Effects of 5-Lipoxygenase Inhibitors on Guinea Pig Trachea in Ovalbumin-Induced Experimental Asthma

Deneysel Astım Modeli Oluşturulmuş Kobay Trakeası Üzerinde 5-Lipoksijenaz İnhibitörlerinin Etkileri

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ABSTRACT Objective: The 5-lipoxygenase products, especially sulfidopeptide leukotrienes take an important role in the pathogenesis of asthma. The effects of two selective 5-lipoxygenase inhibitors, acetohydroxamic acid derivative BWA 4C and a naphtokinon derivative CGS 8515, were investigated for their effect on antigen-induced contraction in ovalbumin sensitized guinea-pig isolated tracheal tissues. **Material and Methods:** The study included 15 male guinea pigs. They were actively sensitized by the Modified Andersson Method, including two standard 0.5 mL injections of a solution containing 20 µg ovalbumin and 50 mg aluminium hydroxide in saline on day 0 and day 14. Twenty-one to 27 days after the injection of ovalbumin, the tracheal rings from sensitized guinea pigs were exposed to antigen, and the possible inhibitor effects of 5-lipoxygenase inhibitors on the antigen-induced contraction were examined in water-jacketed tissue baths containing Krebs buffer. **Results:** Both BWA 4C and CGS 8515 significantly reduced antigen-induced tracheal contraction compared to control groups. **Conclusion:** As both CGS 8515 and BWA 4C inhibit antigen induced tracheal smooth muscle contractions, they may take an important role in the treatment of asthma and related inflammatory events.

Key Words: Asthma; lipoxygenase inhibitors; CGS 8515 (methyl 2-((3,4-dihydro-3,4-dioxo-1-naphthalenyl)amino) benzoate; BWA 4C (N-(3-phenoxycinnamyl) acetohydroxamic acid); trachea; guinea pig

ÖZET Amaç: Günümüzde halen önemli bir sağlık sorunu olan astımın patogenezinde 5-lipoksijenaz ürünleri, özellikle de sülfidopeptit lökotrienler önemli rol oynarlar. İki selektif 5-lipoksijenaz inhibitörünün (bir asetohidroksamik asit derivesi olan BWA 4C ve bir naftikinon derivesi olan CGS 8515) ovalbumine duyarlılaştırılmış kobayların trakea dokusunda antijen aracılı kontraksiyon üzerindeki etkisi incelendi. **Gereç ve Yöntemler:** Çalışmada 15 adet erkek albino kobay kullanıldı. Deneysel astım modeli oluşturmak için her bir kobay Modifiye Andersson Metodu kullanılarak duyarlılaştırıldı. Bu yönteme göre, standart doz olan 0,5 mL serum fizyolojik içinde 20 µg ovalbumin ve 50 mg aluminyum hidroksit içeren solüsyon, 0. gün ve 14. gün olmak üzere iki kez enjekte edildi. Duyarlaştırmanın 21. ve 27. günleri arasında kobayların trakealarından alınan halkalar, ortamda 5-lipoksijenaz inhibitörleri bulunurken ovalbumine maruz bırakıldı ve 5-lipoksijenaz inhibitörlerinin, oluşan kontraksiyon üzerindeki etkileri incelendi. Deneyler Krebs solüsyonun kullanıldığı organ banyosunda yapıldı. **Bulgular:** BWA 4C ve CGS 8515, kontrol grubuna göre antijen aracılı trakea kasılmasını belirgin olarak azalttı. **Sonuç:** 5-lipoksijenaz inhibitörleri olan CGS 8515 ve BWA 4C, astım ve astımla ilişkili yangısal olayların tedavisinde veya başka yangısal hastalıkların tedavisinde önemli bir rol almaya devam edebilir.

