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A STUDY OF CELL MEMBRANES IN NASAL EPITHELIAL CELLS FROM PATIENTS WITH CHRONIC RHINOSINUSITIS WITH NASAL POLYPS BY MEANS OF A FLUORESCENT PROBE

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Abstract

Aim. To assess the state of membranes in nasal epithelial cells obtained from the patients with chronic rhinosinusitis with nasal polyps (CRSwNP) with the help of the fluorescent probe 2-(2′-OH-phenyl)-5-phenyl-1,3-oxazole.

Methods. The state of membrane phospholipid bilayer in suspensions of nasal epithelial cells isolated from ten patients with CRSwNP was evaluated using the fluorescent probe 2-(2′-OH-phenyl)-5-phenyl-1,3-oxazole that reacts on the physico-chemical properties of its microenvironment. Changes in fluorescence spectra were determined using a Thermo Scientific Lumina fluorescence spectrometer (*Thermo Fisher Scientific*) 1 hour after the addition of the probe to nasal epithelial cell suspensions.

Results. CRSwNP was found to be associated with a higher rate of nasal epithelial cell membrane hydration in the region of phospholipid glycerol moiety, carbonyl groups and aliphatic chains of fatty acids attached to the carbonyl groups.

Conclusion. Our findings suggest that CRSwNP is accompanied by the elevated hydration rate of the most polar region, namely polar heads of phospholipids of nasal epithelial cell membranes.

INTRODUCTION

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a long-lasting sinonasal inflammation with the development of inflammatory outgrowths in the nasal tissue called nasal polyps [1]. CRSwNP clinically manifests by non-purulent nasal discharge, nasal congestion, partial loss of olfaction, and facial pain. The symptoms mentioned above should last for at least 12 weeks [2]. The disease is characterized by quite high prevalence (up to 12 % of population in different geographical areas) being a huge socioeconomic burden with substantial healthcare costs and significantly affects the health-related quality of life [3].

Despite numerous efforts to figure out etiology and pathogenesis of CRSwNP, there is no generally recognized theory that explains its pathophysiology. Several hypotheses, including the fungal, super antigen, microbiome, biofilm, eicosanoid and immune barrier ones, have been suggested to explain etiopathogenesis of the disease. However, none of them can fully provide the understanding of mechanisms that underlie CRSwNP development [4]. There is strong evidence that changes in the nasal microbiota, abnormal production of cytokines and inefficient mucociliary clearance may play an important role in the development of CRSwNP [5].

It is worth mentioning that nasal epithelial cells (NECs) significantly contribute to the mucosal barrier integrity playing a protective role against microorganisms. Moreover, NECs express innate immune receptors participating in the immune response regulation [6]. It has been also reported that NECs are capable of secreting cytokines such as IL-25 and IL-33 in response to bacterial agents and cell destruction [7]. Thus, the functional state of NECs may affect the course of CRSwNP. In particular, the cell membrane integrity is crucial for cell viability and proper functioning [8]. Cells can overcome a minor damage to

membranes. However, rupture of cell membrane obligatory results in necrotic cell death. Necrosis is accompanied by the release of the intracellular content in the microenvironment and leakage of intracellular damage-associated molecular patterns (DAMPs). The DAMPs released by injured necrotic cells fuel inflammation interacting with DAMP or pattern recognition receptors located on immune cells [9-11]. Little is known about the functional changes in the cell membrane of NECs in patients with CRSwNP, despite the fact that the loss of phospholipid bilayer integrity of NECs compromises at once the mucosal barrier function.

The aim of our research was to study the state of cell membrane in NECs in patients with CRSwNP using the fluorescent probe 2-(2′-OH-phenyl)-5-phenyl-1,3-oxazole, which non-covalently binds to cell membranes and has a quick response to changes in its microenvironment [12-18].

MATERIALS AND METHODS

Characteristics of patients

Ten patients (6 males; 4 females) from the Kharkiv Regional Clinical Hospital (Kharkiv, Ukraine) were enrolled to the study. All patients were diagnosed with CRSwNP in accordance with the criteria of ‘‘EPOS 2012: European Position Paper on Rhinosinusitis and NPs 2012” guidelines [19]. Their age varied from 29 to 55 years with the mean age of 37.80 ± 2.46 years. Exclusion criteria included any acute inflammatory disease or exacerbation of the chronic one, immunodeficiency, endocrine diseases, chronic cardiovascular pathology, tumors, pregnancy, cystic fibrosis, atopic diseases, and asthma. Ten subjects (7 males; 3 females) whose age ranged from 21 to 52 years with the mean age of 35.60 ± 3.23 years with deviated nasal septum treated surgically under combined general and regional anesthesia without any clinical sign of sinonasal inflammation were enrolled from the Kharkiv Regional Clinical Hospital (Kharkiv, Ukraine) and served as controls.

