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Preproendothelin mRNA Expression, Especially After Allergen Exposure in the Epithelium of the Human Nasal Mucosa

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ABSTRACT

It is known that pre-proendothelin (pre-proET) is a precursor of ET, and that first it is locally broken down to big-ET-1 and further becomes ET-1 by means of endothelin converting enzyme. Investigations of pre-proET in epithelial cells were conducted for the purpose of studying its production, existence, and role in epithelial cells. Pre-proET mRNA was expressed in the nasal mucosal epithelial cells of all normal subjects, chronic sinusitis subjects, and allergic rhinitis subjects. Expression was observed in cultured nasal mucosal epithelial cells also. In combination with previous reports, it is suggested that material is produced in the pre-proET state in the nasal mucosa epithelial cells, that this becomes ET locally, and that it is being released within the cultured supernatant or within the nasal discharge. Furthermore, pre-proET mRNA expression was enhanced in the nasal mucosa epithelial layers as a result of antigen induction reactions as a result of house dust perennial nasal allergies. The degree of this enhancement correlated to the degree of antigen induction reaction. It was thought that pre-proET increased in response to antigen exposure in allergic nasal mucosa, and that it was modifying the allergic reaction. Investigation of the mechanism of pre-proET expression enhancement is needed in the future.

Keyword

Endothelin, Allergic rhinitis, Nasal challenge, mRNA, RT-PCR.

Introduction

The endothelins (ETs) are peptides, which is strong vasoconstrictor and branchoconstrictor. It is composed of 21 amino acid peptide (MW 2490), with the structure of which includes four cytokine residues and two internal disulfate bonds1). Mullol J et al. reported ET-1 and its receptor existence in human nasal mucosa [1,2]. ET-1 immunoreactivity was seen in the submucosal glands, the epithelium, the endothelium of the vein, and myoepithelial cells of small arteries in the nasal mucosa [2]. ET-1 and ET-2 have possible role on nasal secretion via glandular reflex; ET-3 does not by *in vitro* culture experiment [3]. The endothelium of veins and the myoepithelial cells of small arteries may have role on the control of vasomotor tone in the nasal mucosa. We also cleared the high intensity of ET-1 in the epithelium, and release of ET-1 from cultured epithelial cells from allergic nasal mucosa and nasal polyp [3,4]. Some reports on the physiological role of epithelial ETs in the airway [2]. Tamaoki et al. reported that ET-1 in the epithelium correlates with cilially beat frequency in the trachea by *in vitro* study [5]. That study, however, is not focused on correlation of specific airway disease and ET-1 existence. Mullol et al reported cytokine induction in the human bronchial epithelial cell lines by ET-1 [6]. Those studies indicate that ETs in the airway epithelium have some role on the pathophisiology in the nose.

It is known that pre-proendothelin (pre-proET) is a precursor of ET, and that first it is locally broken down to big-ET-1 and further becomes ET-1 by means of endothelin converting enzyme [7]. Investigations of pre-proET in epithelial cells were conducted for the purpose of studying its production, existence, and role in epithelial cells. This time it was investigated whether pre-proET mRNA is being expressed in epithelial cells and the m-RNA expression in allergic or inflamed nasal mucosa scrapings, excised nasal mucosa, and cultured nasal mucosa epithelial cells using RT-

PCR methods. Further, whether this mRNA expression is enhanced in the nasal mucosa epithelial layers after an antigenic induced reaction in allergic rhinitis sufferers was investigated.

Subjects and Methods

Subjects

Twenty-five subjects were engaged in this study. 21 subjects were assessed for house-dust (HD) perennial allergic rhinitis with severe nasal obstruction via screening interviews, rhinoscopy, X-ray examinations, and allergy testing (examination of the eosinophil count in nasal discharge, blood serum total IgE, blood serum specific IgE, skin tests, nasal induction tests) by Japanese practical guideline for management of allergic rhinitis [8].

