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Original Article

The lipid profile depends on the endothelial NO synthase gene promoter T786C-polymorphism in patients with arterial hypertension

Roman Hnizdyukh [1], Svitlana Pidruchna [2], Volodymyr Shmanko [1], Petro Lykhatskyi [2], Liliya Palytsya [2], Iryna Zavarynska [3], Nataliya Tkachuk [4], Oleksandr Tokarskyy [2]*

[1] Department of Pharmacology and Clinical Pharmacology, Ternopil National Medical University, Ternopil, Ukraine
[2] Department of Medical Biochemistry, Ternopil National Medical University, Ternopil, Ukraine
[3] Department of Ukrainian Language and and Slavic Studies, Ternopil National Pedagogical University, Ternopil, Ukraine
[4] Department of Microbiology, Virology, and Immunology, Ternopil National Medical University, Ternopil, Ukraine

* Correspondence to: Oleksandr Tokarskyy, Department of Medical Biochemistry, I.Horbachevsky Ternopil National Medical University, Maidan Voli str., 1, 46001, Ternopil, Ukraine. Phone: +380964102536; E-mail: otokarskyy@tdmu.edu.ua

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Abstract

According to the research data from recent years, genetic factors play an important role in the development and course of arterial hypertension (AHT). The study of the T786C polymorphism of the endothelial NO-synthase (NOS3) gene promoter in the case of arterial hypertension is a promising direction for determining the relationship between heredity, arterial hypertension, and dyslipidemia. A number of 86 AHT patients participated in the experiment, while the control group consisted of 30 practically healthy people. Indicators of lipid metabolism in the blood serum were determined. The T786C promoter of the endothelial NO-synthase (NOS3) gene was analyzed using the PCR method with electrophoretic detection of the results. The level of HDL, as an antiatherogenic factor, in the group of NOS3 gene "CC" genotype carriers was significantly lower by 45.8% than the HDL level of the "TT" genotype carriers, 0.58±0.06 *vs.* 1.07±0.03 mmol/L, respectively. A decrease in the HDL at the expense of an increase in the LDL and VLDL in AHT patients contributes to the formation of a secondary type IV dyslipidemia. "CC" genotype eNOS gene patients had a more pronounced disturbance of the lipid profile compared to patients with the "TT" and "TC" genotypes. Therefore, the "CC" genotype of the eNOS3 gene promoter may predict the development of dyslipidemia in patients with arterial hypertension and additional cardiovascular risk factors for the development of hypertension in patients.

Keywords: gene polymorphism T786C, endothelial NO-synthase gene promoter, arterial hypertension, dyslipidemia.

Introduction

Cardiovascular diseases, including arterial hypertension (AHT), are usually accompanied by changes in the blood lipid profile. Compared to healthy individuals, patients with arterial hypertension have reduced high-density lipoprotein (HDL) cholesterol levels in blood plasma. In contrast, low-density lipoprotein (LDL) cholesterol and triglycerides (TG) increase.

A shift in the lipid profile in the atherogenic direction is a risk factor for cardiovascular complications, such as stroke and myocardial infarction. Cholesterol crystals act as molecules associated with damage (DAMP-danger-associated molecular patterns). DAMPs activate pattern-recognition receptors on the surface of several immune cells, which leads to the emergence and implementation of an inflammatory signal [1].

An increase in the blood plasma level of cytokines, which are produced by several immune cells, promotes the activation of inducible nitrogen synthase (NOS2) and its production of nitric oxide [2].

Nitric oxide (NO), generated by the NOS2 enzyme, interacts with active forms of oxygen (superoxide radical and hydrogen peroxide). The levels of NO in the blood and other tissues of the body increase significantly under conditions of inflammation [3].

As a result, there is a decrease in the bioavailability of NO and the accumulation of active forms of nitrogen, in particular, peroxynitrite. Active forms of oxygen and nitrogen oxidize low-density lipoprotein cholesterol [4].

Going further, a cycle of inflammatory reactions associated with increased LDL content and NO in the blood plasma can continue. However, whether the increase in bloodstream NO level is related to changes in the lipid profile has yet to be fully clarified.

According to some authors, a decrease in the production and bioavailability of this gaseous molecule contributes to the accumulation of the atherogenic fraction of lipids – oxidized forms of cholesterol in LDL, triglycerides (TG), free fatty acids (FFA), and a decrease in the anti-atherogenic high-density lipoprotein (HDL) fraction [5]. A low level of NO in the blood plasma can lead to an increased risk of developing dyslipidemia [6].

