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IMPACT OF INFLAMMATORY CELL ACTIVATION ON NASAL HYPERREACTIVE RESPONSE TO DISTILLED WATER NASAL PROVOCATION

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SUMMARY – The aim of the study was to compare hyperreactive response to nasal distilled water provocation in patients with allergic and non-allergic hyperreactive rhinitis, and to correlate the severity of hyperreactivity with inflammatory cell activation. Cellular activity was measured by the concentration of cellular activation markers in nasal lavage prior to provocation, eosinophil cationic protein (ECP) for eosinophil granulocytes, myeloperoxidase (MPO) for neutrophilic granulocytes and tryptase for mast cells. The study was performed in a group of 78 patients with a history of nasal hyperreactivity, i.e. 48 patients with allergic rhinitis and 30 patients with non-infectious non-allergic rhinitis (NINAR). Prior to provocation, basal nasal airway resistance was measured by active anterior rhinomanometry, and nasal lavage with 5 ccm of saline was taken. Provocation was made by inhalation of 10 ccm of distilled water over 10 minutes. The patients were subdivided into groups according to nasal airway resistance (NAR) increase. In the whole group the provocation induced a significant increase in nasal resistance on the better patent side prior to provocation ($p<0.005$). The only significant difference between allergic and non-allergic patients was recorded in tryptase concentration in nasal lavage, which was significantly higher in the group of allergic patients. No correlation was found between any of the cellular markers and the level of nasal hyperreactivity. The correlation between ECP and MPO in nasal lavage was significant. As no correlation was found between inflammatory cell activation and hyperreactivity, it appears that neural reflexes in addition to inflammation must be involved in the regulation of hyperreactive response.

Key words: *Rhinitis – diagnosis; Rhinitis – physiopathology; Rhinitis, allergic – diagnosis; Nasal provocation tests; Water diagnostic use; Nasal mucosa – drug effects*

Introduction

Different environmental stimuli may induce hyperreactive response in the airways of sensitive individuals. Specific hyperreactive nasal response in an allergic patient following exposure to airborne allergen is caused by the allergen interaction with mast cell-bound IgE, which leads to the increased vascular permeability, glandular hypersecretion, inflammatory cell attraction, and stimulation of irritant nerves and neural pathways[1]. The exposure to irritants or changes in environmental temperature and humidity may sometimes lead to similar changes in the airways of allergic and non-allergic hyperreactive individuals (non-specific nasal hyperreactivity)[2].

Non-isotonic aerosol may be used as a provoking agent for the upper and lower airways[3]. After distilled water provocation as well as after allergen provocation, similar mediator levels in nasal lavages were demonstrated in some patients with allergic rhinitis[4]. It suggests that nasal provocation with distilled water may trigger mast cell degranulation in sensitive subjects. In comparison to normal subjects, patients with non-allergic

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non-infective rhinitis (NINAR) demonstrate hyperreactive response to different environmental stimuli and to lower doses of the provoking agent in nasal provocation tests[5]. Non IgE-dependent mast cell activation is responsible for some of the hyperreactive responses in patients with NINAR[6]. However, literature data indicate that granulocyte activation, presumably eosinophilic[7], is the main cause of non-specific nasal hyperreactivity, due to the release of cytotoxic granular proteins such as eosinophil cationic protein (ECP), major basic protein (MBP) and eosinophil peroxidase (EPO), which induce damage to the respiratory epithelia[8-10].

The aim of the study was to compare the results of nasal challenge with distilled water aerosol between patients with allergic rhinitis and NINAR, and to compare the severity of non-specific nasal hyperreactivity with inflammatory cell activation prior to provocation. Cellular activity was to be estimated by measuring the concentration of cellular activation markers in nasal lavage, ECP for eosinophil granulocytes, myeloperoxidase (MPO) for neutrophilic granulocytes and tryptase (TRY) for mast cells.

