ENGLISH VERSION: THE TLR4 299GLY POLYMORPHIC ALLELE IS ASSOCIATED WITH REDUCED EFFICACY OF AZITHROMYCIN AND PIOGLITAZONE, INCLUDED IN THE COMPREHENSIVE TREATMENT OF PATIENTS WITH CORONARY HEART DISEASE*

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The Asp299Gly polymorphism of the TLR4 gene plays an important role in the pathogenesis of coronary heart disease (CHD). The purpose of the work is to conduct a comparative analysis of the clinical efficacy of azithromycin and pioglitazone against the background of complex therapy taking into account the Asp299Gly polymorphism of the TLR4 gene in patients with CHD. Materials and methods. The study included 40 people with CHD in the age group 45-68 years, divided into 2 groups of observation: the first group (n = 20) received, together with the integrated therapy, azithromycin at a dose of 500 mg/day for 3 days, then 500 mg/week for 6 months, the second group (n = 20) - together with the combined therapy pioglitazone in a dose of 15 mg once daily, in the morning for 6 months. Duration of observation 12 months. Results. Each observation group was divided into two subgroups: the Asp/Asp genotype and the combined genotype Asp/Gly +Gly/Gly. The first observation group with the Asp/Asp genotype was 14 (70%), while the combined genotype Asp/Gly +Gly/Gly was 6 (30%), the second observation group was 17 (85%) and 3 (15%), respectively. The acceptance of azithromycin from Asp (Asp/Asp) allele carriers, led to a more effective reduction in the size of the ACS, the diameter of the stenosis and TSC, partial or complete eradication of the parodontopathogenic microflora in the oral cavity. The use of pioglitazone in carriers of the Asp allele (Asp/Asp) caused a more pronounced decrease in the diameter of the stenosis, moderate anti-inflammatory and pronounced hypoglycemic, hypolipidemic effects. Conclusions The data obtained by us showed that in patients with CHD, carriers of Asp allele, a high susceptibility to treatment was detected, and the presence of the Gly mutant allele of the TLR-4 gene was associated with a reduced therapeutic efficacy of the investigational drugs, against the background of a standard course of treatment, which indicates the pharmacogenetic peculiarity of susceptibility to the action of the investigational drugs and should be taken into account in clinical practice.

Key words: TLR4 299Gly polymorphism, azithromycin, pioglitazone, coronary heart disease

The leading positions in the pathogenesis of coronary heart disease (CHD) and atherosclerosis belong to chronic inflammation with the involvement of the innate link of immune and inflammatory processes, in particular the pattern-recognizing receptors of the Toll-like family type 4 (TLR4). TLR4 has a high level of expression in atherosclerotic plaques (ASPs) and is involved in the protection against bacterial infection, including periodontal pathogenic microflora, which acts as an independent risk factor for the formation and progression of atherosclerosis and coronary artery disease [7].

The polymorphism of TLR4 gene, namely, Asp299Gly (rs4986790), is a single nucleic acid substitution of adenosine (A) for guanine (G) at the position of +896 of exon 3, whose frequency in the human population is slightly more than 5% [5, 8]. As a result of the amino acid replacement of aspartic acid for glycine at the position of 299 of the receptor chain, the extracellular domain of TLR4 is changed, which leads to a decrease in the recognition of the corresponding ligands or intracellular signals with less pronounced activation of the immune cells, and vice versa, with the introduction of pathogens, the formation of different nature of the course of inflammatory response and specific immune responses, reduced proinflammatory cytokine production and the risk of atherosclerosis.

In previous studies, we have shown that the Asp299Gly polymorphism of TLR4 gene plays an important role in the pathogenesis of atherosclerosis and is associated with the persistence of parodontopathogenic microflora in the inflammation site – ASP in CHD [2, 3]. Carriers of the Gly allele are more susceptible to increased microbial contamination of coronary artery (CA) tissue and have a high risk of developing coronary heart disease than those with the Asp allele [12]. However, there are controversial evidence that in the individuals with the Asp299Gly polymorphism of TLR4 gene, the inflammatory reaction in the artery wall develops less intensively, ASP is formed slower, and a lower predisposition to the risk of atherosclerosis is manifested [15]. However, therapeutic strategies for treating coronary heart disease, which would take into account the role of the Asp299Gly polymorphism of TLR4 gene in the pathogenesis of CHD, are still not investigated, which represents the relevant direction of modern cardiology.

