and migration of eosinophils. Apart from the well-known action of antiallergic drugs, they could modify the IgE passage from the intravascular to extravascular por-
tion. However, no report has yet been established whether or not antiallergic drugs do in fact exert effects on the IgE passage from the intravascular to the extravascular tissue. In the present study, we attempted to clarify whether or not the antiallergic drugs (IPD®, azeptin®, and zaditen®) inhibits the increased permeability of rat IgE across cultured rat endothelial cells exposed to histamine.

Materials and Methods

(1) Animals

Wistar strain male rats weighing 250 to 300 g were employed for the experiments. The rats were purchased from Kyudo Co., Ltd. (Kumamoto, Japan).

(2) Animal care and management

We followed the Standards Relating to the Care and Management of Experimental Animals (Notification No. 6, March 27, 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.

(3) Reagents

2, 4-Dinitrobenzene sulfonic acid sodium salt was purchased from Tokyo Kasei Inc., Ltd. (Tokyo, Japan). Nutrient mixture F-12 HAM, Kaighn’s modification (HAM’s F-12K), collagenase type XI (cell culture grade), and 2, 2’-azino-di-[3-ethyl-benzthiazoline-6-sulfonic acid] (ABTS) 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). Aminohexanoyl-biotin-N-hydroxysuccinimide, and peroxidase conjugated streptavidin were obtained from Zymed Laboratories (San Francisco, Calif., USA). Falcon cell culture insert® (diameter : 6 mm, pore size : 3 microns, pore density : $8.0 \times 10^5$ cm$^{-2}$) and its companion plate were purchased from Becton Dickinson and Company (Franklin Lakes, NJ, USA). Fetal bovine serum (FBS) was obtained from Sanko Junyaku (Fukuoka, Japan), and bovine pulmonary artery endothelial cells (CPA47) from the American Type Culture Collection (ATCC). Type I collagen was obtained from Collaborative Biomedical Products (Bedford, MA, USA). Endothelial cell growth supplement (ECG) and Di-I-Ac–LDL were obtained from Harbor Bio-Products (Norwood, MA, USA). Unless otherwise stated, all other chemicals were of reagent grade.

(4) Drugs

Azelastrine hydrochloride (azeptin®, Eisai, Tokyo, Japan), suplatast tosilate (IPD®-1151T, Taiho Pharmaceutical Co., Tokyo, Japan) and ketotifen fumarate (zaditen®, Novartis, Basel, Switzerland) were dissolved in distilled water before use.

(5) Isolation of rat aortic endothelial cells by a primary explant technique and culture of endothelial cells

Isolation of rat aortic endothelial cells was performed according to the method of McGuire and Orkin$^{12}$). The cells obtained were identified as endothelial cells from their uptake of Di-I-Ac–LDL. They were subsequently incubated in the presence of $10 \mu$g/ml of Di-I-Ac–LDL for 4 h at 37°C. Finally, the medium was removed and the cells were washed three times with PBS. The LDL particles were visualized by fluorescence microscopy (Nikon, Tokyo, Japan) with filters adjusted for rhodamine fluorescence.

(6) Chamber preparation

Polyethylene terephthalate track-etched
membranes of the Falcon cell culture insert (upper chamber) were coated with type I collagen from rat tail. Such upper chambers were placed into each well of a 24-well plate (lower chamber). The cells were removed from the tissue flask by treatment with 0.25% collagenase and centrifuged at 1500 rpm for 5 min. The cell pellet was resuspended in complete culture medium and 0.43 ml containing $1 \times 10^5$ cells was seeded onto each collagen-coated membrane. Studies were performed at 72 to 96 h after seeding. Monolayer confluence on the membranes was evaluated by light microscopy.

(7) Preparation of monoclonal DNP-specific rat IgE
The procedure employed for the preparation of monoclonal DNP-specific rat IgE was as described previously.13

(8) IgE-capture ELISA for estimation of monoclonal DNP-specific rat IgE
The IgE-capture ELISA for estimating rat IgE antibodies to DNP-As was performed as described previously.13

(9) Experimental protocol for the IgE passage through the endothelial cell monolayer
0.43 ml of serum free medium containing monoclonal DNP-specific rat IgE was added to the upper chamber and 1 ml of serum free medium was added to the lower chamber. 30 µl samples from the upper chamber were taken and the equal volume of medium was added at different time point (1 h~3 h). 1 ml samples from the lower chamber were taken and the same volume of medium was added to the lower chamber at each time point (1 h~3 h). The IgE in the medium of the upper and lower chambers was determined by IgE-capture ELISA.

(10) Effect of antiallergic drugs on the IgE passage through endothelial cells exposed to histamine
Serum free medium containing histamine and antiallergic drugs at different concentrations was added to the rat endothelial cell monolayer into the upper chamber. The reaction time was 24 h. The degree of passage of IgE through the endothelial cells was evaluated following the addition of histamine and antiallergic drugs.

