H₁-antihistamines and oxidative burst of professional phagocytes

Rado Nosáľ ¹, Katarina Drabíková ¹, Viera Jancinová ¹, Jana Moravcová ², Antonín Lojek ², Milan Cíz ², Tatiana Macíková ¹, Jana Pecísova ¹

¹ Department of Cellular Pharmacology, Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences Bratislava, Slovak Republic
² Department of Free Radical Pathophysiology, Institute of Biophysics, Academy of Sciences Czech Republic, Czech Republic

Correspondence to: Prof. Rado Nosáľ, Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences Bratislava, Dúbravská 9, Slovak Republic.

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Abstract

OBJECTIVES: We analysed and compared the effect of five H₁-antihistamines on stimulated oxidative burst at extra- and intracellular level of isolated and stimulated human polymorphonuclear leukocytes.

DESIGN: Oxidative burst of isolated human neutrophils was studied by means of luminol and isoluminol enhanced chemiluminescence.

RESULTS: The following rank order of potency for H₁-antihistamines to decrease chemiluminescence was evaluated extracellularly: dithiaden> loratadine> chlorpheniramine> brompheniramine> pheniramine and at intracellular site: loratadine> dithiaden.

CONCLUSION: H₁-antihistamines differ substantially according to their chemical structure in suppressing oxidative burst both at extra- and intracellular site of isolated stimulated human neutrophils.

INTRODUCTION

Antihistamines are often viewed as selective antagonists of the histamine H₁-receptor with relatively few other actions. Besides their antihistaminic activities, H₁-receptor antagonists possess other pharmacological properties: anti-inflammatory action, inhibition of blood platelet function and antioxidative effects (Drábková et al. 2002; Nosáľ & Jancinova, 2002, Nosáľ et al. 2002; Macíková et al. 2008). The mechanism(s) responsible for the nonspecific non-receptor operated activity of H₁-antihistamines is not fully understood and may operate both at receptor and non-receptor site of target cells (Church, 2001). The antioxidative effect in cell-free system differs substantially from antiplatelet and antiphagocyte activities, indicating that the H₁-antihistamines studied act both at extracellular and intracellular level. In this study we compared the effect of five H₁-antihistamines and histamine on stimulated chemiluminescence (CL) at extra- and intracellular site of human neutrophils. Moreover, we evaluated the EC₅₀ for histamine and the antihistamines studied at extra- and intracellular site of stimulated neutrophils.

MATERIAL AND METHODS

Chemicals and drugs tested. Luminol, isoluminol, PMA (4-phorbol-12-myristate-13-acetate), superoxide dismutase and histamine dihydrochloride were obtained from Sigma Aldrich Chemie (Düsseldorf, Germany), horseradish peroxidase (HRP) and catalase from Merck (Darmstadt, Germany).
Abbreviations & Units:
CL  chemiluminescence
PA  pheniramine
BPA  brompheniramine
CPA  chlorpheniramine
DIT  dithiaden
HIST  histamine
LOR  loratadine
PMAs 4-phorbol-12-myristate-13-acetate
HRP  horse radish peroxidase
EC50  concentration providing 50% effect

All other chemicals of analytical grade were obtained from available commercial sources. Drugs tested: pheniramine maleate (PA; Hoechst Frantfurt/M, Germany), chlorpheniramine maleate (CPA; Glaxo, Brentdorf, England), brompheniramine maleate (BPA; Wyeth Laboratories, Berks, England), dithiaden (DIT; Leciva Praha, Czech Republic), loratadine (LOR; Slovakofarma Hlohovec, Slovak Republic).

Isolation of polymorphonuclear leukocytes (PMNL). Human blood was collected in 2×9 ml citrate tubes, purified by 3% dextran centrifugation and separated on Lymphoprep (Fresenius, Norway). The erythrocytes were removed with hypotonic, cold haemolysis. Cells were washed with phosphate-buffered saline before counting on Beckman Coulter. Concentration of cells in stock suspension was 10×10⁶ cells/ml (for details see Drabikova et al. 2002).

Extra- and intracellular chemiluminescence. dCL was measured in a microtiter plate computer driven luminometry LM-01T (Immunotech, Czech Republic) at 37°C, using human isolated neutrophils. Measurement of extracellular CL was initiated by addition of PMA (50 µl, final concentration 0.05 µmol/L) to the reaction mixture, which consisted of isoluminol (5 µmol/L), neutrophils (5×10⁵), drug tested (0.01–100 µmol/L) and horseradish peroxidase (HRP, 8 U/ml), in 50 µl aliquots. In experiments where the intracellular light emission was recorded, luminol (5 µmol/L) was used as luminophore, and superoxide dismutase (25 µl, 100 U/ml) and catalase (25 µl, 2000 U/ml) were added instead of HRP. All concentrations are final. CL was recorded continuously for 30 min to obtain kinetic curves and evaluated on the basis of peak values (Drabikova et al. 2006; Jancinova et al. 2006a).