Patients and Methods

The study was performed in a group of 78 patients (21 male and 57 female) aged 18-65 (mean age 30.0±13.9) years, with a history of nasal hyperreactivity. There were 48 patients with perennial or seasonal allergic rhinitis (confirmed by positive skin prick test (SPT) and specific IgE to at least 1 relevant allergen) and 30 patients with NINAR (negative SPT and serum IgE below 20). All patients gave their informed consent and the study was approved by the Ethics Committee of the Sestre milosrdnice University Hospital, Zagreb School of Medicine. Exclusion criteria were polyposis at endoscopy, significant unilateral septal deviation, positive bacteriological swab, recent nasal surgery and uncertain history data on medication taken for six weeks before provocation.

Intervention

Prior to provocation, basal NAR was measured by active anterior rhinomanometry, using a PC 200 rhinomanometer (Atmos, Lenzkirch, Germany). Baseline results represent an average value of 4 measurements within 10 minutes and are expressed as the better side resistance (BSR), worse side resistance (WSR) and total nasal resistance (NAR), calculated with the parallel resistance formula[11]. No medication was taken by the subjects either prior to basal measurements or provocation. Before the provocation, nasal lavage with 5 ccm of saline was taken according to a modified Naclerio technique[12]. Provocation was done using 10 ccm of distilled water at 25 °C delivered through a jet nebulizer over 10 minutes[13]. Anterior rhinomanometry was performed right after the provocation and repeated 3 times within the next 5 minutes. The considered resistance is the mean value of 4 measurements during 5 minutes after provocation.

The resistance figures presented are the values calculated at 150 Pa. According to NAR increase, the patients were subdivided into the following groups: H0, patients with less than 50% of NAR increase; H50, patients with NAR increase between 50% and 100% on the better patent side prior to provocation; and H100, patients with more than 100% NAR increase after provocation on the better patent side[3]. Nasal lavage samples were stored at room temperature for 2 hours, centrifuged at 1000 xg for 10 minutes and placed in a refrigerator at -20 °C. Tryptase and ECP were measured by fluoroenzymeimmunoassay (UniCAP, Pharmacia & Upjohn, Uppsala, Sweden), and IgE and MPO by radioimmunoassay (Pharmacia, Sweden).

Statistics

Data distribution was calculated using Smirnoff-Kolmgorov test. For the parameters demonstrating normal distribution, Student's t-test for paired samples was used. Wilcoxon signed rank test was used for data that showed no normal distribution. Correlations were calculated with paired sample correlation test and Spearman's rank correlation test. All conclusions were based on a significance level of $p<0.05$.

Results

In the whole group, the provocation induced a significant increase (from 0.55 to 0.77 kPa/cm3s^{-1}) in nasal resistance on the better patent side prior to provocation ($p<0.005$). On the worse side the resistance increased non-significantly, from 0.89 to 1.05 kPa/cm3^{s-1}. Results of nasal resistance prior to and after the provocation are presented in Table 1.

Concentrations of cellular activation markers in nasal lavage in allergic and non-allergic patients are presented in Table 2.

Table 1. Results of nasal resistance prior to and after provocation

Nasal resistance (kPa/cm3s-1)	N	Minimum	Maximum	Mean	Standard deviation
BSR basal	78	0.08	5.35	0.55	0.59
BSR provocation	78	0.16	2.34	0.77	0.50
WSR basal	78	0.27	7.50	0.89	0.91
WSR provocation	78	0.24	3.12	1.05	0.64
NAR basal	78	0.09	3.12	0.34	0.36
NAR provocation	78	0.10	2.50	0.43	0.32

BSR, better side resistance; WSR, worse side resistance; NAR, total nasal resistance

Table 2. Concentrations of cellular activation markers in nasal lavage in allergic and non-allergic patients

Marker concentration (μg/L)		N	Mean	Standard deviation	P
ECPL	Allergic	44	50.40	76.13	0.20
	Non-allergic	26	34.59	76.08	
MPOL	Allergic	43	230.21	242.59	0.57
	Non-allergic	27	663.09	1813.97	
TRYL	Allergic	42	1.86	2.85	0.02*
	Non-allergic	27	0.78	0.63	

*p<0.05; ECPL, eosinophil cationic protein in nasal lavage; MPOL, myeloperoxidase in nasal lavage; TRYL, tryptase in nasal lavage

