Association between a TGFβ1 promoter polymorphism and the phenotype of aspirin-intolerant chronic urticaria in a Korean population

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SUMMARY

Background: Chronic urticaria/angioedema is a common phenotype in patients with aspirin sensitivity; however, its genetic mechanism is not understood. Transforming growth factor (TGF)β1 is a key regulatory cytokine involved in allergic inflammation.

Objective: We examined the association of a TGFβ1 genetic polymorphism with aspirin-intolerant chronic urticaria (AICU) and aspirin-tolerant chronic urticaria (ATCU) in a Korean population.

Methods: A promoter polymorphism in the TGFβ1 gene, TGFβ1 -509C>T, was analysed in 112 AICU patients, 153 ATCU patients and 457 normal controls (NC), and the frequency was compared among the groups. Serum TGFβ1 levels were measured by ELISA.

Results: The minor allele frequency of the -509C>T polymorphism was significantly higher in patients with AICU compared with the other two groups (P < 0.02 for AICU vs. NC; P < 0.05 for AICU vs. ATCU). Among the AICU patients, those with the T allele tended to have lower serum TGFβ1 levels.

Conclusion: These findings suggest that the -509C>T polymorphism in the TGFβ1 promoter may contribute to the development of the AICU phenotype.

Keywords: aspirin hypersensitivity, chronic urticaria, genetic polymorphism, transforming growth factor β1

INTRODUCTION

Aspirin ingestion can induce several allergic reactions, including aspirin-intolerant asthma (AIA), aspirin-induced urticaria/angioedema (AIU), anaphylaxis, and rarely, hypersensitivity pneumonitis (1–3). Among these, urticaria/angioedema is the most common phenotype. Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) aggravate pre-existing urticaria in 20–30% of patients with chronic urticaria (2, 4). The prevalence of aspirin-intolerant chronic urticaria (AICU) was reported to be 35.7% in patients with chronic urticaria in a Korean population (5).

The mechanism of AICU is not completely understood. Cutaneous mast cells appear to be centrally involved, but the mechanism by which aspirin/NSAIDs activate them is unclear, as specific IgE to aspirin is not usually detectable. Mast cell homeostasis is controlled by IgE antibodies and cytokines such IL-3, IL-4, IL-10, and transforming growth factor-β1 (TGFβ1) (6, 7). Of these cytokines, TGFβ1 has both positive and negative effects on mast cell function and survival. TGFβ1 can inhibit IL-3-dependent mast cell proliferation, block stem cell factor-mediated rescue of IL-3-deprived mast cells from apoptosis, and alter mast cell effector function. There is also evidence that TGFβ1 can elicit mast cell migration (8) and induce mouse mast cell protease 1 expression (9, 10).

The expression of TGFβ1 can be influenced by polymorphisms in the TGFβ1 gene, some of
which may be associated with asthma (11–13) or other diseases such as cancers (14) and atherosclerosis (15). Previous studies have demonstrated that the T allele of the −509C>T single-nucleotide polymorphism (SNP) in the TGFβ1 promoter is associated with the development and severity of asthma (16) and may contribute to the development of rhinosinusitis in AIA patients in a Korean population (17). However, there are no published data on the association between genetic polymorphisms in TGFβ1 and the phenotype of urticaria or AICU. In this study, we evaluated the association between a TGFβ1 promoter polymorphism and the AICU phenotype, compared with the ATCU phenotype, in a Korean population.

MATERIALS AND METHODS

Subjects

The protocols used in the study were approved by the ethics committee of Ajou University Hospital, Suwon, Korea. All study subjects gave informed consent.

Subjects (n = 722) were enrolled from the Department of Allergy and Rheumatology, Ajou University Hospital, Korea and were classified into three groups: patients with AICU (n = 112), patients with aspirin-tolerant chronic urticaria (ATCU, n = 153) and normal controls (NC, n = 457). Chronic urticaria was defined as suffering from daily urticaria symptoms for more than 6 weeks (1, 2). Based on oral aspirin challenge test results, chronic urticaria patients were classified into two groups: AICU patients, who showed a positive response to an aspirin challenge test and ATCU patients, who showed no response to aspirin. Normal controls (n = 457), who had no personal or family history of allergic diseases and no past history of aspirin and other drug hypersensitivity, were enrolled at random from a local population.

