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Impact of fexofenadine, osthole and histamine on peripheral blood mononuclear cell proliferation and cytokine secretion

Natalia Karolina Kordulewska, Elżbieta Kostyra*, Michał Matysiewicz, Anna Cieślińska, Beata Jarmołowska

Department of Biochemistry, Faculty of Biology & Biotechnology, University of Warmia and Mazury, ul. Oczapowskiego 1A, 10-719 Olsztyn, Poland

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ABSTRACT

This paper compares results of peripheral blood mononuclear cell (PBMC) incubation with fexofenadine (FXF) and osthole. FXF is a third-generation antihistamine drug and osthole is assumed a natural antihistamine alternative. To our best knowledge, this is the first comparative study on FXF, osthole and histamine cytokine secretion and cytotoxicity in PBMC *in vitro* cultures using cell proliferation ELISA BrdU. The cultures were treated 12, 42, 48 and 72 h with FXF and osthole at 150, 300 and 450 ng/ml concentrations and histamine at 50, 100 and 200 ng/ml. Our study results confirm that FXF, osthole and histamine exert no cytotoxic effect on PBMCs and that IL-6, IL-10 and TNF-α cytokine secretion following osthole cell stimulation was similar to that by FXF stimulation.This confirms our hypothesis that osthole is a natural histamine antagonist, and can therefore be beneficially applied in antihistamine treatment.

1. Introduction

Allergy is one of the most common chronic diseases in Europe, and it is rapidly approaching epidemic proportions. Allergic diseases are particularly common in children, adolescents and young adults, causing a serious problem for health care systems in developing and developed countries, including Poland. Current predictions estimate that the number of allergy afflicted in 2020 will equal the healthy population. It is therefore paramount to find new pharmaceuticals which successfully combat allergic disease (Samel-Kowalik et al., 2009; Żukiewicz-Sobczak et al., 2012). Mast cells and basophils are very important in allergic reactions. Their degradation releases histamine – the important mediator of allergic and inflammatory responses – and also several active substances including Th2-type cytokines. Histamine changes vascular permeability, drives inflammatory cell infiltration, and modulates mediator production by these cells (Guzik et al., 2003; Venable and Thurmond, 2006). Histamine also modulates immune responses differently depending on the type of receptor on which it acts, and it plays a major modulatory role in physiology and in pathological conditions through its H_1, H_2, H_3, H_4 G-protein-coupled receptors (Breunig et al., 2007; O'Mahony et al., 2011; Dy and Schneider, 2001; Gschwandtner et al., 2012). In addition, histamine's H_2R receptor stimulation suppresses many immune responses including cytokine production (Żak-Nejmark et al., 2002; Jutel et al., 2009).

Fexofenadine (FXF) is the active metabolite of terfenadine with selective peripheral H_1 receptor antagonist activity (Kasurka et al., 2011; Simpson and Jarvis, 2000; Woosley et al., 1993). It inhibits antigen-induced bronchospasm in sensitized guinea pigs and also histamine release from peritoneal mast cells in rats. FXF is a third-generation antihistamine, non-sedating, rapid and very long acting used in symptoms associated with allergic diseases such as chronic idiopathic urticaria, pruritus, allergic rhinitis, asthma and dermatitis (Aksakal et al., 2010; Kasurka et al., 2011). It is not metabolized by the liver and has no effect on cytochrome P450 (Howarth et al., 1999). In addition, human clinical and animal studies have shown that FXF is free of cardiotoxicity and nervous system effects, such as those witnessed with other non-sedating antihistamines (Hey et al., 1966; Pratt et al., 1999; Rampe et al., 1993).

Our search for anti-allergic agents from natural sources revealed promising properties in the *Cnidium monnieri* dried fruit. This is used in traditional chinese medicine for its major active component – osthole – which has an isopentenoxy-coumarin structure (Gao et al., 2014). Pharmacological studies demonstrate its wide bioactivity as an antibacterial, anticonvulsant, anti-osteoporotic, anti-carcinogenic, anti-diabetic and anti-allergic agent

* Corresponding author.
E-mail addresses: natalia.smulska@uwm.edu.pl (N. Karolina Kordulewska), elzbieta.kostyra@uwm.edu.pl (E. Kostyra), michal.matysiewicz@uwm.edu.pl (M. Matysiewicz), anna.cieslinska@uwm.edu.pl (A. Cieślińska), bj58@wp.pl (B. Jarmołowska).

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(Gao et al., 2014; Lv et al., 2012; Matsuda et al., 2002; Nam et al., 2014; Shi et al., 2013, Zimecki et al., 2009). We hypothesize osthole has potential in allergy treatment; in this is first known the investigation of osthole cytotoxicity and cytokine secretion in *in vitro* cultured human peripheral blood mononuclear cells (PBMC). Here, we evaluate the precise mechanisms and effects of osthole treatment on inflammatory processes and allergic reactions. This research examines fexofenadine, osthole and histamine cytotoxicity on cultured human peripheral blood mononuclear cell proliferation and cytokine secretion.

2. Material and methods

2.1. Study participants

Peripheral blood from ten healthy 25–30 year old non-smoking male volunteers was examined. Selected participants had no allergic signs or symptoms and had not taken antihistamines in the preceding five months. The study was conducted with local Ethic Committee approval and all volunteers gave written informed consent.

