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ARAŞTIRMA RESEARCH ARTICLE

Systemic prostaglandin E2 production in patients with chronic idiopathic urticaria does not discriminate between positive and negative aspirin challenge

Kronik idiyopatik ürtikerli hastalarda sistemik prostaglandin E2 yapımı pozitif ve negatif aspirin provokasyonunu ayırt ettirmez

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ABSTRACT

Objective: We aimed to investigate systemic production of prostaglandin E2 in chronic idiopathic urticaria patients, stratified by positive or negative clinical reaction during oral aspirin challenge.

Materials and Methods: Urinary concentrations of semi-stable prostaglandin E2 metabolite, 13,14-dihydro-15-keto-PGE2, were measured using commercial enzyme immunoassay at baseline and following aspirin challenge.

Results: Aspirin precipitated skin reactions in 14 (63.6%) out of 22 patients with chronic idiopathic urticaria. At baseline, mean urinary prostaglandin E2 metabolite values did not differ between patients who reacted to the drug and those who tolerated it. Following aspirin administration, urinary prostaglandin E2 metabolite excretion significantly decreased in all patients. No correlation was found between urinary prostaglandin E2 metabolite excretion and dose of aspirin precipitating hypersensitivity symptoms.

ÖZET

Giriş: Bu çalışmada kronik idiyopatik ürtiker hastalarında oral aspirin provokasyonu sırasında pozitif ya da negatif klinik reaksiyon gelişmesine göre sınıflandırılmış sistemik prostaglandin E2 yapımını araştırmayı amaçladık.

Gereç ve Yöntem: Prostaglandin E2'nin yarı-stabil bir metaboliti olan 13,14-dihydro-15-keto-PGE2'nin idrar konsantrasyonları, aspirin provokasyonu öncesi ve sonrasında, enzim immünassay kullanılarak ölçüldü.

Bulgular: Kronik idiyopatik ürtikerli 22 hastanın 14 (%63.6)'ünde aspirin, deri reaksiyonlarını tetikledi. Yükleme öncesinde ortalama idrar prostaglandin E2 metabolit düzeyleri ilaca reaksiyon veren ve vermeyen hastalar arasında farklı değildi. Aspirin uygulanması sonrasında, tüm hastalarda idrar prostaglandin E2 metabolit atılımı anlamlı azaldı. İdrar prostaglandin E2 metabolit atılımı ve hipersensitivite semptomlarını tetikleyen aspirin dozu arasında anlamlı ilişki saptanmadı.

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Conclusion: Administration of aspirin decreases systemic production of prostaglandin E2 in chronic idiopathic urticaria patients. This effect is independent of the outcome of aspirin challenge and does not discriminate between patients who develop hypersensitivity symptoms and those who tolerate aspirin well.

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Key words: Aspirin, urticaria, eicosanoids, prostaglandin E2

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Sonuç: Kronik idiyopatik ürtiker hastalarında, aspirin uygulanması sistemik prostaglandin E2 yapımını azaltır. Bu etki, aspirinle provokasyonun sonucundan bağımsızdır ve hipersensitivite semptomları gelişen ve aspirini iyi tolere eden hastaları birbirinden ayırmaz.

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Anahtar kelimeler: Aspirin, ürtiker, eikosanoidler, prostaglandin E2

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INTRODUCTION

Prostaglandin E (PGE)2 is a highly bioactive derivative of arachidonic acid produced by a concerted action of cyclooxygenases (COXs) and specific PGE synthases[1]. The compound can be synthesized during blood sampling and isolation of plasma and its inactivatory metabolism of PGE2 is very rapid[1]. Thus, measurement of stable metabolites is preferred to evaluate the biosynthesis of PGE2. A substantial fraction of these metabolites are excreted in urine, while kidney-derived PGE2 is excreted non-metabolized. Systemic production of PGE2 can be reliably assessed by measuring urinary excretion of inactive metabolites[2]. In humans, PGE2 is continuously produced in small amounts by most of the cells expressing a constitutive isoenzyme-COX-1, while inducible COX-2 can produce much more PGE2 in activated cells of the epithelium, smooth muscles, alveolar cells, macrophages/monocytes, phagocytes, fibroblasts, eosinophils, and lymphocytes[1,3]. Cellular response to PGE2 can vary diametrically in target cells where opposite effects depend on the spectrum of prostaglandin receptors (EP) (EP1, EP2, EP3, and EP4). In vitro PGE2 relaxes smooth muscles and inhibits activation of mast cells, neutrophils or T-cells and synthesis of leukotriene B_4[4-6].