Genotyping the TGFβ1 polymorphism

Seven polymorphisms have been described in the coding and promoter region of the TGFβ1 gene (18). However, no significant polymorphisms were found at positions −988, −800, +72, or at codon 25 or 263 in the unrelated Korean individuals (19). TGFβ1 −509C>T and the L10P polymorphisms showed strong linkage disequilibrium (ID’ = 0.985, d² = 0.919(19). Therefore, we focused on −509C>T as a tagging SNP. The SNP −509C>T in the TGFβ1 gene was genotyped using a single-base extension method. The sequences of the amplifying and extension primers used for genotyping the SNP have been described previously (17). Primer extension reactions were performed with a SNaP shot ddNTP primer extension kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer’s protocol.

Enzyme-linked immunosorbent assay for TGFβ1

Serum TGFβ1 levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Inc., Minneapolis, MN, USA). Before measuring TGFβ1, the serum samples were treated with acid to convert the inactive form of TGFβ1 to the active form. After neutralizing the sample with sodium hydroxide, the levels of TGFβ1 were measured, according to the manufacturer’s protocol.

Statistical analysis

The genotype frequency of the SNP was examined for significant departure from Hardy-Weinberg equilibrium using a chi-square test. A difference in genotype frequency between the patients and controls was assessed by a chi-square test and the calculation of the odds ratio (OR) with a 95% confidence interval (CI). Differences in clinical characteristics between groups were examined using Student’s t-test for continuous variables or a chi-square test for categorical variables. Logistic regression models were used to analyze SNPs and haplotypes, controlling for age, gender, atopy, rhinitis and allergic asthma as covariates, with three alternative models (codominant, dominant and recessive). Differences in the mean values of the phenotypic characteristics within AICU patients were compared using Student’s t-test. Statistical analyses were performed using SPSS (v. 11, SPSS Inc., Chicago, IL, USA). The significance level was set at P < 0.05.
RESULTS

Clinical characteristics of the study subjects

The clinical characteristics of the subjects are summarized in Table 1. The patients with AICU were significantly younger than those with ATCU (P < 0.01), and the rate of atopy and total serum IgE level were significantly higher in AICU patients compared with ATCU patients (P < 0.001 and P < 0.01 respectively). There was no significant difference in the prevalence of serum auto-antibodies, including anti-thyroglobulin antibody, antimicrosomal antibody and anti-nuclear antibodies, between patients with AICU and ATCU.

Frequencies of the TGFβ1 −509C>T genotypes in patients with chronic urticaria

The allele and genotype frequencies of the TGFβ1 promoter polymorphism are shown in Table 2. The genotype distribution of the polymorphism did not depart significantly from Hardy–Weinberg equilibrium (P > 0.05). The frequency of the genotype containing the −509T allele was significantly higher in patients with AICU compared with NC (P = 0.023 in the recessive model). After adjustment for age, sex, atopy, rhinitis and allergic asthma, the genotype containing the −509T allele was significantly associated with phenotype of...
AICU compared with NC (P = 0.044 in recessive model).

Clinical parameters in AICU patients according to the TGFβ1 −509C>T genotype

An analysis of the clinical parameters in AICU patients (Table 3) showed no significant difference in any clinical parameter, including age, gender, total IgE level, duration of urticaria and the presence of autoantibodies, according to the TGFβ1 −509C>T genotype.

Serum TGFβ1 level according to the TGFβ1 −509C>T genotype

Serum TGFβ1 levels in AICU patients were compared according to the TGFβ1 −509C>T genotype. The level of serum TGFβ1 tended to be lower in those possessing the CT or TT genotype than in those with the CC genotype (P = 0.472).

DISCUSSION

This is the first study to demonstrate an association between the genetic polymorphism in the TGFβ1 promoter at −509C>T and the AICU phenotype. We found a significant difference in the genotype frequencies of TGFβ1 −509C>T between AICU patients and normal controls, suggesting that this genetic polymorphism may contribute to the development of the AICU phenotype in a Korean population.

The mechanism of aspirin hypersensitivity in cutaneous reactions remains uncertain. Mast cells appear to be involved, but aspirin-specific IgE was detected in only one study (4). The observation that AICU responds to antihistamines implicates histamine as a key mediator. Moreover, diversion of arachidonic acid metabolism from prostaglandins to leukotrienes may be another mechanism for this type of urticaria (20, 21). There have been a few studies to investigate the genetic mechanisms of AICU (22–24) in which ALOX5 (5-lipoxygenase), FcεRIα (high-affinity IgE receptor) and LTC4S (leukotriene C4 synthase) promoter polymorphisms were suggested, and these results might be useful for differentiating from other types of ASA intolerance such as ASA intolerant asthma.