The aim of this research is to conduct a comparative analysis of the clinical efficacy of azithromycin and pioglitazone against the background of comprehensive therapy taking into account the Asp299Gly polymorphism of TLR4 gene in patients with coronary heart disease.

Materials and methods

The study included 40 people aged 45-68 who suffered from coronary heart disease. The research was conducted in the period from 2012 to 2015 on the basis of the 1st City Clinical Hospital of Poltava and the Research Institute of Genetic and Immunological Foundations of the Development of Pathology and Pharmacogenetics (Higher State Educational Establishment of Ukraine «Ukrainian Medical Stomatological Academy»). Before the study, all participants signed the informed consent forms and the approval from the Bioethics Commission of Ukrainian Medical Stomatological Academy was obtained.

Criteria for inclusion in the study were as follows: stable angina pectoris, type 2 diabetes mellitus (DM), with-

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out injecting hypoglycemic agents. Exclusion criteria: presence of the history of myocardial infarction (MI), surgical intervention, malignant arterial hypertension (AH), chronic heart failure (CHF) of III-IV functional classes (FC), systemic connective tissue diseases, oncological and oncohematological diseases, severe infectious diseases, chronic inflammatory diseases, which require regular antibiotic therapy, presence in history of acute cerebrovascular accident, heart rhythm disturbances by type of auricular fibrillation. The ultimate primary points of the study are: deaths due to MI, revascularization coronary procedures (coronary artery bypass grafting or percutaneous coronary intervention) or hospitalization due to angina pectoris.

The diagnosis of coronary heart disease was confirmed in patients with angina pectoris I-III FC according to the classification of the Canadian Association of Cardiologists and Circulatory Disorders in accordance with the New York Cardiology Classification (NYHA) classification.

Before inclusion in the clinical study, all patients received a common treatment and passed a screening test for the verification of diagnoses of coronary artery disease, type 2 diabetes and inflammatory diseases of dentoalveolar apparatus. After screening, 40 patients were selected who received the standard drug therapy: isosorbide dinitrate 10-20 mg 2 times a day, acetylsalicylic acid 75 mg 1 time nocte, bisoprolol 2.5 mg 1 time per day, rosuvastatin 20 mg 1 once a day, ramipril 5 mg once a day mane. Also, the patients received recommendations about the diet, lifestyle changes and individual oral hygiene. Patients received the common treatment for at least a month until stable parameters were achieved.

In the randomized distribution of patients with coronary heart disease, 2 study groups were formed. Patients in the first study group (n = 20) received, along with the combination therapy, the macrolide group medication – azithromycin (AZV, Tulip Lab Labiwi Limitid, India) at a dose of 500 mg / day for 3 days, then 500 mg / week for 6 months Patients in the second study group (n = 20) received, along with the comprehensive therapy, the thiazolidinedionic group medication – pioglitazone (Piogalar, Ranbaksi, India) – at a dose of 15 mg once daily, mane, for 6 months. After 6 months of treatment, patients in both groups continued to receive only medications of the standard complex of therapy for the next 6 months. The duration of observation in general was 12 months. Monitoring over the effectiveness of treatment was performed at 6 and 12 months.

The examination included collecting anamnestic and objective data, evaluating anthropometric indices, measuring blood pressure (BP), systolic and diastolic blood pressure (SBP and DBP), electrocardiogram (ECG) and bicycle ergometry (BEM). BEM was conducted on Veloergottest 05 (Ukraine) cycling machine according to the method of stepped increasing load with further increase of power every 3 minutes with control of ECG and BP. Criteria for termination of BEM were: generally accepted clinical or ECG signs of myocardial ischemia. Heart rate (HR) parameters of patients were recorded. Consultation of the dentist included a check-up, instrumental examination, verification of diagnoses and taking dentogingival fluid from the periodontal pockets.

