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INFLUENCE OF PHARMACOGENETICALLY DETERMINED TREATMENT ON PARAMETERS OF PERIPHERAL HEMODYNAMICS IN PATIENTS WITH ARTERIAL HYPERTENSION

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Abstract

Aim: *To evaluate daily blood pressure monitoring (DBPM) data changes in patients with essential arterial hypertension (EAH) under the influence of pharmacogenetically determined treatment depending on genes polymorphisms of angiotensin-converting enzyme (ACE, I/D), angiotensin II first type receptor (AGTR1, A1166C), endothelial NO-synthase (eNOS, T894G), peroxisome proliferators-activated receptor-γ2 (PPAR-γ2, Pro12Ala), β_1-adrenergic receptor (ADRB1, Arg389Gly).*

Material and Methods: *249 patients with EAH I-III stages (48.2% – women, 51.8% – men, mean ages 50.5±10.4 years) and 50 practically healthy persons were observed. Alleles of polymorphic locus were assessed by polymerase chain reaction (PCR) based method. DBPM was performed according to standard protocol.*

Patients were split by genotypes and treatment type into 6 groups:

- *1st group: I-allele of ACE gene carriers (n=60) took Hydrochlorothiazide (HCTZ) and angiotensin II receptor blocker (ARB II);*
- *2nd group: I/D-genotype carriers (n=34) took HCTZ+β1-adrenobloker (β1-AB);*
- *3rd group: I/D-genotype carriers (n=50) took HCTZ+ACE-inhibitor (ACEI);*
- *4th group: DD-genotype carriers (n=15) took calcium channels bloker (CCB)+ ARB II;*
- *5th group: DD-genotype carriers (n=15) took CCB+β1-AB;*
- *6th group: DD-genotype carriers (n=27) took CCB+ACEI.*

Treatment efficacy was analyzed according to European Guidelines (2007).

Results: *Pharmacogenetically determined treatment of EAH patients during 9-12 months caused daily, day, and night blood pressure (BP) decrease below the "threshold" level in 154 (76.6%) patients. That did not significantly differ from the frequency of "target" office BP achievement: 149 (74.1%). The number of patients with normal BP daily profile "dipper" increased reliably by 10.1% ($p<0.001$), with "non-dipper" and "night-peaker" patients amount diminishing ($p<0.05$). Long-term combined therapy caused achievement of "target" average daily BP_{24} (DBPM) depending on types of treatment combination: after HCTZ+ARB II – in 55 (91.7%) patients; after HCTZ+β1-AB – in 25 (73.5%) persons; after HCTZ+ACEI – in 33 (66.0%) patients; after CCB+ARB II – in 11 (73.3%) persons, without a reliable difference between genotypes; after CCB+β1-AB – in 11 (73.3%) patients (easier in Ala-allele carriers of PPAR-γ2 gene, $p=0.002$); after CCB+ACEI – in 19 (70.4%) patients (easier in AlaAla-genotype carriers of PPAR-γ2 gene, $p=0.007$).*

Conclusions: *Prescription of HCTZ+ARB II drugs combination is more effective in treatment of EAH I-allele carriers of ACE gene patients than HCTZ+β1-AB or HCTZ+ACEI – 91.7% vs 66.0 or 73.5%, accordingly ($p<0.001$); for DD-genotype carrier hypertensive patients the combination of CCB+ARB II and CCB+β1-AB is more effective than CCB+ACEI – 73.3% vs 70.4%, accordingly.*

Keywords: pharmacogenetics, arterial hypertension, daily blood pressure monitoring.

