Is the Role of Tumor Necrosis Factor-Alpha in Pathogenesis of Asthma Mediated by Oxidative Stress?

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Summary

In vitro studies indicate that tumor necrosis factor-alpha (TNF-α), a proinflammatory cytokine, plays role in bronchial hyperresponsiveness and airway remodelling in asthma; however, the mechanism of this effect is not clear yet. The purpose of this study is to assess whether the role of TNF-α in the pathogenesis of bronchial asthma is mediated by oxidative stress.
Thirty-four asthmatic outpatients (mean age 50.23±15.21) and 20 healthy controls of the same age group were included in the study. After clinical evaluation, serum TNF-α concentration was measured by ELISA asssay using BioSource human TNF-α kit; and serum malonyldialdehyde (MDA) concentration, which is an indirect marker of oxidative stress, was measured spectrophotometrically by Yoshioka-Kawada method.
Mean serum TNF-α concentration was 17.37±8.15 pg/ml, and MDA concentration was 0.93±0.13 nmol/L in asthmatic patients; whereas they were 6.03±3.94 pg/ml and 0.87±0.10 nmol/L consecutively in control group. When two groups were compared, there was no significant difference in serum MDA level (p=0.219), but TNF-α concentration was higher in asthmatics (p=0.001). However, there was no correlation between serum MDA and TNF-α concentrations (p=0.352). Serum MDA concentrations were found to be higher in moderate and severe asthmatics compared to mild disease (p=0.037).
The results of this study indicate that serum TNF-α concentration is increased in asthmatics compared to healthy people. However, there was no correlation between serum TNF-α and MDA concentrations; that is why further studies are indicated to explore the mechanisms of TNF-α induced changes in asthma pathogenesis.
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Key Words: Asthma, cytokines, malonyldialdehyde, tumor necrosis factor

Özet

Tümör Nekroz Faktör-Alfa'nın Astım Patogenezindeki Rolü Oksidan Stres Aracılı mı?
In vitro çalışmalarda proinflamatuvar bir sitokin olan tümör nekroz faktör-alpha'nın (TNF-α) astımda bronş hiperreaktivitesinde ve havayolu yeniden yapılanmasında rol oynadığı gösterilmişse de bu etkinin mekanizması henüz açık değildir. Bu çalışmanın amacı TNF-α'nın bronş astması patogenezindeki rolünün oksidan stres aracılı olup olmadığını araştırmaktır.
Otuz dört astmatik poliklinik olgusu (ortalama yaş 50.23±15.21) ve aynı yaş grubundan 20 sağlıklı kontrol çalışmaya dahil edilmiştir. Klinik değerlendirme sonrası serum TNF-α konsantrasyonu ELISA asssay ile BioSource human TNF-α kiti kullanılarak ve oksidan stresin dolaylı bir göstergesi olan serum malonildialdehid (MDA) konsantrasyonu spektrofotometrik olarak Yoshioka-Kawada metodu ile ölçüldü.
Astmatik olgularda ortalama serum TNF-α konsantrasyonu 17.37±8.15 pg/ml ve MDA konsantrasyonu 0.93±0.13 nmol/L iken; kontrol grubunda sırası ile 6.03±3.94 pg/ml ve 0.87±0.10 nmol/L bulundu. İki grup karşılaştırıldığında serum MDA düzeyinde anlamlı fark yoktu (p=0.219), TNF-α konsantrasyonu ise astım grubunda daha yüksekti (p=0.001). Ancak serum MDA ve TNF-α konsantrasyonları arasında korelasyon saptanmadı (p=0.352). Serum MDA konsantrasyonu orta ve ağır astımlılarda hafif olgulara kıyasla daha yüksekti (p=0.037).
Bu çalışmanın sonuçları serum TNF-α konsantrasyonunun astımlı olgularda sağlıklı kişilerden yüksek olduğunu göstermektedir. Ancak serum TNF-α ve MDA konsantrasyonları arasında korelasyon gösterilememiştir, bu nedenle astım patogenezinde TNF-α'ya bağlı değişikliklerin mekanizmasını açıklamak için farklı çalışmalar yapılmalıdır.
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Anahtar Kelimeler: Astma, sitokinler, malonildialdehid, tümör nekroz faktör

Introduction

Asthma is a chronic inflammatory disorder of the airways, characterized by reversible airflow obstruction. Oxidative stress was reported to play an important role in the pathophysiology of asthma and may be a final common pathway leading to tissue damage (1). Oxidative stress has many detrimental effects of on airway including airway

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smooth muscle contraction, induction of airway hyperresponsiveness, mucus hypersecretion, epithelial shedding and vascular exudation (1, 2, 3). Oxidants can induce cytokine and chemokine production through induction of the oxidative-stress sensitive transcription of nuclear factor-κB in bronchial epithelial cells (4).