Aspirin hypersensitivity manifests as asthma and/or urticaria/angioedema[7-10]. A specific mechanism triggering aspirin-induced asthma (AIA) symptoms upon inhibition of PGE2 synthesis has been proposed. However, AIA patients challenged with aspirin did not show any decrease in urinary PGE2 metabolites[11]. Progress in understanding urticaria/angioedema sensitive to aspirin has been slow. No studies on the influence of aspirin ingestion upon PGE2 metabolism have been carried out so far in aspirin-induced urticaria (AIU) patients. To our knowledge, this is the first study in which such investigations have been conducted.

The mechanism of urticaria and/or angioedema precipitated by aspirin is not based on antigen-antibody reactions, but results from the pharmacological inhibition of COX by the drug[12]. Currently, the name aspirin-exacerbated chronic urticaria best describes both the precipitation and aggravation of pre-existing chronic urticaria (defined as daily or almost daily recurrence for at least six weeks). The symptoms follow ingestion of aspirin and most other non-steroidal antiinflammatory drugs. Patients with AIU have a similar profile of eicosanoid mediators as patients with AIA[13,14]. The diagnosis can be confirmed by oral aspirin challenge test. Recently, a standardized score for skin eruptions triggered by aspirin has been described[15].

We studied urinary excretion of a PGE2 metabolite, reflecting systemic production of this prostaglandin. The study was carried out on

chronic idiopathic urticaria (CIU) patients with positive (CIU+) or negative (CIU-) outcome to the aspirin challenge. The metabolite was measured in urine both at baseline and after the aspirin provocation test.

MATERIALS and METHODS

Subjects

The study population consisted of 22 CIU patients. The patients' characteristics are presented in Table 1.

On the day of the aspirin challenge, the patients had no clinical symptoms of urticaria and their baseline forced expiratory volume in 1 second (FEV_1) was > 70% of the predicted value. None had experienced any exacerbation of the disease within two weeks preceding the aspirin test. The subjects were instructed to withhold medications that decrease skin responsiveness prior to the aspirin challenge. Short-acting antihistamines were stopped five days before the challenge. None of the patients had been treated with systemic corticosteroids or leukotriene-modifying drugs four weeks before the aspirin test.

The patients gave informed consent and the study was approved by the university ethics committee.

Study Design

The single-blind, placebo-controlled oral challenge test with aspirin was carried out on two consecutive days[15]. The challenge procedure with aspirin was interrupted if the skin reaction occurred and/or FEV_1 dropped at least 20%, or the maximum cumulative dose of aspirin was reached. Skin symptoms and FEV_1 were recorded at baseline, before the challenge, and then every 30 minutes until six hours after the last dose of placebo and aspirin.

In patients with positive aspirin challenge, urine samples were collected for 13,14-dihydro-15keto-PGE2 (PGE2-M) measurement at baseline, at the time of appearance of the skin symptoms (time 0), and then two and four hours later. In patients with negative aspirin challenge, urine samples were collected at baseline, one hour after the last aspirin dose, i.e. when the cumulative dose of 500 mg was reached (time 0), and then two and four hours later.