Operation and Scraping

These patients with severe nasal obstruction were operated. From these 21 subjects, scrapings of the mucosal epithelial layer and thin slices of the lamina propria mucosa were carried out for nasal mucosal tissue surgically excised. The patients had not had a recent infection. At the time of surgery, 2% xylocaine and 0.01% epinephrine were applied topically with nasal packs and the turbinates were injected 2-4 ml of 1% xylocaine and 0.002% epinephrine. An incision was made from the lateral wall of the inferior turbinate at the level of the infundibulum through the inferior conchal bone, and then inferiorly along the medial aspect of that bone. The medial flap of the turbinate remnant was wrapped superiorly and laterally to close the wound. Within 20 min of surgical excision, the resected nasal mucosa was dissected from the inferior conchal bone. Mucosal specimens were scraped with a small surgical curette in order to collect the epithelial layers. And after scraped out of the epithelial layer, the lamina propria of nasal mucosa was dissected to small piece by scissor, then homogenate and centrifuged to collect the mRNA of nasal lamina propria. The surgically excised nasal mucosa tissues from same two cases were cultured monolayer epithelium *in vitro* using the method of Ohnishi et al. [4].

The Cultured Nasal Mucosa Epithelial Cells Were Used as Samples

Five patients without any provocation, the nasal mucosal specimens were scraped with a small surgical curette in order to collect the epithelial layers. These specimens were also used as samples. Two subjects were assessed as normal via the same interviews, the X-ray examinations noted above, and allergy testing. The mucosa epithelial layers of these two normal cases and the mucosa epithelial layers of nasal mucosa tissue from two cases of subjects with chronic infective sinusitis also obtained by same method were used as samples for the purpose of prepro-ET mRNA detection. The nasal polyp from one patient with chronic infective sinusitis mentioned above was obtained by surgery. This polyp was also cultured using same culture method [4]. This cultured nasal polyp epithelium was used as samples.

Collection Mucosal Epithelial Layer of Allergic Rhinitis after Antigen Stimulation

The induction was carried out by means of a control disc provocation (two discs on the right side) in rest 14 patients aged from 18 to 54 (39.4 ± 11.4) assessed as having house dust perennial allergic rhinitis via interviews, rhinoscopy, and allergy testing including nasal provocation test by also Japanese practical guideline for management of allergic rhinitis, and it was confirmed that the control induced responses were negative. At this time, the part of the right inferior turbinate mucosa adhering to the control disk was scraped with a surgical curette, and control mucosa epithelial layer cells were obtained. After this, two disks for inducing house dust mite (PNU) were placed on the left inferior turbinate mucosa, and induction was carried out for 15 minutes. After this, the same nasal epithelial layer cells were secured as controls.

The provocation reactivity of nasal allergy was assigned based on the severity of each symptom in accordance with the Severity Classification of the Clinical Practice Guideline for Nasal Allergy. See Table 1: Severe (3 plus), moderate (2 plus), and mild (1 plus) [9].

All of the test samples at the time of collection were samples in which permission was granted for use in nasal mucosa tissue testing. The study was designed and monitored in accordance with the principles of Good Clinical Practice, as set out in the Declaration of Helsinki and its amendments. Written informed consent was obtained from all study participants. Ethical approval was obtained from the institutional review board of Nippon Medical School H16-15-49.

RT-PCR of pre-proET mRNA

The mRNA from these collected samples was homogenized using TrisolvTM and extracted via the guanidine method. For PCR, one cycle was denaturation at 94°C 1 min, annealing at 60°C 2 min, and extension at 72°C 3 min, and the number of amplifications was 30. The amplified product was observed to be approximately 600 bp. For primer, sense: 5'-CTGCTGTTTGTGGCTTGCCAAGGA-3' and antisense: 5'-TCGGTTGTGGGTCACATAACGCTC-3' were used. Further, the density of β-actin for each band was compared using a densitometry.

Statistics

All analyses were conducted Two-way RM ANOVA using SAS software version 9.3.