(11) Data analysis
The degree of IgE passage through the endothelial cells was expressed as the upper chamber volume which was cleared IgE through the endothelial cell monolayer according to the method of Cooper et al. 14 The clearance volume of IgE at different time point ($V_{IgE(t)}$) was calculated by summing the incremental clearance volumes up to that time point:

$$V_{IgE(t)}=\Sigma(V_{L(t)} \cdot \Delta [L]/[U])$$

where $V_{L(t)}$ is the volume of the lower chamber at different time point, $\Delta [L]$ is the increase in concentration of IgE between time points, and $[U]$ is the concentration of IgE in the upper chamber at different time point. The change in $V_{IgE}$ over time ($dV_{IgE}/dt$), equal to the clearance rate in microliters per minute, was determined by weighted least-squares nonlinear regression for the experimental period. Furthermore, if ($PS \cdot (V_L + V_U)/(V_L \cdot V_U)) \cdot t < 0.1$, then with an error of <5%, $dV_{IgE}/dt =PS$ where $P$ is the permeability constant (PC), $S$ is the surface area, and $V_U$ is the volume of the upper chamber. The total permeability of the system with the endothelial monolayer ($P_{total}$) can be related to the permeabilities of the endothelial monolayer alone ($P_{ee}$), the permeability of the system with the collagen-coated membrane devoid of endothelial cells ($P_{filt}$) by

$$1/P_{total} = 1/P_{ee} + 1/P_{filt}$$
Thus permeability of the endothelial cell to protein independent of the collagen-coated membrane and unstirred layers was calculated.

(12) Statistical analysis
Statistical analysis was carried out by the unpaired Student's t-test for between-group comparisons. Data are expressed as the means ± standard deviation (SD). When a p-value of less than 0.05 was obtained, the means were considered to be significantly different.

Results
(1) Cellular characterization
Cells which were translated from the tissue culture dish to the culture flask exhibited a "cobblestone" morphology (Fig. 1). Cultures of rat aortic endothelial cells were also treated with the metabolic probe, acetylated low density lipoprotein (Di-I-Ac-LDL), which has been used as a probe to identify endothelial cells. When incubated in the presence of Di-I-Ac-LDL, cells from the rat aortic explants demonstrated fluorescent deposits throughout their cytoplasm and the fluorescent deposits were similar to that of bovine pulmonary endothelial cells (Fig. 2).

(2) Correlation of clearance volume and reaction time of IgE
Continuous lines formed the weighted least-squares regression curves for the reaction time of IgE irrespective of treatment with antiallergic drugs or histamine (Fig 3 a, b, c). The slope...
Fig. 3 Clearance volume–reaction time curves for rat IgE

The clearance volume–reaction time curves for rat IgE were examined after treatment of rat endothelial cells with histamine (10^-10 M) and/or antiallergic drugs at the different concentrations for 24 h. 0.43 ml of IgE was added to the upper chamber at the start of the assay. The concentration of IgE in the upper and lower chambers were respectively determined at different time points. The clearance volume was calculated as described under Materials and Methods. (a) azelastine hydrochloride. (b) suplatast tosilate. (c) ketotifen fumarate. Data are expressed as the means±SD (n=5).
Fig. 4  Effect of antiallergic drugs on the permeability of rat IgE across rat endothelial cells exposed to histamine

Serum free medium containing histamine (10^{-10} M) and/or antiallergic drugs at the different concentration was added to the rat endothelial monolayer in the upper chamber. The reaction time was 24 h. The permeability constants were calculated on the basis of the clearance rate as described under Materials and Methods. *p<0.05 (vs. the case of histamine exposure). Data are expressed as the means±SD (n=5).

The regulatory mechanism on the barrier function of rat endothelial cells remains to be elucidated. However, the barrier function of endothelial cells against albumin has been reported to be enhanced by a decrease of [Ca^{++}]_{i} in human umbilical vein endothelial cells (HUVECs)\textsuperscript{14-16}, and by an increase of cyclic AMP in bovine aortic endothelial cells (BAECs)\textsuperscript{14,16,18}. It has been known that sulplastas tosilate did not modulate Ca^{++} influx and the IP3 pathway\textsuperscript{6}. In contrast, it has been found that azelastine hydrochloride increased the cyclic AMP levels in macrophages\textsuperscript{19} and decreased the concentration of [Ca^{++}]_{i} in mast cells\textsuperscript{20}. The inhibition of azelastine hydrochloride on the permeability of IgE across endothelial cells
elial cells exposed to histamine, alternatively the enhancement of the barrier function by azelastine hydrochloride could be responsible for the decrease of Ca++ influx and/or the increase of cyclic AMP in rat endothelial cells. We conclude from our present data that the clinical efficacy of azelastine hydrochloride may be based not only on the previously described effects but also on an inhibition on the IgE passage through endothelial cells. We propose a new concept whereby allergic reactions can be controlled through inhibition of the IgE passage through endothelial cells.

References


