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Original Article

Theophylline and silymarin are potent inhibitors of IL-4 secretion from activated human basophils

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Abstract. Basophils are the most important cells in allergic reactions and secret interleukin 4 (IL-4) and tumor necrosis factor α (TNFα) mediators. Theophylline as a phosphodiesterase 4 inhibitor and silymarin with its anti-inflammatory effects seem to be useful drugs in treatment of allergic reactions. The aim of the present study was comparing effects of theophylline and silymarin on IL-4 and TNFα secretion by human basophils. After isolation of peripheral blood mononuclear cells, three cultures were designed; theophylline, silymarin and control cultures. Cultures were incubated for 15 minutes with theophylline, silymarin and buffer before challenging with bacterial lipopolysaccharide and anti-IgE antibody. IL-4 and TNFα mediators were assessed by ELISA assay and values of each culture were compared with control group. Silymarin and theophylline as potent inhibitors decreased secretion of IL-4 from human basophils significantly ($P<0.01$ and $P<0.01$, respectively). However, neither theophylline nor silymarin had significant effect on TNFα secretion ($P=0.14$ and $P=0.08$, respectively). These data suggest that silymarin and theophylline could regulate the generation of IL-4 from human basophils.

Keywords: Theophylline, silymarin, interleukin-4, tumor necrosis factor α, basophil

Introduction

Basophils are well known for their pivotal role in allergic reactions. These cells are at least abundant leukocytes in peripheral blood (less than 1 percent). Basophils like mast cells have high-affinity IgE receptors on cell surface and also contain granules in their cytoplasmic space that will be exocytose when cross-linked antigen-specific IgE occurred. Granules consists of histamine, leukotrienes, cytokines, chemokines and other mediators that are responsible for early and late allergic reactions [1]. In allergic disorders, basophils promotes T helper2 (Th2) differentiation by IL-4 production and other co-stimulatory molecules such as CD40, CD80 and CD86 [2, 3]. Basophils would consider as a key role in allergic reaction for major source of cytokines such as IL-4 and Tissue necrosis factor alpha (TNFα) after activation through IgE receptors [4-6]. Production of these cytokines could be induced either by IgE-dependent mechanism or non-IgE dependent mechanism.

Silymarin is a flavonoid derived from milk thistle, silybum marianum L. It is an antioxidant agent with protective effects on liver organ and prescribed clinically in liver diseases [7, 8]. In addition, silymarin has been described to represent antioxidant, immunomodulatory, anti-proliferative, anti-fibrotic, and antiviral activities. The mechanisms of silymarin is elusive and its clinical efficacy is currently uncertain. Several studies, have reported immunomodulatory actions of silymarin *in vitro*. In addition, silymarin could modulate immune system and stabilizes cell membrane. Previously, it was shown that silymarin could reduce histamine release from basophils and also promotes of Th2 cytokines and suppresses of Th1 cytokines [9, 10]. However, data on its effects on Th1/Th2 balance is in controversy.

Cyclic monophosphate phosphodiesterases (PDE) are an enzyme family that degrade cyclic adenosine monophosphate (cAMP) and guanosine monophosphate. PDE-4 enzyme, is a member of PDE enzyme that exist mostly in immune cells including neutrophils, monocytes, eosinophils and lymphocytes. Also, PDE-4 express highly in epithelial cells of lung and play a crucial role in pathogenesis of inflammatory lung diseases such as chronic obstructive pulmonary disease (COPD). PDE inhibitors prevent degradation of cyclic adenosine monophosphate (cAMP) and guanosine monophosphate. Moreover, PDE-4 inhibition results to accumulation of

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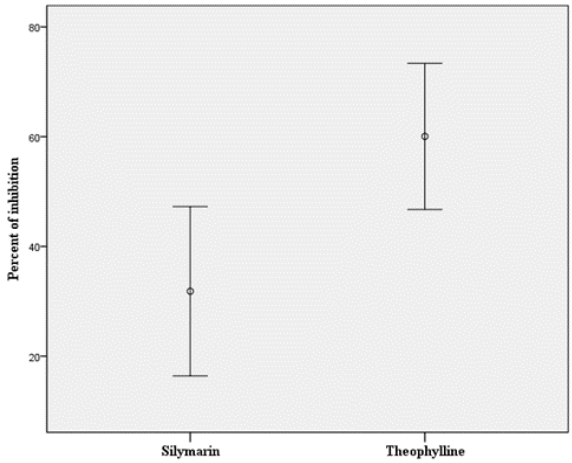

Figure 1 Effects of theophylline and silymarin on IL-4 secretion. Cells were incubated for 15 minutes before challenging with anti-IgE and LPS for 4 hours (n=5). Mean value of IL-4 was 60.06±10.73 and 31.83±12.43 in cultures treated with theophylline and silymarin respectively.

cAMP and consequently leads to suppressing expression of pre-inflammatory cytokines and chemokines [11, 12].