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INTRODUCTION

Arterial Hypertension (AH) treatment is one of the most actual problems of modern medical science. Far beyond the limits of cardiologic pathology, AH became a topical problem in medical practice of internal disease doctors and general practitioners. In Ukraine, almost 10 million people have a high blood pressure (BP). Epidemiology research of the National Ukrainian Scientific Centre "Institute of cardiology named after M.D. Strazhesko" indicates that AH is diagnosed in 35% of adult Ukrainian population [*Sirenko Yu. et al., 2006*]. Last years the pandemic character of AH was mostly predefined by the life style of population. However, the influence of environmental factors could be realized in connection with the individual human genotype. Despite the numerous studies devoted to the discovery of potential genes-candidates of AH or ischemic heart disease (IHD), accumulated information on their genetic origin is not yet sufficient [*Tiret L. et al., 1998; Cadman P., O'Connor D., 2003; Mooser V. et al., 2003; Ioannidis J., 2009*]. A several tens of genes, which encode the synthesis of certain enzymes in the conditions of RAAS or eNO-systems activating, are known nowadays. They regulate vessels tone, endothelium function, salt, hydrocarbon and lipids metabolisms, etc. [*Headley A. et al., 2007; Hiltunen T. et al., 2006; Jankowska K. et al., 2007; Karlsson J. et al., 2004*]. However, in Ukraine likewise other countries all over the world, similar studies were limited [*Ohmichi N. et al., 1997; Celuyko V., Peleckaya O., 2008; Dzyak G.V., Kolesnyk T., 2008; Ioannidis J., 2009; Parkhomenko A.N. et al., 2008; Sydorchuk L.,2008; . Sydorchuk L. et al., 2008; 2009; 2010; Tykhonova S., Litovkin K., 2008*], whereas AH related morbidity in population is still high and efficacy of treatment does not exceed 18.7% among the urban and 8.0% among rural population of Ukraine [*Sirenko Yu. et al., 2006*]. Low efficacy of existing methods of AH treatment, insufficiently personalized drugs sensitivity, drugs side-effects compelled an urgent necessity to improve and develop new therapy approaches based on individual pharmacogenetics and pharmacogenomics [*Cadman P., O'Connor D., 2003; Guidelines of ESC/ESH, 2007; Ioannidis J., 2009; Stavroulakis G. et al., 2000; Sydorchuk L., 2008; Sydorchuk L. et al., 2008; 2009; 2010*].

The aim of the present study is evaluation of daily blood pressure monitoring (DBPM) data changes in patients with essential arterial hypertension (EAH) under pharmacogenetically determined treatment depending on five genes polymorphism: insertion/deletion of angiotensin-converting enzyme gene (ACE, I/D), the first type receptor of angiotensin II gene (AGTR1, A1166C), endothelial NO-synthase gene (eNOS, T894G), Peroxisome proliferators-activated nuclear receptor-γ2 gene, associated with insulin resistance (PPAR-γ2, Pro12Ala), β1-adrenergic receptor (ADRβ1, Arg389Gly).

MATERIAL AND METHODS

In Prospective Research Study took part 370 patients with EAH I-III severity stages (WHO, 1999), in which mean office BP exceeded 140/90 *mm* Hg in 7 days after antihypertensive drugs abolition. BP was measured in accordance to European Societies of Hypertension and Cardiology Guidelines requirements [ESH/ESC, 2007] and 249 persons were included into study after screening [*Sydorchuk L., 2008*]. Among them were: women made 48.2%, men – 51.8%, mean age was 50.5±10.4 years. EAH I stage was recorded in 66 (26.5%) patients, EAH II in 114 (45.%), EAH III in 69 (27.7%) subjects. The mild BP increase was evaluated in 66 (26.5%) patients, moderate in 105 (42.2%), severe in 78 (31.3%). In all EAH patients of II stage left ventricle hypertrophy (LVH) was revealed (evaluated with ECG and Echo-CG); in 14 persons transitory proteinuria was recorded, in 2 patients – slight plasma creatinine increase: 115-133 *μmol/L*, in 50 patients – "intimae-media" thickness (IMT) of common carotid arteries (CCA) enlarged (evaluated with Doppler-Ultrasound scanning, like >0.9 *mm*); in 15 persons the co-morbidity state was *diabetes mellitus* type 2 (DM 2). Among the EAH III stage patients (n=69) in 28 persons there was Stable Angina of I- II functional class (FC), Chronic Heart Failure (CHF) I-II (NYHA) with preserved LV systolic function, in 12 persons – CHF II (NYHA) was recorded as EAH complication, not a concomitant state; in 6 persons there was Q-myocardial infarction in anamnesis; 14 patients had transitory ischemic attacks in anamnesis (TIA); 3 underwent ischemic stroke; 42 persons had LVH; all patients had increased IMT of CCA >0.9 *mm* or an atherosclerotic plaque; in 2 persons mild-to-moderate plasma creatinine increase 134-150 *μmol/l* was recorded; in 21 patients DM 2 was diagnosed. Control group included 50 practically healthy persons of comparable age and gender (p>0.05).

Office systolic/diastolic BP (SBP/DBP) and heart rate (HR) were measured in accordance to European requirements [*Guidelines of ESH/ESC, 2007*]. Daily (24-hour) BP monitoring (DBPM) was done on the portable devices "ABPE-02" ("SOLVAIG", Ukraine-France) and "ABPM" ("Meditech", Hungary) according to standard protocol (40-55 daily measuring). The DBPM data analysis was performed with appropriate software of mentioned devices. In addition, patients underwent the basic clinical-instrumental inspections: ECG in the 12 leads, Echo-CG, basic clinical and biochemical blood analyses; immune-enzyme and genetic analysis; ophthalmologist and neurologist consultations.