Table 1. Clinical characteristics of all study patients (n= 22), and patients stratified according to positive (n= 14) and negative (n= 8) aspirin challenge

	CIU (n= 22)	CIU (+) with positive aspirin challenge (n= 14)	CIU (-) with negative aspirin challenge (n= 8)	p CIU (+) vs. CIU (-)
Age (years)	49.0 ± 12.5 53 (40-56)	47.6 ± 11.3 52.5 (40.0-55.0)	51.5 ± 14.8 54.5 (44.5-61.0)	ns
Female/Male	16/6	11/3	5	ns
Duration of urticaria (years)	12.2 ± 9.5 7.0 (5-19)	11.4 ± 7.1 7.5 (5.0-19.0)	13.5 ± 13.0 6.0 (4.8-24.0)	ns
Urinary PGE_2-M at baseline (pg/mg creatinine)	689.3 ± 427.9 621.5 (400-827)	760.6 ± 489.0 659.5 (400-842)	564.4 ± 278.0 543.5 (324-718.5)	ns
Total IgE (IU/mL)	184.3 ± 257.4 116.5 (39.1-218)	118 ± 77.0 116 (39.1-193.0)	299.4 ± 404.8 157.0 (32.5-390.5)	ns
Blood eosinophil count	225.8 ± 224.5 181 (90-293)	239.7 ± 247.1 212 (90.0-303.0)	201.4 ± 191.8 167 (72.0-257.5)	ns

Values are expressed as mean ± SD, and median (25% and 75% percentiles). CIU: Chronic idiopathic urticaria patients, CIU (+): Patients with positive aspirin test, CIU (-): Patients with negative aspirin test. Baseline values of eicosanoids in CIU, CIU (+) and CIU (-) patients (values represent means of two estimations performed on placebo and aspirin day).

Lung Function

Pulmonary function tests were recorded using a flow-integrating computerized pneumotachograph (pneumoscreen, E. Jaeger, Germany).

Assay of Urinary PGE2-M

Urinary PGE2-M was measured in unpurified urine samples by direct enzyme immunoassay (EIA) (Cayman Chemical, prostaglandin E metabolite EIA kit)[16]. Urinary levels of PGE2-M were expressed in picograms per mg of creatinine.

Assessment of Severity of Skin Eruption

In order to standardize the assessment of severity of the skin eruptions, a modified Psoriasis Area and Severity Index (PASI) score was used[17]. PASI score > 10 was considered as a severe skin reaction.

Statistical Analysis

Summary statistics were expressed as the mean and standard deviation for symmetrically distributed data or the geometric mean, and 25% and 75% percentiles for non-symmetrically (skewed) distributed data. Multi-way ANOVA model was used for multiple-group comparisons. Logarithmic transformation was used when needed as variance stabilizing transformation. Fisher's exact test was used for dichotomous data for two independent random samples. A ρ-value ≤ 0.05 was considered statistically significant.

RESULTS

Clinical Reactions

There was no statistical difference in clinical characteristics between the patients with CIU and positive aspirin challenge test (CIU+), and those who tolerated aspirin well (CIU-) (Table 1). None of the patients developed symptoms after administration of placebo.

In CIU positive patients, skin reactions developed after 188 mg of cumulative dose of aspirin in six subjects, and following 500 mg in eight. Those patients had skin rash, angioedema, or both, but dyspnea was absent, and spirometric values remained stable throughout the observation period. Severe skin reaction (PASI score > 10) developed in 50% of patients. All the symptoms were relieved by short-acting antihistaminic. Rescue administration of systemic corticosteroid was required in one case.

Urinary Prostaglandin E2-M

At baseline, urinary levels of PGE2-M did not differ significantly between the patients with positive and negative aspirin challenge ($p= 0.9$) (Table 1).

The day of placebo challenge: After placebo administration, no significant differences in urinary PGE2-M levels were found in either study group when compared to baseline values (ANOVA, $p> 0.5$) (Figure 1a).

