ORIGINAL ARTICLE

Arch. Immunol. Ther. Exp., 2006, **54**, 283–287
PL ISSN 0004-069X

DOI 10.1007/s00005-006-0032-0

Circadian variations of histamine binding to lymphocytes and neutrophils and skin reactivity to histamine in atopic and healthy subjects

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Received: 2005.08.18, **Accepted:** 2006.02.03, **Published online first:** 2006.07.25

Abstract

Introduction: Numerous pathophysiological conditions change during 24-hour periods. Histamine, the main mediator in allergic reactions, exerts a multiplicity of pathophysiological actions through binding to specific receptors on effector cells. Nocturnal exacerbation of symptoms occurs in many atopic diseases in which histamine is an important mediator. Nocturnal wheezing is a very common symptom of asthma. The aim of this study was to determine whether the binding of (fluorescein-labeled) histamine to cells participating in allergic-inflammatory processes (lymphocytes, neutrophils) and skin reactivity to histamine undergo circadian changes and to compare these phenomena in atopic asthmatic and healthy subjects.
Materials and Methods: Blood samples were collected at 8 am, 2 pm, 8 pm, 2 am, and 8 am the next day. Histamine skin-prick tests were performed at the same times.
Results: It was found that skin reactivity to histamine (wheal, erythema) in healthy subjects underwent significant circadian changes with acrophase at 8 am (wheal) or 8 pm (erythema), the lowest values being at night (2 am, $p=0.017$), in contrast to atopics, in whom the highest reactivity was found at night (2 am, $p=0.002$). Significant differences in the binding of fluorescein-labeled histamine between day (8 am–2 pm) and night (2 am) were observed for lymphocytes ($p=0.006$) and neutrophils ($p=0.018$).
Conclusions: In the asthmatic group these changes were not significant. Circadian changes in both the binding of histamine by effector cells and skin reactivity to histamine were different in healthy and asthmatic subjects, and this may play a role in the pathomechanism, course, and chronopharmacotherapy of atopic diseases.

Key words: histamine, circadian rhythms, lymphocytes, neutrophils, skin-prick test.

Abbreviations: FLH – fluorescein labeled histamine, PBS – phosphate buffered saline, fMLP – N-formyl-methionyl-leucyl-phenylalanine.

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INTRODUCTION

Numerous physiologic processes undergo biological rhythms, mostly circadian. Disturbances in circadian rhythms can manifest as the appearance or exacerbation of pathological reactions. Among atopic diseases, nocturnal occurrence or exacerbation of symptoms has been frequently observed in asthma [3, 12], rhinoconjunctivitis [10, 18], and atopic dermatitis [6, 8]. Histamine, the main mediator in allergic reactions, also has an immunoregulatory function and can exert various pathophysiological influences through interaction with its receptors. Histamine has a proinflammatory influence following interaction with receptors of H1 type, clinically manifested as constriction of the smooth muscles of the bronchi and intestines, dilation of peripheral vessels, increased vascular permeability, pruritus, and a suppression of immunological response through H2 receptors on target cells [7, 21].

The aim of this study was to compare the binding of histamine to cells participating in allergic-inflammatory processes (lymphocytes and neutrophils *in vitro*, endothelial cells *in vivo*) in atopic and in healthy subjects and to determine whether this binding is influenced by circadian rhythms.

MATERIALS AND METHODS

The study was performed on 6 healthy subjects aged 19–46 years and 8 patients aged 23–40 years with clinically diagnosed atopic asthma, accompanied in 5 cases by rhinoconjunctivitis. Asthma was defined according to American Thoracic Society criteria. The atopic status of the subjects was confirmed by elevated total IgE, positive skin-prick tests with common airborne allergens, and a family history of atopic diseases. The study was approved by the Bioethics Committee of the Wrocław Medical University and all subjects gave written informed consent. The patients' experience was a worsening of symptoms at night or in the early morning, at least from time to time. Blood samples were collected and skin-prick tests with histamine hydrochloride (Roussel Uclaf, France) solution (10 mg/ml physiological saline) were performed at 8 am, 2 pm, 8 pm, 2 am, and 8 am the next day. Physiological saline alone served as a control.