Results

As in Figure 1, the same message as cultured epithelial cells was seen at 600 bp for the pre-proET mRNA of all nasal allergy patients, chronic sinusitis patients, and normal subjects. The pre-proET mRNA / β-actin mRNA ratio was 54.7 ± 24.8% for the mucosa epithelial scrapings of nasal allergy subjects (n=7), 45.0 ± 1.4% for the concha nasalis inferior lamina propria mucosa of nasal allergy subjects (n=2), 28.3 ± 25.3% for the nasal mucosa epithelial scrapings of chronic sinusitis subjects (n=2), 36.9 ± 19.6% for the nasal mucosa epithelial scrapings of normal subjects (n=2), and 74.0 ± 24.0% for cultured epithelial cells from allergy mucosa and polyp mucosa (n=3).

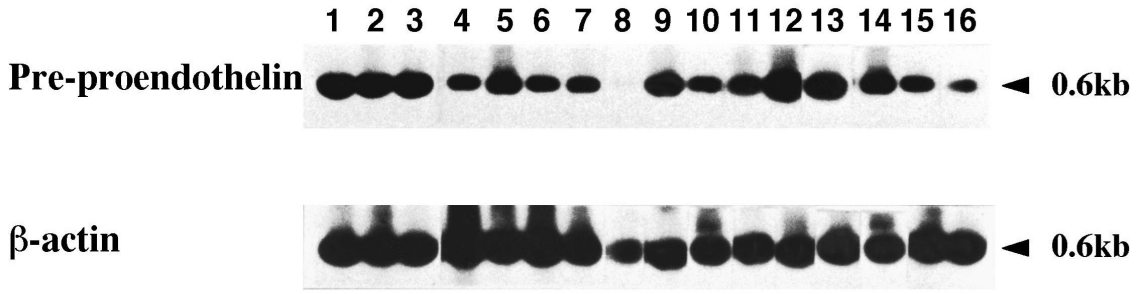

Figure 1: mRNA expression for pre-proET in nasal mucosa and its cultured cells.

Lane 1,3, 5-9 shows the mRNA expression for pre-proET of allergic nasal mucosal surface by scraping. Lane 2 and 4 shows the mRNA expression for pre-proET of allergic nasal mucosal lamina propria from surgical procedure. Lane 10 and 11 shows the mRNA expression for pre-proET of sinusitis mucosa from surgical procedure. Lane 14 shows cultured nasal epithelial cells. Lane 15 and 16 hows the mRNA expression for pre-proET of normal nasal mucosal surface by scraping for control.

The pre-proET band was seen at 600 bp in allergic nasal mucosa epithelial layers irrespective of whether before or after induction. Pre-proET expression in nasal mucosa before and after antigen induction was assessed as a percentage of β-actin mRNA expression. The pre-proET expression before induction was 21.4 ± 2.37% and after induction was 35.8 ± 4.48% (Figure 2). The respective β-actin mRNA expression was assessed as the ratio of pre-proET expression after induction/expression before induction, and it was 1.93 ± 0.47 units. Though there was a difference in before and after expression in each subject, the degree of this difference correlated with the degree of antigen induction reaction according to the classifications of Practical Guideline Management of Allergic Rhinitis [8]. In Table 1, the respective backgrounds of cases as well as the antigen induction reaction and nasal mucosa condition are tabulated. Pre-proET mRNA expression did not correlate to any factor other than the degree of this induction reaction.

14 patients were challenged dust-mite allergen. Figure were shown each patient's nasal surface mRNA expression for pre-proET before and after nasal antigen challenge.