Table 3. Correlation between cellular markers and level of nasal hyperreactivity

Spearman's rank correlation test		ECPL	MPOL	TRYL	H50	H100
ECPL	ro	1.000	0.472	0.004	0.054	0.102
	p		0.000*	0.975	0.658	0.399
	N	70	68	67	70	70
MPOL	ro	0.472	1.000	-0.017	-0.058	0.105
	p	0.000*		0.894	0.635	0.387
	N	68	70	67	70	70
TRYL	ro	0.004	-0.017	1.000	-0.075	-0.029
	p	0.975	0.894		0.542	0.812
	N	67	67	69	69	69

*p<0.05; ECPL, eosinophil cationic protein in nasal lavage; MPOL, myeloperoxidase in nasal lavage; TRYL, tryptase in nasal lavage; H50, patients with NAR increase between 50% and 100% on the better patent side; H100, patients with more than 100% NAR increase on the better patent side

The only significant difference between allergic and non-allergic patients was recorded for tryptase concentration in nasal lavage, which was significantly higher in allergic patients. No correlation was found between any of the cellular markers and the level of nasal hyperreactivity. However, the correlation between ECP and MPO was significant. These data are presented in Table 3.

Discussion

Literature data on lower airways indicate that an increased number of eosinophils correlates with bronchial hyperreactivity, comparing bronchoalveolar lavage fluid with airway resistance following bronchoprovocation tests[14]. These findings are explained by the action of

eosinophilic granular proteins, which, by causing damage to the respiratory epithelia, are considered to be the main cause of non-specific hyperreactivity in patients with allergic rhinitis and non-allergic rhinitis with eosinophilia syndrome (NARES). As we found no correlation between NAR and ECP, either between basal or provoked NAR, or percentual NAR increase, our results did not confirm this hypothesis. The H0, H50 and H100 subgroups showed no difference in ECP concentration in nasal lavage. Rasp and Hochstrasser report on increased tryptase in nasal lavage of allergic patients[15]. The lack of difference in nasal ECP between allergic and non-allergic patients suggested that a high proportion of non-allergic patients had local activation of eosinophils, presumably NARES patients. The absence of hyperreactive response in patients with up-regulated eosinophil activation may be explained by the potentially high local levels of histaminase, which is mainly synthesized by eosinophils[16]. Another explanation might be the production of anti-inflammatory prostanoids, which are also produced by eosinophils, as demonstrated in animal model of eosinophilic tracheobronchitis induced by polymyxin B inhalation[17].

An unexpected correlation was found between ECP and MPO, eosinophilic and neutrophilic markers, which are regulated by different T-helper cell profile of cytokines. In allergic and asthmatic patients ECP is usually increased by Th-2 profile cytokine up-regulation, while MPO is increased by Th-1 cytokine up-regulation. Similarly, a significant correlation between ECP and MPO was also found in the bronchoalveolar lavage of allergic and non-allergic asthmatics[18] as well as in serum of patients with chronic bronchitis with airway obstruction[19]. A high correlation was found between ECP, MPO and IL-8 in the bronchial lavage of chronic bronchitis patients, and the value of these markers was highest in patients with pneumococcal infection[20]. A significant correlation between IL-8, MPO and ECP was found in nasal lavage fluid following experimental rhinovirus infection and allergen challenge[21], while no correlation was observed between ECP and IL-8 after allergen provocation[22]. Our data suggest simultaneous activation of neutrophils and eosinophils. This means that in addition to IL-8, the process may also be regulated by GM-CSF.

Although hyperreactive response to distilled water inhalation is probably induced by mast cell degranulation[4], this process may be primarily dependent on neurogenic stimuli. The absence of correlation between inflammatory cell mediators and the severity of nasal hyperreactivity suggests that the level of hyperreactive response is not dependent on either intensity of local inflammation or epithelial damage. As non-isotonic aerosol stimulates C-fiber endings in nasal mucosa, the level of hyperreactive response to distilled water may depend on the level of neurogenic inflammation induced by such inhalation, and receptors responsible for such response are probably transient receptor potential (TRP) channels[23].