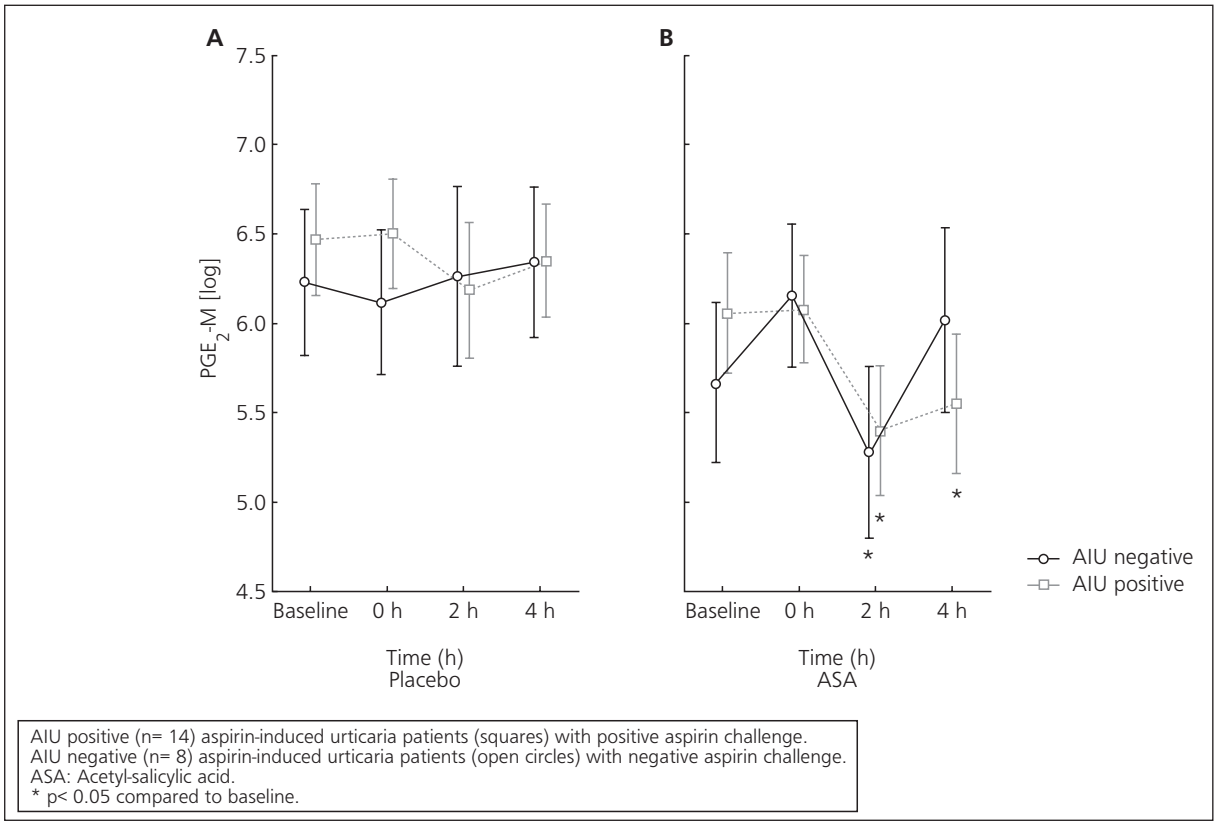
The day of aspirin challenge: On the day of aspirin challenge, mean values of urinary PGE2-M did not differ significantly between CIU positive and CIU negative patients (ANOVA, $p= 0.98$). During the six hours observation period following aspirin challenge, a decrease in PGE2-M excretion was noted in both study groups (ANOVA, $p< 0.001$). In the CIU positive group, urinary PGE2-M concentrations were significantly lowered after two ($p= 0.007$) and four hours ($p= 0.038$) following aspirin-precipitated reaction, when compared to baseline values. The lowest values were seen two hours after the onset of symptoms. In the CIU negative group, urinary PGE2-M excretion decreased significantly only two hours after the last dose of aspirin ($p= 0.001$), when compared to baseline values (Figure 1b).

The cumulative dose of aspirin had no effect on the magnitude of the response of PGE2-M in CIU positive patients.

No correlation was found between urinary PGE2-M levels and severity of skin reactions after aspirin challenge (expressed as PASI score).