Theophylline (1, 3 dimethylxanthine) is a non-selective PDE inhibitor with anti-inflammatory and bronchodilator effects. Previously, it has been demonstrated that theophylline administration could leads to statistically improvement of forced expiratory volume in 1 second, forced vital capacity, and FEV1/FVC [13]. Also, long term administration of theophylline leads to reduction of interleukin 8 and tissue necrosis factor alpha in patients with chronic obstructive pulmonary disease [14]. Furthermore, theophylline administration in asymptomatic atopic asthmatic patients could reduce the serum level of IL-4 and also interleukin 5 [15].

The aim of the present study was to determine whether silymarin and theophylline regulates the generation of the cytokines, IL-4 and TNFα, from activated basophils.

Materials and Methods

Preparation of inhibitors and stimuli

Silymarin (Sigma, Poole, U.K.) was dissolved in 100% dimethyl sulfoxide (Sigma, Poole, U.K.) to achieve a 100mM stock solution. We used 10^{-2} concentration of silymarin stock solution. Theophylline (Sigma, Poole, UK) was prepared daily as stock solutions (2 mM) in the buffer. Escherichia coli bacterial lipopolysaccharide (LPS) (Enzo Life Sciences, Exeter, UK) was diluted with sterile HEPES buffer (2000ng/ml). Neat polyclonal goat anti-human IgE antibody (Sigma, Poole, UK) was made up in distilled water and stored at 4ºC. All drugs were diluted to desired concentration in the buffer just before use.

Isolation of human basophils

Human peripheral blood mononuclear cells (PBMCs) were collected by vein puncture in heparinized tubes from whole blood obtained from fifteen healthy volunteers, who gave informed written consent. The study protocol was approved by Ethics Committee of Isfahan University of Medical Sciences. Our exclusion criteria were as follows: any acute or chronic infectious disease, any neurological disorder, addiction and current smokers.

The cells were isolated by using standard Ficoll density-gradient centrifugation. Shortly, heparinized blood (50ml) was mixed with phosphate-buffered saline (PBS) (50ml) and after coating samples with Ficoll, the centrifugation (2800 rpm for 20 min) was performed and the interface layer of PBMCs was harvested and washed twice with PBS. Cell viability was assessed by trypan blue dye exclusion (0.4% trypan blue in PBS). Cells with viability of 95% and more were selected for further experiments.

Cell culture and mediator release

The cells were categorized in three cultures; control, theophylline and silymarin cultures. The cultures contained RPMI 1640 buffer (Gibco BRL, Dundee, U.K.) supplemented with BSA (Sigma, Poole, U.K.) (1 mg/ 1ml), gentamicin (Gibco BRL, Dundee, U.K.) (10 mg /ml) and calcium chloride (made up to 1 mM). Cultures were incubated with theophylline, silymarin and buffer for duration of 15 minutes before challenging with LPS and anti-IgE antibody. Cells incubated in buffer alone were considered as values of spontaneous mediator release, and all values cited for other cultures were corrected by subtracting values of spontaneous mediator release.

In experiments monitoring mediator generation, basophils were activated for 4 hours to assess IL-4 generation and 24 hours to assess TNFα generation. These conditions for optimal generation of IL-4 and TNFα generation have been reported by others [16].

After activation, the cells were centrifuged at 450 g for 4 min and the supernatants saved and analyzed for mediator release. The supernatant was assayed for IL-4 and TNFα mediators by available commercial enzyme linked immunosorbent assay (ELISA) kit (Biolog Life Science Institute, Bremen, Germany). The OD of samples was measured at 450 nM using a Dynatech plate reader.

Statistical analysis

Wilcoxon test were applied for comparing data between study groups. Data are expressed as means ± standard deviation. A P value of less than 0.05 was considered statistically significant. All of the analysis was performed by using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Effects of theophylline and silymarin on IL-4 secretion

The means ± SEM generation of IL-4 concentration inhibited by theophylline and silymarin were 5.68±1.53 ng/mL and 9.69±1.77ng/mL respectively. Both theophylline and silymarin had significant effects on reduction of IL-4 secretion ($P<0.01$ and $P<0.01$ respectively) (Figs. 1 and 2). As figures 1 depicts, theophylline is more effective inhibitor than silymarin.