TRP channels include a superfamily of non-selective cation channels with at least seven subfamilies, which correspond to differences in the activation mechanisms and functions. Cation channels activated by extracellular hypo-osmoticity are TRPM3 (TRP melastatin 3) and TRPV4 (TRP vanilloid 3). The metabolites of arachidonic acid as well as alpha-isomers of phorbol esters known to be ineffective in stimulating proteins of the protein kinase C family activate TRPV4, while TRPM3 respond to sphingosine derivatives. TRPV4 could be found in many epithelial cells, which suggests its important role in epithelial physiology. Multiple cellular responses are triggered by TRPV4 activation and subsequent elevation of intracellular calcium. On the other hand, paracellular permeability may allow the cells to adjust to changes in extracellular osmolarity. Accordingly, TRPV4 plays a central role in epithelial homeostasis by modulating epithelial barrier function[23].

As most of the mechanisms involved in the response to non-isotonic aerosol have not yet been elucidated, further research on neurogenic inflammation and detection of responsible receptors involved is still expected.

Conclusion

Nasal provocation with distilled water aerosol in patients with allergic and non-allergic nasal hyperreactivity leads to a significant increase in nasal resistance. The intensity of hyperreactive response to such a challenge does not significantly correlate with inflammatory cell (eosinophil, neutrophil and mastocyte) activity prior to provocation. Neurogenic mechanisms involved in response to such a provocation are still to be clarified.

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Sažetak

UČINAK AKTIVNOSTI UPALNIH STANICA NA NOSNU HIPERREAKTIVNOST U ODGOVORU NA NOSNU PROVOKACIJU DESTILIRANOM VODOM

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Cilj istraživanja bio je usporediti hiperreaktivni odgovor na nosnu provokaciju destiliranom vodom u bolesnika s alergijskim i nealergijskim hiperreaktivnim rinitisom i stupanj hiperreaktivnosti s aktivnošću upalnih stanica. Stanična aktivnost mjerena je koncentracijom biljega stanične upalne aktivnosti u nosnom ispirku prije provokacije. To su eozinofilni kationski protein (ECP) za eozinofilne granulocite, mijeloperoksidaza (MPO) za neutrofilne granulocite i triptaza za mastocite. Istraživanje je obuhvatilo skupinu od 78 bolesnika s nosnom hiperreaktivnošću u anamnezi, 48 bolesnika s perenijalnim ili sezonskim alergijskim rinitisom i 30 bolesnika s neinfektivnim nealergijskim rinitisom (NINAR). Provokaciji je prethodilo mjerenje bazalnog nosnog otpora pomoću aktivne prednje rinomanometrije i uzimanje nosnog ispirka s 5 cm^3 fiziološke otopine. Provokacija je provedena inhalacijom 10 cm^3 destilirane vode tijekom 10 minuta. Prema razini porasta nosnog otpora (NAR) bolesnici su podijeljeni u tri skupine: bez odgovora, srednje do umjereno jak i vrlo jak odgovor. U svih ispitanika provokacija je izazvala značajan porast nosnog otpora na strani koja je prije provokacije bila bolje prohodna ($p<0,005$). Jedina značajna razlika među ispitanicima s alergijskim i nealergijskim rinitisom bila je u koncentraciji triptaze u nosnom ispirku, koja je bila značajno viša u skupini ispitanika s alergijskim rinitisom. Nije nađena korelacija između staničnih biljega i razine nosne hiperreaktivnosti, ali je zabilježena značajna korelacija između ECP i MPO u nosnom ispirku. Kako nema korelacije između aktivnosti upalnih stanica i hiperreaktivnosti, čini se da su u regulaciji hiperreaktivnog odgovora uz upalne uključeni i neuralni mehanizmi.

Ključne riječi: *Rinitis – dijagnostika; Rinitis – fiziopatologija; Rinitis, alergijski – dijagnostika; Nosni provokacijski testovi; Dijagnostička primjena vode; Nosna sluznica – učinci lijekova*