Lymphocytes and neutrophils were isolated on Gradisol gradient [22]. The viability of the cells, assessed by trypan blue exclusion. was >97%. Twenty μl of fluorescein-labeled histamine (FLH, Molecular Probes, USA) was added to 4×10^5 viable cells in 200 μl PBS to a final concentration 10^{-5} M and the samples were incubated for 15 min at room temperature. The number of positive cells was determined using an Olympus BX-50 fluorescence microscope. The specificity of FLH binding was tested by competitive inhibition with either unlabeled histamine hydrochloride or histamine receptor antagonists or agonists. A 100-fold excess of both H2R agonist (Dimaprit) and antagonist (Ranitidine) as well as unlabeled histamine strongly inhibited FLH binding. However, concentrations $>10^{-5}$ M of a number of commonly used H1R antagonists (Diphenhydramine, Promethazine, Phenazoline, Tripelennamine, Levocabastine, Cetirizine) and an agonist (2 Thiazolylethylamine) were toxic to the cells and could not be used for testing. We observed that FLH did not bind to erythrocytes which were reported not to express histamine receptors on their surface [14].

Skin-prick tests with histamine were performed by the same person. The reaction was evaluated after 10 min. Skin reaction was determined by measuring the wheal and erythema diameters in 2 perpendicular directions. Their sum divided by 2, and expressed in mm, was the measure of the reaction.

The data were analyzed by multivariate repeated-measures analysis of variance ANOVA to test for differences in time and group and group-by-time interactions. Differences between times within groups were tested by individual linear contrasts. Differences between groups of specific times were tested by the multiple comparison procedure. Data are expressed as the mean ±SEM. A value of $p<0.05$ was considered significant for all tests.

RESULTS

The binding of FLH by lymphocytes of atopic and healthy subjects at the time points described in Materials and Methods is shown on Fig. 1. The percentage of labeled cells in healthy subjects was significantly lower at night, falling from 12.3±3.6% at 8 pm to 8.5±2.9% at 2 am ($p=0.006$), with a subsequent significant ($p=0.002$) increase to 13.5±2.8% at 8 am the next day. In atopics the binding of FLH over the 24-hour period was not significantly different. Comparing FLH binding during the 24-hour period in both groups, we found significant differences in the intervals 8 am–2 pm ($p=0.008$, healthy subjects: 13.6±3.3–15.1±3.3, patients: 13.1±2.9–11.6±3.2) and 2 pm–8 pm ($p<0.05$,

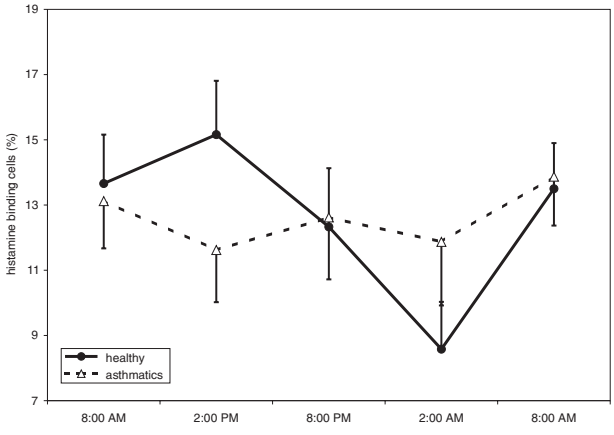

Fig. 1. Binding of histamine to lymphocytes of healthy and asthmatic subjects. In healthy subjects the binding changed significantly, from the highest values at 2 pm (15.1±3.3%) to the lowest at 2 am (8.58±2.9%; $p=0.006$), which subsequently increased to 13.5±2.8% at 8 am the next day ($p=0.002$). In asthmatic patients, the binding of histamine remained at a similar level: 8 am 13.2±2.9%, 2 am 11.8±3.9%.

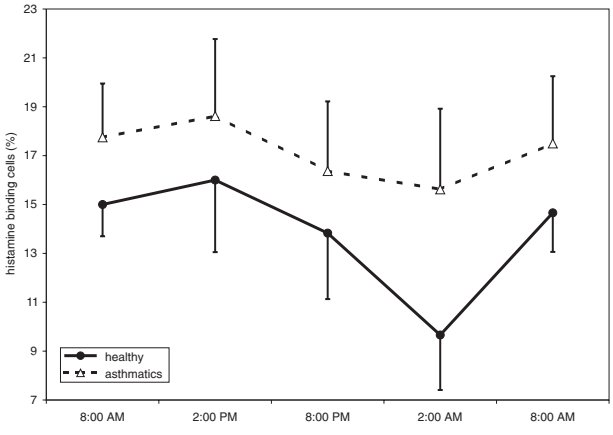

Fig. 2. Binding of histamine to neutrophils of healthy and asthmatic subjects. The highest percentage of FLH-labeled neutrophils was observed in both healthy (16.0±5.9%) and the atopic group (18.6±6.3%) at 2 pm. A significant decrease to 9.6±4.5% at 2 am occurred in the healthy group only ($p=0.0018$). The changes in histamine binding by neutrophils of atopics were not significant.

