ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Some Aspects of Bronchial Asthma and Essential Hypertension Comorbidity.

OM Uryasyev, AV Shakhanov, YA Panfilov*, and LV Tverdova.

Ryazan State Medical University 9 Vysokovoltnayast., Ryazan, Russian Federation, 390026.

ABSTRACT

There exists an interest in studying polymorphism of nitric oxide synthase genes in patients with bronchial asthma and essential hypertension. To study clinical and pathogenetic significance of polymorphism of nitric oxide synthase genes (NOS1 84G/A and NOS3 786C/T) in formation of comorbid pathology of bronchial asthma and essential hypertension. In the study 71 patients participated who were treated for bronchial asthma or essential hypertension. Themaingroup consisted of 24 patients with comorbid pathology of bronchial asthma and essential hypertension. The group of patients with isolated bronchial asthma included 23 individuals. The group of patients with isolated essential hypertension included 24 individuals. In all patients polymorphism of nitric oxide synthase genes NOS1 84G/A and NOS3 786C/T was determined. The studied groups did not differ in distribution of the genotypes ($\chi2 = 2.13$, $p = 0.712$) of NOS1 84G/Apolymorphism. The studied groups reliably differed in distribution of alleles ($\chi2 = 10.55$, $p = 0.005$) and of separate genotypes ($\chi2 = 10.49$, $p = 0.03$) of NOS3 786C/Tpolymorphism which evidences the relationship between the given polymorphism and development of bronchial asthma and essential hypertension. Inpatients with comorbid pathology of bronchial asthma and essential hypertension the frequency of occurrence of T-allele is higher and that of C-allele is lower($\chi2 = 4.24$, $p = 0.04$) than in patients with isolated bronchial asthma. T-alleleofNOS3 786C/T polymorphism2.4 times increases the probability for comorbid pathology of bronchial asthma and essential hypertension in comparison with isolated bronchial asthma (OR = 2.40, 95% CI: 1.04 – 5.56). Evaluation of NOS3 786C/Tpolymorphism can be used for identification of patients with bronchial asthma with a high risk for development of essential hypertension.

Keywords: bronchial asthma, genetic polymorphism, nitric oxide synthase, essential hypertension.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Recently the attention of researchers has been increasingly attracted by the problem of comorbidity. Study of comorbid pathology permits to better understand the mechanism of diseases and develop pathogenetically justified therapy. Of interest is study of comorbidity of such important and socially significant diseases as bronchial asthma and essential hypertension. A share of patients with bronchial asthma suffering from cardio-vascular diseases reaches 50%.Here, the predominating disease is essential hypertension with the incidence among the patients with bronchial asthma from 12.9% to 37.6% increasing with age [1]. At present many questions concerning the clinical course and pathogenesis of comorbid pathology of bronchial asthma and essential hypertension still remain open. However, there is no doubt in the existence of some pathogenetic mechanisms of the mutual influence of these two diseases. One of probable mechanisms consists in the alteration of physiological synthesis of nitric oxide (NO) which regulates the tone of vessels and arterial pressure, adhesion of platelets and proliferation of smooth muscle cells, ion transport and barrier function of the epithelium, secretion of mucus and the mucociliary clearance [2, 3]. Reduction in the endothelial production of nitric oxide leads to development and progress of endothelial dysfunction and to increase in the arterial pressure [4, 5]. Besides, NO participates in formation of inflammation in bronchial asthma through inhibition of production of anti-inflammatory mediators in the epithelium and inhibition of the functional activity of T-lymphocytes [6].

In an organism nitric oxide is synthesized from L-arginine by a group of cytochrome P-450-type hemoproteins – NO-synthases that include three isoforms: neuronal (nNOS), macrophagal (iNOS) and endothelial (eNOS)[7]. TheseisoformsareproductsofexpressionofNOS1, NOS2 and NOS3genes, respectively. In physiological conditions neuronal and endothelial nitric oxide synthases are constitutive, but in different pathological conditions their expression is induced which is accompanied by enhanced production of NO. NitricoxideproducedbyNOS1 gene is known as a neurotransmitter at nerve synapses and also as a regulator of physiological respiratory processes. Genetic data show the significance of NOS1gene in the pathogenesis of bronchial hypersensitivity in asthma. Nitric oxide produced by eNOS coded for by NOS3 gene is the most potent of the known endogenous vasodilators, and its connection with cardiovascular pathology is out of doubt. Activity of NOS expression directly depends on the nucleotide composition of the coding genes. A significant amount of research is conducted to study different variants of polymorphism of NOS genes and their role in development of diseases.