In vitro studies indicate that tumor necrosis factor-alpha (TNF-α), a proinflammatory cytokine, plays role in bronchial hyperresponsiveness and airway remodelling in asthma (5, 6). The mechanism of this effect is not clear yet; however, it was reported that contractility of airway smooth muscle increases by producing oxidants when stimulated by TNF-α (7). TNF-α was reported to induce the production of reactive oxygen species and chymokines in various cell systems (8, 9).

The purpose of this study is to assess whether the role of TNF-α in the pathogenesis of bronchial asthma is mediated by oxidative stress.

Material and Methods

Patients

Thirty-four asthmatic outpatients (17-72 years-old, mean age 50.23±15.21) were included in the study. The diagnosis of asthma had previously been established by a respiratory physician on the basis of American Thoracic Society (ATS) criteria (10). Severity of asthma was classified according to the National Institutes of Health/World Health Organization (NIH/WHO) guidelines (11).

The asthmatic patients had been clinically stable for at least a month. Mild-moderate-severe persistent asthmatics were receiving inhaled bronchodilatory therapy in the form of long-acting β2 agonists and inhaled corticosteroids; whereas intermittent asthmatics were receiving short-acting β2 agonists on demand. Patients who had conditions known to affect serum TNF-α levels (infection, heart failure, cancer and collagen vascular diseases) were excluded (12).

Control subjects

The control group consisted of 20 voluntary healthy subjects (18-70 years-old, mean age 49.5±8.10). They were nonsmokers with no history of lung disease and had normal pulmonary function. None was receiving any medications.

The study was approved by the local ethics committee and informed consent was obtained from all subjects.

Pulmonary Function Evaluation

Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were measured with standard spirometric techniques according to the ATS criteria with Minato AutoPal Spirometry, Japan (13). The highest value from at least three spirometric maneuvers was used.

Measurement of Serum TNF-α and MDA Concentrations

Fasting blood samples (approximately 10 ml) were collected by venipuncture into plain tubes. Sera were obtained by centrifugation at 1000Xg for 5 min at room temperature. The samples were stored at –70 °C until analysis.

Serum TNF-α concentration (pg/ml) was measured by a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) using hTNF-α kit (BioSource International inc, Cat No: KHC 3012; Nivelles, Belgium) (14, 15, 16). Serum malonyldialdehyde (MDA) concentration was measured as an indirect marker of oxidative stress in terms of TBARS (thiobarbituric acid reactive substances), spectrophotometrically (17, 18).

Statistical Analysis

Correlations between parameters were evaluated using Pearson correlation analysis. Data of patients and control groups were compared by Mann-Whitney U test and data of asthma subgroups were compared by Kruskal-Wallis test. All data were presented as mean±SD and significance was defined as a value of $p<0.05$.

Results

The characteristics and data of pulmonary function tests of asthmatics and control subjects were shown in table I. Study groups were at the same age interval (p=0.936).

Mean serum concentrations of TNF-α and MDA in asthmatics and control group were shown in table II. When two groups were compared, there was no significant difference in serum MDA level (p=0.219), but TNF-α concentration was higher in asthmatics (p=0.001). However, there was no correlation between serum MDA and TNF-α levels in asthmatics in correlation tests (r = 0.165, p=0.352). FEV_1% of asthmatic patients was not correlated to either TNF-α (r = 0.196, p=0.273); nor MDA (r = 0.269, p=0.130).

When the asthmatics were subgrouped according to the severity of disease as mild intermittent (group 1, n=6), mild persistent (group 2, n=8), moderate persistent (group 3, n=11) and severe persistent (group 4, n=9) asthma, there was statistically significant difference in serum MDA concentrations (p=0.037); whereas the difference in TNF-α was not significant (p= 0.149) (table 3). In dual analyses of these four groups, MDA of group 1 differed

Table I: The characteristics and pulmonary function tests of asthmatics and control subjects (mean±SD)

	Asthma (n=34)	Control (n=20)
Age (years)	50.23 ± 15.21	49.5 ± 8.10
Female/Male	25/9	16/4
Smoking	--	--
FEV_1%	69.18 ± 17.06	88.63 ± 6.12
FEV_1/FVC%	67.45 ± 10.07	78.24 ± 5.45

FEV1%: forced expiratory volume in one second, %predicted
FVC%: Forced vital capacity

Table II: The comparison of serum TNF-α and MDA concentrations of asthmatics and control subjects (mean±SD)

	Asthma	Control	P value
TNF-α	17.37 ± 8.15	6.03 ± 3.94	0.001
MDA	0.93 ± 0.13	0.87 ± 0.10	0.219

TNF-α: Tumor necrosis factor-alpha (pg/ml)
MDA: Malonyldialdehyde (nmol/mL)

significantly from MDA of group 2, 3 and 4 (p= 0.02, 0.02 and 0.005 consecutively). There was no statistically significant difference in dual analyses of remaining groups.

