

Research Article

Level of Arachidonic Acid and State of Peroxidation Processes in Patients with Aspirin-Intolerant Polypous Rhinosinusitis

Ivanna Koshel

Abstract

The main peculiarity of aspirin-intolerant polypous rhinosinusitis pathogenesis is the presence of “genetic block” of constitutive cyclooxygenase being the key enzyme of the arachidonic acid metabolism. It justifies the necessity of studying its metabolic peculiarities.

The objective of the research was to determine the level of arachidonic acid as well as the state of lipid and protein peroxidation processes in patients with aspirin-intolerant polypous rhinosinusitis.

Materials and methods. The levels of arachidonic acid, malondialdehyde and oxidative modification of serum proteins were studied in 20 patients with aspirin-intolerant polypous rhinosinusitis and 7 healthy individuals.

Results. Significantly elevated levels of arachidonic levels were observed. The search for alternative metabolic pathways stimulated lipid and protein peroxidation processes and led to the increase in the levels of malondialdehyde and oxidative modification of serum proteins. The peculiarities of biochemical changes indicated pro-inflammatory orientation of lipid metabolism.

Conclusions. The obtained data confirmed the hypothesis of “genetic block” of the arachidonic acid metabolism as the main pathogenetic component of aspirin-intolerant polypous rhinosinusitis and allowed us to clearly interpret biochemical picture of the disease.

Keywords

aspirin-intolerant polypous rhinosinusitis; arachidonic acid

Ivano-Frankivsk National Medical University, Ukraine

*Corresponding author: ivannakoshel@gmail.com

Problem statement and analysis of the recent research

Over the past decade, particular importance has been given to the problem of pathogenesis, diagnosis and treatment of chronic polypous rhinosinusitis being one of the urgent problems of modern medicine, in both Ukraine and Europe [17]. There is a wide range of variants of clinical course and different responses to traditional methods of treatment. All these peculiarities are combined into one diagnosis of “polypous rhinosinusitis”. It suggests the heterogeneity of a group of patients with the aforementioned diagnosis and the need for a detailed study of various clinical variants of nasal polyposis. It has been clinically observed that aspirin-intolerant polypous rhinosinusitis is one of the most clinically damaging pathogenetic variants of respiratory damage which is characterized by progressive relapsing course and less effectiveness of conservative and surgical treatment [15].

From the perspective of current view on the etiopathogenesis, aspirin-intolerant polypous rhinosinusitis is associated with changes in the arachidonic acid metabolism and belongs to a group of metabolic diseases (MD) [4, 19, 20]. The main peculiarity of pathogenesis is the presence of genet-

ically determined reduction in the activity of the key enzyme of the metabolism of unsaturated fatty acids, arachidonic acid in particular - constitutive cyclooxygenase (COX-1) [5, 12]. “Genetic block” develops resulting in the disruption of the biochemical chain and, therefore, pathogenetic processes of MD occur at the molecular level; however, it is the initial link of the complex pathogenetic chain. The pathogenesis at the cellular level means that the main pathological processes typical for aspirin-intolerant polypous rhinosinusitis occur in the cells, mucosal cells lining the respiratory tract in particular. Any block is characterized by the fact that all products up to the level of block are not involved in the exchange. As a result, arachidonic acid (ARA) accumulates in the body [11]. The body tends to metabolize an excessive amount of ARA and searches for alternative ways. High serum levels of fatty acids are known to activate NADPH oxidase through the activation of protein kinase C, thereby activating free radical formation [22]. Free-radical reactions are the initial stage of the generation of reactive oxygen species (ROS) [8]. ROS are involved in the metabolic processes of the body related to lipid, protein and nucleic acid metabolism as well as in the synthesis of prostaglandins, leukotrienes, thromboxanes. They initiate

lipid peroxidation (LPO) which is characterized by the accumulation of toxic byproducts of LPO, oxidative modification and damage to protein molecules and DNA [3]. ROS are able to abstract hydrogen atoms from a CH_2 - group of ARA and convert them to free radical groups - CH^* . Such fatty acid radical easily absorbs a molecule of oxygen and is converted to peroxy radical which initiates further development of a chain reaction. The products of such reactions are conjugated diene and triplets; they are rather unstable and break down to form aldehydes. Significant amount of malondialdehyde (MDA) is observed; its concentration in the tissues can serve as the indicator of LPO intensity [13]. The byproducts of LPO can impair protein synthesis. The aggression of free radicals against proteins results in changes in their physicochemical properties. Oxidative modification of proteins (OMP) affects the indicators of the isoelectric point, thermal stability and proteolytic ability resulting in impaired enzymatic activity. Enzymes may lose their catalytically active groups as well. Such mechanism may occur in secondary impairment of enzyme synthesis. It has been proven that under oxidative stress as well as due to ROS, not lipids but proteins of the plasma membranes primarily undergo oxidation which results in their depolymerization and cell lysis [8]. Under physiological conditions, the intensity of alternative metabolic processes is low. Under pathological conditions, the intensification of these processes is one of the universal etiopathogenetic mechanisms of MD [7].