Alleles of polymorphic sites of I/D in ACE gene, A1166S in AGTR1 gene, T894G in eNOS gene, Pro12Ala in PPAR-γ2 gene, Arg389Gly in ADRβ1 gene were studied by a selection of genomic DNA from the leucocytes of peripheral blood with following amplification by polymerase chain reaction (PCR) on programme amplificator "Amply" (Moscow, Russia). Alleles Discrimination of AGTR1, eNOS, PPAR-γ2 and ADRβ1 genes performed with restriction enzymes Ddel, BanII, CseI and FaqI accordingly. The amplificated DNA fragments were divided by the gel-electrophoresis based method, stained with bromine ethidium, visualized with UV-transluminator in presence of molecular marker ladder (100-1000 *bp*) [*Sydorchuk L., 2008*].

For elucidation of personal drugs sensitivity we conducted a 2-3 weeks empiric antihypertensive "sequential" monotherapy trial with the first-line drugs, then performed the comprehensive analysis of treatment results depending on the analysed genes genotype [*Sydorchuk L. et al., 2009*] and took into account the adequate "responder BP rate" (SBP/DBP reduction ≥20/10 *mm* Hg), or "target" BP (<140/90 *mm* Hg) achievement, in accordance to European recommendations [*Guidelines of ESH/ESC, 2007*]. Then all patients underwent pharmacogenetically determined treatment correction depending on ACE I/D gene polymorphism by setting the fixed-dose of antihypertensive drugs combinations recommended by ESC, ESH [*Guidelines of ESH/ESC, 2007*].

Patients were split by genotypes and treatment type into 6 groups:

- 1st group: I-allele of ACE gene carriers (n=60) took Hydrochlorothiazide (HCTZ) and angiotensin II receptor blocker (ARB II) Telmisartan;
- 2nd group: I/D-genotype carriers (n=34) took HCTZ+β1-adrenobloker (β1-AB – Metoprolol, Nebivolol, Bisoprolol or Atenolol), from this group we excluded patients with DM 2 and metabolic syndrome (MS);
- 3rd group: I/D-genotype carriers (n=50) took HCTZ+ACE-inhibitor (ACEI – Ramipril, Enalapril or Perindopril);
- 4th group: DD-genotype carriers (n=15) took calcium channels bloker (CCB – Normodipin, Amlodipin-S or Amlodipin) + ARB II;
- 5th group: DD-genotype carriers (n=15) took CCB+β1-AB;
- 6th group: DD-genotype carriers (n=27) took CCB+ACEI.

The drugs were administered one-two times per day at individual doses. Drugs' doses and reception numbers were corrected, at necessity, in a week of prescription. The total treatment time duration was 9-12 months, period of supervision: 24-30 months. During the treatment period, the office BP and HR control, complaints, therapy efficacy, drugs side effects were evaluated. At the beginning and at the end of treatment the DBPM and above mentioned complex of instrumental-laboratory analysis were conducted. Totally, 201 patients finished the study; 48 persons "dropped out" during treatment period for different reasons (migration, refused the offered treatment, did not appear on the repeated inspections and so on).

The treatment efficacy was analyzed according to European Guidelines [*Guidelines of ESH/ESC, 2007*]. Therapy was considered primary effective at achievement of "target" office BP <140/90 *mm* Hg to the end of supervision. Secondary efficiency was estimated with number of patients with "target" office BP or adequate BP reduction (SBP and/or DBP ≥20 and/or 10 *mm* Hg, accordingly); average daily BP decrease (BP_{24}) <125-130/80 *mm* Hg, average day BP (BPday) of <130-135/80 *mm* Hg, average night BP (BPnight) <120/70 *mm* Hg [*Guidelines of ESH/ESC, 2007*].

Statistical processing was conducted with MS Excel 2003, Primer of Biostatistics 6.05 and Statistica 7.0 (StatSoft Inc., USA) programs software. Data authenticity before/after treatment was calculated with unpaired/pair Student *t*-criteria (patients' distribution was near to normal on Kolmogorova-Smirnova test); quality signs analysed with χ^2-criterion (at frequencies less than 5 an exact Fisher-test was used), after treatment – Mak-Nimara-criterion. Differences were considered reliable at $p<0.05$.

