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Theophylline induces a reduction in circulating interleukin-4 and interleukin-5 in atopic asthmatics

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ABSTRACT: Theophylline, a known phosphodiesterase inhibitor, has been widely used as an additional bronchodilator in asthmatic patients who are not adequately controlled on high-doses of inhaled steroids. However, there is growing evidence that theophylline may also have anti-inflammatory or immunomodulatory effects in asthma. This study investigated whether theophylline administration has an impact on serum levels of interleukin (IL)-4 and IL-5 in asthmatic patients.

Eight asymptomatic patients aged 30±1.5 yrs (mean±SEM) with mild atopic asthma were given a single daily dose of theophylline 150 mg or placebo in an on (theophylline)–off (placebo)–on (theophylline)–off (placebo) protocol with a 3-week duration of each on- or off- interval. Determination of serum IL-4 and IL-5 was done at baseline for all subjects and on the last day of each 3-week interval for the patients under study.

Serum IL-4 levels were: 35±6 (baseline), 19±3 (on-1 interval), 29.5±4 (off-2), 15±2 (on-3) and 26±4 pg·mL^{-1} (off-4), while IL-5 levels were 27±5, 18±4, 28±5, 17±4 and 28±5 pg·mL^{-1}, respectively. Spirometry was unchanged during the study and serum theophylline levels at the end of the two on-periods were 4.5±0.05 and 4.2±0.07 μg·mL^{-1}, while all patients remained asymptomatic.

In conclusion, the administration of a low, single, daily dose of oral theophylline in asymptomatic patients with mild atopic asthma seems to reduce circulating interleukin-4 and interleukin-5.

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Theophylline, a nonselective phosphodiesterase (PDE) inhibitor, has been used as a bronchodilator in the treatment of bronchial asthma for >50 yrs [1]. It has been suggested that theophylline may owe part of this therapeutic activity to the inhibition of PDE enzymes. It has recently become apparent that several inflammatory cells, involved in the inflammatory process which characterizes asthma, possess PDE isoenzyme IV [2], raising the possibility that inhibition of this particular enzyme by theophylline or other PDE inhibitors may result in the diminution of their inflammatory effects. In support of this theory, a substantial body of evidence has accumulated to show that theophylline and other more selective PDE inhibitors exhibit anti-inflammatory and immunomodulatory actions beyond their well-recognized action on airway smooth muscle function [3–5].

The cytokines interleukin (IL)-4 and IL-5, produced by the T-helper (Th)-2 subset of T (CD4+) lymphocytes, have been shown to play a key role in the cascade of immune events leading to allergic tissue response [6]. Furthermore, HUMBERT *et al*. [7] have demonstrated an increase in messenger ribonucleic acid (mRNA) encoding IL-4 and IL-5 and increased numbers of IL-4 and IL-5-immunoreactive cells within the bronchial mucosa of patients with both atopic and nonatopic asthma, compared with nonasthmatic subjects.

There is evidence that PDE IV inhibitors impede, at least *in vitro*, the expression of IL-4 and IL-5 genes in Th-2 cells [8] and, more recently, it has been shown that theophylline induces a reduction in the expression of IL-4, with a similar tendency in the expression of IL-5 on airway submucosal cells in asthmatic patients [9]; therefore, it was hypothesized that theophylline may have an impact on the levels of these cytokines in serum. Hence, the objective in this study was to assess the *in vivo* effect of theophylline on circulating serum levels of IL-4 and IL-5 in patients with bronchial asthma.

Subjects and methods

Subjects

The study group was recruited from the Asthma Clinic of A. Fleming General Hospital, Athens, and fulfilled the following selection criteria: 1) age range 25–40 yrs; 2) lifelong nonsmoking status; 3) an established diagnosis of