2.2. Chemicals

Fexofenadine (FXF; PubChem CID: 63002), osthole (PubChem CID: 10228) and histamine (PubChem CID: 774) were obtained from Sigma-Aldrich. FXF was dissolved in 8% dimethylsulfoxide (DMSO, Sigma) to final concentration under 0.1%; thus not affecting cell viability. Osthole was dissolved in 96% ethyl alcohol (AbChem) and histamine in RPMI-1640 (Sigma-Aldrich). All solutions were sterilized through a 0.22 μg/ml filter and stored at 4 °C as stock solutions for later dilution.

2.3. PBMC isolation

Participant peripheral blood samples were collected between 9.00 a.m. and 10.00 a.m. on the first study day and concentrated in grouping tubes with K3EDTA (BD Vacutainer). PBMC isolation was initiated after tube transport to the laboratory, where monocytes, lymphocytes and dendritic cells were isolated by Ficol density gradient centrifugation (Histopaque, 1077, Sigma). The PBMC layer was double-washed with RPMI-1640 (Sigma-Aldrich), counted, and diluted with RPMI-1640 supplemented with 1% heat inactivated human AB serum, 1% gentamycin and 0.25% phytohemagglutinin-L (PHA-L, Roche Diagnostics). The isolated cells were seeded on 96-well plates at 0.25×10^5, and active reagents were added to each well after 24 h. The effect of 150, 300 and 450 ng/ml fexofenadine and osthole and 50, 100 and 150 ng/ml histamine on PBMC proliferation was determined. Peak FXF serum concentrations were compared with the 100% registered for unstimulated control PBMC in pure culture.

2.4. BrdU PBMC proliferation assessment

Proliferated PBMC were prepared as in the manufacturer's instructions (Roche Diagnostic, Cell Proliferation ELISA BrdU), and absorbance was measured at 492 nm λ after 12, 24, 48 and 72 h incubation with each substrate solution.

2.5. Cytokine determination

Cells were seeded at 2×10^6 cells/ml with RPMI-1640 and supplemented with 1% heat-inactivated human AB serum, 1% gentamycin and 0.25% PHA. Pure medium formed the control for each substance concentration added after 24 h incubation. Cells were harvested after a further three days and the supernatant was collected and stored at −80 °C. Commercial ELISA kits determined interleukin IL-6, IL-10 and tumor necrosis factor (TNF-α) output, *via* quantitative sandwich immunoassay. Tripicate samples were run and the results were equalized by comparison with standard curves expressed in pg/ml.

2.6. Statistical analysis

Statistical analysis comprised one-way ANOVA comparisons of control and test culture proliferation. Experimental values were expressed as mean ± standard error at $P < 0.001$ significance level. GraphPad Prism 6 for Windows software was utilized (GraphPad Software, Inc., USA).

3. Results

3.1. BrdU test results

Bromodeoxyuridine (BrdU) determines cell proliferative changes over time by measuring cell DNA incorporation.

The concentrations of 150, 300 and 450 ng/ml FXF and osthol and 50, 100 and 200 ng/ml histamine were evaluated to determine PBMC toxicity. These concentrations were chosen because this is the FXF human serum level following its administration; and similar osthol concentrations were required for comparison.

The BrdU FXF test effects on PBMC are presented in Fig. 1A. Dose-dependent increases were observed in 24 h incubations, with the largest recorded for 300 ng/ml administration. No statistically significant decreases were detected when compared with controls, and cell incubation in osthol initiated no statistically significant BrdU incorporation in cellular DNA (Fig. 1B). While this was also true for all histamine administations (Fig. 1C), PBMC proliferation in 50 ng/ml histamine only registered the same level as the control after 48 h incubation. The absence of statistically significant decrease in BrdU tests for all substances and concentrations confirmed the expected lack of PBMC cytotoxicity.

3.2. Fexofenadine, osthole and histamine effect on PBMC cytokine secretion

The influence of the above active substances on cytokine secretion was assessed: cytokines comprised IL-6 and IL-10 interleukins and TNF-α Tumor Necrosis Factor, and cytokine levels secreted by untreated PBMC in pure culture medium formed the controls.

3.2.1. Single substance application

3.2.1.1. IL-6 secretion. (a) PBMC incubation with 150 ng/ml FXF had the greatest IL-6 increase (Fig. 2A1 and C1), (b) histamine incubation significantly increased IL-6 at each of its 50, 100 and 200 ng/ml concentrations at $P < 0.001$ (Fig. 2 C1) and (c) no osthole concentration made any difference to control PBMC IL-6 secretion (Fig. 2A1 and B1).

3.2.1.2. IL-10 secretion. All substance concentrations initiated a statistically significant $P < 0.01$ increase in PBMC IL-10 secretion compared to the control effect (Fig. 2A2–C2).

3.2.1.3. TNF-α secretion. Incubation with all examined substances caused inhibition of PBMC TNF-α secretion compared to pure medium controls (Fig. 3A–C). (a) There was no change in TNF-α secretion in PBMC incubated in different fexofenadine concentrations. (b) 150 and 300 ng/ml osthole incubation exhibited statistical differences to controls (Fig. 3B); with 450 ng/ml having