Anahtar Kelimeler: Astım; lipooksijenaz inhibitörleri; CGS 8515 methyl 2-((3,4-dihydro-3,4-dioxo-1-naphthalenyl)amino) benzoat; BWA 4C (N-(3-phenoxycinnamyl)acetohydroxamic acid); trakea; kobaylar

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The 5-lipoxygenase products, especially sulfidopeptide leukotrienes have an important place in the pathogenesis of asthma. Recently, it has been evident that sulfidopeptide leukotrienes may contribute to the pathophysiology of asthma. After an endobronchial allergen challenge, the leukotriene levels increased in bronchoalveolar lavage fluid of asthmatic patients.[1,2] Bronchial provocations with allergens, aspirin and exercise in asthmatic patients caused an increase in urinary leukotrien levels.[3,4] The mechanism of aspirin sensitive asthma appears to be dependent on 5-lipoxygenase pathway metabolites.[5,6] The comparative action of inhaled leukotriene C4 (LTC4) and leukotriene D4 (LTD4) has shown that they are more potent bronchoconstrictors than histamine, with sustained action.[7] In addition, moderate to severe asthmatic persons who remain poorly controlled have various treatment options including leukotriene receptor antagonists.[8]

In this study, the antiasthmatic effects of the naphtokinon derivate CGS 8515 and acetohydroxamic acid derivate BWA 4C, two selective 5-lipoxygenase inhibitors, were assessed and compared on the ovalbumin sensitized guinea-pig tracheal tissue.

MATERIAL AND METHODS

The Decleration of Helsinki recommendations and internationally and locally accepted principles were followed for the care and use of the animals in this study. After the approval of the Faculty Ethics Committee (Fatih University, Local Ethical Committee 06.10.2010/6), fifteen male albino guinea pigs, weighing 180-250 g, were included in the study. Guide for the Care and Use of Laboratory animals (1996) was reviewed and care was taken to use minimum number of subjects in the study. Animals were obtained from the Hıfzıssıhha Hygiene Center.

Fifteen male albino guinea pigs, weighing 180-250 g, were actively sensitized with a standard dose of 20 μg ovalbumin and 50 mg aluminium hydroxide in saline administered by intraperitoneal injections on day 0 and day 14, according to the Modified Andersson Method.[9] Guinea pigs were used for the study 21-28 days after the immunization. Guinea pigs were anesthetized with intraperitoneal injection of 50 mg/kg sodium pentobarbital and were sacrificed by cutting the iliac artery. The tracheas were excised immediately and were cleaned of other tissues. The central part of each trachea was cut into four rings. Each ring had 3-4 cartilages. Each animal also served as its own control by two rings. The tracheal rings were suspended under 1 g of resting tension in 10 ml water-jacketed tissue baths containing Krebs buffer of the following composition (in mM/L): NaCl, 112; $NaHCO_3$, 26; NaH_2PO_4 1; KCl, 5; $MgCl_2$, 0.5; $CaCl_2$, 2.5; and dextrose, 11,5. The buffer was kept at 37^0C and was aerated continuously with 95% O_2 and 5% CO_2. The tracheas were connected to Grass Polygraph (Model 7E) FT3 force displacement transducer in order to record isometric tensions.

After a 90 min equilibration period (during which all tissues were washed for 15 minutes with Krebs buffer) one preparation was exposed to 1 μg/ml ovalbumin. After the development of ovalbumin-induced contraction the tracheal ring was considered sensitized and was removed from the experiment. Fifty percent of maximal ovalbumin contraction was considered reference contraction for sensitization.

After the equilibration period, each preparation was treated with carbachol (10^{-8}M–$3x10^{-5}$M) in cumulative doses to achieve maximum contraction. Seventy five percent of carbachol contraction was considered reference contraction for each tracheal ring. Following a 30-minute equilibration period, each tissue was incubated for 45 min with acetylsalicylic acid (ASA) (10 μM), pyrilamin maleate (10 μM) and one dose of selective lipoxygenase inhibitors (10^{-8}, 10^{-7} or 10^{-6}M). For the DMSO group, various concentrations of dimethylsulfoxide (DMSO) ($1.4x10^{-3}$ M, $1.4x10^{-4}$M and $1.4x10^{-5}$ M) that correspond to the concentrations in which 5-lipooxygenase inhibitors were dissolved were used.