RESULTS

The DBPM data in EAH patients before treatment depending onanalyzed genes polymorphism shown in a table 1. Linear Echo-CG parameters of LV back wall thickness in diastola (LVTd) and intraventricle septum thickness in diastola (IVTd) in EAH II-III patients exceeded analogical data in EAH I patients by 10.6% and 18.0% ($p<0.05$) and 25.0% and 39.0% ($0.005=p<0.03$) accordingly, reliably differed between itself ($p<0.05$). Left Ventricle Mass in EAH II patients was 264.90±20.58 *g*, in EAH III – 325.60±27.30 *g*, that was more than in EAH I patients on 24.9% ($p<0.04$) and 53.6% ($p=0.005$), accordingly, with a reliable intergroup difference of 22.9% ($p<0.05$).

Combined pharmacogenetically determined treatment with HCTZ+ARB during 9-12 months assisted reliable SBP_{24} and DBP_{24} reduction in II-genotype carriers of ACE gene by 11.0% and 12.7% ($p<0.04$) accordingly, in I/D-genotype carriers – by 12.7% ($p<0.01$). AGTR1 gene analysis demonstrated SBP_{24} and DBP_{24} decreasing in AA-genotype patients – by 15.9% and 23.2% ($p<0.05$), in AC-genotype patients – by 16.3% and 14.3% ($p<0.01$), in CC-genotype carriers – by 18.8% and 16.4% ($p<0.01$), accordingly. In patients with GG-genotype of eNOS gene SBP_{24} and DBP_{24} reduced by 13.9% and 25.0% ($p<0.01$), in TG-genotype carriers – by 14.6% and 16.9% ($p<0.01$), in TT-genotype carriers – by 13.5% and 14.1% ($p<0.01$) accordingly. In patients with the AlaAla-genotype of PPAR-γ2 gene SBP_{24} and DBP_{24} decreased by 13.1% and 14.1% ($p<0.01$), in ProAla-genotype carriers – by 19.9% and 19.8% ($p<0.01$), in ProPro-genotype carriers – by 19.3% and 18.7% ($p\leq0.003$), accordingly. Under treatment in GlyGly-genotype patients of ADRβ1 gene SBP_{24} and DBP_{24} diminished by 16.7% and 25.0% ($p<0.001$), in ArgGly-genotype carriers – by 16.8% and 19.4% ($p<0.01$), in ArgArg-genotype carriers – by 19.3% and 18.8% ($p<0.01$), accordingly. The reliable declining of average daily pulse BP (PBP_{24}) after treatment was in C-allele carriers of AGTR1 gene, ProPro-genotype of PPAR-γ2 gene and ArgGly-genotype carriers of ADRβ1 gene ($p<0.05$).

Table 1.

Daily Blood Pressure Monitoring (DBPM) and Heart Rate (HR) data in patients with essential arterial hypertension before treatment depending on genes polymorphism of ACE (I/D), AGTR1 (A1166C), ADRB1 (Arg389Gly), eNOS (T894G) and PPAR-γ2 (Pro12Ala)

Genes	Genotypes, (n=249) %	N n/n	SBP_{24}, *mm* Hg	DBP_{24}, *mm* Hg	PBP_{24}, *mm* Hg	HR_{24}, *b/min*
Control group, (n=20)			111.06±4,88	68.33±4.06	42.73±2.18	78.57±3.64
ACE	II, (n=50) 20.08%	1	134.60±3.70 p	78.55±3.25 p	47.45±3.05	74.90±1.50
	I/D, (n=130) 52.21%	2	141.90±3.57 p	85.81±3.16 p	54.70±3.01 p*	76.93±3.28
	DD, (n=69) 27.71%	3	155.64±6.25 p*#	96.70±4.20 p*#	64.90±4.75 p*#	89.55±1.95 p*#
AGTR1	AA, (n=123) 49.40%	1	141.90±8.09p	88.80±5.49 p	48.97±5.03	93.57±4.33 p
	AC, (n=96) 38.55%	2	139.80±5.03p	87.68±3.74 p	53.57±3.19 p	73.59±4.66*
	CC, (n=30) 12.05%	3	160.62±9,43 p*#	93.30±6.24 p	68.57±2.12 p*#	81.03±7.58
eNOS	GG, (n=94) 37.75%	1	138.80±4.34p	92.50±4.39 p	46.0±5.42	65.80±5.97 p
	TG, (n=134) 53.82%	2	143.0±4.06 p	86.02±4.34 p	55.29±4.89 p	76.60±6.61
	TT, (n=21) 8.43%	3	144.50±5.04p	88.97±5.56 p	56.67±4.58 p	79.21±6.17*
PPAR–g2	12Ala, (n=72) 28.92%	1	142.50±5.60p	82.30±2.49 p	57.33±7.85 p	78.25±1.85
	Pro12Ala (n=162) 65.06%	2	149.70±6.64p	88.65±4,24 p	58.56±4.61 p	78.02±3,07
	Pro12, (n=15) 6.02%	3	161.50±4.32 p*#	90.56±1.98 p	72.24±6.14 p*	76.52±3.17
ADRB1	389Gly, (n=25) 10.0%	1	142.46±5.07p	90.14±2.49 p	52.80±3.72 p	67.75±4.95 p
	Arg389Gly, (n=102) 41.0%	2	147.59±5.28p	90.49±2.16 p	57.34±2.50 p	74.93±5.91
	Arg389, (n=122) 49.0%	3	153.45±7.0 p	91.25±2.95 p	61.70±9.80 p	83.11±6.05*

