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INFLUENCE OF THE PPARγ2 GENE POLYMORPHISM ON SOME METABOLIC INDICES IN PATIENTS WITH ESSENTIAL ARTERIAL HYPERTENSION ACCOMPANIED BY ISCHEMIC HEART DISEASE AND TYPE 2 DIABETES MELLITUS

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Abstract

Introduction. Currently, numerous investigations have been conducted to study the role of various genes in the development of arterial hypertension, but still they do not contain data concerning patients with comorbid pathology.
Objectives. To investigate the effect of Pro12Ala polymorphism of the PPARγ2 gene on metabolism rates in patients with essential arterial hypertension accompanied by stable ischemic heart disease and type 2 diabetes mellitus.
Methods. Indices of carbohydrate, lipid metabolism and oxidative-antioxidant protective activity in blood were studied in 50 patients with essential arterial hypertension accompanied by stable coronary heart disease (group I) and in 62 patients with essential hypertension accompanied by stable coronary heart disease and type 2 diabetes (group II).

Résumé

L'influence du polymorphisme du gène PPARγ2 sur certains indices métaboliques chez les patients avec de l'hypertension artérielle essentielle accompagnée d'une maladie cardiaque ischémique et de diabète sucré de type 2

Actualité. Actuellement, le rôle de divers gènes dans le développement de l'hypertension artérielle est activement étudié, mais pas assez chez les patients présentant une comorbidité.
Objectifs. Étudier l'effet du polymorphisme Pro12Ala du gène PPARγ2 sur les paramètres du métabolisme chez les patients souffrant d'hypertension essentielle accompagnée d'une cardiopathie ischémique stable et de diabète sucré de type 2.
Méthodes. Des indices de métabolisme des glucides, de métabolisme lipidique et d'activité

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Results. In the Ist group of patients Pro/Pro genotype (82%) predominated, and in the II-nd group of patients Pro/Pro genotype was found in 85.5%, Pro/Ala – in 14.5% of the patients.
Significant impairments ($p<0.05$) of carbohydrate, lipid metabolism and indices of oxidant-antioxidant homeostasis, with the exception of glutathione peroxidase and catalase in patients with Pro/Pro genotype, were detected in patients with essential hypertension accompanied by stable ischemic heart disease and type 2 diabetes with Pro/Pro and Pro/Ala genotypes of PPARγ2 gene compared to the controls.
Conclusions. The most pronounced disorders of carbohydrate, lipid metabolism and oxidant-antioxidant homeostasis indices are observed in patients with essential hypertension accompanied by stable ischemic heart disease and type 2 diabetes with the Pro/Pro genotype compared with the control and Pro/Ala genotype.

Key words: essential arterial hypertension, ischemic heart disease, type 2 diabetes mellitus, metabolism, PPARγ2 gene polymorphism.

oxydante-antioxydante dans le sang ont été étudiés chez 50 patients présentant une hypertension artérielle essentielle accompagnée d'une cardiopathie coronarienne stable (groupe 1) et chez 62 patients atteints d'hypertension essentielle accompagnée d'une maladie coronarienne stable et de diabète de type 2 (groupe 2).
Résultats. Le génotype Pro/Pro (82%) prédominait dans le premier groupe de patients et 18% des patients avait le génotype Pro/Ala. Dans le deuxième groupe de patients, le génotype Pro/Pro a été retrouvé à 85.5%, Pro/Ala – chez 14.5% des patients.
Des altérations significatives ($p<0.05$) des glucides, du métabolisme lipidique et des indices d'homéostasie oxydante-antioxydante, à l'exception de la glutathion peroxydase et de la catalase chez les patients avec le génotype Pro/Pro, ont été détectées chez des patients souffrant d'hypertension artérielle essentielle accompagnée de cardiopathie ischémique stable et de diabète de type 2 avec les génotypes Pro/Pro et Pro/Ala du gène PPARγ2 comparés au groupe témoin.
Conclusions. Les troubles les plus prononcés de métabolisme des glucides et des lipides et des indices de l'homéostasie oxydante-antioxydante sont observés chez les patients souffrant d'hypertension essentielle accompagnée d'une cardiopathie ischémique stable et d'un diabète de type 2 avec le génotype Pro/Pro par rapport au groupe témoin et génotype Pro/Ala.

Mots-clés: hypertension artérielle essentielle, cardiopathie ischémique, diabète sucré de type 2, métabolisme, polymorphisme du gène PPARγ2.