Thus, the ability of NO to act as a physiological regulator or as a probable toxic agent may be determined by the activity of nitric oxide synthase isoforms which changes in the presence of different mutations of NOS genes. Among these mutations of interest is single nucleotide polymorphism of NOS1–84G/A gene and NOS3–786C/T gene. The aim of this research was to study clinical and pathogenetic significance of these mutations in comorbid pathology of bronchial asthma and essential hypertension. For this, our goal was to study the incidence of NOS1 84G/A and NOS3 786C/Tpolymorphisms in patients with combined bronchial asthma and essential hypertension and to evaluate their connection with the development and course of comorbid pathology.

MATERIALS AND METHODS

The study was performed in the period from 2014 to 2016 at the Department of Intermediate Level Therapy with courses of endocrinology, clinical pharmacology, occupational diseases of acad. I.P. Pavlov State Medical University of Ryazan. The study was approved by Local Ethics Committee of RyazSMU and complies with the requirements of Good Clinical Practice (GCP) and the World Medical Association Declaration of Helsinki “Ethical Principles for Medical Research Involving Human Subjects”.

Into the study 71 patients were included who were receiving medical treatment for bronchial asthma or essential hypertension in SBI Ryazan Regional Clinical Hospital (Ryazan). All patients were representatives of Caucasian race, were residents of Ryazan region and were not relatives.

Criteria for inclusion into the research: signed voluntary informed consent for participation in the research; ability to understand procedures of the research and to adequately co-operate with the researcher; age from 45 to 69; diagnosed with“bronchial asthma, mixed form”, and/or “essential hypertension” according

to the requirements of the global strategy for treatment and prevention of bronchial asthma and to recommendations of Society of Cardiology of Russian Federation/Russian Society of Cardiology, respectively.

Patients with decompensated cardiovascular pathology, severe disorders of the liver and kidneys in history, and also with other conditions that could significantly influence the function of the respiratory and cardiovascular systems and the studied parameters, were not included into the study.

Patients included into the study - 33 (46%) males and 38 (54%) females, were divided into 3 groups depending on the disease. The first group included 24 patients with comorbid pathology of bronchial asthma and essential hypertension. Comparison groups - a group of patients with isolated bronchial asthma a group of patients with isolated essential hypertension included 23 and 24 patients, respectively. The groups were comparable by sex and age.

Determination of the genetic polymorphism of nitric oxide synthase genes NOS1 84G/A and NOS3 786C/Twas conducted on the basis of Central Research Laboratory of RyazSMU. The material for molecular-genetic analysis wasDNA samples isolated from leukocytes of the whole blood using DNA-Express-Blood reagent of OOO SPCLitekh manufacture (Moscow). For analysis of genetic polymorphism a method of allele-specific PCR was used with the subsequent electrophoretic separation of amplification products. Amplification products were visualized by electrophoresis in 3% agarose gel with the addition of ethidium bromide exposed to the ultraviolet light. The results of analysis of fluorescent signal for each sample gave information about the presence or absence of each allele in hetero- or homozygous form.

The obtained results were statistically processed using Microsoft Excel 2016, StatSoftStatistica 10 and DoctorStat 1.9 programs. Distribution of characteristics was evaluated using Shapiro-Wilk statistics. Taking into consideration the fact that distribution of the studied characteristics differed from normal, the data were presented in Meform [Q25; Q75], where Me is median, and Q25 and Q75 are lower and upper quartiles. For comparison of groups by a quantitative characteristic, Kruskall-Wallis and Mann-Whitney tests were used. Comparison of relative qualitative characteristics (frequencies and shares) was performed using χ^2 Pearson test. For the quantitative evaluation of the probability of outcome vs. the presence of the factor, the odds ratio parameter was used with 95% confidence interval. Interconnection of the quantitative characteristics was evaluated using Spearman's correlation coefficient r. Statistically significant were considered differences with $p < 0.05$.

Comparability of allele distribution of the studied polymorphisms in the examined selected subset relative to the population was evaluated by correspondence with Hardy-Weinberg equilibrium. The relationship between polymorphism and development of the disease was evaluated using general and multiplicative model of inheritance depending on the correspondence with Hardy-Weinberg equilibrium.