Notes: SBP_{24}, DBP_{24}, PBP_{24} – average daily systolic, diastolic, pulse blood pressure; *p* – differences compared to control ($0.001<p<0.05$); * – differences compared to homozygous patients of each gene (II, AA, GG, 12Ala, 389Gly) $p<0.05$-0.001; # – differences compared to heterozygous patients (I/D, AC, GT, Pro12Ala, Arg389Gly) $p<0.05$-0.001; n (%) – number (percentage) of observations; N – groups number of each gene.

The patients' treatment with HCTZ+β1-AB caused SBP_{24} and DBP_{24} decreasing in I/D-genotype patients of ACE gene by 11.7% and 13.1% ($p<0.02$), accordingly, that did not differ reliably from therapy of analogical patients with HCTZ+ARB II combination. In AA-genotype carriers of AGTR1 gene SBP_{24} and DBP_{24} diminished by 15.4% and 18.9% ($p<0.01$), in AC-genotype carriers – by 9.1% and 13.4% ($p<0.02$), accordingly. The analogical decline of SBP_{24} and DBP_{24} was found: after eNOS gene analysis – significantly better in TT-genotype carriers by 16.0% and 17.2%, accordingly ($p<0.01$), after PPAR-γ2 gene – better in Pro-allele carriers by 17.8% ($p<0.01$) and 17.4% and 16,3% accordingly ($p<0.001$), after ADRβ1 gene – reliably better in Arg-allele carriers by 16.0% and 19.1% ($p<0.001$) and 16.6% and 16.8% ($p<0.01$) accordingly, These results did not differ certainly from the results of treatment with HCTZ+ARB II combination. The reliable reduction of PBP_{24} under the treatment was observed in TT-genotype carriers of eNOS gene, Pro-allele of PPAR-γ2 gene of and in all genotypes' carriers of ADRβ1 gene ($p<0.05$).

Under the treatment with HCTZ+ACEI combination during 9-12 months SBP_{24}, DBP_{24} and PBP_{24} decreased in I/D-genotype carriers of ACE gene by 11.1%, 12.6% ($p<0.02$) and 6.5%, accordingly. SBP_{24} and DBP_{24} diminishing was reliable under the therapy with HCTZ+ACEI in other genotypes-carriers of analysed genes, unsignificantly weaker than under HCTZ+β1-AB treatment combination, and comparable to HCTZ+ARB II combination ($p>0.05$).

Under long-term therapy with CCB+ARB II combination SBP_{24}, DBP_{24} and PBP_{24} reduced in DD-genotype carriers of ACE gene by 18.3%, 22.0% ($p<0.001$) and 20.5% ($p<0.05$) accordingly, that differed reliably from therapy with HCTZ ($p<0.05$). SBP_{24} and DBP_{24} decreasing was significantly deeper under CCB+ARB II treatment combination, and reliably differed from all combinations with HCTZ for all genotypes-carriers of AGTR1 ($p<0.04$), eNOS ($p<0.03$), PPAR-γ2 ($p<0.03$) and ADRβ1 genes ($p<0.01$).

Combined therapy with CCB+β1-AB during 9-12 months caused decrease of SBP_{24}, DBP_{24} and PBP_{24} in DD-genotype carriers of ACE gene by 18.8%, 23.4% ($p<0.001$) and 18.4% ($p<0.05$) accordingly, that was more reliable influence than drugs combinations with HCTZ ($p<0.05$), however did not differ from results of CCB+ARB II treatment combination. The analogical picture was observed in other analysed genes, where SBP_{24}, DBP_{24} and PBP_{24} declining under treatment with CCB+β_1-AB was stronger than under treatment with HCTZ drugs combinations, however, did not differ significantly in the genotypes of AGTR1, eNOS, PPAR-γ2 and ADRβ1genes.