ASA was added to the solution as it inhibits prostaglandin synthesis without inhibiting lipoxygenase and pyrilamine was used to inhibit H1 receptor related effects of histamine.

The DAP is a group in which the tissues were incubated with DMSO ($1.4x10^{-3}$M), ASA (10 μM) and pyrilamin maleate (10 μM) without lipoxygenase inhibitors. To constitute the control group, following a 30-minute equilibration period, 1 μg/ml ovalbumine response was obtained in a group of preparations without incubating it with either lipoxygenase inhibitors or DMSO, ASA and pyrilamine maleate.

At the end of the incubation period, 1 μg/ml ovalbumine was added in all groups to obtain contraction, and all data were expressed as a percentage of the carbachol-induced reference contraction response.

The following drugs were used in the study. Pyrilamine maleate, carbachol and ovalbumin Grade5 were purchased from Sigma Chemical Co. (USA), acetylsalicylic acid was provided from BAYER (Germany), CGS 8515 was provided from Ciba-Geigy (Switzerland), BWA 4C was provided from Wellcome (U.K.) and $AlOH_3$ was provided from the market. The stock solutions of pyrilamine maleate, ovalbumine, asetylsalicylic acid and carbachol were prepared by dissolving them in distilled water. Daily dilutions of carbachol were prepared with Kreb's solution. CGS 8515 and BWA 4C were dissolved in DMSO and were used daily. AlOH3 was prepared in (%0.9 NaCl) physiological saline solution.

STATISTICAL ANALYSIS

Data were presented as means±standard error of mean (SEM). Ovalbumine responses were expressed as percent of reference carbachol contraction. Data regarding similar concentrations of 5-lipoxygenase inhibitors and DMSO and control group were analysed by Kruskal Wallis nonparametric analysis of variance and post hoc Dunn's test. The p values less than 0.05 were considered statistically significant.

RESULTS

Both BWA 4C and CGS 8515 significantly reduced antigen-induced tracheal contractions. A significant difference was noted between the groups in all concentrations (10^{-8} M p=0.0006, 10^{-7} M p=0.0019, 10^{-6} M p=0.0001) (Figure 1).