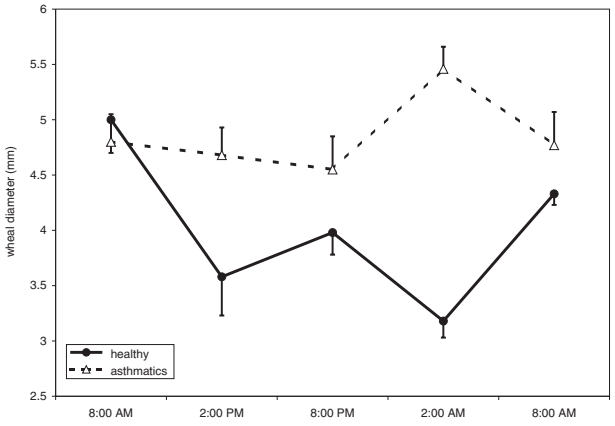

Fig. 3. Circadian variations in the size of the histamine wheal in healthy and atopic subjects. Histamine wheals in the healthy group changed significantly, from the largest in the morning (8 am, 5.0±0.6 mm) to the smallest at night (2 am, 3.1±0.3 mm; p=0.017). In contrast, in asthmatic patients the wheal size not only did not decrease at night, but rose significantly: (8 am 4.8±0.5 mm, 2 am 5.4±0.4 mm; p=0.003).

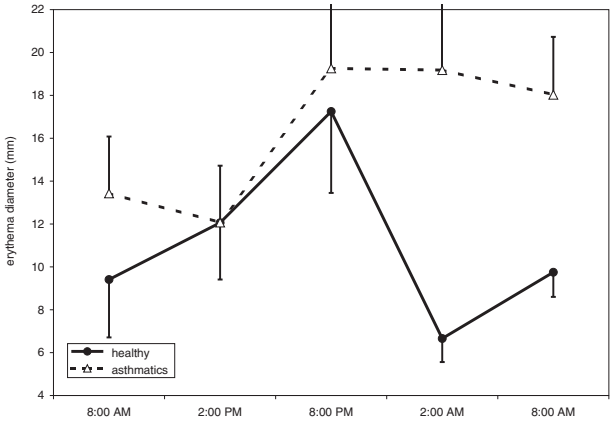

Fig. 4. Circadian variations in the size of erythema in healthy and atopic subjects. In both groups the largest size of erythema occurred at 8 pm (healthy: 17.2±7.6 mm, atopics: 19.2±6.7 mm). In healthy subjects the size decreased significantly to 6.6±2.2 mm at 2 am (p=0.001), whereas in atopics it remained on a similar level (19.1±7.2 mm).

healthy subjects: 15.1±3.3–12.3±3.6, patients: 11.6±3.2–12.6±3.8).

The binding of FLH to the neutrophils of healthy subjects (Fig. 2) significantly decreased at night, from 13.8±5.4% at 8 pm to 9.6±4.5% at 2 am (p=0.018), and subsequently rose at 8 am the next day to 14.6±3.2% (p=0.013). No significant changes in FLH binding by the neutrophils of atopics were observed at the investigated time points. Differences in FLH binding during the 24-hour period were also not found when comparing both groups.

Circadian changes in the size of the histamine wheal following the skin-prick test in both groups are presented on Fig. 3. In healthy subjects the size of the wheal decreased significantly during the day, from 5.0±0.6 mm at 8 am to 3.5±0.7 mm at 2 pm (p=0.003), reaching the lowest value of 3.1±0.3 mm (p=0.017) at 2 am at night and significantly rising o 4.3±0.2 mm at 8 am next day (p=0.001). In contrast, the size of the wheal in atopics was significantly larger at night (p=0.003) than at the remaining time points. Comparison of wheal size between the groups showed significant differences at 2 pm (p=0.007), 8 pm (p=0.01), and 2 am (p=0.001).

Figure 4 illustrates circadian changes in the erythematous reaction. In healthy subjects the largest erythema appeared at 8 pm, with 17.2±7.6 mm, and the smallest at 2 am, with 6.6±2.2 mm (p=0.001). No nocturnal decrease in erythema size was observed in the atopic subjects. Comparing the erythema size between groups, during the 24-hour period we found a significant difference at 2 am (p=0.002).