DISCUSSION

The levels of PGE2 metabolite decreased during the six hours observation period following aspirin challenge in CIU patients regardless of



AIU positive (n= 14) aspirin-induced urticaria patients (squares) with positive aspirin challenge.
AIU negative (n= 8) aspirin-induced urticaria patients (open circles) with negative aspirin challenge.
ASA: Acetyl-salicylic acid.
* p< 0.05 compared to baseline.

Figure 1. Urinary PGE_2-M (13,14-dihydro-keto-PGE_2) levels before and following placebo (Figure 1a) and aspirin (ASA) (Figure 1b) challenges in the two study groups.

the appearance of clinical symptoms in 14 out of 22 subjects. Deficiency i PGE2, a prostaglandin inhibiting cysteinyl leukotrienes production, has been proposed to play a role in aspirin hypersensitivity[18,19]. Kowalski et al. observed that epithelial cells derived from surgically removed nasal polyps from patients with AIA produce less PGE2 than cells from patients who suffer from asthma and tolerate aspirin well. Peripheral blood leukocytes from AIA patients produced more 15-HETE after aspirin challenge, and misoprostol (synthetic analogue PGE_1) inhibited this production[20-22]. Some studies have suggested that epithelial cells of the airway, bronchial fibroblasts and peripheral blood cells produce less PGE2 in AIA patients[23].

It is well known that the antiinflammatory effect of PGE2 results from stimulation of the E-prostanoid 2 (EP2) receptor on leukocytes. Ying et al. observed a decrease in the number of inflammatory cells (neutrophils, mastocytes, eosinophils and T-lymphocytes) expressing EP2 receptor in tissue samples from the nasal mucosa of patients with rhinitis and aspirin hypersensitivity, as compared to aspirin-tolerant patients with rhinitis[24]. Jinnai et al. found a genetic association between aspirin hypersensitivity and polymorphism in the gene encoding the EP2 receptor[25].

Recently, we demonstrated in patients with AIA that urinary levels of PGE2 metabolites measured by two different laboratory methods did not change after aspirin challenge. In contrast, in the aspirin-tolerant asthmatics, both PGE2 metabolites in urine decreased following a 500 mg dose of aspirin[11]. A puzzling result of this study prompted us to measure urinary PGE2-M in patients with cutaneous manifestation of aspirin hypersensitivity, i.e. AIU.

In contrast to our previous results on aspirin challenge in AIA subjects, AIU patients challenged with aspirin showed decreased PGE2-M excretion in urine[11]. This opposite reaction during a positive aspirin challenge between asthmatic and urticaria patients is not clear.

Aspirin administered during the challenge procedure exerted its pharmacological action, and a significant decrease in urinary PGE2-M was noted, regardless of the hypersensitivity status. However, some differences between CIU positive and CIU negative groups were noted as well. Mainly, depression of PGE2-M lasted longer in CIU positive than in CIU negative subjects, even though all CIU negative patients were given a 500 mg dose of aspirin.

It is plausible that mediators released during aspirin provocation are different in bronchial and cutaneous forms of aspirin hypersensitivity. Even more likely is that mast cells responsible for their release are distributed differently. In asthmatic subjects, inflammatory mediators released from activated mast cells following the positive aspirin challenge can up-regulate PGE2 biosynthesis in other cells, including epithelial, smooth muscle and alveolar cells, macrophages, phagocytes, lymphocytes, and eosinophils. This secondary inflammatory reaction is evidently absent in CIU positive patients.

Some examples of PGE2 modulation by inflammatory cytokines released during aspirin hypersensitivity reaction are interleukin (IL)-13 mediated inhibition of PGE-1 synthase, and at the same time up-regulation of PGE2 15-dehydrogenase, which catabolizes the prostaglandin. In addition, IL-13 released by Th_2 lymphocytes depresses the cellular expression of COX-2[26]. It would be particularly interesting to study the role of IL-13 on the prostanoid pathway of PGE2 in skin cells.

In conclusion, aspirin changed systemic PGE2 production in CIU patients in a way consistent with its pharmacological action. PGE2 systemic production is transiently depressed by aspirin in urticaria patients, regardless of their positive or negative aspirin challenge outcome.

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