RESULTS

Figure 1 demonstrates the effect of H₁-antihistamines and histamine on extracellular CL of human neutrophils stimulated with PMA. All drugs tested decreased dose-dependently CL of neutrophils with significant inhibition at 10 µmol/L concentration. Pheniramines and histamine decreased CL by 15%, loratadine by 39%. The most potent was dithiaden, decreasing CL by 58%. Increase of the concentration to 100 µmol/l resulted in further suppression of neutrophil CL.

The effect of H₁-antihistamines on intracellular CL of isolated human neutrophils is demonstrated in Figure 2. Pheniramines in concentrations of 0.1 and 10 µmol/L did not change intracellular CL, in 100 µmol/l concentration PA, CPE and BPE potentiated CL by 11, 23 and 13 %, respectively. Histamine did not change intracellular CL in any concentration used. DIT and LOR decreased significantly intracellular chemiluminescence in 10 µmol/L concentration by 42 and 37%, respectively. Increase of the concentration of DIT and LOR to 100 µmol/L resulted in the inhibition of intracellular CL by 95 and 96%, respectively.

Figure 3 shows EC50 values for pheniramines, HIST, DIT and LOR calculated for inhibition of extra- and intracellular CL. The respective rank order of potency for EC50 of BPH, CPH, DIT and LOR was 50.9, 98.4, 8.6 and 12.1 µmol/L. EC50 values for PA and histamine were over 100 µmol/L. Since BPH, CPH and PA potentiated intracellular CL, the EC50 values were irrelevant (negative). The same was true for HIST.

DISCUSSION

All H₁-antihistamines tested decreased dose-dependently the CL of whole human blood, both when stimulated by membrane-operating or by membrane-bypassing stimuli (Jancinova et al. 2006c; Kralova et al. 2008b; Nosal et al. 2002b). Inhibition of CL at extracellular site of neutrophils seems to be the result of scavenging free radicals due to molecules of H₁-antihistamines and HIST, as demonstrated for drugs studied in a cell free system (Kralova et al. 2008b). DIT and LOR significantly suppressed stimulated CL inside neutrophils, indicating an interaction with the regulatory pathway stimulated with PMA. This may suggest an interaction at protein kinase C, as recently demonstrated for diferuloylmethane (Jancinova et al. 2009). As expected, HIST operated extracellularly and was not effective inside neutrophils since it does not enter living cells. On the other hand, pheniramines potentiated stimulated CL of neutrophils at intracellular site in the highest concentration used (100 µmol/L). The wide variety in the suppressive effect of H₁-antihistamines on stimulated neutrophil CL is demonstrated by EC50 values of the drugs tested and HIST.

When examining the possible mechanisms by which antihistamines exert their anti-inflammatory effects, both receptor-independent and receptor-dependent mechanisms need to be taken into consideration. Although the number of recently published results (Jancinova et al. 2006c, Kralova et al. 2006, Nosal et al. 2002b, 2005, 2006) demonstrated a very important role of DIT and other H₁-antihistamines in the regulation of reactive oxygen species production by professional phagocytes and in the regulation of myeloperoxidase activity (Kralova et al. 2008a,b), further information is needed to provide insight into the effect of H₁-antihis-
Oxidative stress and H1-antihistamines on the mechanism of reactive oxygen species generation.

The effect of the pheniramines tested on CL of oxidative burst of whole human blood was structure-dependent and related to lipophilicity, partition coefficient and other physico-chemical parameters (Jancinova et al. 2006b). BPA and CPA differ from PA by halogenisation of the molecule with Br and Cl. Moreover, the affinity and binding kinetics of H1-AH were found to be differently affected by an acidic environment, which can be encountered during inflammation (Gillard & Cahealain, 2006).

Thus it appears likely that antihistamines exert anti-inflammatory effects by both receptor-dependent and receptor-independent mechanisms. The receptor-independent mechanisms, which require higher drug concentrations, appear to include the release of preformed mediators from inflammatory cells, such as HIST and eosinophil proteins, eicosanoid generation, and particularly oxygen free radical production (Church, 2001).
The mechanism of CL inhibition of professional phagocytes due to H₁-antihistamines differs according to their chemical structure. Suppression of intracellular CL suggests interaction of H₁-antihistamines with regulatory pathways responsible for oxidative burst of neutrophils, like protein kinase C (Jancinova et al. 2009), NADPH-oxidase and others.

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