Genetic Determinants of Efficiency of Magnetic Laser Therapy of Essential Hypertension

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The efficiency of magnetic laser therapy was evaluated in patients with essential hypertension (stages I and II). The role of angiotensinogen, angiotensin-converting enzyme, type II bradykinin receptor, and endothelial nitrogen oxide synthetase gene polymorphism in the realization of hypotensive effect of magnetic laser therapy was evaluated. The hypotensive effect of magnetic laser therapy depends on the polymorphism of the studied genes and is maximum in patients with MM polymorphism of angiotensinogen gene and DD polymorphism of angiotensin-converting enzyme gene. Additive interaction between angiotensin-converting enzyme and angiotensinogen genes in the formation of hemodynamic effects of magnetic laser therapy was detected.

Key Words: *magnetic laser therapy; essential hypertension; angiotensin-converting enzyme gene; angiotensinogen gene; endothelial nitrogen oxide synthetase gene*

Essential hypertension is one of the most prevalent diseases; its treatment is a pressing problem of modern medicine [7]. There is evidence that essential hypertension is a genetically heterogeneous disease characterized by multifactorial inheritance type [2].

Essential hypertension as a multifactorial disease modulates the pathogenetic factors of numerous genes, each of which has several polymorphic markers. When certain combination of "unfavorable" variants of these genes in the genotype appears, the so-called "risk threshold" for the disease development can be achieved. The following genes are considered to be involved in the pathogenesis of essential hypertension: angiotensinogen (AGT, angiotensin I precursor), angiotensin-converting enzyme (ACE, determines the synthesis of angiotensin II, modifies blood pressure and water-salt homeostasis) [3], type II bradykinin receptor (BDKRB2, regulates vasodilatory potential), and endothelial nitrogen oxide synthetase (eNOS, determines the production of nitrogen oxide by the endothelium) [12,14].

Different lengths of restriction fragments of these genes are responsible for their polymorphism and, hence, determine changes in the synthesis and metabolic activity of the products encoded for by them. Polymorphism of AGT, ACE, BDKRB2, and eNOS gene restriction fragments is responsible for differences in the degree of the risk of development, severity, course and prognosis of essential hypertension.

Recent reports describe different effects of hypotensive drugs on the central hemodynamics, depending on gene polymorphism [5,13]. Pronounced hypotensive effect was observed in patients with AGT gene TT polymorphism and ACE gene DD polymorphism. These variants of AGT and ACE genes polymorphism are responsible for higher activity of the renin-angiotensin system.

ACE gene polymorphism is essential for the efficiency of physical methods of treatment, for example, magnetic laser therapy [4]. The involvement of other genes in determination of the effects of therapeutic physical factors have not been discussed in literature.

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The relationship between the hypotensive effects of physical factors and polymorphism variants of the genes involved in the pathogenesis of essential hypertension seems to be obvious.

We evaluated the hypotensive effect of magnetic laser therapy with consideration for polymorphism of the genes involved in the pathogenesis of essential hypertension.

MATERIALS AND METHODS

The hypotensive effect of magnetic laser therapy was evaluated in 101 patients with essential hypertension (stages I and II) with different variants of AGT, ACE, BDKRB2, and eNOS genes polymorphism. Mean patient age was 44.0±2.6 years, mean disease duration 7.0±2.2 years. The diagnosis of essential hypertension was verified on the basis of WHO criteria using two-staged diagnostic protocol in order to rule out symptomatic hypertension [1].

Gene polymorphism in the patients was studied after they gave their informed consent to participation in the study. Venous blood was collected from the ulnar vein (5 ml). Genome DNA was isolated from peripheral blood lymphocytes by phenol chloroform extraction [8] using Chelex-100 chelate primer (Bio-Rad). Variants of AGT gene T235M, ACE gene I/D, BDKRB2 gene 0/+9, and eNOS gene Glu298Asp polymorphism were analyzed by PCR [8,10,11].

Magnetic laser therapy was carried out using RIKTA-04 device generating infrared radiation with 8 W pulse power at λ=890 nm in permanent magnetic field with 60 mT induction. The procedures were carried out daily, the course consisted of 10 sessions. Paravertebral points at C_{IV}-C_{VI} level (1 min each, 5 Hz), projection of the vasculomotor center of the brain (occipital fossa, 1 min at 1000 Hz frequency), sinocarotid vessels (1 min each, 50 Hz), heart apex region (5 min, 5 Hz), and renal projection areas (5 min each, 1000 Hz) were exposed in succession. Total dose of absorbed energy per procedure was 0.56 J.