DISCUSSION

Our parallel studies on the binding of histamine to peripheral lymphocytes and neutrophils and its action on the skin reactivity of healthy subjects have shown that it is characterized by a specific circadian rhythm. Maximal values were observed at 8 am (wheal) and 8 pm (erythema), and were minimal at night. In contrast, in atopics the binding of histamine to lymphocytes and neutrophils did not decrease at night and significantly larger histamine wheal compared with the values before noon was observed (p=0.003). As we found in our previous study, one of the effects of lymphocyte and neutrophil activation, both specific (allergen) and nonspecific (fMLP), was an increased binding of histamine [23].

It was shown that the plasma concentration of histamine in allergic asthmatics was higher than in healthy subjects [1, 4]. In the latter group, the concentration of histamine did not change over the 24-hour period, although it was markedly lower than in asthmatics, in whom the lowest concentration occurred between noon and at 4 pm, the highest at night (2 am), and correlated with the intensity of bronchospasm [1]. Murdoch and Pollock [15] described significant differences in the levels of infused histamine required to obtain a clinical endpoint between atopic and healthy subjects. The authors suggest that the occurrence of clinical symptoms of histamine action in spite of its lower infused concentrations in atopic subjects may be caused by impairment of histamine degradation or increased expression of histamine receptors on target cells.

De Vries et al. [5] reported that circadian changes in bronchial reactivity to histamine occur in asthmatics. In around-the-clock investigations of spirometric parameters, a significantly lower threshold value for histamine was found in asthmatics between 8 pm and 5 am. It is also known that nocturnal bronchospastic dyspnea is accompanied by an increased number of leukocytes in bronchoalveolar lavage and an increase in bronchial reactivity [13]. Panzer et al. [16] demonstrated circadian variations in the number of airway leukocytes in induced sputum of

asthmatics. A significant increase in leukocytosis was observed in induced sputum at 7 am in comparison with the 4 pm value. Peak lung function in healthy subjects occurred near 4 pm and minimal lung function near 4 am [2]. Hetzel and Clark [9], in a comparative study on patients and healthy subjects, found that the average amplitude of circadian rhythms in peak respiratory flow rate was 8.3% in healthy and up to 50.9% in asthmatic subjects. Skin reactivity to histamine and specific allergens also shows circadian variability. Seery et al. [19] reported that the reactions in atopic patients were significantly higher at night than during the day. Lee et al. [11], observing the circadian rhythm of skin reactivity to histamine and allergens, found that acrophase occurred between 7 pm and 11 pm, with minimal response at 7 am, and that urinary hydrocortisone excretion curves exhibited agreement with antiphase timing.

Glucocorticosteroids are currently the most efficient drugs in allergic-inflammatory diseases, including asthma. It is known that plasma cortisol levels show circadian fluctuation, being highest around 7 am and lowest at midnight, and that they correlate with the degree of bronchospasm [1, 12]. It was found in our previous observations that glucocorticosteroids (cortisol, dexamethasone) caused a concentration-dependent decrease in histamine binding by unstimulated and specifically stimulated lymphocytes as well as modulated histamine receptor mRNA expression in both healthy and allergic subjects [24, 25].

It is currently assumed that the mechanism of nocturnal exacerbation of disease symptoms requires the interaction of many factors, including circadian variation in hormone and mediator levels, inflammatory cells, and vagal tone [20]. In about 2/3 of asthmatics, a higher degree of circadian activation of inflammatory cells is reported. The circadian rhythms of these processes influence the course of disease. More than 90% of dyspnoeic episodes take place during the night (between 10 pm and 7 am, peak at 4 am), as do 53% of asthma deaths [2]. These data prompted the selection of the most appropriate time for corticosteroid administration (3 pm–5 pm), enabling a reduction in overnight disease symptoms [2, 12, 17]. Elevated severity of symptoms in rhinoconjunctivitis and atopic dermatitis at night and early morning can also be efficiently reduced through individually optimized dosing times of antihistamines [10, 18]. The significantly different circadian expressions of histamine binding to lymphocytes, neutrophils, and vascular skin matrix in atopics from those in healthy subjects may participate in the mechanism of nocturnal exacerbation of disease symptoms mediated by histamine.

Acknowledgment: The authors are grateful to Grażyna Nadobna, M.S., and Conrad Knapski for skillful technical help.

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