

Original Article

Oxatomide modifies membrane fluidity of polymorphonuclear leukocytes from children with allergic asthma

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ABSTRACT

Background: Plasma membrane fluidity of polymorphonuclear leukocytes (PMN) was investigated in 10 allergic asthmatic children before and after oxatomide treatment.

Methods: Membrane fluidity was studied by measuring the steady state fluorescence anisotropy of 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) incorporated into polymorphonuclear leukocyte (PMN) plasma membranes.

Results: There was an increase in membrane fluidity at the surface of PMN from asthmatic children. Oxatomide treatment significantly decreased PMN membrane fluidity.

Conclusions: These data suggest that oxatomide may induce changes in the physicochemical properties of the PMN plasma membrane in asthmatic subjects. These changes may modify the functional activities of PMN.

Key words: bronchial asthma, leukocyte, membrane fluidity, oxatomide.

INTRODUCTION

Since the introduction of oxatomide, a second-generation histamine H₁ receptor antagonist, clinical and experimental data have provided a better understanding of its efficacy, safety and mode of action. Oxatomide is effective in the treatment of allergic rhinitis, urticaria and atopic dermatitis.^{1–4} Moreover, there is now evidence that it may be useful in the treatment of asthma.⁵

Various studies have demonstrated that oxatomide possesses a variety of anti-inflammatory activities beyond H₁ receptor antagonism. Several *in vitro* and *in vivo* studies performed on different cell types involved in inflammation have demonstrated that oxatomide modulates the inflammatory response. In particular, it has been shown that oxatomide reduces epithelial intercellular adhesion molecule (ICAM)-1 expression⁶ and inhibits the release of histamine, leukotriene C₄ and tryptase by basophils and mast cells,⁷ activation of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system in polymorphonuclear leukocytes (PMN),⁸ extracellular calcium influx in a mast cell model⁹ and the release of inflammatory mediators and agents from PMN and human lung tissue.^{10,11} The mechanisms by which oxatomide acts remain unknown.

The plasma membrane is a focal point for the control of cellular activity. Cell membranes act as macromolecular assemblies that respond to both internal and external changes and integrate enzymatic responses and functions, as well as sensitivities to other stimuli, by rearrangement of membrane configuration and, in some cases, also by altered composition.¹² In the present study, we evaluated the effect of oxatomide treatment on

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Received 12 October 2000. Accepted for publication 26 February 2001.

membrane fluidity of PMN obtained from children with allergic asthma.

METHODS

Subjects

Ten children with mild allergic asthma (six females and four males; age range 7.4–11.3 years; mean (\pm SD) age 9.4 ± 2.0 years) were included in the study after appropriate informed consent had been obtained. Patients were selectively allergic to rye grass. The study was not performed during the pollen season. Oxatomide in liquid formulation, 15 mg twice daily, was administered for 14 days. Patients were not permitted to take other antihistamines or systemic or inhaled anti-inflammatory drugs. Patients were allowed to use salbutamol as rescue medication in case of asthma exacerbation.

The control group consisted of 10 healthy children (five females and five males; age range 6.8–11.3 years; mean age 9.7 ± 1.9 years). None of the children in the control group had a family or personal history of allergy, asthma or immunologic disorders. Adequate informed consent was obtained from all subjects enrolled in the study.

All subjects were without signs of acute infection at the time of the study.

After overnight fasting, 10 mL heparinized blood was obtained for PMN isolation. Simultaneously, we measured blood cholesterol, triglycerides and phospholipids.

Isolation of PMN

Polymorphonuclear leukocytes were separated using a MonoPoly resolving Medium (ICN Biomedicals, Aurora, OH, USA), as described previously.¹³ Cells were resuspended in Krebs'–Ringer phosphate (KRP) solution supplemented with 5 mmol/L glucose.