The patients were examined before and after the course of magnetic laser therapy. The hemodynamic parameters were evaluated using 24-h blood pressure monitoring (BPM) by the oscillometrical method with a TONOPORT V device (GE Medical System) and by exercise test (bicycle ergometry) by the protocol of step-by-step constantly increasing load using a Cardiodata bicycle ergometer and Cardio perfect software.

Clinical efficiency of magnetic laser therapy was evaluated by the time course of arterial pressure (BP) before and after therapy (BP was measured by 24-h monitoring, during submaximal exercise on a bicycle ergometer, and by the double product analysis). The data were statistically processed by methods of variation statistics.

RESULTS

AGT genotypes on the T235M polymorphic locus were distributed as follows: 19 (19%) MM, 54 (53%) MT, and 28 (28%) TT. All the patients were divided into 3 groups by the detected AGT gene polymorphism.

All BP values, shown by BPM, decreased significantly under the effect of magnetic laser therapy in patients with MM polymorphism (Table 1). A lesser number of significant shifts in BPM values was observed in patients with AGT gene MT and TT polymorphism. The decrease in the mean 24-h and mean day-time systolic BP values was the greatest in patients with MM polymorphism, while the mean nocturnal levels of systolic BP decreased to a greater extent in patients with MT polymorphism. Changes in BPM values were the least of all variants of AGT gene polymorphism in patients with TT polymorphism.

The maximum decrease in the threshold systolic BP and double product (according to bicycle ergometry test) was observed in patients with MM polymorphism and the minimum decrease was noted in patients with TT polymorphism (Table 1).

Hence, magnetic laser therapy caused a significant decrease of BP in patients with all variants of AGT gene polymorphism, but the maximum hypotensive effect (according to BPM and bicycle ergometry) was detected in patients with MM polymorphism and the minimum in those with TT polymorphism. It seems that magnetic laser therapy modifies predominantly the mean 24-h and mean day-time systolic BP in patients with MM polymorphism and the mean nocturnal BP in patients with MT polymorphism.

ACE gene II polymorphism was detected in 18 (18%) patients, DD polymorphism in 33 (33%) patients, and ID polymorphism in 50 (49%) patients, which is comparable with the incidence of ACE gene polymorphic alleles distribution in the Russian population [6].

Magnetic laser therapy caused a significant decrease in all BPM parameters in patients with DD and ID polymorphism (Table 1). The decrease in the majority of BP parameters was more pronounced in patients with DD polymorphism, while in the group of patients with II polymorphism only 24-h and day-time systolic BP decreased significantly. Changes in the majority of parameters in patients with II polymorphism were minimum according to BPM, differing significantly (p<0.05) from the decrease in the same parameters in other groups.

The range of values in bicycle ergometry test in the patients with different ACE gene polymorphisms was maximum in patients with DD polymorphism and minimum in patients with II polymorphism (Table 1). The trend of changes in BP was the same in 24-h monitoring and exercise test.

TABLE 1. Time Course of BP during Magnetic Laser Therapy of Patients with Essential Hypertension with Different AGT and ACE Gene Polymorphism ($M \pm m_x$, mm Hg)