asthma based on the presence of a clinical history of intermittent wheeze, cough, chest tightness or dyspnoea and documented reversible airflow obstruction either spontaneously or with treatment during the preceding year [10]; 4) atopic status, based on the seasonal appearance of symptoms, evidence of allergic rhinitis, elevated serum immunoglobulin (Ig)E and positivity of skin tests; 5) mild level of asthma severity according to the Global Initiative for Asthma criteria [10], such as symptom frequency less than once a week, nocturnal symptoms less than twice a month, intermittent exacerbations lasting from hours to a few days, symptom-free prolonged intervals with normal lung function, forced expiratory volume in the first second of expiration (FEV_1) or peak expiratory flow (PEF) rate >80% of predicted values and diurnal variation <20%; 6) stable and asymptomatic condition for at least the past 3 months; 7) absence of other immunological or chronic inflammatory diseases that might interfere with the serum levels of the cytokines under study; and 8) regular treatment with β_2-agonists on demand and low-dose inhaled steroids for the last 3 weeks (beclomethasone <1,000 μg, budesonide <1,000 μg or fluticasone <500 μg daily).

In addition, serum levels of IL-4 and IL-5 were determined in a population of healthy control subjects recruited from the personnel of the hospital.

The study was approved by the Institutional Ethics Committee and written informed consent was signed by all subjects.

Methods

A single-blind, placebo-controlled, on–off–on–off pattern of protocol was used in terms of administration and withdrawal of theophylline. Each on- or off-interval lasted for 3 weeks and the total duration of the study was 12 weeks for each patient.

Patients visited the Asthma Clinic between 08:00 and 09:00 h at baseline (1st day of the study) and on the last day of each interval thereafter (a total of five visits). Initially, a venous blood sample of 10 mL was drawn from each subject, which was centrifuged for 15 min at 650×*g*. Serum was stored at -70C and the levels of IL-4 and IL-5 were determined within 48 h. Determination was carried out by enzyme-linked immunosorbent assay (ELISA) using specific anti-IL-4 and anti-IL-5 monoclonal antibodies (Endogen, Boston, MA, USA), according to the manufacturer's instructions. An additional blood sample was drawn only at the baseline visit for measurements of IgE concentration and eosinophil count.

Immediately after blood sampling, the patients performed three acceptable and reproducible forced vital capacity manoeuvres according to the American Thoracic Society criteria, using an electronic spirometer (MicroLab 3300; Micromedical, Rochester, Kent, UK) which was calibrated before each spirometry.

After blood sampling and performing baseline spirometry, patients were instructed to take 150 mg of slow-release anhydrous theophylline (Theodur®; Lavipharm Hellas AE, Paiania Attica, Greece) once daily at 20:00–21:00 h for the next 3 weeks (on-1 interval). On the last day of the on-1 interval, patients visited the Asthma Clinic, where blood was sampled for IL-4 and IL-5 measurements and spirometry was performed. They were given placebo for the next 3 weeks and taken off theophylline (off-2 interval). The same procedure was followed for the on-3 (theophylline) and off-4 (placebo) intervals, respectively. All patients were given inhaled steroids as a 3-week pretreatment and during the whole period of the study.

Symptoms were assessed before and during the study by the use of a daily diary card that contained daytime asthma symptom and night-time awakening scales [11]. The four daytime asthma symptom scales (regarding symptom frequency, discomfort perceived by the patient, level of daily activities and activity limitations due to asthma, on a seven-point scale of 0–6, where 0 is the best and 6 the worst) were combined into a mean daily score. Night-time awakenings owing to asthma were evaluated by the response to a single question. Daily usage of rescue β_2-agonists and PEF rate values self-measured immediately after morning awakening, at noon and just before bedtime were also recorded.

On the last day of the on-1 and on-3 intervals an additional sample of venous blood was drawn for measurement of serum theophylline concentration. Theophylline levels were estimated by the fluorescence polarization immunoassay (FPIA) method using a commercially available kit (IMX; Abbott Diagnostics, Dallas, TX, USA).

Data analysis

Results are expressed as mean values±SEM. The classical Student′s t-tests was used for the comparison between serum levels of IL-4 and IL-5 in the study group with those in the control group. The Student′s t-test for paired observations and Wilcoxon matched-pairs signed-ranks test were applied for the comparison of cytokine levels within the study group between successive intervals (baseline *versus* on-1, on-1 *versus* off-2, off-2 *versus* on-3 and on-3 *versus* off-4). Statistical significance was set at a level of $p<0.05$.