The average BP daily data changes under CCB+ACEI drugs combinations' treatment during 9-12 months caused decline of SBP_{24}, DBP_{24} and PBP_{24} in DD-genotype carriers of ACE gene by 17.8%, 22.0% ($p<0.001$) and 19.2% ($p<0.03$) accordingly, that was stronger, than under treatment with HCTZ drugs' combinations ($p<0.05$), but did not differ substantially from the therapy with other CCB drugs combination. SBP_{24}, DBP_{24} and PBP_{24} reduction were significant under CCB+ACEI treatment combination in all genotypes-carriers of AGTR1 gene (better in CC-genotype patients – by 19.7%, 18.3% and 23.1%, $p<0.02$, accordingly), eNOS, PPAR-γ2 and ADRβ1 genes, but unreliably differed from treatment with HCTZ drugs combinations.

DBPM data (with average daily, day and night BP analysis) after pharmacogenetically combined treatment achieved the "threshold" in 154 (76.6%) patients (Table 2) that reliably did not differ from frequency of "target" office BP achievement – 149 (74.1%) persons. The type of treatment combination caused "target" BP_{24} (with DBPM) achievement in following rates: after HCTZ+ARB II combination – in 55 (91.7%) patients (better in II-genotype carriers of ACE gene ($p=0.019$), CC-genotype of AGTR1 gene ($p<0.001$), G-allele of eNOS gene ($p=0.002$), Ala-allele of PPAR-γ2 gene and GlyGly-genotype of ADRβ1 gene ($p<0.001$); after HCTZ+β1-AB combination – in 25 (73.,5%) persons (better in T-alelle carriers of eNOS gene ($p<0.001$), AlaAla-genotype of PPAR-γ2 gene and GlyGly-genotype of ADRβ1 gene ($p<0.001$); after HCTZ+ACEI – in 33 (66.0%) patients (better in TG-genotype carriers of eNOS gene ($p=0.016$), Ala-allele of PPAR-γ2 gene and GlyGly-genotype of ADRβ1 gene ($p<0.001$); after CCB+ARB II combination – in 11 (73.3%) persons, without a reliable difference between genotypes; after CCB+β1-AB – in 11 (73.3%) patients (easier in Ala-allele carriers of PPAR-γ2 gene ($p=0.002$); after CCB+ACEI – in 19 (70.4%) patients (easier in AlaAla-genotype carriers of PPAR-γ2 gene ($p=0.007$). These results did not differ significantly from the frequency of "target" BP achievement ($p>0.05$).

Table 2.

"Target" BP achievement (according to Daily Blood Pressure Monitoring data) in patients with essential arterial hypertension under pharmacogenetically determined treatment during 9-12 months depending on AH severities and type of treatment

Drugs combination	"Target" BP (DBPM)	BP (DBPM) below "threshold"			
		EAH I, n=60 (%)	EAH II, n=82 (%)	EAH III, n=59 (%)	Total, n (%)
HCTZ+ARB II, n=60 (%)	[1]BP_{24}	29 (48.3)	20 (33.3)	6 (10.0)	55 (91.7)
	[2]BPd	29 (48.3)	20 (33.3)	6 (10.0)	55 (91.7)
	[3]BPn	29 (48.3)	19 (31.7)	7 (11.7)	55 (91.7)
HCTZ+β_1-AB, n=34 (%)	[1]BP_{24}	10 (29.4)	10 (29.4)	5 (14.7)	25 (73.5)
	[2]BPd	10 (29.4)	9 (26.5)	5 (14.7)	24 (70.6)
	[3]BPn	9 (26.5)	10 (29.4)	5 (14.7)	24 (70.6)
HCTZ+ACEI, n=50 (%)	[1]BP_{24}	10 (20.0)	15 (30.0)	8 (16.0)	33 (66.0)
	[2]BPd	10 (20.0)	15 (30.0)	8 (16.0)	33 (66.0)
	[3]BPn	9 (18.0)	15 (30.0)	7 (14.0)	31 (62.0)
CCB+ ARB II, n=15 (%)	[1]BP_{24}	4 (26.7)	4 (26.7)	3 (20.0)	11 (73.3)
	[2]BPd	4 (26.7)	4 (26.7)	3 (20.0)	11(73.3)
	[3]BPn	4 (26.7)	4 (26.7)	3 (20.0)	11 (73.3)
CCB + β_1-AB, n=15 (%)	[1]BP_{24}	3 (20.0)	4 (26.7)	4 (26.7)	11 (73.3)
	[2]BPd	3 (20.0)	5 (33.3)	4 (26.7)	12 (80.0)
	[3]BPn	3 (20.0)	5 (33.3)	5 (33.3)	13 (86.7)
CCB + ACEI, n=27 (%)	[1]BP_{24}	4 (14.8)	9(33.3)	6 (22.2)	19 (70.4)
	[2]BPd	4(14.8)	9(33.3)	6(22.2)	19 (70.4)
	[3]BPn	4(14.8)	10 (37.0)	6(22.2)	20 (74.1)
Total, n (%)	[1]BP_{24}	60 (100.0)	62 (75.6)	32 (54.2)	154 (76.6)
	[2]BPd	60 (100.0)	62 (75.6)	32 (54.2)	154 (76.6)
	[3]BPn	58 (96.7)	63 (76.8)	33 (55.9)	154 (76.6)