Parameter	AGT gene polymorphism			ACE gene polymorphism		
	MM	MT	TT	II	ID	DD
	1	2	3	1	2	3
24-h BP						
systolic	-9.2±0.9***^{o}	-8.3±1.0***	-6.3±0.8***^{o}	-4.7±1.4*^{o}	-6.7±0.8***^{x}	-9.7±0.8***^{ox}
diastolic	-5.8±1.3***	-4.9±1.5***	-3.7±1.2**	-2.1±1.9	-5.0±1.3***	-5.3±1.2***
mean	-6.8±1.1***	-7.3±3.3	-3.6±0.9***	+2.9±1.1^{+o}	-5.4±0.7***$^{+}$	-8.7±0.9***^{o}
Day-time BP						
systolic	-9.6±0.9***^{o}	-7.8±1.4***	-6.4±0.7***^{o}	-5.2±1.5*^{o}	-6.9±1.1***	-9.3±1.1***^{o}
diastolic	-5.9±1.5**	-5.5±1.5***	-4.0±1.3**	-3.2±2.0	-5.0±1.3***	-6.1±1.2***
mean	-7.2±1.3***	-6.8±2.8***^{x}	-3.0±2.2^{x}	-3.9±1.3^{o}	-5.7±1.1***^{x}	-7.7±0.9***^{ox}
Nocturnal BP						
systolic	-9.1±2.5**	-12.2±2.1***^{x}	-5.6±1.6***^{x}	-2.4±2.5^{o}	-6.1±1.6***^{x}	-12.2±2.1***^{ox}
diastolic	-5.8±2**	-3.2±2.5	-3.4±1.5*	+1.6±2.4	-5.0±1.6**	-5.1±2.0*
mean	-6.7±2.0**	-9.1±3.7**	-4.1±1.4**	-1.8±1.2^{o}	-5.3±1.5***	-9.9±3.1***^{o}
Threshold systolic BP	-22.1±3.8^{+}	-19.4±5.6	-12.4±2.2^{o}	-13.2±5.4	-16.2±2.6	-18.5±3.5
Double product of exercise test, arb. units	-36.6±8.9^{+o}	-30.8±11.0^{+}	-18.3±4.3^{o}	-18.3±9.7	-21.4±5.6	-30.3±5.1

Note. Here and in Table 2: 1-3: groups of patients, *p<0.05, **p<0.01, ***p<0.001 compared to initial values; p<0.05 significant differences between groups: $^{+}$1 and 2, ^{o}1 and 3, ^{x}2 and 3.

TABLE 2. Time Course of BP during Magnetic Laser Therapy in Patients with Essential Hypertension with Different BDKRB2 and eNOS Genes Polymorphism ($M \pm m_x$, mm Hg)

Parameter	BDKRB2 polymorphism			eNOS polymorphism		
	00	0/+9	+9/+9	AspAsp	AspGlu	GluGlu
	1	2	3	1	2	3
24-h BP						
systolic	-8.3±1.1***	-6.8±0.8***	-7.3±1.2***	-8.8±2**	-6.2±0.8***	-8.7±0.8***
diastolic	-6.6±1.6***^{o}	-4.2±1.2**	-2.5±1.4^{o}	-5.5±1.6**	-4.3±1.1***	-4.2±1.4**
mean	-6.3±1.6***	-6.9±1.0***	-3.6±3.7	-6.9±1.6***	-5±1***	-6.3±2.1
Day-time BP						
systolic	-7.6±1.5***	-7.1±0.9***	-7.5±1.4***	-8.1±1.9**	-6.3±1.0***	-8.2±1.0***
diastolic	-6.7±1.6***	-4.5±1.2**	-2.8±1.5	-5.6±2.2*	-4.3±1.2**	-4.9±1.3***
mean	-3.8±3.8	-7.6±2.5***	-4.5±1.4**	-6.6±2.0*	-5.1±1.1***	-6.0±3.4
Nocturnal BP						
systolic	-8.7±1.9***	-8.5±3.3**	-6.7±2.0**	-10.0±3.9**	-5.7±1.9**	-9.8±2.9***
diastolic	-6.5±1.8**	-3.0±1.9	-1.7±1.9	-5.6±1.3**	-4.5±1.5**	-2.7±1.9
mean	-7.2±1.6***	-6.6±2.6**	-3.4±1.8	-7±2**	-4.9±1.5**	-6.8±2.5**
Threshold systolic BP	-20.2±3.7^{+}	-12.9±4.2^{+}	-15.8±3.8	-10.9±5.9	-14.9±2.9	-19.1±2.8
Double product of exercise test, arb. units	-34.4±10.7	-22.7±4.4	-22.6±6.3	-17.5±7.8	-19.5±5.0	-33.6±7.0

Hence, magnetic laser therapy led to a significant decrease in all BP parameters in patients with essential hypertension with DD and ID polymorphism, the maximum hypotensive effect was observed in patients with DD polymorphism and the minimum in patients with II polymorphism.