Results

From a total of 27 consecutive asthmatic patients who were referred to the Asthma Clinic, 10 patients fulfilled the selection criteria and agreed to participate. Two of these patients dropped out owing to an asthma attack during the study period, one on the 16th day of the off-2 interval (37th day of the study) and the other on the 2nd day of the on-3 interval (44th day of the study). Both of them presented at the Emergency Department with shortness of breath, chest tightness, wheezing and cough and they were given parenteral steroids, antibiotics, nebulized salbutamol and *i.v.* aminophylline. Finally, eight patients (three males; five females, aged 30±1.5 yrs) out of the 10 patients who initially entered the study were able to complete the protocol. Seventeen healthy subjects (seven males and 10 females, aged 36±3 yrs) volunteered to participate as the control subjects. The serum IL-4 and IL-5 levels in the control group were 7.6±0.7 and 6.0±0.7 $pg \cdot mL^{-1}$, respectively, while the baseline serum values of IL-4 and IL-5 in asthmatic patients were 35±6 and 27±5 $pg \cdot mL^{-1}$, respectively ($p<0.001$ for both IL-4 and IL-5).

Table 1. – Demographic data, baseline lung function, serum immunoglobulin E (IgE) concentration at baseline, eosinophil count in peripheral blood at baseline and daily dosage of inhaled steroids

Patient No.	Sex	Age yrs	FEV1 % pred	PEF diurnal variation %	IgE ng·mL^{-1}	Eosinophils cells·μL^{-1}	Inhaled steroids μg·day^{-1}
1	F	27	89	10	175	480	500 becl
2	M	33	94	11	205	530	400 bud
3	F	36	91	8	144	180	500 becl
4	F	24	90	6	127	125	250 flut
5	M	29	89	6	178	310	250 flut
6	F	28	93	10	227	455	500 becl
7	M	31	94	9	156	312	500 becl
8	F	35	90	4	117	175	250 flut
Mean±SEM		30.0±1.5	91.0±0.8	8.0±0.9	166±13	321±54	

FEV1: forced expiratory volume in one second; PEF: peak expiratory flow; F: female; M: male; becl: beclomethasone; bud: budesonide; flut: fluticasone.

The demographic data of the patients, lung function at baseline, serum IgE concentration, eosinophil count in peripheral blood, and dosage of inhaled steroids are shown in table 1.

The administration of theophylline for 3 weeks (on-1 and on-3 intervals) resulted in a substantial reduction in circulating IL-4 and IL-5 in all patients ($p<0.01$, table 2, figs. 1 and 2), while withdrawal of theophylline for 3 weeks (off-2 and off-4 intervals) was accompanied by an increase in both IL-4 and IL-5, reaching pretreatment levels ($p<0.01$, table 2, figs. 1 and 2). It must be noted that, although the use of theophylline was associated with a significant decline in both IL-4 and IL-5 serum levels (on-1 and on-3 intervals), those levels continued to be significantly higher than control values ($p<0.001$ for both cytokines). Serum levels of IL-4 were 35±6 (baseline), 19±3 (on-1), 29.5±4 (off-2), 15±2 (on-3) and 26±4 pg·mL^{-1} (off-4), while those of IL-5 were 27±5 (baseline), 18±4 (on-1), 28±5 (off-2), 17±4 (on-3) and 28±5 pg·mL^{-1} (off-4) (table 2).

The determined serum theophylline concentrations at the end of on-1 and on-3 intervals were well below the therapeutic bronchodilator range of 10–20 μg·mL^{-1} (4.5±0.05 and 4.2±0.07 μg·mL^{-1}, respectively).

Baseline FEV1 was 91±0.8% pred (ranging 89–94% pred). None of the patients had any evidence of spirometric improvement or deterioration during theophylline administration or theophylline withdrawal (placebo administration), respectively. Furthermore, all patients were and remained without clinical symptoms during the study period and without any need for additional use of their rescue treatment.