According to the data of other research groups, the level of NO production is not related to the cholesterol content of LDL, HDL, or TG [7, 8].

Analyzing the research data from recent years, genetic factors, such as gene polymorphisms, play an important role in the development and course of arterial hypertension [9–11].

It can be assumed that allelic variations of the NOS3 gene by several polymorphic markers are associated with the NO content in the blood plasma of healthy people and patients with AHT. These allelic variations may probably also affect the peculiarities of the LDL and HDL distribution in blood plasma. To quantify these possible changes, it is important to assess the relationship between this gene polymorphism, high blood pressure, and dyslipidemia.

The role of the rs2070744 (T-786C) polymorphism of the NOS3 gene was first actively investigated in cohorts of patients with cardiovascular pathology and/or metabolic cardiovascular risk factors. In particular, in a number of prospective studies, the correlation of the "CC" genotype with coronary heart disease was revealed [12], as well as with prinzmetal's angina pectoris [13], arterial hypertension [14, 15], and insulin resistance in patients with ischemic and nonischemic cardiomyopathy [16].

The clinical-pathogenetic significance of the T786C promoter polymorphism of the endothelial NO-synthase (NOS3) gene under conditions of arterial hypertension remains debatable.

The current research aimed to investigate the parameters of the blood plasma lipid profile depending on the T786C polymorphism of the endothelial NO-synthase gene promoter in AHT patients.

Material and methods

A number of 86 AHT patients who were treated and examined at the therapeutic department of Kozova Central Regional Hospital, aged between 45 and 76 years old, among them – 47 (or 55%) females and 39 (or 45%) males, were included in the study. The average age of the AHT patients was (61.35±13.3) years. The control group consisted of 30 practically healthy people without signs of AHT. The criterion for inclusion in the study was the presence of AHT in the 1st to the 3rd degree. The AHT diagnosis was carried out in accordance with the orders of the Ministry of Health of Ukraine No. 54 and No. 436 and the Recommendations of the Ukrainian Association of Cardiologists based on anamnestic data, complaints, physical and clinical-instrumental examination, and laboratory data.

The research was conducted according to the principles of bioethics outlined in the Declaration of Helsinki, "Ethical principles of medical research involving human subjects", the General Declaration on Bioethics and Human Rights (UNESCO), and the order of the Ministry of Health of Ukraine, "On approval of the procedure for conducting clinical trials of medicinal products and examination of materials", No 690, dated 23.09.2009.

Table 1: Characteristics of examined patients (n=86).

Investigated parameter	Value
Average age, number of years	61.35±13.3
Males, n (%)	39 (45)
Females, n (%)	47 (55)
Average AHT duration, number of years	12.6±1.8
BMI, kg/m^2	32.6±1.1

Table 1: Continued.

Investigated parameter	Value
Obese patients (BMI >30 kg/m^2), n (%)	30 (34.9)
Smokers, n (%)	27 (31.4)
Alcohol abusers, n (%)	11 (12.8)
Weighted heredity regarding the early development of cardiovascular diseases (for men aged <55 years, for women ≤65 years), n (%)	47 (54.7)
AHT 1st degree patients, n (%)	21 (24.4)
AHT 2nd degree patients, n (%)	47 (54.6)
AHT 3rd degree patients, n (%)	18 (21)

All individuals involved in the research gave written informed consent for the study (conclusion of the Commission on Biomedical Ethics of I. Horbachevsky Ternopil National Medical University No 69, dated April 12, 2022).

Patients with a history of myocardial infarction and stroke, secondary arterial hypertension, congenital or acquired heart defects, rhythm and conduction disorders, heart failures of III-IV functional classes according to NYHA, chronic obstructive pulmonary disease, diabetes, chronic kidney disease, oncological and mental diseases were not included in the study.

The clinical and anamnestic characteristics of the patients are shown in Table 1.

The following data were collected from the patients: body weight and height measurements, blood pressure data, electrocardiograms (ECG), and lipid profile indicators; the T786C polymorphism of the endothelial NO-synthase gene promoter in each human subject was also determined.

The parameters of the lipid profile in the blood serum were measured in the laboratory of the Kozova Central District Hospital. The concentrations of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL) were determined using commercially available kits on a Biochem FC-200 analyzer (High *et al.*, USA). The cholesterol coefficient of atherogenicity (CA) was calculated according to the Klimov equation: CA=(TC - HDL)/HDL.