Type II bradykinin receptor gene 00 polymorphism was detected in 31 (31%) patients with essential hypertension treated by magnetic laser therapy, 0/+9 polymorphism in 45 (44%), and +9/+9 polymorphism in 25 (25%) patients.

Analysis of the hypotensive effect of magnetic laser therapy in patients with BDKRB2 00 and 0/+9 polymorphism showed a significant decrease in virtually all BPM values (Table 2). The most pronounced changes in the mean 24-h diastolic BP were recorded in patients with 00 polymorphism.

Changes in the threshold systolic BP and double product in bicycle ergometry test were characterized by a greater range in patients with BDKRB2 gene 00 polymorphism, though were negligible (Table 2). Minimum changes in these values were detected in patients with +9/+9 polymorphism.

Hence, the maximum decrease of virtually all BP types was detected in patients with BDKRB2 gene 00 polymorphism according to BPM and bicycle ergometry test. Just less than half of the recorded values differed significantly from the initial values after magnetic laser therapy in patients with BDKRB2 gene +9/+9 polymorphism.

The incidence of eNOS gene polymorphism variants was as follows: AspAsp in 13 (13%), AspGlu in 41 (41%), and GluGlu polymorphism in 47 (46%) patients, which was comparable with the incidence of type Glu298Asp eNOS gene polymorphic alleles in the European population [15].

Magnetic laser therapy led to a significant decrease in all BPM values in patients with AspAsp and AspGlu polymorphism (Table 2). Maximum shifts in the majority of BPM values were observed in patients with AspAsp polymorphism and minimum number (about 60%) and intensity in patients with GluGlu