Table 2. – Mean values (±SEM), range and statistics of circulating levels of interleukin (IL)-4 and IL-5 baseline and on the last day of the various intervals of the study

	Baseline	On-1	Off-2	On-3	Off-4
IL-4 pg·mL^{-1}					
Mean±SEM	35±6	19±3	29.5±4	15±2	26±4
Range	17–66	10–32	17–49	8–26	14–41
Statistics	$p<0.001$ *vs* control	$p<0.01$ *vs* baseline	$p<0.01$ *vs* on-1	$p<0.01$ *vs* off-2	$p<0.01$ *vs* on-3
IL-5 pg·mL^{-1}					
Mean±SEM	27±5	18±4	28±5	17±4	28±5
Range	11–51	5–36	13–48	5–31	13–52
Statistics	$p<0.001$ *vs* control	$p<0.01$ *vs* baseline	$p<0.01$ *vs* on-1	$p<0.01$ *vs* off-2	$p<0.01$ *vs* on-3

On-1, on-3: theophylline administration; off-2, off-4: theophylline withdrawal.

Discussion

The main findings of this study are as follows: 1) Circulating levels of IL-4 and IL-5 were found to be significantly elevated in asthmatic patients compared with nonasthmatic control subjects, despite the asthmatic patients being asymptomatic, with a normal spirometry and taking inhaled steroids for the last 3 weeks. 2) The short-term administration of a single, oral, low daily dose of theophylline, producing subtherapeutic serum levels of the drug, resulted in a significant reduction in serum IL-4 and IL-5, but this was not accompanied by any obvious spirometric improvement. 3) This theophylline-related reduction in serum IL-4 and IL-5 was not complete, since suppressed cytokine levels remained well above the control values. 4) Finally, withdrawal of theophylline administration for 3 weeks was associated with an increase in IL-4 and IL-5 to baseline pretreatment values, still with no detection of a spirometric alteration.

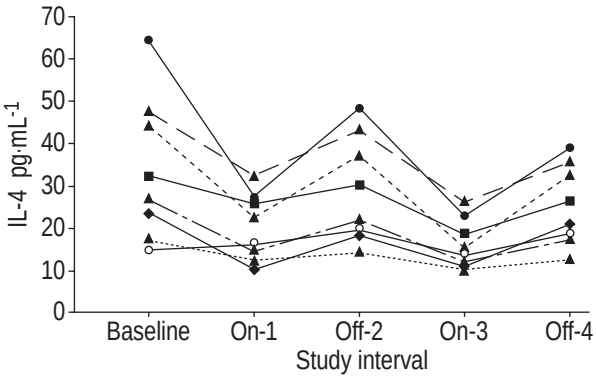

Fig. 1. – Graph showing an evident reduction in serum levels of interleukin-4 (IL-4) in each patient on the last day of the on-1 and on-3 intervals (theophylline administration). In contrast, a clear elevation in serum levels of IL-4 was found in each patient on the last day of the off-2 and off-4 intervals (theophylline withdrawal). Each point represents the serum level of IL-4 in a single patient at the respective intervals. Each line represents the variation in circulating IL-4 in a single patient.

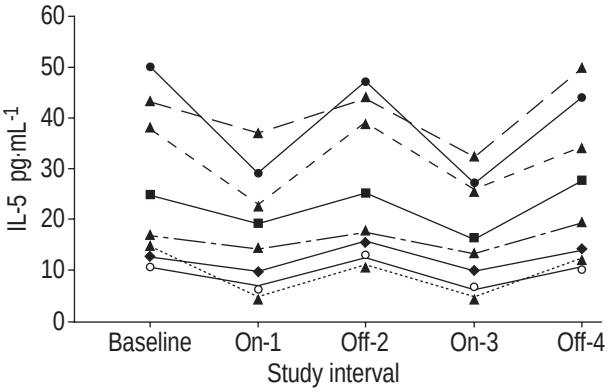

Fig. 2. – Graph showing an evident reduction in serum levels of interleukin-5 (IL-5) in each patient on the last day of the on-1 and on-3 intervals (theophylline administration). In contrast, a clear elevation in serum levels of IL-5 was found in each patient on the last day of the off-2 and off-4 intervals (theophylline withdrawal). Each point represents the serum level of IL-5 in a single patient at the respective intervals. Each line represents the variation in circulating IL-5 in a single patient.

Concerning the study design, the decision to use low-dose inhaled steroids as a 3-week pretreatment and during the study, even though the patients were classified as mild asthmatics according to currently recognized criteria [10], was based on the following two considerations.