Chemiluminescence measurements

Luminol-amplified chemiluminescence was measured in the Autolumat LB 953 (Berthold, Wilbad, Germany) and PMN were activated by the addition of 10^{-6} mol/L phorbol myristate acetate (PMA; Sigma Chemical Co., St Louis, MO, USA). Chemiluminescence measurements were taken over the next 60 min, as described previously.¹⁴ Chemiluminescence was measured as counts per minute (c.p.m.).

Fluorescence measurements

Polymorphonuclear leukocytes were labeled in the dark. 1-[4-Trimethylammoniumphenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH; Molecular Probes, Eugene, OR, USA) was added to a 2.5 mL quartz cuvette containing 10^6 cells/mL PMN in 2 mL KRP, to give a final concentration of 10^{-6} mol/L TMA-DPH. Steady state fluorescence anisotropy (r_s) measurements were performed at 37°C with a spectrofluorimeter (model MPF-66; Perkin Elmer, Seer Green, UK) equipped with a Perkin Elmer personal computer (model 7300) for data acquisition and elaboration using TMA-DPH as the fluorescent probe, as described previously.^{15,16} The computer program calculated fluorescence anisotropy using the following equation:

$$(I_{||} - I_{\perp} \times g)/(I_{||} + (2I_{\perp} \times g))$$

where g is an instrumental correction factor and $I_{||}$ and I_{\perp} are the emission intensities with the polarizers parallel and perpendicular to the direction of the polarized exciting light, respectively.

RESULTS

During the treatment period none of the asthmatic children used the rescue medication. Blood cholesterol values, triglycerides and phospholipids from the control and asthmatic groups were not significantly different ($P > 0.5$). Luminol-amplified chemiluminescence was used, as described previously,¹⁵ to verify that, in isolated PMN, the NADPH oxidase system is dormant under resting conditions and can be activated by PMA. All samples used in the present study demonstrated an activatable NADPH oxidase system. Peak activity was noted within 15–20 min of addition of PMA and there was no significant difference in the peak value of PMA-stimulated chemiluminescence between the two groups ($(7.94 \pm 0.68$ vs $8.10 \pm 0.82) \times 10^7$ c.p.m., respectively). These data indicate that PMN in both groups, asthmatic and control children, were under resting conditions.

The background phospholipid fluorescence of PMN was checked prior to each measurement and was less than 0.1% of the fluorescence when TMA-DPH was added. The fluorescence intensity of the free probe in KRP, in the absence of PMN, was negligible.

Table 1 shows r_s values in the control and asthmatic groups before and after oxatomide treatment. In the asthmatic group, r_s values of TMA-DPH in the PMN were

Table 1 Mean \pm SD of fluorescence anisotropy, r_s , of 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene at 37°C in polymorphonuclear leukocytes from control and asthmatic children before and after oxatomide treatment

	Control (n = 10)	Asthmatic (n = 10)	
		Before treatment	After treatment
r_s	0.287 \pm 0.003	0.255 \pm 0.004*	0.276 \pm 0.002**

* $P < 0.001$ compared with polymorphonuclear leukocytes (PMN) from the control group; ** $P < 0.001$ compared with PMN from the asthmatic group before treatment.

significantly lower than in the control group. Treatment with oxatomide induced a significant increase in r_s values in the asthmatic group.

DISCUSSION

Although PMN are not the dominant cells in asthma and allergic diseases, they contribute to the inflammatory process underlying the disease. Thus, PMN are increased in bronchial biopsies, as well as in airway washings from asthmatic subjects.¹⁷ Moreover, several inflammatory mediators involved in asthma can prime or activate PMN. Leukocyte–endothelial adhesion molecules are involved in the recruitment and migration of inflammatory leukocytes from the circulation to sites of inflammation. Polymorphonuclear leukocytes are believed to play an important role in several models of airway hyperactivity.¹⁷ Metzger *et al.* reported that superoxide generation by PMN from asthmatics correlates with the severity of bronchial constriction.¹⁸