The bloodstream condition was determined in all patients by ultrasound examination (US) of the neck vessels by the apparatus «ULTIMA PA», with L5 sensor – 12/40 according to standard protocols in the B mode on the three levels of the vasculature and bilaterally at the end of the diastole: in proximal, medial and distal points at a distance of 1 cm from the bifurcation along the back wall of the right and left common carotid artery (RCCA and LCCA, respectively) as more distant from the sensor. The contour of carotid arteries, their internal lumina were recorded, and indicators of the presence of signs of ASP (size, localization) were registered. The intima-media thickness (IMT) of the carotid arteries was defined as the distance between the first and second echogenic lines of the locative area by the method of P. Pignoli and coauthors. IMT less than 0.9 mm was considered as normal; IMT of the common carotid artery > 1.4 mm was regarded as ASP, and within 1.0-1.3 mm – as thickening of IMT (ESH, ESC, 2007).

Determining the DNA of the representatives of parodontopathogenic microflora of dentogingival fluid of periodontal pockets and the alleles of the Asp299Gly polymorphous site of TLR4 in patients with CHD was performed as previously described [2].

The scope of laboratory studies included complete blood count biochemical blood tests. The determination of the inflammatory response was carried out using the main biomarkers – high-sensitivity C-reactive protein (hs-CRP, «DRG», USA) and tissue inhibitor of metalloprotease-1 human (TIMP-1, «eBioscience», Austria) by the immunoenzyme method. The condition of carbohydrate metabolism was determined by the glycemic profile of glucose: fasted and 1 hour after receiving 75 g glucose (oral glucose tolerance test, OGTT) by glucose oxidase method; the hyperglycemic coefficient was calculated. Lipid spectrum was determined by the indices of total cholesterol (CH), triglycerides (TG) and high density lipoproteins cholesterol (HDL) («Diakon-DS», Russia), the cholesterol content was calculated as a part of low density lipoproteins (LDL cholesterol), cholesterol content in very low density lipoproteins (VLDL), the atherogenicity factor (AF) was calculated.

Statistical processing was performed using STATISTICA 6.0 (StatSoft, USA), calculating the average (M) and standard error (m). The reliability was determined using the Student's t-test, Fischer's tests and $\chi 2$. Differences between the groups were considered statistically significant at $p < 0.05$.

Results and discussion

We conducted the comparative assessment of clinical efficacy and safety of inclusion of a medication from the macrolides group and a medication from the thiazolidinediones group in the comprehensive therapy of patients with coronary heart disease, which can serve as an important criterion in making a reasonable decision as to the choice of treatment. The reason for using, for the purpose of correction, the selected medications from the antibiotics group – azithromycin, and from the insulin sensitizers group – pioglitazone, was us earlier changes in the spectrum of parodontopathogenic microflora in ASP as well as in the withdrawn content of the periodontal pocket of the oral cavity, the presence of endothelial dysfunction (ED), systemic inflammation and disturbance of carbohydrate metabolism in patients with coronary heart disease [3, 13].

Each study group, depending on Asp299Gly genotype of TLR-4 gene was divided into two subgroups: the Asp / Asp genotype and the combined genotype Asp/Gly + Gly/Gly. 14 patients (70%) were included in the first study group with the Asp / Asp genotype, and 6 (30%)

with the combined Asp / Gly + Gly / Gly genotype, whereas the second study group included 17 patients (85%) and 3 persons (15%), respectively.

Determination of the effect of azithromycin and pioglitazone against the background of standard therapy on the incidence of atherosclerotic processes in RCCA, LCCA and the external carotid artery (ECA) in CHD is presented in Table 1. It was found that in patients who are carriers of Asp (Asp / Asp) allele under the influence of azithromycin after 12 months, there is a significant decrease in the IMT in RCCA and LCCA as compared with the initial values (p = 0.04; p = 0.01, respectively).