Firstly, the patients had to be maintained in a steady clinical condition for the period needed for completion of the study, in order to determine whether fluctuations in ILs were related to theophylline administration or withdrawal and not due to possible exacerbation of asthma during the study period. Should this happen, the hypothesized cause-and-effect relation between theophylline and IL levels would be obscured. Furthermore, in a study group not receiving inhaled steroids, possible exacerbation would lead to either an increased rate of dropouts (to maintain the homogeneity of the patients) or the necessity for addition of inhaled steroids at an unpredictable time during the study period; this would prevent any conclusion related to the cause-and-effect hypothesis under study.

Secondly, although no direct evidence of decreased production or release of IL-4 and IL-5 has been clearly reported, there is evidence that steroids inhibit the transcription of several cytokines which are relevant in asthma, including IL-4 and IL-5 [12]. Therefore, patients already receiving inhaled steroids can be thought of as having a steroid-related suppression of IL-4 and IL-5 to some extent. The concern in this study was to investigate whether intermittent administration of theophylline (on–off periods) would result in an additional and independent effect on cytokines beyond the anticipated suppression caused by steroids.

Asthma is characterized by infiltration of the bronchial mucosa predominantly with activated T-lymphocytes and eosinophils [13–17]. T-lymphocytes are thought to be major cells involved in the orchestration of the allergic inflammatory response [12–14]. Th (CD4+) lymphocytes, and particularly the Th-2 subset, respond to the processed antigen and through the release of IL-4 and IL-5 are involved in the regulation of IgE production by B-cells and the recruitment of other inflammatory cells, such as eosinophils [13, 16, 17].

Increased local elaboration of the Th-2-type cytokines IL-5 and IL-4 has been clearly implicated in the pathogenesis of atopic asthma [6, 18–20]. IL-5 has selective biological effects on eosinophils and their precursors and may regulate selective accumulation of these cells in the asthmatic bronchial mucosa [6, 18]. IL-4 is an essential cofactor for IgE switching in B-lymphocytes [6, 21] and is, therefore, likely to be involved in situations in which there is inappropriate IgE synthesis, such as atopic asthma. There is evidence for increased mRNA encoding of IL-4 and IL-5 and increased numbers of IL-4 and IL-5-immunoreactive cells within the bronchial mucosa of patients with both atopic and nonatopic asthma, compared with nonasthmatic subjects [7]. Furthermore, it has been reported that local IL-4 and IL-5 mRNA expression correlated with disease severity and response to treatment in atopic asthma [22, 23]. Apart from the increased local expression of IL-4 and IL-5 production in the bronchial mucosa and bronchoalveolar lavage fluid [7, 16], the present study clearly demonstrates an *in vivo* elevation of circulating levels of IL-4 and IL-5 in patients with mild atopic asthma compared with nonasthmatic control subjects.

Theophylline has been in clinical use for the treatment of bronchoconstriction due to bronchial asthma for well over 50 yrs [1]. It is widely held that the bronchodilator effect of theophylline is due to the inhibition of PDE, thereby preventing the decrease in the intracellular concentrations of the cyclic nucleotides 3′,5′ adenosine monophosphate (cAMP) and 3′,5′-guanosine monophosphate (cGMP), which are important messengers involved in the regulation of smooth muscle tone in the airways, activation of inflammatory cells and mediator secretion [24]. The intracellular concentrations of cAMP and cGMP are determined by PDE, which catalyses the hydrolysis of cAMP and cGMP within the cytoplasm of cells. Airway smooth muscle cells express PDE isoenzymes III, IV and V, while inflammatory cells, including T-cells, eosinophils and mast cells, appear to express predominantly PDE IV [2, 24]. Since these particular cells are involved in the pathogenesis of asthmatic inflammation, being an important source of inflammatory mediators, it is believed that PDE inhibitors, such as theophylline, may have an important regulatory effect on them. Therefore, in addition to the well-recognized effect of theophylline on airway smooth muscle function, there is a growing amount of evidence to support the theory that theophylline also possesses anti-inflammatory or immunomodulatory properties [2–5, 25, 26]. Theophylline, even at low plasma concentrations, inhibits the late asthmatic reaction following allergen challenge [25, 26]. Furthermore, it is currently recognized that theophylline has other anti-inflammatory activities relevant to asthma, including the inhibition of cytokine synthesis and release, the inhibition of inflammatory cell activation and microvascular leakage and the prevention of airway hyperresponsiveness induced by airway inflammation [26].