Introduction

Arterial hypertension (AH) is the most common cardiovascular disease in the world[1]. Today, medical specialists around the world state the fact of the dynamic progression of the relative importance of comorbid pathological conditions. Comorbid pathology in patients with AH, which is most often combined with chronic ischemic heart disease and type 2 diabetes mellitus (DM 2) is supposed to be particularly urgent[2]. The combination of AH and DM 2 is considered to be the most aggressive in the context of cardiovascular diseases and mortality associated with earlier development of affection of target organs and subsequent cardiovascular catastrophes[3]. Pathogenetic mechanisms that predetermine the development of AH, ischemic heart disease and DM 2, intersect in many respects and lead to the disease progression and complications development[4].

At present, numerous studies are conducted to study the role of various genes in the development of AH[5]. Much attention is paid to the study of polymorphisms of peroxisome proliferator-activated receptors (PPARα, PPARβ/σ, PPARγ) – transcription factors from the family of nuclear hormonal receptors[6,7].

It has been established that Pro/Pro genotype carriers of PPARγ2 gene have a more pronounced immunoreactivity and dyslipidemia, and these individuals have a higher risk of developing AH and DM 2 compared to carriers of the Ala/Ala genotype PPARγ2[8,9]. At the same time, a small amount of works is devoted to the study of the effect of this polymorphism on the comorbid pathology, therefore the study of the effect of Pro12Ala polymorphism of the PPARγ2 gene on the development of metabolic disorders in patients with AH accompanied by ischemic heart disease and DM 2 needs to be studied in detail.

The aim of the study

To investigate the effect of Pro12Ala polymorphism of PPARγ2 gene on carbohydrate, lipid metabolism, oxidant-antioxidant homeostasis indices in

patients with essential arterial hypertension (EAH) of stage II in combination with stable ischemic heart disease and DM 2.

Material and methods

The study involved 112 patients with EAH of stage II accompanied by IHD (stable angina of the II-III functional class) and DM 2 of moderate severity, subcompensated. The patients' age was from 42 to 73 years.

Exclusion criteria: uncontrolled AH (malignant), type 1 diabetes, cardiac failure of the III-IV functional class according to NYHA classification, infectious diseases, oncological diseases, bronchial asthma, chronic obstructive pulmonary disease, sub- and decompensated liver, kidney diseases, mental illness.

The control group consisted of 26 practically healthy persons, representative by age and sex.

The patients underwent clinical, laboratory, instrumental, genetic examinations. Blood for a biochemical study was taken from the elbow vein in the morning on an empty stomach 12 hours after the last meal.

Carbohydrate metabolism disorders diagnosis was carried out on the basis of determining the glucose rate in the blood serum on an empty stomach. Blood glucose level determination on an empty stomach was performed using a set of test systems (BIO-LA-TEST, Erba Lachema, Czech Republic).

The insulin level in blood on an empty stomach was determined using standard Monobind Inc. sets (USA) by immunoassay analysis method (ELISA). Normal values of insulin concentrations on an empty stomach were considered up to 25 μU/ml for men and up to 23 μU/ml for women[10].

To assess the degree of insulin resistance a small Homeostasis Model Assessment (HOMA) was used determining the HOMA-IR index (D. Matthews et al, 1985), which was calculated using the formula: Blood Insulin on an empty stomach (μU/ml) x Blood Plasma Glucose on an empty stomach (mmol/l) / 22.5. Insulin resistance was verified at the HOMA-IR index value above 2.77 μU/ml x mmol/L.

The state of lipid metabolism was studied by determining the total cholesterol, high-density lipoprotein cholesterol, and triacylglycerol using standard diagnosis sets of the Felisit-Diagnostics Company. The level of low-density lipoprotein cholesterol was estimated by W. Friedewald formula.

The content of malonic aldehyde in plasma and erythrocytes and the activity of reduced glutathione, glutathione peroxidase, catalase in plasma were determined.

To determine the alleles of the polymorphic site (Pro12Ala) of the PPARγ2 gene (rs 1801282), the polymerase chain reaction method was used[11]. The studied areas of the genes were amplified using specific primers ("Metabion", Germany). Specific fragments of PPARɣ (Pro12Ala) genes were amplified using the commercial 5x FIREPol® Master Mix Ready to Load (7.5 mM $MgCl_2$) set ("Solis BioDyne" Company, Estonia).

Genetic studies were conducted at the State Institution „Reference Center for Molecular Diagnostics of the Ministry of Health of Ukraine".

The statistical processing of the results of the study was carried out by determining the mean arithmetic values (M) and standard error (m). The verification of the distribution of samples to normality was carried out according to the Shapiro-Wilk test. The significance of changes in the case of normal distribution in the samples was determined according to the Student's test; in other cases the Wilcoxon criterion was used. The difference between the samples was considered statistically significant at $p<0.05$.