RESULTS AND DISCUSSION

Distribution of genetic information of NOS1 84G/A polymorphism among the patients: GG-genotype – 18 individuals (25%), GA-genotype – 24 individuals (34%), AA-genotype – 29 individuals (41%), allele G – 42% (n=60), allele A – 58% (n=82). The distribution did not correspond to Hardy-Weinberg equilibrium ($\chi2 = 6.71$, $p = 0.01$). Distribution of genetic information of NOS3 786C/T NOS1 84G/A polymorphism among the patients: CC-genotype – 9 individuals (12%), CT-genotype – 31 individuals (44%), TT-genotype – 31 individuals (44%), allele C – 35% (n=49), allele T – 65% (n=93), which corresponds to Hardy-Weinberg equilibrium($\chi2 = 0.08$, $p = 0.77$). Distribution of genetic information in the studied groups is given in Table 1.

ISSN: 0975-8585

Table1: Distribution of Genetic Information of NOS1 84G/A and NOS3 786C/T Polymorphisms in Studied Groups

Genotype / Allele	Bronchial asthma and Essential hypertension	Bronchial asthma	Essential hypertension	p
NOS1 84G/A				
GG	0.250	0.174	0.330	χ2 = 2.13, p = 0.712
GA	0.375	0.391	0.250	
AA	0.375	0.435	0.417	
G	0.437	0.370	0.458	χ2 = 4.19, p = 0.123
A	0.563	0.630	0.542	
Correspondence with Hardy-Weinberg equilibrium	χ2 = 1.36, p = 0.24	χ2 = 0.59, p = 0.44	χ2 = 5.92, p = 0.02	
NOS3 786C/T				
CC	0.083	0.261	0.042	χ2 = 10.49, p = 0.03
CT	0.458	0.522	0.333	
TT	0.458	0.217	0.625	
C	0.313	0.522	0.208	χ2 = 10.55, p = 0.005
T	0.688	0.478	0.792	
Correspondence with Hardy-Weinberg equilibrium	χ2 = 0.11, p = 0.74	χ2 = 0.05, p = 0.83	χ2 = 0.00, p = 0.96	

In the analysis of distribution of genetic information of NOS1 84G/A polymorphism in the examined groups no reliable difference in the genotype depending on the group of patients was determined ($\chi2 = 2.13$, $p = 0.712$), however, a tendency was noticed to an increase in the share of GG genotype of NOS1 84G/A polymorphism in the series bronchial asthma<bronchial asthma and essential hypertension<essential hypertension(0.174 – 0.250 – 0.330) which may be indicative of its connection with the studied pathology.

In the evaluation of distribution of genetic information of NOS3 786C/T polymorphism it was found that the examined groups reliably differed from each other both in distribution of alleles($\chi2 = 10.55$, $p = 0.005$) and in distribution of separate genotypes ($\chi2 = 10.49$, $p = 0.03$) which suggests connection of the given polymorphism with development of bronchial asthma and essential hypertension. It is interesting that distribution of genetic information in the main group occupies the intermediate position between patients with isolated bronchial asthma and isolated essential hypertension which permits to suggest that the given polymorphism changes the activity of nitric oxide synthase in bronchial asthma and essential hypertension in different ways. Comparison of the group of patients with a comorbid pathology of bronchial asthma and essential hypertension with the group of patients with isolated bronchial asthma showed a reliable increase in the incidence of T-allele and a decrease in that of C-allele ($\chi2 = 4.24$, $p = 0.04$). Calculation of relative risk showed that T-allele of NOS3 786C/T polymorphism 2.4 times increases the probability for com or bid pathology of bronchial asthma and essential hypertension than of isolated bronchial asthma (OR = 2.40, 95% CI: 1.04 – 5.56). The obtained results on the whole compare with those described earlier. Thus, R.E. Kalinin's investigation shows that TT genotype is more common in patients with trophic ulcers and severe trophic disorders associated with a chronic venous insufficiency of the lower limbs, which, like arterial hypertension, is also connected with reduced production of nitric oxide in the endothelium of patients with the given polymorphism [8]. And an examination conducted in Czechia shows that T-allele of NOS3 786C/T polymorphism may be associated with development of arterial hypertension[9]. This permits to recommend evaluation of polymorphism to determine the risk for development of essential hypertension in patients with bronchial asthma.

ForevaluationoftheclinicalsignificanceofNOS1 84G/A and NOS3 786C/T polymorphisms the patients were divided into groups by the extent of severity and the stage of the baseline therapy of bronchial asthma, and also by stages and degrees of essential hypertension. The results are given in Tables 2 and 3.