Table 1

Ultrasonography parameters of the neck vessels in patients with coronary heart disease in the first and second study groups

Parameters	Prior to treatment	In 6 months	In 12 months	p_1	p_2	p_3	Prior to treatment	In 6 months	In 12 months	p_4	p_5	p_6
The first study group												
Genotype	Ala/Ala (n=14)						Ala/Gly + Gly/Gly (n=6)					
Right common carotid artery: thickness of ASP, mm	1.43±0.45	0.88±0.21	0.77±0.2	0.1	0.08	0.2	0.77±0.31	0.48±0.19	0.33±0.23	0.07	0.03	0.2
IMT	1.08±0.06	1.04±0.04	1.01±0.04	0.3	0.04	0.1	1.05±0.06	1.0±0.06	0.95±0.03	0.07	0.04	0.2
Diameter of stenosis: 2 – 15 % 16-49%	 3 6	 3 6	 3 5	 1.0 1.0	 1.0 0.43	 1.0 0.43	 0 3	 2 1	 1 1	 0.45 0.54	 1.0 0.54	 1.0 1.0
Left common carotid artery: thickness of ASP, mm	0.74±0.27	0.59±0.19	0.39±0.15	0.4	0.1	0.06	0.27±0.27	0.22±0.22	0.15±0.15	0.4	0.4	0.4
TKIM	1.04±0.03	1.01±0.02	0.98±0.03	0.1	0.01	0.1	1.05±0.04	0.98±0.03	0.95±0.02	0.02	0.07	0.4
Diameter of stenosis: 2 – 15 % 16-49%	 2 4	 1 6	 3 2	 1.0 0.2	 1.0 0.65	 0.6 0.21	 1 1	 0 1	 0 1	 1.0 1.0	 1.0 1.0	 - 1.0
External carotid artery thickness of ASP, mm	0.21±0.21	0.07±0.07	0.07±0.07	0.3	0.3	-	0.18±0.13	0.05±0.05	0	0.2	0.2	0.4
The second study group												
Genotype	Ala/Ala (n=17)						Ala/Gly + Gly/Gly (n=3)					
Right common carotid artery: thickness of ASP, mm	0.92±0.53	0.3±0.14	0.94±0.76	0.2	0.99	0.4	1.07±0.67	0.73±0.5	0.87±0.49	0.2	0.4	0.4
IMT	1.06±0.04	1.03±0.03	1.0±0.02	0.6	0.2	0.2	1.23±0.09±	1.16±0.03 ±	1.1±0.06±	0.4	0.2	0.4
Diameter of stenosis: 2 – 15 % 16-49%	 1 5	 0 4	 2 2	 1.0 1.0	 1.0 0.4	 0.5 0.65	 1 1	 1 1	 1 1			
Left common carotid artery: thickness of ASP, mm	0.72±0.2	0.56±0.19	0.3±0.14	0.3	0.03	0.02	0.4±0.4	0.4±0.4	0.37±0.37	--	0.2	0.4
TKIM	1.07±0.04	1.04±0.03	1.0±0.02	0.4	0.054	0.02	1.23±0.09	1.16±0.03	1.1±0.06	0.4	0.2	0.4
Diameter of stenosis: 2 – 15 % 16-49%	 0 8	 5 3	 2 2	 0.04 0.14	 0.5 0.057	 0.4 1.0	 - -	 - -	 - -			
External carotid artery thickness of ASP, mm	0.24±0.11	0.17±0.09	0.09±0.06	0.3	0.07	0.1	0.5±0.5	0.5±0.5	0.33±0.33	--	0.4	0.4

Note (here and in Tables 2-3): P_1 – the comparison in the first study group prior to the beginning and after 6 months of treatment with azithromycin;

P_2– the comparison in the first study group before and after 12 months of treatment with azithromycin;

P_3– the comparison in the first study group after 6 months and after 12 months of treatment with azithromycin;

P_4– the comparisons in the second study group before and after 6 months of treatment with pioglitazone;

P_5– the comparison in the second study group before and after 12 months of treatment with pioglitazone ;

P_6 – the comparison in the second study group after 6 months and after 12 months of treatment with pioglitazone

Patients who are carriers of Gly (Asp / Gly + Gly / Gly) allele under the influence of azithromycin after 6 months showed a significant decrease in IMT in LCCA as compared with initial indicators (p = 0.02); after 12 months, there is a reliable decrease in ASP and IMT in RCCA as compared initial indicators (p = 0.03; p = 0.04, respectively).