Notes: 1. "Threshold" BP_{24} according to DBPM – average daily SBP and DBP <125-130 and 80 *mm* Hg, accordingly [*Guidelines of ESH/ESC, 2007*].
2. "Threshold" BPd – overage SBP and DBP in day time according to DBPM <130-135 and 85 *mm* Hg, accordingly [*Guidelines of ESH/ESC, 2007*].
3. "Threshold" BPn – overage SBP and DBP in night according to DBPM <120 and 70 *mm* Hg, accordingly [*Guidelines of ESH/ESC, 2007*].
4. DBPM – Daily Blood Pressure Monitoring.
5. HCTZ – hydrochlorothiazide; ARB II – angiotensin II receptor blocker; β_1-AB – β_1-adrenergic blocker; ACEI – angiotensin converting enzyme inhibitor; CCB – calcium channel blocker.
6. n (%) – number (percentage) of observations.

Pharmacogenetically combined treatment caused growth of patients' number with daily BP "dipper" profile to 75.6% *vs* 65.5% before treatment ($p<0.001$): among CC-genotype carriers of AGTR1 gene by 13.1% ($p=0.005$), TT-genotype of eNOS gene by 12.3% ($p<0.001$), AlaAla- and ProPro-genotypes of PPAR-γ2 gene by 20.0% ($p<0.001$) and 13.75% ($p=0.047$), accordingly; GlyGly-genotype of ADRβ1 gene by 25.8% ($p<0.001$). The number of patients with daily BP "non-dipper" profile diminished to 19.9% *vs* 26.5% before treatment ($p<0.01$): among D-allele carriers of ACE gene by 7.9% ($p=0.049$) and 8.7% ($p=0.005$), accordingly, CC-genotype of AGTR1 gene by 6.7% ($p<0.01$), T-allele of eNOS gene by 7.0% ($p=0.035$) and 9.8% ($p<0.01$), accordingly; Ala-allele of PPAR-γ2 gene by 13.3%

(p<0.001) and 8.0% (p=0.017), accordingly, and among GlyGly-genotype carriers of ADRβ1 gene by 6.4% (p<0.01). The number of patients with daily BP "night-peaker" profile decreased to 4.0% *vs* 7.2% before treatment, p=0.026: significantly only in TT-genotype carriers of eNOS gene (p=0.039).

DISCUSSION

Ideas regarding the efficacy of ACEI administration in EAH patients depending on I/D polymorphism of ACE gene are extremely contradictory. In Rotterdam Study a higher death rate (general and cardio-vascular) was revealed in EAH patients of ACE gene D-allele carriers, but just these patients gave the best response to ACEI therapy [*Bleumink G. et al., 2005*]. G.A. Stavroulakis and co-workers [*Stavroulakis G. et al., 2000*] also revealed certainly deeper SBP and DBP decrease especially in DD-genotype carriers under Fosinopril administration in the dose of 20 *mg/day* during 6 months. However, our results do not conform to some authors upshots, which did not reveal the hemodynamics changes after treatment with ACEI depending on I/D ACE gene polymorphism [*Sciarrone M. et al., 2003*], or someone set more reliable BP decline under treatment with ACEI or ARB II in EAH patients with II-genotype than in such with DD-genotype (p<0.05) [*Ohmichi N. et al., 1997; Kurland L. et al., 2001; Celuyko V.J., Peleckaya O.V., 2008*]. These were mostly patients with mild or moderate EAH without complications.