Sample collection and preparation of NECs suspension

Specimens of mucosa from polyps were obtained from ten patients with CRSwNP by scraping. Mucosal NECs from ten control subjects with deviated nasal septum were also collected by scraping. Human NECs were used to prepare suspensions. The content of cells in suspensions and their viability was evaluated microscopically. During the entire period before the measurement of fluorescence spectra, the temperature was maintained at 37 °C.

Characteristics of the fluorescent probe

To assess the state of cell membranes in NECs of patients with CRSwNP, we used 2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole. This fluorescent probe is able to bind non-covalently to phospholipid bilayer of cell membranes and respond to changes in its microenvironment [12-18]. Its fluorescence characteristics depend on the physicochemical properties (e.g., proton-donor ability, polarity, and viscosity of the microenvironment) of the medium where the probe locates [20-23]. Fluorescence of the probe 2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole was measured in cell suspensions prepared from NECs isolated from patients with CRSwNP and from conditionally healthy subjects with deviated nasal septum. The latter were used as controls. Ten suspensions of NECs isolated from the patients with CRSwNP were mixed to obtain one common suspension. The same procedure was performed for the control samples. Given that the solubility of 2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole in water is limited, we used a 0.2 mM stock solution of the probe in acetonitrile. An aliquot of 0.2 mM stock solution of the probe in acetonitrile was added to NECs suspensions obtained from healthy controls and from patients with CRSwNP (i.e. acetonitrile final concentration was ≤ 0.5% vol.) to obtain final probe concentrations of 1 μM (thus, the lipid-to-probe molar ratio was 1000:1). Then suspensions were incubated with the probe at room temperature (25 °C) for 1 hour avoiding the light. After the incubation fluorescence measurements were performed.

Fluorescence spectra were measured using a Thermo Scientific Lumina fluorescence spectrometer (*Thermo Fisher Scientific*) 1 hour after the addition of the probe to NECs suspensions. The slits on excitation and emission monochromators were 5 nm, while an excitatation wavelemgth was selected to be 330 nm.

Bioethics

The design of the current study was approved by the local Ethical and Bioethical Committee at Kharkiv National Medical University (Kharkiv, Ukraine). The research was conducted in accordance with the principles of the seventh revision of the Declaration of Helsinki (2013). All patients and control subjects signed a written informed consent.

RESULTS

In this study, we used a fluorescent probe 2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole, which has been already shown to be efficient for assessing the state of cell membrane phospholipid bilayer [12-18]. Location and orientation of the probe 2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole in the cell membrane is demonstrated in Figure 1. Its location and orientation was judged based on its fluorescent properties in lipid membranes [16-18], calculations of its location using a method of molecular dynamics [24] and its structural resemblance to the fluorescent probes whose location in lipid membranes has been already known [25]. The probe 2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole is located in the area of glycerol moiety of phospholipids (close to the center of lipid bilayer), in the area of phospholipid carbonyl groups, and in the areas of alkyl fatty acid chains adjacent to the region of carbonyl groups (**Figure 1**).

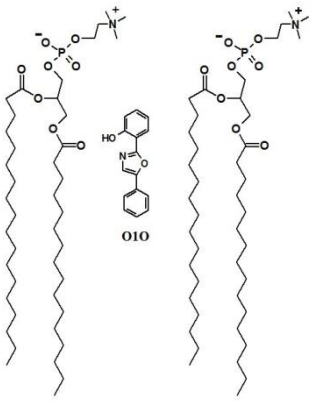

Figure 1. Location and orientation of the fluorescent probe 2-(2′-OH-phenyl)-5-phenyl-1,3-oxazole in phospholipid membranes. Two molecules of phosphatidylcholine from the outer leaflet are shown to denote the location of the probe.

Our findings on the measurement of the probe fluorescence in NECs suspensions isolated from the conditionally healthy control subjects and from the patients with CRSwNP are represented in **Figure 2** and **Table 1**. It was found that there are changes in the fluorescence spectra of the probe 2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole bound to the lipid membranes of NECs from the patients with CRSwNP compared with the corresponding spectra of the probe bound to the membranes of the cells from the control subjects.

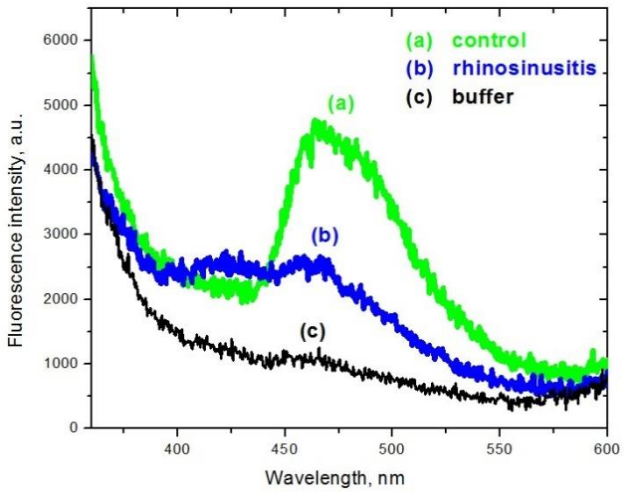

Figure 2. Fluorescence spectra of the probe 2-(2′-OH-phenyl)-5-phenyl-1,3-oxazole in suspensions of NECs isolated from the control subjects (a), from the patients with CRSwNP (b) and in a buffer solution that lacks NECs (c).