The patients who are carriers of Asp (Asp / Asp) allele, under the influence of pioglitazone after 12 months there is a reliable decrease in the size of ASP and the diameter of the stenosis in LCCA as compared with the initial values (p = 0.03; p = 0.04), as well in 12 months, a reliable decrease in the size of the ASP, the diameter of the stenosis and IMT in the LCCA is observed in comparison with the indicators after 6 months (p = 0.02; p=0.057; p = 0.02, respectively).

Patients who are carriers of Gly allele (Asp/Gly + Gly/Gly) under the influence of pioglitazone did not display any significant changes of the parameters in the treatment dynamics after 6 and 12 months.

Thus, intake of azithromycin and pioglitazone against the background of standard therapy during 12 months caused more effective inhibition of atherosclerotic processes in the carotid vessels of the neck in carriers of Asp (Asp / Asp), Asp / Gly (Asp / Gly + Gly / Gly) allele, by reducing the size of the ASP, stenosis diameter and IMT. However, azithromycin most strongly influenced the reduction of ASP thickness, while pioglitazone reduced the diameter of stenosis in carotid arteries in Asp (Asp / Asp) allele carriers, which may indicate a more pronounced effect of the first medication in the early stages of atherosclerosis, whereas the second drug – at somewhat later stages.

In determining the spectrum of parodontopathogenic microflora in the withdrawn content of periodontal pockets, after azithromycin administration in patients (Table 2), carriers of Asp (Asp / Asp) allele and Gly (Asp / Gly + Gly / Gly) allele in 6 months, a tendency to decrease in the frequency of detection of almost all representatives, with the exception of *Treponema denticola*, was observed. However, in the first study group after 12 month, only two patients who are carriers of Gly allele (Asp / Gly + Gly / Gly) displayed a twofold increase in the percentage contents of *Porphyromonas gingivalis* from 33.3% to 66.7% (p = 0.3).

Table 2

The frequency dynamics of indicators of parodontopathogenic microflora in the contents extracted from periodontal pockets of patients with ischemic heart disease in the first and second study groups

Parameters	Prior to treatment	In 6 months	In 12 months	p_1	p_2	p_3	Prior to treatment	In 6 months	In 12 months	p_4	p_5	p_6
The first study group												
Genotype	Ala/Ala (n=14)						Ala/Gly + Gly/Gly (n=6)					
Porphyromonas gingivalis	5 / 35.7%	3 / 21.4%	4 / 28.6%	0.34	0.5	0.5	5 / 83.3 %	2 /33.3%	4 / 66.7%	0.12	0.5	0.3
Actinobacillus actinomycetemcomitans	2 / 14.3 %	0	0	0.2	0.2	-	1 / 16.7 %	0	0	0.5	0.5	-
Prevotella intermedia	1 / 7.14 %	0	0	0.5	0.5	-	-	-	-			
Bacteroides forsythus	1 / 7.14 %	0	0	0.5	0.5	-	-	-	-			
Treponema denticola	-	-	-	-	-	-	-	-	-			
The second study group												
Genotype	Ala/Ala (n=17)						Ala/Gly + Gly/Gly (n=3)					
Porphyromonas gingivalis	5 / 29.4%	5 / 29.4%	3 / 17.6%	0.65	0.34	0.34	1 / 33.3%	1 / 33.3%	3 / 100.0%	0.8	0.2	0.2
Actinobacillus actinomycetemcomitans	-	-	-				-	-	-			
Prevotella intermedia	-	-	-				-	-	-			
Bacteroides forsythus	-	-	-				-	-	-			
Treponema denticola	-	-	-				-	-	-			

After 6 weeks of taking pioglitazone, of Asp (Asp / Asp) alleles and Gly (Asp / Gly + Gly / Gly) alleles did not display changes in the presence frequency of periodontal pathogenic microflora in the contents of periodontal pockets. However, in the second study group in 12 months, only in carriers of Gly allele (Asp / Gly + Gly / Gly), 3% increase in the percentage contents of *Porphyromonas gingivalis* was observed from 33.3% to 100.0% (p = 0.2).