Our results showed better influence of HCTZ on SBP and DBP decrease in II-genotype carriers of ACE gene with the doubtful sensitiveness in patients with DD-genotype that conform to research data of M.T. Sciarrone and associates [*Sciarrone M. et al., 2003*], but partly agree with prospective cross double-blind pharmacogenetic GENERS Study results performed in Finland (n=233 men; 35-60 years old; patients with moderate EAH), where no clear dependence was proved on BP changes (office and DBPM) depending on polymorphism of α-adducin (G460W), AGT (M235T), ACE (I/D) and AGTR1 (1166 A/C) genes under 4-week treatment course with thiazide diuretic HCTZ (25 *mg*), Amlodipine (5 *mg*), Bisoprolol (5 *mg*) or Losartan (50 *mg*) [*Hiltunen T.P. et al., 2006*].

In 60% EAH patients administration of β1-AB in monotherapy does not cause an adequate antihypertensive response [*Mason D. et al., 1999; Humma L., Terra S., 2002*] that conforms to our results before pharmacogenetic correction [*Sydorchuk L. et al., 2009*]. D.A. Mason and co-workers [*Mason D. et al., 1999*] supposed that one of the reasons of low sensitivities to β1-AB is in genetic polymorphism of β-adrenergic receptors: Arg389 mutation of ADRβ1 gene accompanied by greater basal and agonists mediated adenylatecyclase activity in comparison with Gly-allele carriers. Therefore, probably, EAH patients especially with Arg389-allele gave 3 times better response (according to DBPM data) under treatment with Metoprolol during 4-weeks therapy, especially ArgArg-genotype carriers (BP decrease was by 13.3±8.4%), than such ones with GlyGly-genotype (by 4.5±8.2%) accordingly (p=0.018) [*Karlsson J. et al. , 2004*] that conforms to our results. These researches did not coincide with the results of K.M. O'Shaughnessy and colleagues [*O'Shaughnessy K. et al., 2000*], who did not discover any connections between BP and myocardial sizes diminishing in response to the Atenolol or Bisoprolol prescription depending on Arg389Gly polymorphism of ADRβ1 gene. We set the reliably better answer of D-allele carriers of ACE gene to monotherapy with β1-AB that correlates with the results of J Karlsson. and associates [*Karlsson J. et al., 2004*]; they explained that with the increase of blood/tissue concentration of angiotensin II and, accordingly, vegetative nervous system activity rise especially in DD-genotype carriers, but hereby DBP decline was significantly better in Insertion homozygote-carriers of ACE gene.

Thus, pharmacogenetic approaches in treatment of EAH patients enable to individualize treatment, promote its efficiency, increase sensitivity, and decrease the number of drugs side effects [*Cadman P., O'Connor D., 2003; Ioannidis J., 2009; Sydorchuk L.P. et al., 2008; 2010*].

CONCLUSION

Combined pharmacogenetically determined treatment during 9-12 months caused decrease of average daily, day and night BP (DBPM) below "threshold" in 154 (76.6%) patients that did not differ significantly from frequency of "target" office BP achievement – 149 (74.1%) persons. The number of patients with normal BP daily profile "dipper" increased reliably by 10.1% (p<0.001), with "non-dipper" and "night-peaker" patients amount diminishing (p<0.05).

Long-term combined therapy caused achievement of "target" average daily BP_{24} (DBPM) depending on types of treatment combination:
after HCTZ+ARB II – in 55 (91.7%) patients;
after HCTZ+β1-AB – in 25 (73.,5%) persons;
after HCTZ+ACEI – in 33 (66.0%) patients;
after CCB+ARB II – in 11 (73.3%) persons,

without a reliable difference between genotypes;
after CCB+β1-AB – in 11 (73.3%) patients (easier in Ala-allele carriers of PPAR-γ2 gene, p=0.002);
after CCB+ACEI – in 19 (70.4%) patients (easier in AlaAla-genotype carriers of PPAR-γ2 gene, p=0.007).

For EAH I-allele carriers' patients of ACE gene more effective is combination with HCTZ+ ARB II, than HCTZ+β1-AB or HCTZ+ACEI – 91.7% *vs* 66.0 or 73.5%, accordingly (p<0.001), for DD-genotype carrier patients more effective are combinations of CCB+ARB II and CCB+β1-AB than CCB+ACEI – 73.3% *vs* 70.4%, accordingly.

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