Biological membranes are highly heterogeneous. They consist of mixtures of different lipids and a variety of different proteins. Different organizational modes of compositionally and functionally differentiated domains correspond to different physicochemical states of the membrane.¹⁹ Many biological processes and biochemical events originate directly or indirectly within the cell membrane. Chemical and physical events that take place within the membrane allow the cells to carry out their specific functions.²⁰

Fluorescence spectroscopy encompasses a diverse set of techniques capable of probing a wide range of fundamentally different physical, chemical and biological processes. Fluorescence spectroscopy has been demonstrated to be a useful tool in the study of the structure and dynamics of biological membranes. This technique offers several advantages for the study of membranes. Among these advantages are the high sensitivity and virtual

absence of perturbation of membrane structure due to the probes themselves. Also important is the responsiveness of the fluorescence parameters to the physical properties of the environment and the possibility of resolving spectroscopic parameters arising from sample heterogeneity. Particularly interesting are those probes with environmentally sensitive steady state fluorescence parameters.^{21,22} 1,6-Diphenyl-1,3,5-hexatriene (DPH) is one such probe and a number of derivatives of DPH have become popular as fluorescent probes in membrane research. In particular, TMA-DPH has been used extensively for studying membrane structure and fluidity because of its advantageous structural and photochemical properties. TMA-DPH is incorporated into the membrane, but remains at the lipid–water interface region because of its cationic residue. The r_s of TMA-DPH reflects the packing of the membrane lipid fatty acid chain and can be related to the order parameter S , if certain precautions are taken. Lipid fluidity may be defined as the reciprocal of the lipid structural order S and, thus, an increase in the TMA-DPH r_s value corresponds to a decrease in membrane fluidity.^{23,24}

In the present study, we observed that the r_s value for PMN from the asthmatic group was significantly lower than in the control group. This indicates an increase in membrane fluidity, which reflects a decrease of the lipid order in the exterior part of the membrane. Although the origin of this alteration is not known yet, we can speculate that the inflammatory process induces these changes. After oxatomide treatment, a significant increase in r_s value was observed, indicating a decrease in fluidity of the plasma membrane. This observation suggests that oxatomide may interact with the plasma membrane of PMN. Oxatomide is a lipophilic molecule and, thus, could interact with the lipid bilayer. A recent study by Fischer *et al.*²⁵ has demonstrated that oxatomide is able to interact with plasma membrane lipid components, although we cannot exclude the possibility that oxatomide acts indirectly by reducing the influence of inflammatory processes on PMN.

Numerous studies have demonstrated that asthma is associated with alterations in plasma membrane composition, function and fluidity of various cell types, including leukocytes.^{26–29} Evidence has accumulated that anti-inflammatory drugs can exert their action by interacting with the plasma membrane of inflammatory cells. Steroids are lipophilic, suggesting that they may intercalate into the bilayer of target cell plasma membranes, potentially altering the fluidity and function of the

membrane.³⁰ Lamche *et al.* demonstrated that steroids cause a decrease in membrane fluidity of PMN.³¹ Recently, we have demonstrated that nedocromil sodium can induce changes in membrane fluidity of PMN plasma membrane.³² These changes can induce functional changes in the PMN from asthmatic subjects.

The present study demonstrates that oxatomide treatment affects the physicochemical properties of PMN plasma membranes in asthmatic subjects. These changes may induce functional modifications of PMN. The plasma membrane is a complex structure exhibiting specific and dynamic interactions between its constituents. Experimental evidence indicates that each integral membrane protein interacts with its neighboring 'boundary' lipids in a specific manner.¹² Changes in the composition of this lipid boundary may lead to changes in enzyme activity, as well as ligand specificity and affinity.³³ In contrast, functional activities of membrane proteins are associated with changes in the composition and organization of boundary lipids.³⁴

Further studies investigating the effect of oxatomide on plasma membrane fluidity of PMN could elucidate the anti-inflammatory activity of this drug in asthma.

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