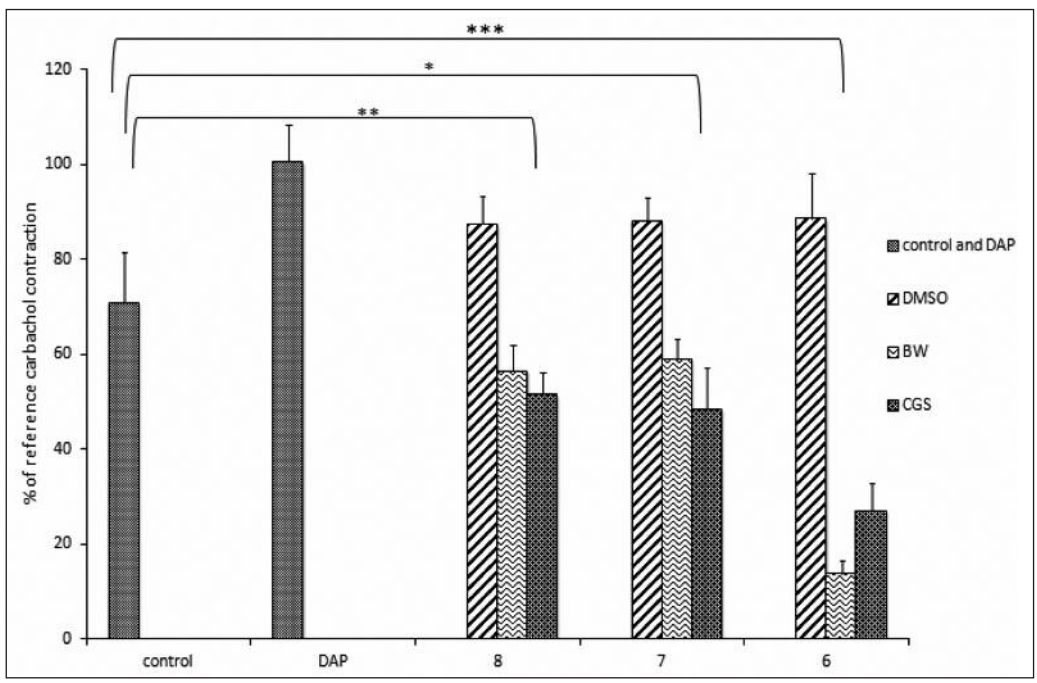

FIGURE 1: Inhibition of tracheal contractions during incubation period with different concentrations of 5-lipoxygenase inhibitors after the ovalbumin (1 μg/mL) challenge. Each point represents mean ± standard error of mean. All responses are expressed as percent of reference carbachol contraction of each ring (10^{-8}, 10^{-7} or $^{-6}$ M). *** p<0.0001, ** p=0.0006, *p=0.0019 according to the Kruskal Wallis nonparametric analysis of variance.

Although there was a slight increase in the ovalbumine (1 μg/mL) induced contractions of tracheal smooth muscles in the DMSO and DAP groups, they were not significantly different from the results of the control group ($p>0.05$) (Figure 1).

BWA 4C significantly reduced antigen-induced tracheal contractions compared to control groups; the reduction with BWA 4C was greater than the reduction with CGS 8515, especially at higher concentrations (10^{-6} M).

DISCUSSION

Guinea pigs are a well-known model for immediate hypersensitivity to irritants and have pharmacological responses similar to humans.[10] IgG and IgE antibodies can be induced by this sensitization method in the organism, and this is similar to the human allergic reaction.[9,11]

5-Lipoxygenase inhibitors are used in some countries to prevent or treat diffuse airway obstruction in asthma.[12,13] BWA 4C and other acetohydroxamic acid derivates BW AI37C and BWA 797C were investigated in this regard. BWA 4C did not produce significant inhibition compared to the control response at anaphylactic concentration on guinea pig lung parenchyma.[14] In some other investigations, all acetohydroxamic acid derivates were effective on bronchial anaphylaxis in anaesthetized guinea pigs.[15] The result of another study suggested that BWA 4C and BWA 797C inhibited leukocyte migration to the peripheral inflammation area.[16] In our study, BWA 4C significantly reduced antigen-induced tracheal contractions compared to control groups; the reduction was greater compared to the reduction with CGS 8515, especially at higher concentrations (10^{-6} M).

In atopic asthmatic persons, mast cell and lymphocyte-induced eosinophil survival were completely reversed by SB201146, SKF104353, BWA 4C and MK886. These findings provide evidence for the involvement of an autocrine cysteinyl leukotriene pathway that supports eosinophil survival in response to a range of survival stimuli.[17]

The naphtokinon derivate, selective 5-lipoxygenase inhibitor CGS 8515 reduced the exudate volume, and inhibited leukocyte migration to the region in carragenin-induced pleurisy in rats.[18] Another study suggested that it had a beneficial effect during endotoxic shock in the rat.[19] Our study revealed that CGS 8515 had an inhibitor activity on the antigen-induced tracheal contraction. In the future, the effects of CGS 8515 on inflammatory cells and the tracheal smooth muscle may take an important role in the treatment of asthma and some other inflammatory events.