Results

In the given study, the genotypes of PPARγ2 were distributed as follows: in the group of patients with EAH of the IInd stage accompanied by ischemic heart disease (50 persons) Pro/Pro genotype (82%) predominated, 18% of the people had Pro/Ala genotype. In 62 patients with EAH of the IInd stage accompanied by ischemic heart disease and DM 2 the following distribution of genotypes was noted: Pro/Pro – 53 (85.5%), Pro/Ala – 9 (14.5%).

The status of metabolic rate in the blood of patients with EAH of the IInd stage accompanied by ischemic heart disease depending on the polymorphism of the PPARγ2 gene is presented in Table 1.

Study of metabolic rates in the examined patients with EAH of the IInd stage accompanied by ischemic heart disease, the pro-allele PPARγ2 gene (Pro/Pro) homozygous carriers showed significant ($p<0.05$) higher carbohydrate, lipid metabolism rates, lipid peroxidation and antioxidant protection, with the exception of glutathione peroxidase. Carbohydrate metabolism rates and high density lipoprotein cholesterol content in heterozygous Pro/Ala alleles of the PPARγ2 gene carriers, unlike the Pro/Pro genotype did not significantly differ ($p>0.05$) from those in healthy individuals. Homozygous Pro-allele PPARγ2 (Pro/Pro) gene carriers had significantly ($p<0.05$) higher levels of glucose on an empty stomach, immunoreactive insulin, HOMA-IR index, total cholesterol and triacylglycerols compared to those in heterozygous patients with Pro/Ala allele of PPARγ2 gene (Table 1). There was

Table 1. The state of some metabolic indices in the blood of patients with EAH of the II[nd] stage accompanied by ischemic heart disease (n=50) depending on the Pro12Ala polymorphism genotype of the PPARγ2 gene

Indices	*Healthy individuals (n=26)*	*Patients (n=50)*	
		Pro/Pro (n=41; 82%)	*Pro/Ala (n=9; 18%)*
Glucose on an empty stomach, mmol/l	4.56±0.07	5.34±0.19*	4.76±0.16**
Immunoreactive insulin on an empty stomach, μU/ml	11.06±1.14	19.72±1.34*	14.55±1.56**
HOMA-IR index	2.37±0.23	4.54±0.31*	2.98±0.37**
Total cholesterol, mmol/l	4.09±0.23	6.19±0.28*	5.52±0.26*/**
Triacylglycerol, mmol/l	1.14±0.07	1.98±0.13*	1.53±0.17*/**
High density lipoprotein cholesterol, mmol/l	1.39±0.03	1.03±0.04*	1.28±0.14
Low density lipoprotein cholesterol, mmol/l	2.48±0.08	4.12±0.28*	3.98±0.32*
Malonic aldehyde in plasma, mmol/l	2.49±0.26	6.25±0.33*	5.87±0.56*
Malonic aldehyde in erythrocytes, mmol/l	6.69±0.37	8.99±0.34*	7.77±0.28*/**
Reduced glutathione, mmol/l	0.86±0.04	0.59±0.05*	0.66±0.04*
Glutathione peroxidase, nmol GSH/min per g Hb	184.56±8.86	204.49±8.03	218.55±8.32*
Catalase, mmol/min per g Hb	16.84±0.76	20.98±1.52*	26.67±1.87*/**

Note: * – p<0.05 compared to control,
** – p<0.05 comparison between groups with different polymorphisms

also a tendency to an increase in the concentration of anti-atherogenic high density lipoprotein cholesterol and a decrease in low density lipoprotein cholesterol in patients with Pro/Ala allele of the PPAR-γ2 gene compared to Pro/Pro allele.

Comparison of the lipid peroxidation and antioxidant protection values of the examined patients, depending on the polymorphism of the PPARγ2 gene, demonstrated significant changes of the malonic aldehyde in erythrocytes and catalase (Table 1). Therefore, the malonic aldehyde content in erythrocytes in patients with EAH of the IInd stage accompanied by ischemic heart disease with Pro/Pro genotype is 14% higher than in patients with Pro/Ala genotype. The catalase activity, which is included in the system of antioxidant enzymes, in patients with Pro/Pro genotype is 19% lower than in patients with Pro/Ala genotype. There was no statistically significant influence of alleles on other indices of oxidant-antioxidant homeostasis.

The status of metabolic rate in the blood of patients with EAH of the IInd stage accompanied by ischemic heart disease and DM 2 depending on the polymorphism of the PPARγ2 gene is presented in Table 2.