Many researchers agree that pro-inflammatory reaction in the nasal epithelium induced by free radical oxidation is the main cause of polyp formation [16, 21, 23]. Nevertheless, the role of these processes in the pathogenesis of polyposis in patients with aspirin intolerance has not yet been fully understood [14]. However, in order to implement treatment due to the intervention in the pathogenesis it is necessary to interpret biochemical phenotype of the disease, i.e. to detect disrupted metabolic components and biochemical mechanisms which trigger the pathological process. Certainly, purposefully intervention in the pathogenesis of the disease may be performed only on this basis.

The objective of the research was to determine the level of arachidonic acid as well as the state of lipid and protein peroxidation processes in patients with aspirin-intolerant polypous rhinosinusitis.

1. Materials and methods

The study included 20 patients at the age of 24-57 years (the average age was 45.7 ± 0.85 years) with chronic polypous rhinosinusitis associated with aspirin intolerance who were treated in the ear, nose, and throat (ENT) department of Ivano-Frankivsk Regional Clinical Hospital during 2014-2015. The control group included 7 somatically healthy donors at the age of 20-50 years (the average age was 42.4 ± 1.16 years).

In patients, blood was collected in the morning (on an empty stomach). Heparin solution was used as an anticoagulant. The object of the study was quarantine fresh-frozen

plasma stored at -20°C .

Serum levels of arachidonic acid (20:4 ω -6) were determined using capillary gas-liquid chromatography. The identification of chromatographic peaks was performed using individual standard solutions of fatty acid methyl esters. Registering and processing of chromatograms were performed using the HP ChemStation software.

The study was carried out using 7890A gas chromatograph (Agilent Technologies) of the Institute of Animal Biology, National Academy of Agrarian Sciences of Ukraine.

The analysis of LPO products – MDA was made on the basis of determining the concentration of TBA-active products through incubating the samples with the thiobarbituric acid, extracting reaction products with butanol and detecting coloured complex by spectrophotometry. The indicators of MDA were studied according to the method proposed by V.P. Gavrilov [2].

The analysis of LPO products – OMP was based on the interaction between oxidized amino acid residues and 2,4-dinitrophenylhydrazine with the formation of derivatives having characteristic absorption spectra. Aldehyde and ketone derivatives of a neutral character were registered at a wavelength of 370 nm, aldehyde and ketone derivatives of the main character were registered at a wavelength of 430 nm. The indicators of OMP were studied according to the method proposed by E.E. Dubinina et al. [9].

To assess the differences between groups one-factor dispersion analysis with subsequent use of the Tukey's multiple comparison test at a significance level of 0.05 was applied [6].

Statistical software IBM SPSS Statistics 22.0 was used. Pairwise comparisons of means in studied groups were performed. To perform a sufficiently large number of pairwise comparisons without loss of the statistical power the Tukey HSD criterion was used. Pairwise comparison was performed at a significance level of $p=0.05$, i.e. when performing all pairwise comparisons the probability of obtaining one or more false-negative results was 0.05. Correspondingly, if $p>0.05$, one could conclude that the groups were not statistically different by the studied parameter and if $p<0.05$ – they were statistically different.

2. Results and Discussion

According to current views, the main peculiarity of aspirin-intolerant polypous rhinosinusitis pathogenesis is the presence of genetically determined defect of COX-1 being the key enzyme of the arachidonic acid metabolism (Fig. 1) [12]. In our previous works, the reduction in the expression of COX-1 in the mucosa of the nasal cavity and polyps has been proven using the methods of immunohistochemistry [5].

Any block is characterized by the fact that all products up to the level of block are not involved in the exchange and one of the most important and earliest mechanisms of pathology development is the accumulation of products which are not involved in the metabolism, i.e. those being located above “genetic block”. Reduced COX-1 expression expectedly leads

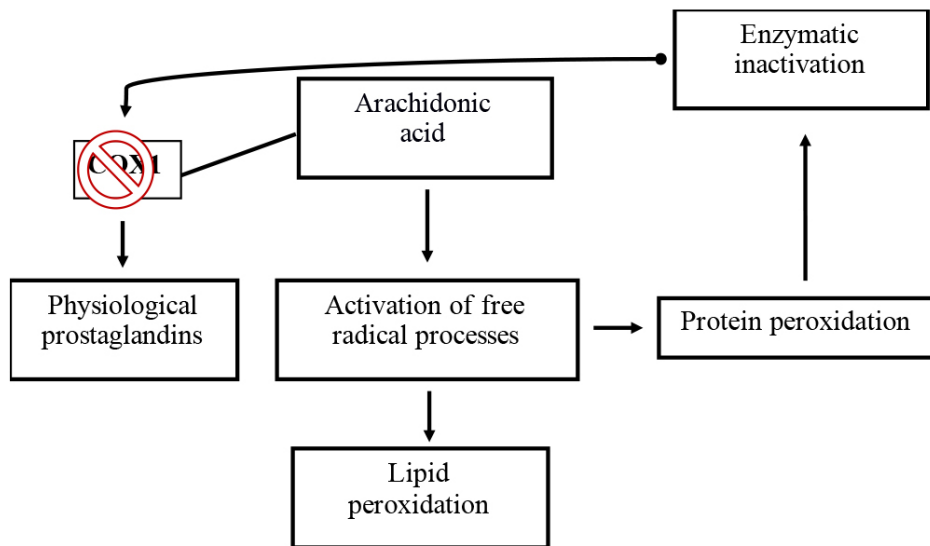


Figure 1. Arachidonic acid metabolism.