The polymerase chain reaction method with the electrophoretic detection of the results was used to study the T786C allelic polymorphism of the endothelial NO-synthase gene promoter using SNP-EXPRESS reagent sets (Litech LLC, Ukraine).

The obtained data were statistically processed using the commercially available software Statistica 8.0 (StatSoft Inc., USA) and Microsoft Office Excel 2003 (Microsoft Corp., USA).

Results

Overall, lipid metabolism correlated well with the T786C promoter polymorphism of the endothelial NO-synthase (NOS3) gene in AHT patients. The presence of pathological changes in blood plasma lipid profile was revealed in all AHT patients; however, in AHT patients with the "CC" genotype compared to the "TT" genotype of the T786C – endothelial NO-synthase gene promoter, lipid homeostasis disorders were more profound, confirmed by a statistically significant increase in the levels of TC, TG, LDL, and VLDL (Table 2). When analyzing the lipid profile of AHT patients, higher cholesterol values were noted in all groups compared to control (Table 2).

Table 2: Lipid profile of AHT patients depending on the T786C polymorphism of the promoter of the endothelial NO-synthase gene.

Indexes	Control group, n=30	"TT" genotype, n=24	"TC" genotype, n=31	"CC" genotype, n=31
TC, mmol/L	4.17±0.14	5.03±0.01* (p<0.01)	7.1±0.09* (p<0.001)	7.54±0.07*# (p<0.01)* (p<0.02)#

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Table 2: Continued.

Indexes	Control group, n=30	"TT" genotype, n=24	"TC" genotype, n=31	"CC" genotype, n=31
TG, mmol/L	1.01±0.06	1.12±0.02* (p<0.01)	2.54±0.04* (p<0.01)	3.25±0.05*# (p<0.01)* (p<0.05)#
LDL, mmol/L	2.69±0.12	3.54±0.01* (p<0.01)	4.78±0.04* (p<0.01)	5.9±0.03*# (p<0.001)* (p<0.05)#
VLDL, mmol/L	0.27±0.05	0.42±0.02* (p<0.01)	0.74±0.03* (p<0.01)	1.92±0.13*# (p<0.01)* (p<0.05)#
HDL, mmol/L	1.21±0.04	1.07±0.03* (p<0.05)	0.72±0.04* (p<0.01)	0.58±0.06*# (p<0.01)* (p<0.05)#
CA, units	2.4±0.23	3.7±0.3* (p<0.01)	8.86±0.4* (p<0.01)	12.0±0.2*# (p<0.01)* (p<0.01)#

Note: * – indicates a significant difference in indicators compared to the control group; # – indicates a significant difference in indicators in relation to homozygotes ("TT" genotype).

As a result of the study, an increase in the level of all atherogenic fractions in blood was found in the group of patients with "CC" genotype carriers compared to carriers of the "TT" genotype of the NOS3 gene (Table 2). The level of TC in the group of "CC" genotype carriers increased by 33.3% compared to the carriers of the "TT" genotype and was almost twice as high as the control values. Similar changes were observed for TG (Table 2). The level of LDL and VLDL in AHT patients with the "CC" genotype significantly increased by 1.6 and 4.6 times, respectively, compared to the carriers of the "TT" genotype.

Regarding the level of HDL as an anti-atherogenic factor, in the "CC" genotype AHT group patients, this indicator was significantly lower by 45.8% than the value in the AHT patients with the TT genotype: 0.58±0.06 mmol/L against 1.07±0.03 mmol/L, respectively. A decrease in HDL against the background of an increase in LDL and VLDL in patients with hypertension contributes to the formation of a secondary type IV dyslipidemia, according to the D.S. classification Fredrickson.

Discussion

A long-term increase in blood pressure leads to proatherogenic lipid metabolism disorders, such as an increase in LDL and a decrease in HDL fractions in blood plasma.

Analyzing the indicators of lipid status, we found the presence of pathological changes in lipid metabolism in all AHT patients; however, in patients with the "CC" genotype compared to the carriers of the "TT" genotype of the T786C – promoter of the endothelial NO-synthase gene, lipid homeostasis disorders were more profound, which was confirmed by statistically significant increases in levels of TC, TG, LDL, and VLDL. While analyzing the lipid profile of AHT patients, higher TC values were noted in all groups compared to controls.

In addition, AHT patients usually have an elevated level of nitric oxide metabolites in the bloodstream [2]. Both of these processes are a consequence of the development of hypertension and can act as etiological factors of this disease.