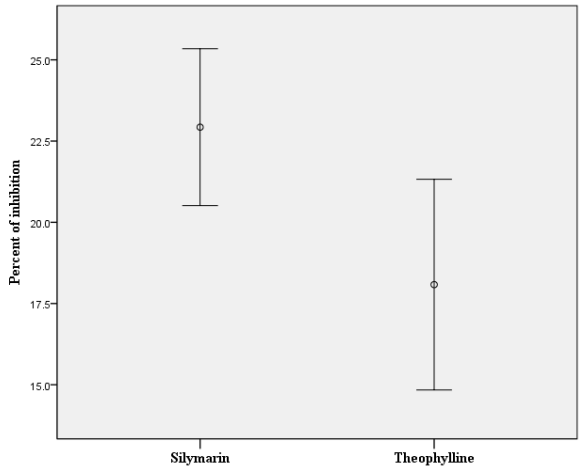

Figure 2 Effects of theophylline and silymarin on TNFα secretion. Cells were incubated for 15 minutes before challenging with anti-IgE and LPS for 24 h (n=5). Mean value of TNFα was 18.08±2.61 and 22.93±1.94 in cultures treated with theophylline and silymarin, respectively.

Effects of theophylline and silymarin on TNFα secretion

The mean value of TNFα inhibition in silymarin and theophylline cell cultures were 1.53±0.04 ng/mL and 1.62±0.52 ng/mL respectively. Neither silymarin nor theophylline had significant effects on reduction of TNFα secretion (P=0.08 and P=0.14 respectively) (Fig. 2).

Discussion

In the present study, we have investigated the effects of silymarin and theophylline on IL-4 and TNFα generation from human basophils.

A recent study showed topical administration of silymarin in NC/Nga mice with atopic dermatitis showed significant decrement of serum level of IL-4 [16]. Bakhshaee et al. have shown that prescription of silymarin with anti-histamine had significantly better improvement in clinical symptom severity of allergic disorders and the level of IL-4 decreased numerically in the sera of patients [17]. In the present study, it was shown that silymarin is effective inhibitor of the IL-4 generation from basophils, confirming observations made by others [16]. Previously inhibition of histamine releasing by theophylline from basophils has been reported [18]. Our data demonstrate the inhibitory effects of theophylline on IL-4, as reported by others [19, 20].

Further studies were performed to determine silymarin and theophylline effects that regulate cytokine generation from human basophils. Both compounds inhibited the IgE-mediated generation of TNFα. Earlier, it has been shown that long term treatment with theophylline in COPD patients lead to significant reduction of TNFα in sputum and could be associated with FEV1 increase [21]. In addition, theophylline could enhance basophil apoptosis and maybe this effect is one of the explanations of its affectivity in allergic diseases [9].

The mechanism of theophylline on decrement of pro-inflammatory cytokines generation is inhibition of NF-kappaB activation via protection of IkappaBalpha protein [19]. Many properties of silymarin has been reported; some of them are anti-oxidant activity, anti-inflammatory activity, immuno-suppressive and immunomodulatory effects [22, 23]. The large body of data showed that Silymarin increases lymphocyte proliferation, interferon gamma (IFN-γ), IL-4 and IL-10 secretion by activated lymphocytes, in a dose-dependent manner [24]. Previously Han and colleagues reported that silymarin inhibits chemical-induced irritant contact dermatitis in mice [25].

In individual studies, administration of silymarin in patients with peritoneal dialysis leads to decreasing in serum level of TNFα. In another study in patients with β-thalassemia major it was shown that silymarin could significantly decrease TNFα in serum of patients [26, 27].

Inhibition of TNFα expression by silymarin had been reported by Han et al. and Zi and colleagues [25, 28]. The recent meta-analysis showed that administration of silymarin in patients with hepatitis B can decrease TNFα, TGF-β1 and IL-6 [29]. Our results showed numerically but not significantly decrement of TNFα secretion by human basophils. This data could suggest that silymarin affects other sources of TNFα secretors except basophils; however more studies are suggested in this regard.

Silymarin exerts its anti-inflammatory effects via phosphorylation of IκBa and consequently it leads to inhibition of NF-κB. NF- κB influence production of cytokines, chemokines and other mediators related to inflammation [30, 31]. Therefore it could be concluded that PDE-4 inhibitors exerts their effect via inhibition on NF- κB and due to wide range of activity of NF- κB. It seems that more inflammatory mediators will be affected by PDE-4 inhibitors treatment.

Our study limitations were low number of cell cultures due to financial problems and lack of assessment of other involved cytokines in allergic reactions such as interleukin13 and interleukin 5. We recommend future studies to investigate inhibitory effects of silymarin and theophylline on IL-6, IL-13, IL-25 and other involved biomarkers in allergic reactions secreted from human basophils.

Conclusion

In conclusion, the present study demonstrates significant effects of silymarin on mediator releasing from human basophils, and it seems that theophylline and silymarin could be useful drugs in allergic diseases.

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Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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