The ability of theophylline to inhibit the late asthmatic response has indicated the possible inhibition of mechanisms regulating the influx and activity of inflammatory T-cells and eosinophils into the airways. Theophylline modifies lymphocyte behaviour, inhibits lymphocyte proliferation [15] and reduces the *in vivo* recruitment of lymphocytes into the airways of antigen-challenged asthmatics [27]. Withdrawal of theophylline in asthmatic patients is

associated with an increase in the number of activated CD4+ and CD8+ lymphocytes in the airway [28]. Based on these studies, it can be stated that theophylline prevents T-cell trafficking from the blood into the airways. Furthermore, theophylline significantly attenuates the antigen challenge-induced recruitment of activated eosinophils into the airways of asthmatic patients [29] and inhibits the *in vitro* Ig-induced eosinophil degranulation and the release of reactive oxygen species, eosinophil cationic protein and other basic proteins [30, 31].

The effect of theophylline on the Th-2 cells and on the cytokines IL-4 and IL-5 which regulate the inflammatory cell recruitment has been studied recently. ESSAYAN *et al.* [8] have shown that theophylline and other PDE IV inhibitors impede the *in vitro* expression of IL-4 and IL-5 genes in Th-2 cells. Theophylline reduces significantly the eosinophil airway infiltration induced by the intratracheal administration of recombinant human IL-5 [32]. In keeping with these studies, DJUKANOVIC *et al*. [33] have shown that theophylline decreases the numbers of epithelial T-cells containing IL-4 and IL-5. More recently, FINNERTY *et al*. [9] reported that theophylline induces a reduction in the expression of IL-4 and a tendency towards reduction in the expression of IL-5 on airway submucosal cells in asthmatic patients. In the present study, a theophylline-related downregulation and a theophylline withdrawal-related re-upregulation of serum IL-4 and IL-5 were observed, findings which are in close agreement with the effect of theophylline on the local expression of IL-4 and IL-5.

In summary, the results showed that asthmatic patients, although symptom-free, with normal spirometry and on inhaled steroids, have increased circulating levels of IL-4 and IL-5 which are substantially suppressed by a short-term administration of sub-bronchodilator dosage of theophylline. Withdrawal of theophylline results in the recovery of circulating IL-4 and IL-5 to baseline (pretreatment) values and this observation further strengthens the attribution of IL-4 and IL-5 downregulation to theophylline use. The findings support the current knowledge that theophylline exerts at least part of its anti-inflammatory/immunomodulatory action by affecting those particular cytokines. It would, therefore, appear that theophylline may contribute to asthma control through its ability to reduce the cytokines, which are relevant to allergic mucosal responses.

Along with the results of this study, considerable evidence has accumulated that theophylline has anti-inflammatory or immunomodulatory effects on serum levels well below 10 $\mu g \cdot mL^{-1}$. Theophylline can alter lymphocyte infiltration of the airways and attenuate the activation of eosinophils and the IgE response, probably through its effect on IL-4 and IL-5. Although the exact mode of action of theophylline remains to be determined, current available data suggest that theophylline has quite potent effects on the inflammatory cells and cytokines which are central among the pathogenetic mechanisms of asthma. Therefore, theophylline may deserve a role in the control treatment of asthma, mainly through its bronchoprotective and anti-inflammatory properties rather than its bronchodilatory action.

Most recent work has focused on the development and clinical use of selective inhibitors of the isoenzyme phosphodiesterase IV, such as denbufylline, nitraquazone, rolipram, tibenelast, Ro 20-1724 and BRL-61063, based on the overwhelming literature regarding the anti-inflammatory action of theophylline in asthma [34]. This is because inhibitors of this isoenzyme family have an appealing therapeutic profile: a broad-spectrum anti-inflammatory activity coupled with additional bronchodilatory and neuromodulatory actions. It is almost certain that information on the biological role of phosphodiesterases, along with convincing evidence that theophylline exerts anti-inflammatory action through nonselective phosphodiesterase inhibition, has already opened additional avenues towards the development of novel and more potent therapeutics for asthma, such as selective phosphodiesterase IV inhibitors.

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