Discussion

Oxidative stress and disturbed antioxidant status in asthmatics are well established (1, 19-23). Elevated levels of MDA, one of lipid peroxidation products, have been observed both in plasma and breath condensate in asthmatics (20, 21) as well as ethane, carbon monoxide, nitric oxide and H_2O_2 (21-23).

Direct measurement of oxidants is difficult since they are highly reactive, short-lived species. Oxidative stress is often measured as damage of oxygen radicals upon biomolecules, including lipids. Thiobarbituric acid-reactive substances (TBARS) measure the concentration of MDA, an end product of the oxidation and decomposition of polyunsaturated fatty acids containing three or more double bonds (1).

Cytokines play a critical role in orchestrating and perpetuating inflammation in asthmatic airways and several specific cytokine and chemokine inhibitors are now in development in treatment of asthma (24). Elevated levels of TNF-α, the proinflammatory cytokine, have been detected in sputum, bronchoalveolar lavage fluid and biopsy samples from asthmatics (25, 26). TNF-α was reported to induce cytokine and chemokine synthesis (27) and plays role in airway remodelling inducing smooth muscle cell proliferation (5, 6). Inhalation of TNF-α causes airway hyperresponsiveness (28), and its level is increased during asthma attacks (29).

TNF-α increases the contractility of airway smooth muscle and the underlying mechanisms are being investigated yet. (7, 30, 31). Thabut et al (7) proposed that TNF may act indirectly, via the release of other inflammatory or bronchoconstricting agents such as leukotrienes, by inflammatory cells; or directly on airway smooth muscle cells that express TNF receptors. This direct effect of TNF on airway smooth muscle contractility could be mediated by reactive oxygen species synthesized by muscular cells. In previous experimental studies it was reported that TNF leads to the generation of reactive oxygen species in various cell systems (8, 9).

In the present study we searched for serum TNF-α concentration in association with MDA in asthmatic patients in order to explore whether the role of TNF-α in the pathogenesis of bronchial asthma is mediated by oxidative stress. We found that serum TNF-α concentration was higher in asthmatics compared with healthy controls, in consistency with previous studies (24-28). However, serum MDA level in asthmatics was similar to controls and there was no correlation between MDA and TNF-α concentrations. The reason for this indifference, and consequently for no correlation between MDA and TNF may be that, serum MDA concentrations were found to be increased during asthma attacks significantly, and decreased partially at 24-48 hours with treatment, and decreased further after 3 weeks (32). Ozaras et al (33) searched for changes in MDA levels in serum and BAL by the treatment of asthma. They found that MDA levels decreased significantly by one-month treatment with inhaled steroid and beta2-agonist, but stayed still higher than healthy controls. However, our study group consisted of clinically stable outpatients receiving regular treatment as inhaled steroid and/or beta2-agonist.

Although the mean serum concentration of the whole group was not higher than controls statistically, when the results were evaluated according to the clinical stage of asthma, it was observed that serum concentrations of MDA increased in parallel to the severity of disease. Similarly, Wood et al (34) had reported that lipid peroxidation (measured as plasma 8-iso-PG F2 alpha) was related to the clinical asthma severity. Montuschi et al (35) measured 8-isoprostane levels in breath condensate of 34 asthmatic patients and they also stated that 8-isoprostane levels were significantly increased in subjects with severe as compared with mild to moderate asthma.

In dual analyses of our four study groups, MDA of group 1 differed significantly from MDA of group 2, 3 and 4; so we can conclude that MDA is higher in persistent asthmatics than intermittent ones.

In our study, when the asthmatics were subgrouped according to the severity of disease it was detected that there was no statistical difference in TNF-α concentrations of mild and severe asthmatics. Likewise, no correlation was seen between TNF-α and FEV_1%. Hughes et al studied serum levels of the proinflammatory cytokines IL-5 and TNF-α in asthmatic children and found them to be unrelated to either serum eosinophilia and asthma severity. Serum TNF-α was not correlated to FEV_1%, markers of asthma severity and composite asthma score (made of PEF, symptoms and medication use). They concluded that number of circulating eosinophils remained to be a better index of asthma severity than serum cytokine levels (36).

The results of this study indicate that serum TNF-α concentration is increased in asthmatics compared to healthy people. However, there was no correlation between serum TNF-α and MDA concentrations; that is why further studies are indicated to explore the mechanisms of TNF-α induced changes in asthma pathogenesis.

Table III: Serum TNF-α and MDA concentrations in asthmatics grouped according to the severity of asthma.

	Mild intermittent	Mild persistent	Moderate persistent	Severe persistent	P Value
TNF-α	11.87±5.99	15.77±5.46	19.62±8.41	21.17±10.54	0.149
MDA	0.80±0.10	0.87±0.12	0.97±0.16	0.95±0.14	0.037

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