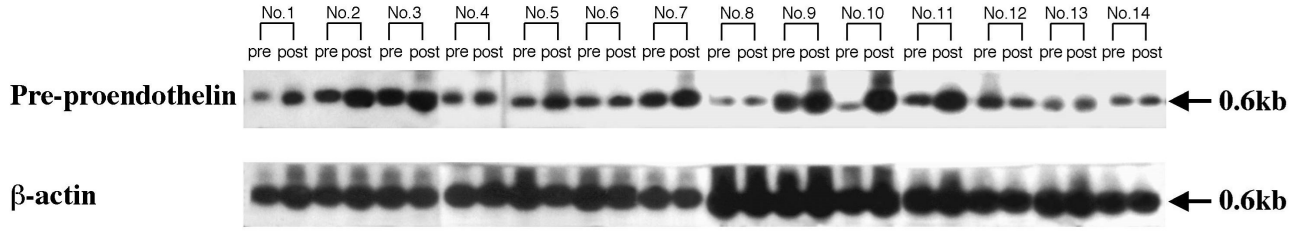

Figure 2: mRNA expression for pre-proET in nasal scrapings from perennial allergic rhinitis patients before and after antigen challenge.

Table 1: Patient Characteristics and Immunological Response to Antigen Challenge.

Patient No	Inferior turbinate swelling	With asthma	Mite specific IgE	Reactivity of Ag challenge	Pre-challenging expression	Post-challenging expression	Unit (post/pre)
1	++	+	>100	+++	19.7	30.1	1.53
2	++	+	>100	+	27.5	54.2	1.97
3	-	+	>100	++	24.4	42.8	1.75
4	+++	-	51	+++	15.6	25.8	1.65
5	+	-	>100	+	19.2	22.8	1.19
6	+	-	96	+	10.6	14.1	1.33
7	+++	-	>100	++	41.8	62.2	1.48
8	++	+	>100	+	11.6	12.1	1.04
9	+	-	2.8	++	28.8	45.2	1.58
10	+++	-	16	+++	6.8	55.2	8.12
11	++	-	11	++	26.3	62.0	2.36
12	++	-	69	++	21.3	22.6	1.07
13	+++	-	66	+	28.2	26.2	0.93
14	+++	-	>100	+	25.1	25.8	1.03

Discussion

Endothelin (ET) is thought to be an important mediator in inflammatory reactions of the lower respiratory tract [10,11], and its role in the upper respiratory tract including the nasal mucosa is not still all known [12]. In looking at ET localization in the nasal mucosa by the immune system last time, it was thought ET it was involved in the activation of epithelial cells and nasal discharge including ciliary movement [5] and cytokine induction [11], but on the other hand, in ET in-situ hybridization, there was mRNA only in vascular endothelial cells, and regulation of vascular permeability was suggested [3]. Based on the fact that signals have not been observed in the epithelial cells by in-situ hybridization analysis, this time the production in nasal mucosa epithelial cells of pre-proET was studied. Though pre-proET mRNA was confirmed, it was known previously that ET is released in cultured nasal mucosa epithelial cells (ref), and conversion from pre-proET to ET in those locations in cultured nasal mucosa epithelia is suggested. In addition, in allergic nasal mucosa epithelial cells, a trend of greater pre-proET expression in the nasal mucosa epithelial cells of normal subjects than in sinusitis subjects was observed. Further, the autonomous production in epithelial cells brought about due to an allergic reaction of the nose was made clear in regard to the production in epithelial cells after antigen induction of pre-proET-1, and it was thought there was some role in the immediate phase of the allergic reaction of the nose. In every antigen induction of the lower respiratory system, the ET-1 level within the respiratory tract ravage after induction increased for 10 minutes after induction, and it has been reported that this increase was not observed in groups using local steroid medication [13,14]. Nasal allergy subjects produced the presence or absence of antigen notwithstanding, pre-proET, but this production due to an antigen stimulus was increasing in response to the reaction to that antigen. The results of this study and reported literature on the lower respiratory tract suggest that in an allergic reaction in the respiratory tract mucosa, ET can indeed be produced in the reaction process in question. In addition, the positively of nasal challenge reflects the symptoms of the immediate phase of the allergic reaction, and there is a strong possibility that ET is involved in any of the sneezing, nasal discharge, or nasal mucosa swelling or multiple reactions brought about in the immediate phase in the upper respiratory tract.

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