to intense accumulation of ARA as it is the substrate of COX-1 (Fig. 1).

The results revealed that in patients with aspirin-intolerant polypous rhinosinusitis serum levels of ARA increased significantly to $16.99 \pm 0.17\%$ compared to $4.69 \pm 0.27\%$ under normal conditions (Table 1).

Decreased function of constitutive COX-1 and the accumulation of ARA result in the search for possible bypass routes of the metabolism as well as stimulate its possible directions. If ARA metabolism via the cyclooxygenase pathway is disrupted, it is included in other metabolic processes, the intensity of which, under normal conditions, is low. In particular, peroxidation or lipid peroxidation is activated (Fig. 1) [1]. Significant amount of MDA is observed; its serum concentration can serve as the indicator of LPO intensity. According to our data, serum levels of MDA in healthy individuals were 2.77 ± 0.26 nmol/ml; in people with aspirin-intolerant polypous rhinosinusitis they increased to 4.1 ± 0.15 nmol/ml (Table 1).

Free radicals affect not only lipids but also proteins (Fig. 1). All enzymes providing the metabolic and regulatory processes are known to be proteins. The products of such reactions are OMP. In healthy individuals, serum levels of oxidative modified proteins (aldehyde and ketone derivatives) were: OMP₃₇₀ – 3.47 ± 0.09 mmol/g of protein, OMP₄₃₀ – 1.93 ± 0.04 mmol/g of protein. Our results showed significant increase in OMP concentration in blood plasma of patients with aspirin-intolerant polypous rhinosinusitis: OMP₃₇₀ – to 4.93 ± 0.16 mmol/g of protein, OMP₄₃₀ – to 6.81 ± 0.94 mmol/g of protein (Table 1). Thus, the results obtained when treating patients with aspirin-intolerant polyposis showed a sharp increase (by nearly 4 times) in serum levels of ARA. Significantly elevated ARA levels lead to the activation of

free radical processes and significant increase in the level of LPO and OMP products.

The aggression of free radicals against proteins results in changes in their physicochemical properties. Enzymes may lose their catalytically active groups as well, which results in impaired enzymatic activity and metabolic disorders. Such mechanism may occur in secondary impairment of enzyme responsible for ARA metabolism synthesis (Fig. 1).

Modified proteins are not restored; they are destroyed through proteolytic degradation. It should be considered that the reduction in enzymatic activity is accompanied by an increase in the level of OMP in the cell [10]. It contributes to the slowing down of the apoptotic and proteolytic processes in such cells. The slowing down of apoptotic death, eosinophils in particular is known to be one of the key events in the pathogenesis of eosinophilic nasal polyposis [18].

3. Conclusions

1. In patients with aspirin-intolerant polypous rhinosinusitis serum levels of ARA increased to $16.99 \pm 0.17\%$ significantly exceeding normal values ($4.69 \pm 0.27\%$).
2. The activation of alternative routes, free radical lipid oxidation in particular resulted in the increase in MDA level compared to the control group (4.1 ± 0.15 nmol/ml versus 2.77 ± 0.26 nmol/ml).
3. The increase in LPO intensity resulted in the elevation of OMP₃₇₀ level to 4.93 ± 0.16 mmol/g of protein versus 3.47 ± 0.09 mmol/g of protein under normal conditions and OMP₄₃₀ level to 6.81 ± 0.94 mmol/g of protein versus 1.93 ± 0.04 mmol/g of protein under normal conditions.

Table 1. Levels of ARA and major metabolites of peroxidation.

Metabolism	Control group	Study group
Serum levels of ARA (%)	4.69±0.27	16.99±0.17*
MDA (nmol/ml)	2.77±0.26	4.1±0.15*
OMP ₃₇₀ (mmol/g of protein)	3.47±0.09	4.93±0.16*
OMP ₄₃₀ (mmol/g of protein)	1.93±0.04	6.81±0.94*

Note.

* – a statistically significant difference between groups (p<0.05).

4. The aforementioned changes confirmed the hypothesis of “genetic block of COX-1” as the main pathogenetic component of aspirin-intolerant polypous rhinosinusitis.

4. Prospects for further research

The possibility of using the study of the concentration of ARA and peroxidation products to control the effectiveness of therapy when treating patients with aspirin-intolerant polypous rhinosinusitis is promising.

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