ISSN: 0975-8585

Table 2: Distribution of Genetic Information of NOS1 84G/A Polymorphism depending on Course of bronchial asthma and essential hypertension

	Genotype			Allele		Correspondence with Hardy-Weinberg equilibrium
	GG	GA	AA	G	A	
Extent of severity of bronchial asthma						
Moderate asthma	0.154	0.538	0.308	0.423	0.577	$\chi2 = 0.14$, $p = 0.71$
Severe asthma	0.235	0.324	0.441	0.397	0.603	$\chi2 = 3.58$, $p = 0.06$
p	$\chi2 = 1.84$, $p = 0.40$			$\chi2 = 0.05$, $p = 0.82$		
Stage of therapy of bronchial asthma						
3	0.154	0.538	0.308	0.423	0.577	$\chi2 = 0.14$, $p = 0.71$
4	0.190	0.330	0.476	0.357	0.643	$\chi2 = 1.58$, $p = 0.21$
5	0.308	0.308	0.385	0.462	0.538	$\chi2 = 1.89$, $p = 0.17$
P	$\chi2 = 2.54$, $p = 0.64$			$\chi2 = 0.78$, $p = 0.68$		
Stage of essential hypertension						
I	0.100	0.500	0.400	0.350	0.650	$\chi2 = 0.10$, $p = 0.75$
II	0.300	0.400	0.300	0.500	0.500	$\chi2 = 0.40$, $p = 0.53$
III	0.357	0.214	0.429	0.464	0.536	$\chi2 = 9.07$, $p < 0.01$
P	$\chi2 = 4.21$, $p = 0.38$			$\chi2 = 0.76$, $p = 0.69$		
Degree of essential hypertension						
I	0.333	0.500	0.167	0.583	0.417	$\chi2 = 0.00$, $p = 0.94$
II	0.211	0.316	0.474	0.368	0.632	$\chi2 = 1.96$, $p = 0.16$
III	0.348	0.261	0.391	0.478	0.522	$\chi2 = 5.24$, $p = 0.02$
P	$\chi2 = 2.67$, $p = 0.61$			$\chi2 = 2.00$, $p = 0.36$		

Table 3: Distribution of Genetic Information of NOS3 786C/T Polymorphism depending on Course of bronchial asthma and essential hypertension

	Genotype			Allele		Correspondence with Hardy-Weinberg equilibrium
	CC	CT	TT	C	T	
Extent of severity of asthma						
Moderate asthma	0.154	0.385	0.462	0.346	0.441	$\chi2 = 0.29$, $p = 0.59$
Severe asthma	0.174	0.529	0.294	0.654	0.559	$\chi2 = 0.18$, $p = 0.67$
p	$\chi2 = 1.21$, $p = 0.55$			$\chi2 = 0.70$, $p = 0.40$		
Stage of therapy of asthma						
3	0.154	0.385	0.462	0.346	0.441	$\chi2 = 0.29$, $p = 0.59$
4	0.143	0.476	0.381	0.381	0.538	$\chi2 = 0.00$, $p = 0.96$
5	0,231	0,615	0,154	0,619	0,462	$\chi2 = 0.74$, $p = 0.39$
p	$\chi2 = 3.10$, $p = 0.54$			$\chi2 = 2.34$, $p = 0.31$		
Stage of hypertension						
I	0.000	0.500	0.500	0.250	0.750	$\chi2 = 1.11$, $p = 0.29$
II	0.100	0.400	0.500	0.300	0.700	$\chi2 = 0.02$, $p = 0.88$
III	0.071	0.357	0.571	0.250	0.750	$\chi2 = 0.06$, $p = 0.80$
p	$\chi2 = 1.38$, $p = 0.85$			$\chi2 = 0.21$, $p = 0.90$		
Degree of hypertension						
I	0.000	0.667	0.333	0.333	0.667	$\chi2 = 1.50$, $p = 0.22$
II	0.000	0.421	0.579	0.211	0.789	$\chi2 = 1.35$, $p = 0.25$
III	0.130	0.304	0.565	0.283	0.717	$\chi2 = 1.43$, $p = 0.23$
p	$\chi2 = 5.44$, $p = 0.24$			$\chi2 = 0.94$, $p = 0.63$		

It was found that the studied polymorphisms do not produce any influence on the course of essential hypertension. Besides, no influence of NOS3 786C/T polymorphism was found on bronchial asthma and on the volume of baseline therapy required to control bronchial asthma. It was also determined that polymorphism of NOS1 84G/A gene does not produce any statistically reliable influence on the course of bronchial asthma, however, a tendency was revealed for increased detection of GG genotype with increase in both severity of

bronchial asthma and the volume of baseline therapy which may indicate a certain role of this polymorphism in the pathogenesis of bronchial asthma.