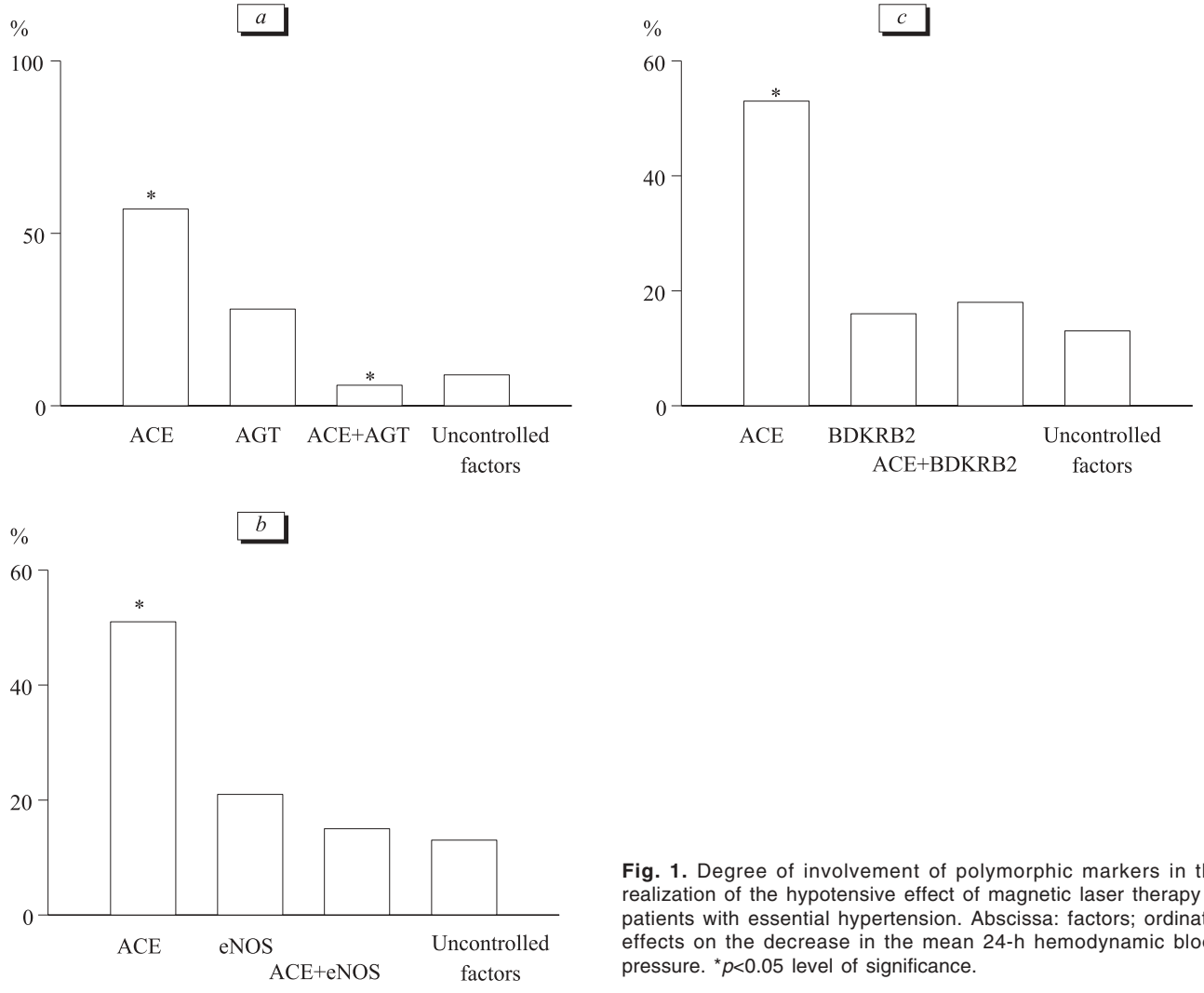

Fig. 1. Degree of involvement of polymorphic markers in the realization of the hypotensive effect of magnetic laser therapy in patients with essential hypertension. Abscissa: factors; ordinate: effects on the decrease in the mean 24-h hemodynamic blood pressure. *p<0.05 level of significance.

polymorphism, though the differences in the degree of BP decrease in the patients with eNOS gene different polymorphisms were negligible.

More appreciable shifts in the threshold systolic BP and double product during bicycle ergometry test were observed in patients with GluGlu polymorphism (Table 2). The changes were minimum in patients with AspAsp polymorphism.

eNOS gene polymorphism was less significant for the hypotensive effect of magnetic laser therapy than other variants of gene polymorphism. The hypotensive effect was maximum in patients with AspAsp polymorphism.

Interactions between different genes in the formation of the hypotensive effect of magnetic laser therapy were evaluated by factor dispersion analysis of factor pairs (Fig. 1). Full-factor model, in which the genes and their interactions account for the major part of dispersion of 24-h hemodynamic BP (ΔCHAP) is statistically correct only for combination of gene polymorphisms in ACE—AGT, ACE—BDKRB2, and ACE—eNOS pairs.

ACE gene polymorphism was the most significant for ΔCHAP dispersion in all cases. ACE—AGT genes polymorphism determined the dispersion of ΔCHAP by only 6%, this attesting to a strong interaction of gene alleles. The degree of dispersion was higher for other pairs: 18% for ACE—BDKRB2 and 15% for ACE—eNOS. The impact of ACE and AGT genes polymorphism on ΔCHAP was maximum and was significantly greater than the effects of ACE—BDKRB2 or ACE—eNOS interactions. The percentage of errors in dispersion of BP decrease in all three factor models did not surpass 13. The results of analysis of dispersions indicate an additive effect of ACE and AGT genes on the formation of the hypotensive effect of magnetic laser therapy in patients with essential hypertension.

Evaluation of the significance of differences in the mean ΔCHAP values at three levels formed in our study, corresponding to three variants of each gene polymorphism and their combinations, was carried out by the LSD (linear square distance) test. Significant ($p<0.05$) differences in the mean degree of BP changes by the LSD test were detected at all three levels (for all polymorphism variants) for ACE and AGT genes, but not for BDKRB2 or eNOS genes polymorphism.

Hence, magnetic laser therapy had a significant hypotensive effect in the majority of patients with essential hypertension. A more pronounced hypotensive effect was observed in patients with AGT gene MM polymorphism and the minimum effect was noted in patients with TT polymorphism.

The results of evaluation of paired interaction of polymorphism of different genes necessitate studies on an appreciably greater sample (up to 1000 subjects) because of pronounced heterogeneity in the incidence of this or that gene polymorphism and require additional analysis. However, even at this stage of analysis we can say that dispersion in the mean hemodynamic BP and the amplitude of its reduction is largely determined by the combination of ACE gene DD polymorphism and AGT gene MM polymorphism. These data once more demonsrate that the AGT—ACE axis is the key factor in the pathogenesis of essential hypertension and hence, the most sensitive to therapeutic physical factors.

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