Thus, the accumulation of atherogenic lipid fractions contributes to the development of atherosclerosis, an important factor in increasing blood pressure. Our findings confirmed this. An increase in the level of all atherogenic blood fractions was established in the group of patients carrying the "CC" genotype of the NOS3 gene compared to carriers of the "TT" genotype.

The level of TC in the group of carriers of the "CC" genotype T786C – the promoter of the endothelial NO-synthase gene – increased by 33.3% compared to the carriers of the "TT" genotype and was almost twice as high as the control values. Similar changes were observed for the TG blood lipid fraction: in the "CC" genotype AHT patients group, the TG levels were

significantly higher than in the "TT" genotype group, namely, by 2.9 times. The level of LDL and VLDL in AHT patients with the "CC" genotype significantly increased by 1.6 and 4.6 times, respectively, compared to the carriers of the "TT" genotype.

A high level of NO as a source of active forms of nitrogen promotes damage to proteins, DNA and inflammation of blood vessel walls [3].

Nitric oxide can directly or indirectly affect the lipid spectrum. Exogenous NO has been shown to activate the lipase of some bacteria and yeast [17].

An important role in lipid metabolism is played by lipoprotein lipase, which cleaves triacylglycerols of the largest and most lipid-rich blood plasma lipoproteins – chylomicrons and very low-density lipoproteins. A decrease in its activity can lead to dyslipidemia. In the culture of adipocytes, which produced a large amount of tumor necrosis factor (TNFα), an increase in NO and suppression of lipoprotein lipase activity in the cells were observed [18]. The suppressive effect of enhanced NO synthesis on the activity of this enzyme was weakened when NOS inhibitors were added to the culture medium. Since the cGMP inhibitor also reduced lipoprotein lipase activity, the authors concluded that elevated NO affects the activity of this enzyme by regulating cGMP production [18].

In addition, arterial hypertension is accompanied by an inflammatory process in which the level of proinflammatory cytokines in the blood plasma increases [19]. Proinflammatory cytokines activate NOS2, leading to increased NO production [2, 20].

As a result, in hypertension, the increased level of NO can obviously also inhibit lipoprotein lipase activity. Interestingly, not only the high level of NO produced due to the activity of NOS2 but also its decrease due to the suppression of the functions of NOS3 in hypertensive rats contribute to a decrease in the activity of lipoprotein lipase and an increase in the level of triacylglycerols, low-density lipoprotein cholesterol, free fatty acids, phospholipids [5].

Another mechanism through which NO can flow to different fractions of lipids is the regulation of the transcriptional activity of genes encoding proinflammatory proteins. Under inflammation conditions, NO can activate the transcription factors NF-κb and AP-1, which regulate the synthesis of proinflammatory proteins and NOS2 [21].

Proinflammatory cytokines, such as interleukin 6 and TNFα, can modulate the activity of a number of lipid metabolism enzymes and affect the synthesis and metabolism of triacylglycerols, fatty acids, and cholesterol [22].

An increase in the level of TNFα in the blood helps to increase the production of reactive oxygen species and reactive nitrogen species, which activate lipoperoxidation processes [23].

Under inflammation, the adhesion molecules and permeability of the endothelium for leukocytes and cholesterol in low-density lipoproteins on the surface of endothelial cells increase [24].

These processes contribute to the damage and inflammation of the walls of blood vessels, increasing blood pressure. The NO level in the plasma of AHT patients is higher than that of normotensives and may be determined by the carrier of allelic variants of the eNOS3 gene.

Regarding the level of HDL as an antiatherogenic factor, in the group of carriers of the "CC" genotype of the NOS3 gene, this indicator was statistically significantly lower by 45.8% than the indicator of the group with carriers of the "TT" genotype and was (0.58±0.06) mmol/L against (1.07±0.03) mmol/L. A decrease in HDL against the background of an increase in LDL and VLDL in AHT patients contributes to the formation of secondary dyslipidemia type IV, according to the D.S. Fredrickson's classification.

Conclusions

Therefore, the "CC" genotype of the eNOS3 gene promoter may be considered a genetic predictor of the formation of several hemodynamic factors of the cardiovascular risk of developing hypertension in our patients. The study showed that the carriers of the "CC" genotype of the eNOS gene had a more pronounced disturbance of the lipid profile compared to the carriers of the "TT" and "TC" genotypes. This allows us to believe that the "CC" genotype predicts the development of dyslipidemia in patients with arterial hypertension.

Conflict of interest

The authors declare no conflict of interest.

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