Consequently, only azithromycin, against the background of standard therapy for 6 months, exhibits an antibacterial effect predominantly in patients with coronary heart disease, Asp (Asp / Asp) allele carriers, unlike Gly (Asp / Gly + Gly / Gly) allele carriers, due to partial or complete eradication of parodontopathogenic microflora in the oral cavity.

The analysis of inflammation markers, carbohydrate and lipid metabolism (Table 3) showed that after 6 months, in patients with Asp allele (Asp / Asp) under the influence of azithromycin, there was a reliable decrease in cholesterol and HDL as compared to initial indicators (p = 0.04; p = 0.04, respectively), after 12 months there is a reliable decrease in the level of ESR, glucose and LDL in comparison with initial indicators (p = 0.04; p = 0.02; p=0.04, respectively), as well as in 12 months there is a reliable decrease in the level of ESR and CA, increase in the level of HDL as compared with the indicators in 6 months (p = 0.04; p = 0.0003; p = 0.003, respectively).

Table 3

Parameters of laboratory examination depending on the polymorphism of TLR4 gene in the treatment of patients with coronary heart disease in the first and second study groups

Parameters	Prior to treatment	In 6 months	In 12 months	p_1	p_2	p_3	Prior to treatment	In 6 months	In 12 months	p_4	p_5	p_6

The first study group												
Genotype	Ala/Ala (n=14)						Ala/Gly + Gly/Gly (n=6)					
ESR, *mm / h*	16.14±4.34	9.07±1.02	7.57±0.86	0.08	0.04	0.04	14.0±5.01	9.33±1.2	6.83±0.8	0.28	0.18	0.053
hs-C-reactive protein, *mg / l*	5.33±1.69	3.26±0.85	6.57±1.53	0.27	0.58	0.09	5.22±2.58	3.65±0.85	4.27±1.6	0.58	0.77	0.5
TIMP-1, *pg / ml*	1713.8±115.3	1926.5±116.7		0.26			1559.9±190.4	2060±234.1		0.006		
Blood glucose, *mmol / l*	6.42±0.6	5.65±0.3	5.4±0.34	0.17	0.02	0.35	6.59±1.04	5.42±0.23	5.25±0.19	0.33	0.25	0.58
Oral glucose tolerance test, *mmol / l*	9.91±1.33	8.73±0.47		0.27			9.32±0.64	8.59±0.37		0.21		
Hyperglycemic coefficient	4.89±0.29	4.14±0.18	4.4±0.18	0.04	0.13	0.31	4.57±0.38	4.25±0.19	4.1±0.15	0.46	0.36	0.5
Cholesterol, *mmol / l*	0.82±0.14	0.79±0.12	0.89±0.09	0.62	0.49	0.27	0.63±0.06	0.65±0.07	0.77±0.09	0.8	0.06	0.3
Triglycerides, *mmol / l*	1.05±0.07	0.88±0.09	1.11±0.07	0.04	0.26	0.0003	0.8±0.07	0.99±0.12	1.09±0.2	0.16	0.11	0.46
HDL, *mmol / l*	3.52±0.28	2.9±0.2	2.9±0.16	0.07	0.04	0.9	3.49±0.39	2.96±0.18	2.66±0.16	0.13	0.08	0.21
LDL, *mmol / l*	0.35±0.06	0.36±0.05	0.41±0.05	0.92	0.27	0.27	0.27±0.03	0.29±0.03	0.35±0.04	0.55	0.02	0.3
VLDL, *mmol / l*	4.09±0.67	4.23±0.47	3.17±0.32	0.78	0.06	0.003	5.02±0.92	3.49±0.36	3.3±0.67	0.08	0.04	0.8
The second study group												
Leucocytes, *x 10^9/l*	6.46±0.3	6.56±0.3	6.6±0.2	0.7	0.6	0.9	6.27±0.77	7.45±0.35	5.08±0.22	0.35	0.35	0.03
ESR, *mm / h*	16.65±2.9	11.06±0.8	9.65±0.9	0.03	0.01	0.04	8.67±4.25	8.67±1.33	6.67±1.67	1	0.5	0.32
hs-C-reactive protein, *mg / l*	3.41±0.61	3.99±0.74	7.74±1.63	0.55	0.01	0.04	11.77±4.9	4.6±2.07	6.9±5.4	0.22	0.4	0.7
TIMP-1, *pg / ml*	1447±118.9	1549.2±97.4		0.5			1217±229.03	1920±203.3		0.007		
Blood glucose, *mmol / l*	6.02±0.15	6.06±0.26	5.49±0.25	0.91	0.09	0.07	5.9±0.03	5.5±0.5	5.03±0.25	0.49	0.09	0.6
Oral glucose tolerance test, *mmol / l*	12.45±0.69	9.52±0.53		0.0001			10.9±1.44	7.99±0.4		0.25		
Hyperglycemic coefficient	2.07±0.11	1.59±0.08		0.002			1.84±0.25	1.47±0.11		0.21		
Cholesterol, *mmol / l*	5.21±0.19	4.48±0.17	4.6±0.2	0.006	0.06	0.6	5.63±0.47	4.97±0.52	4.5±0.4	0.57	0.006	0.7
Triglycerides, *mmol / l*	0.72±0.09	0.72±0.05	0.92±0.09	1.0	0.12	0.02	0.53±0.09	0.7±0.06	0.87±0.09	0.04	0.06	0.13
HDL, *mmol / l*	0.86±0.06	1.06±0.04	1.12±0.07	0.01	0.002	0.4	0.72±0.06	0.85±0.1	1.03±0.1	0.52	0.18	0.16
LDL, *mmol / l*	4.03±0.2	3.1±0.16	3.06±0.17	0.001	0.004	0.87	4.69±0.4	3.8±0.4	3.07±0.5	0.4	0.009	0.5
VLDL, *mmol / l*	0.32±0.04	0.33±0.02	0.42±0.04	0.79	0.08	0.02	0.23±0.04	0.32±0.03	0.39±0.04	0.03	0.06	0.13
Atherogenicity coefficient	5.42±0.5	3.39±0.2	3.29±0.2	0.001	0.0006	0.77	6.83±0.32	4.94±0.48	3.5±0.74	0.14	0.051	0.26