Table 1. The fluorescence intensities* (in arbitrary units, a.u.) at different wavelengths for the probe 2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole bound to the lipid membranes of NECs isolated from the patients with CRSwNP and to the membranes of the cells from the control subjects

Wavelength	Buffer solution	Control group (n = 10)	CRSwNP (n = 10)
420 nm	1210	2111	2526
465 nm	1036	4632	2508

*The relative error of the fluorescence intensity measurements was less than 5%.

In case of CRSwNP, the spectra are characterized by an increase in the intensity of a short-wavelength (~ 420 nm) fluorescence band of the probe (the fluorescence of the normal form of the probe [20]). In addition, a ~ 5 nm short-wavelength (i.e. hypsochromic) shift of the long-wavelength (~ 470 nm) fluorescence band of the probe (the fluorescence of the phototautomer form of the probe [20]) was observed in suspensions of NECs obtained from the patients with CRSwNP. While determining the fluorescence of the probe 2-(2'-OH-phenyl)-5-phenyl-1,3-oxazole in NECs suspensions from the patients with nasal polyposis and from control subjects, a significant (almost double) decrease in the intensity of the long-wavelength fluorescence band was revealed in case of CRSwNP.

DISCUSSION

The detected changes in the intensity of the short-wavelength (~ 420 nm) fluorescence band of the probe indicate a higher proton-donor ability of the probe microenvironment, whereas the short-wavelenth (i.e. hypsochromic) shift of the long-wavelength (~ 465 nm) fluorescence band of the probe observed by us is indicative of the increased polarity of the microenvironment. An almost two-fold decrease in the intensity of long-wavelength band fluorescence points to both the increased proton-donor ability and the reduced viscosity of the probe microenvironment in the lipid membrane.

The aforementioned augmented proton-donor ability, enhanced polarity and reduced viscosity of the probe microenvironment point to the increased hydration of NECs cell membranes collected from the patients with CRSwNP in the area of glycerol moieties, phospholipid carbonyl groups, and fatty acid chains in the area located next to phospholipid carbonyl groups.

Thus, our research indicates alteration in plasma membrane homeostasis in NECs as a result of CRSwNP development in the form of the increased fluidity or reduced viscosity. Such changes in plasma membranes in patients with CRSwNP may significantly affect functionality and viability of cells. In particular, it has been reported that cell death is promoted by the enhanced membrane fluidity [26]. For instance, Moulin et al. demonstrated that higher membrane fluidity may trigger TRAIL-induced apoptosis [26]. These data are consistent with the reports concerning activation of NECs apoptosis, including the TRAIL-induced one, in chronic rhinosinusitis [27, 28]. Thus, we have hypothesized that the changes in the state of NECs membrane observed in this study may result in the cell death of NECs. However, this hypothesis requires to be tested.

In addition, it is not clear whether our findings contribute to the development of NECs apoptosis or are supposed to be its outcome, since there is strong evidence that the membrane fluidity increases and the membrane order decreases in cells undergoing apoptosis [29-31]. It is worth noting that the membrane fluidification in apoptotic cells may be due to phosphatidylserine (PS) translocation from the cytosolic leaflet to the outer one, which is characteristic of apoptosis and serves as a so-called “eat-me” signal for phagocytes to promote efferocytosis, i.e. removal of cells dead by apoptosis [32].

Thus, we believe that fluidification of NECs membrane in patients with CRSwNP either may contribute to intensification of NECs apoptosis or can be considered a result of apoptosis-associated membrane changes. The cause-and-effect relationship between the increased membrane fluidity and apoptosis of NECs in nasal polyposis remains to be elucidated.

Given that NECs as a component of nasal mucosa barrier play a crucial role in the protection from detrimental environmental factors and microorganisms, their membrane integrity is of huge importance for the nasal barrier maintenance [33] The intact membrane is also critical for diffusion of molecules across it and its changes may be associated with mucosal barrier dysfunction and affect its permeability. In particular, the increased fluidity is associated with higher membrane permeability [34]. The increased NECs membrane permeability may in turn contribute to the epithelial barrier dysfunction in upper airways of patients with CRSwNP.

CONCLUSIONS

Our findings support that CRSwNP is accompanied by the enhanced hydration of NECs membranes in the region of glycerol backbones of phospholipids, in the region of phospholipid carbonyl groups and in the region of fatty acid tails directly adjacent to the carbonyl groups. Our findings allow us to suggest that the development of CRSwNP is associated with an increase in the hydration of the most polar region of cell membranes in NECs, i.e. the area of phospholipid polar heads.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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