In patients with EAH of the IInd stage accompanied by ischemic heart disease and DM 2, with genotypes Pro/Pro and Pro/Ala of the PPARγ2 gene all metabolic rates with the exception of glutathione peroxidase and catalase differed from those in healthy individuals (Table 2). As in the previous group of the examined (see Table 1), more significant metabolic disturbances were detected in patients with Pro/Pro genotype compared to those with Pro/Ala genotype. They had significant higher (p<0.05) levels of glucose on an empty stomach, immunoreactive insulin, HOMA-IR index, triacylglycerols. Total cholesterol concentration was also higher in patients with Pro/Pro genotype, but the difference is statistically insignificant. In addition, the genetic polymorphism of PPARγ2 influenced the activity of the antioxidant protection system, as evidenced by the significantly higher levels of erythrocyte malonic aldehyde (p<0.05) and lower levels of glutathione peroxidase and catalase (p<0.05) in Pro/Pro genotype compared to Pro/Ala one.

According to the sources[12-14], Ala allele is considered to be protective against the development of type 2 diabetes. The polymorphism Pro12Ala (amino acid replacement Pro> Ala in position 12), which moderately reduces the function of this receptor, is an indicator of a decrease in the risk of developing type 2 diabetes mellitus, hyperinsulinemia, insulin resistance and atherosclerosis[15].

More pronounced disturbances of carbohydrate and lipid metabolism in patients with EAH accompanied by DM 2 and genotype Pro/Pro compared with the genotype Pro/Ala are noted by Shalimova A.S. (2015)[16], which is consistent with the results of our study.

Conclusions

1. Patients with EAH of the IInd stage accompanied by ischemic heart disease, heterozygous carriers of Pro/Ala allele of the PPARγ2 gene compared to

Table 2. The state of some metabolic indices in the blood of patients with EAH of the II[nd] stage accompanied by ischemic heart disease and DM 2 (n=62) depending on the Pro12Ala polymorphism genotype of the PPARγ2 gene

Indices	Healthy individuals (n=26)	Patients (n=62)	
		Pro/Pro (n=53; 85.5%)	Pro/Ala (n=9; 14.5%)
Glucose on an empty stomach, mmol/l	4.56±0.07	8.84±0.44*	7.56±0.32*/**
Immunoreactive insulin on an empty stomach, μU/ml	11.06±1.14	32.41±2.03*	25.29±1.73*/**
HOMA-IR index	2.37±0.23	13.02±1.39*	8.55±1.45*/**
Total cholesterol, mmol/l	4.09±0.23	6.3±0.31*	5.88±0.39*
Triacylglycerol, mmol/l	1.14±0.07	2.29±0.21*	1.86±0.18*/**
High density lipoprotein cholesterol, mmol/l	1.39±0.03	0.75±0.06*	0.89±0.07*
Low density lipoprotein cholesterol, mmol/l	2.48±0.08	4.36±0.19*	4.18±0.22*
Malonic aldehyde in plasma, mmol/l	2.49±0.26	6.47±0.41*	6.06±0.37*
Malonic aldehyde in erythrocytes, mmol/l	6.69±0.37	9.82±0.46*	8.57±0.32*/**
Reduced glutathione, mmol/l	0.86±0.04	0.54±0.04*	0.57±0.04*
Glutathione peroxidase, nmol GSH/min per g hgb	184.56±8.86	195.33±5.98	212.55±6.82*/**
Catalase, mmol/min per g Hb	16.84±0.76	18.66±1.44	22.48±1.27*/**

Note: * – p<0.05 compared to control,
** – p<0.05 comparison between groups with different polymorphisms

the control showed a significant (p<0.05) increase in the concentration of total cholesterol, triacylglycerol, low density lipoprotein cholesterol, plasma and erythrocytes malonic aldehyde, glutathione peroxidase and catalase, a decrease in high density lipoprotein cholesterol content, and plasma reduced glutathione at almost normal carbohydrate metabolism rates. Significant disturbances of the carbohydrate metabolism along with this were detected in patients – homozygous carriers of Pro/Pro allele of the PPARγ2 gene.

2. Patients with EAH of the II[nd] stage accompanied by ischemic heart disease and DM 2, with genotypes Pro/Pro and Pro/Ala of the PPARγ2 gene compared to control had significant higher (p<0.05) concentration of glucose on an empty stomach, immunoreactive insulin, HOMA-IR index, triacylglycerol, low density lipoprotein cholesterol, plasma and erythrocytes malonic aldehyde, a decrease in high density lipoprotein cholesterol content, and plasma reduced glutathione. In this case, in patients with Pro/Ala genotype of PPARγ2 gene, activation of antioxidant protection was noted: a significant increase in glutathione peroxidase and catalase activity compared with the control.

3. The most pronounced disturbances of carbohydrate and lipid metabolism and oxidant-antioxidant homeostasis indices in patients with EAH accompanied by ischemic heart disease and DM 2 were observed in patients with genotype Pro/Pro genotype compared to Pro/Ala one (p <0,05).

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article"

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study"

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