In carriers of Gly (Asp / Gly + Gly / Gly) allele, under the influence of azithromycin after 6 months, a significant increase in TIMP-1 (p = 0.006) was observed; after 12 months, a significant decrease in CA (p = 0.04), increase in VLDL (p = 0.02) as compared to initial indicators, were observed.

The analysis of effect of pioglitazone in patients with coronary heart disease showed that in carriers of Asp (Asp / Asp) allele under the influence of pioglitazone after 6 months there was a significant decrease in ESR levels (p = 0.03), OGTT (p = 0.0001), hypertension (p = 0.002), cholesterol (p = 0.006), LDL (p = 0.001) and CA (p = 0.001), increase in HDL (p = 0.01) as compared to initial indicators, after 12 months (P = 0.03), LDL (p = 0.004) and CA (p = 0.0006), an increase in the level of hs-CRP (p = 0.01) and HDL (p = 0.002) as compared with initial indicators, as well as in 12-month a reliable decrease in the level of ESR (p = 0.004), an increase in the level of hs-CRP (p = 0.04), triglycerides (p = 0.02) and VLDL (p=0.02) as compared to 6 months.

In carriers of Gly (Asp / Gly + Gly / Gly) allele, under the influence of pioglitazone, in 6 months a significant increase in TIMP-1 (p = 0.007), triglycerides (p = 0.04), and VLDL (p = 0.03) was observed as compared to initial indicators; after 12 months there was a significant decrease in cholesterol levels (p = 0.006) and LDL (p=0.009) as compared to initial indicators; after 12 months, a significant reduction in leukocyte levels (p = 0.03) as compared with indicators in 6 months was detected.

Thus, in patients with coronary heart disease, Asp (Asp / Asp) allele carriers, azithromycin had a more pronounced effect on inflammation and lipid metabolism rates, whereas pioglitazone influenced on indicators of inflammation and carbohydrate metabolism.

The obtained data suggest that as a result of treatment, both azithromycin and pioglitazone, used against the background of standard therapy, have common and separate mechanisms of influence on the course of coronary heart disease. The use of antibiotics, azithromycin, against the background of standard therapy for 6 months in carriers of Asp (Asp / Asp) allele led to a more effective inhibition of atherosclerosis by reducing the size of ASP, antibacterial effect, a more pronounced anti-inflammatory and satisfactory hypolipidemic effect than in Gly allele (Asp / Gly + Gly / Gly) carriers.