In a previous study comparing clinical features of AICU and ATCU patients (5), AICU patients required higher doses of systemic steroids to control their urticaria symptoms, indicating that AICU patients might have more severe urticaria symptoms. Given that the mast cell has a central role in the pathogenesis of urticaria, and that mast cell homeostasis can be influenced by IgE levels and several cytokines, including TGFβ1 (6, 7), we suggest that mast cells may be more activated in the cutaneous tissue of AICU patients than in ATCU patients. This TGFβ1 polymorphism might influence cutaneous mast cell function in AICU patients (8).

The TGFβ1 has been reported to be a multifunctional cytokine with both pro-inflammatory and anti-inflammatory effects (25–27) and has been implicated in the pathogenic mechanisms of...
chronic inflammatory allergic diseases such as asthma and atopic dermatitis (11–13, 28, 29). However, it is still unclear that how TGFβ1 is involved in the skin lesions of chronic urticaria. Although there are no published data demonstrating that blood tryptase and histamine levels are elevated after aspirin challenge in AICU patients, the observation that aspirin-induced urticaria responds to antihistamines implicates histamine as a mediator. Thus, mast cell activation may play an important role in AICU with exposure to aspirin. Both an increased mast cell releasability and a decreased mast cell activation threshold have been reported in the skin of chronic urticaria patients (30). According to a number of in vitro studies, TGFβ1 is a potent inhibitor of mast cells and can diminish IgE-mediated histamine release and tumour necrosis factor alpha (TNF-α) production, inhibit in vivo mast cell responses and mast cell FcεRI expression, and induce mast cell apoptosis (31–35). TGFβ1 can be produced by human skin keratinocytes in the basal cell layer of the epidermis, which has an important role in chemotaxis of mast cells in atopic dermatitis patients (8, 29). TGFβ1 localized in the skin as well as serum TGFβ1 may be associated with the development of skin lesions in atopic dermatitis (8, 28, 29). A study using an animal model of atopic eczema (28) demonstrated that the subcutaneous injection of recombinant TGFβ1 suppressed macrorscopic eczematous skin lesions in NC/Nga mice, indicating that TGFβ1 can suppress atopic dermatitis-like skin lesions. Thus, we suggest that dysregulation of TGFβ1 in AICU patients carrying the TGFβ1−509 CT or TT genotype facilitates cutaneous mast cell degranulation.

In the present study, we observed that the frequencies of the −509CT and TT genotypes were significantly higher in AICU patients than in normal controls. TGFβ1 gene regulation and expression level could be affected by three TGFβ1 SNPs [−509C>T, +869T>C (codon 10) and +915G>C (codon 25)] (36), however, in the Korean population, TGFβ1 at −509C>T was the only significant polymorphism (19). An in vitro functional study showed that this polymorphism could influence TGFβ1 gene expression (37–40) through enhancement of the binding affinity of Yin-yang 1 transcription factor, leading to increased TGFβ1 transcription and higher circulating concentrations of TGFβ1 in the plasma (37, 39). However, Shah et al. (40) suggested that the molecular mechanism for differences in the plasma TGFβ1 level involves transcriptional suppression by AP1, via binding to the TGFβ1−509 C allele. Taken together, these findings indicate that the TGFβ1−509C>T polymorphism can affect the activity of TGFβ1, which may increase cutaneous mast cell releasability and lead to the development of the AICU phenotype.

In this study, we observed that T allele carriers tended to have a lower serum level of TGFβ1 (data not shown). This is consistent with our previous study (17), in which AIA patients carrying the TGFβ1 −509 CT or TT genotype showed a lower serum level of TGFβ1, compared with those carrying the TGFβ1 −509 CC genotype; however, this finding contradicts another previous study reporting that this polymorphism was associated with higher levels of TGFβ1 in plasma (39). Although there are no published data demonstrating the expression of TGFβ1 mRNA or protein in the cutaneous tissues of patients with chronic urticaria, Matsui and Nishikawa (29) reported that peptidoglycans from Staphylococcus aureus may induce an increase in mast cell numbers in the skin through TGFβ1 production by epidermal keratinocytes, in atopic dermatitis patients. We suggest that these findings may be the result of TGFβ1 being localized and compartmentalized within cutaneous tissue, with strong inflammatory responses in AICU patients. Further investigation is needed to examine how this genetic variant may increase histamine release and/or augment mast cell signalling in AICU patients.

In conclusion, a TGFβ1 promoter polymorphism may contribute to the development of the AICU phenotype in a Korean population.

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REFERENCES