The mechanism of bronchoconstriction was investigated in the Brown Norvay rat model of allergic asthma. The augmented response to bradykinin was not affected by 5-lipoxygenase inhibitor CGS 8515. However, mast cell activation, the products of cyclooxygenase or 5-lipoxygenase pathways and tachykinins were not involved.[20] Allergen induced airway edema in actively sensitized rats has been studied earlier by magnetic resonance imaging (MRI) to monitor the consequences of non-immunological mast cell activation induced by 48/80 in the rat lungs in vivo. Pretreatment with wortmanin, capsazepin or the antiallergy drug CGS 8515, but not indomethacin, resulted in partial inhibition.[21]

DMSO is a good vehicle for the two compounds but it also has some biological effects such as radical scavenging, enzyme inhibition or stimulation on some enzyme systems, and antibacterial and anti-inflammatory effects.[22-24] In all these studies, it has been used in *in-vivo* conditions at higher doses. In our study, DMSO was used in *in-vitro* conditions and at very low concentrations (1.4×10^{-3} M, 1.4×10^{-4}M and 1.4×10^{-5} M dissolving for the 10^{-6}, 10^{-7} or 10^{-8}M concentrations of compounds). In a study, DMSO was used at 0.1% dilution in organ baths to avoid anti-inflammatory effect.[24] In our study, it was used only at 0.01%, 0.001% and 0.0001% dilutions. In this study, the DMSO group had no anti-inflammatory effect compared to the control group.

Type I hypersensitivity reactions occur with the release of various mediators from the sensitized tissue cells. Some of these mediators-histamine, prostaglandins, thromboxanes, kinins, 5-hydroxytriptamine, dopamine and platelet aggregating factor (PAF)-have various direct effects on the airway

tissues such as contraction of smooth muscles, dilation and increase in the permeability of vascular system, stimulation of exocrine glands and vagal stimulation.[25] In a stduy, challenge of the sensitized trachea with an antigen resulted with a product of the epithelium dependent relaxing factor(s) (EpDRF(s) and not the cyclooxygenase or lipoxygenase pathways.[26] This contention was supported by some investigators as well.[27,28] Under our study conditions, ovalbumin-induced bronchial contractions in sensitized trachea and prevention of this contraction by the 5-lipoxygenase inhibitors BWA 4C and CGS 8515 might have been influenced by some unknown factors and mediators as mentioned above.

In our study, despite the presence of a lipoxygenase inhibitor and a cyclooxygenase inhibitor with a histamine receptor antagonist in the organ bath, complete inhibition did not happen in antigen-induced tracheal contraction. This may be attributed to several factors. One is that the EpDRF(s) releasing effect of histamine may be via a non H1 and H2 receptor and perhaps an unknown histamine receptor is responsible for this effect.[29] In this case, our H_1 receptor antagonist might not prevent this effect of histamine. The cyclooxygenase inhibition in the study with ASA might have caused inhibition of bronchodilator effects of some prostaglandins. However, some *in-vivo* studies showed that despite the corticosteroid treatment some cysteinil leukotrienes and their precursors, arachidonic acid and its metabolites, could be released into the airway.[30,31] The second cause may be the inhibition of bronchoconstrictor response to the endogen mediators and antigens.[32] The contribution of PAF on bronchoconstriction may be another inducing factor.

The treatment of asthmatic attacks with 5-lipoxygenase inhibitors is a current debate. Clinical use of these drugs may offer a prophylactic treatment of mild asthma. In addition, 5-lipoxygenase inhibitors may be promising in the treatment of aspirin sensitive asthma.[33] In addition, the patients who have rhinitis and asthma, intranasal corticosteroids, antihistamines, and anti-leukotrienes can reduce asthma symptoms and in some instances improve pulmonary function and bronchial hyperresponsiveness.[34]

Introduction of more potent 5-lipoxygenase inhibitors may be a better option in the treatment of asthma to prevent antigen-induced bronchoconstriction.[4,8,35]

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