Treatment with thiazolidinedion and pioglitazone, against the background of standard therapy for 6 months in carriers of Asp (Asp / Asp) allele caused a more pronounced reduction of atherosclerosis by reducing the stenosis diameter, moderate anti-inflammatory and expressed hypoglycemic effect, as well as the hypolipidemic effect by preventing formation of atherogenic dyslipidemia than in carriers of Gly allele (Asp / Gly + Gly / Gly).

Analyzing the literary data, one can conclude that among modern therapeutic agents in CHD, macrolides, in particular azithromycin, have an effective anti-inflammatory effect, and thiazolidinediones, in particular, pioglitazone, exhibit alternative effects. This effect of both medications is implemented at the level of molecular mechanisms by suppressing the expression of the proinflammatory nuclear factor NF-kB [14, 16], which may form different clinical efficacy in the treatment with these drugs in patients with Asp299Gly polymorphism of TLR4 gene.

The positive effect of azithromycin obtained over half a year against the background of standard therapy in patients with CHD, carriers of Asp (Asp / Asp) allele is probably due to the direct action on parodonotopathogenic microorganisms in the inflammation site, namely, in the oral cavity and in the ASP [1]. Azithromycin stimulates phagocytosis, inhibits the production of active forms of oxygen, infiltration of leukocytes in the tissue [11], and also activates the activity of TIMP-1, which can prevent the initiation of instability and rupture of ASP [4].

Our study found positive effects of pioglitazone over a six-month period against the background of standard therapy in patients with CHD, carriers of Asp (Asp / Asp) allele, which was expressed in suppressing the atherosclerotic and inflammatory processes, insulin resistance and dyslipidemia in CHD patients. The mechanism of this effect is associated with blocking of proinflammatory NF-kB-dependent mechanisms, which leads to a decrease in the level of CRP, adiponectin, MMP-9, monocyte-chemotactic protein (MCP-1) and soluble marker CD40L, which can contribute to the prevention of atherosclerosis and macrovascular complications in CHD [6]. It is known that the total impact of factors influenced by pioglitazone has a cardioprotective effect by improving the diastolic function of the left ventricle in patients with hypertension, glucose utilization by cardiomyocytes, increased blood flow, prevention of CA inflammation and coronary vasospasm, as well as minimizing ischemic-reperfusion myocardial damage [9, 10].

Our data showed that patients with CHD, carriers of Asp (Asp / Asp) allele, are more responsive to the conducted treatment, while the presence of Gly (Asp / Gly + Gly / Gly) mutant allele of TLR-4 gene was associated with a risk of reducing the effectiveness of therapy with the studied medications against the background of standard course of treatment, indicating at the pharmacogenetic peculiarities of responsiveness to the action of the studied medications which should be taken into account in clinical practice.

Conclusions

1. Intake of azithromycin against the background of standard therapy for 6 months in carriers of Asp allele (Asp / Asp), in contrast to carriers of Gly allele (Asp / Gly + Gly / Gly) caused more effective inhibition of atherosclerotic processes by reducing the size of ASP, stenosis diameter and IMT, antibacterial effect is mainly due to partial or complete eradication of parodontopathogenic microflora in the oral cavity, more pronounced anti-inflammatory and satisfactory hypolipidemic effects.

2. Intake of pioglitazone against the background of standard therapy for 6 months in carriers of Asp allele (Asp / Asp), in contrast to carriers of Gly allele carriers (Asp / Gly + Gly / Gly) caused a more pronounced reduction in atherosclerosis by reducing the diameter stenosis, moderate anti-inflammatory and expressed hypoglycemic and hypolipidemic effects by preventing the formation of atherogenic dyslipidemia.

Prospects for further research. In our study, it has been shown for the first time that the efficacy of azithromycin and pioglitazone in the course of standard therapy depends on the presence of Asp299Gly polymorphism of TLR4 gene, which may have broad prospects for use in modern cardiology and therapy.

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