CONCLUSIONS

- In patients with the comorbid pathology of bronchial asthma and essential hypertension the frequency of occurrence of T-allele of NOS3 786C/T polymorphism is higher, and that of C-allele is lower in comparison with patients with the isolated bronchial asthma.
- Carriers of T-allele of NOS3 786C/T polymorphism are under 2.4 times higher risk for development of comorbid pathology of bronchial asthma and essential hypertension (OR = 2.40, 95% CI: 1.04 – 5.56).
- To detect patients with bronchial asthma with an increased risk for essential hypertension, evaluation of NOS3 786C/T polymorphism can be used.
- The studied polymorphisms produce no influence on the course of essential hypertension and bronchial asthma.

REFERENCES

[1] BelovaIV, KulaginOL, ZhestkovAV. Epidemiologijasochetanijaserdechno-sosudistyhzabolevanij i bronhial'nojastmy u vzroslyhpacientov (naprimeregorodaNovokujbyshevska) [Epidemiology of combination of the cardiovascular diseases and bronchial asthma in adult patients (on the example of Novokuybyshevsk city)]. *IzvestijaSamarskogonauchnogotsentraRossijskojakademiinauk [News of the Samara Scientific Center of the Russian Academy of Sciences]*. 2013;15(3–6):1728–30. (in Russian)

[2] Ghosh S, Erzurum SC. Modulation of asthma pathogenesis by nitric oxide pathways and therapeutic opportunities. *Drug discovery today. Disease mechanisms*. 2012; 9 (3–4): e89–94.

[3] Aytekin M, Aulak KS, Haserodt S, Chakravarti R, Cody J, Minai OA et al. Abnormal platelet aggregation in idiopathic pulmonary arterial hypertension: role of nitric oxide. *AJP: Lung Cellular and Molecular Physiology*. 2012;302(6): L512–20.

[4] Belozerov VK, Rubanova MP, Veber VR, Gubskaja PM, Zhmajlova SV, Kulik NA. Endotelij-zavisimajavazodilatacija i sostojaniestenkisonnojarterii [Endothelium-dependent vasodilation and the condition of the carotid artery wall]. *Kardiovaskuljarnajaterapijaiprofilaktika [Cardiovascular therapy and prevention]*. 2014;13(S2):16. (in Russian)

[5] Kumar R, Kohli S, Mishra A, Garg R, Alam P, Stobdan T, et al. Interactions between the genes of vasodilatation pathways influence blood pressure and nitric oxide level in hypertension. *American journal of hypertension*. 2015;28(2): 239–47.

[6] Hernansanz-Agustín P, Izquierdo-Álvarez A, García-Ortiz A, Ibiza S, Serrador JM, Martínez-Ruiz A. Nitrosothiols in the immune system: signaling and protection. *Antioxidants & redox signaling*. 2013;18(3): 288–308.

[7] Uryasev OM, Rochagikov AI. Rol' oksidaazota v reguljaciidyhatel'nojsistemy. [Role of nitric oxide in regulation of respiratory system] *Naukamolodykh(Eruditiojuvenium) [Science of young (EruditioJuvenium)]*. 2014;(2):133–40. (in Russian)

[8] Kalinin RE, Gryaznov SV, Nikiforov AA, Kamaev AA, Shvalb AP, Slepnev AA. Polimorfizmgenasintazyazotai endotelina-1 prihronicheskojvenoznojnedostatochnostinizhnihkonechnostej [Nitric oxide synthase and endothelin-1 gene polymorphism in lower limb chronic venous insufficiency]. *Rossijskijmediko-biologicheskijvestnikimeniakademika I.P.Pavlova [I.P. Pavlov Russian Medical Biological Herald]*. 2015;(4):97–102. (inRussian)

[9] Seidlerová J, Filipovský J, Mayer Jr O, Kučerová A, Pešta M. Association between endothelial NO synthase polymorphisms and arterial properties in the general population. *Nitric Oxide.* 2015; 44: 47–51.