**Antihypertensive treatment effects on systemic inflammation, oxidative stress and proinflammatory cytokines**

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**Abstract**

Hypertension in its origin is a heterogeneous and multisystemic disease. Evaluatation of the oxidative stress activity based on the level of 8-iso-PgF2α, of proinflammatory cytokines based on tumor necrosis factor-α and its type I soluble receptor and C-reactive protein is relevant for further understanding of pathogenesis of hypertension and improvement of the early diagnostics of heart failure.186 hypertensive patients have been observed during the 2-months course of treatment, aged 30 to 65 years. The serum levels of 8-iso-PgF2α (n = 34), tumor necrosis factor-α and its type I soluble receptor were determined by ELISA before and after course of treatment. C-reactive protein level was determined by biochemical method. The control group included 16 practically healthy individuals, aged 27 to 55 years. Hypertensive patients enrolled into study had been randomized into 3 arms that received different protocols of combined anti-hypertensive therapy. I clinical group – а combination of a bisoprolol and indapamid, II – а combination of a lacidipine and candesartan, III – а combination of а fosinopril sodium and hydrochlorothiazide. On the background of combined antihypertensive therapy, we observed favorable dynamics of 8-iso-PgF2α, tumor necrosis factor-α and its type I soluble receptor and C-reactive protein. Taking into account the insignificance of the correlations revealed, a one-factor dispersion analysis was applied which allows to determine with certainty the influence of the grade and duration of hypertension on the dynamics of the studied indices levels. It has been found that the grade of hypertension is related to the increase in TNF-α and 8-iso-PgF2α serum levels, but not on to TNF-α type I soluble receptor, and the duration of hypertension is related to the increase in activity of the C-reactive protein, TNF-α and its type I soluble receptor, with no relation to the level of 8-iso-PgF2α. Thus, oxidative stress possibly promotes the activation of potentially damaging immune mechanisms mediated by pro-inflammatory cytokines, nonspecific inflammation and drives the further progression of the target organs lesions.

**Key words:** 8-iso-PgF2α; tumor necrosis factor-α; type I soluble receptor; C-reactive protein; blood pressure.

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**Introduction**

Hypertension occupies one of the leading places in the structure of cardiac pathology and presents a complex medical and social problem considering its high prevalence and early development of complications. Hypertension in its origin is a heterogeneous and multisystemic disease. In the last decade, the role of immune inflammatory activation mediated by proinflammatory cytokines, systemic inflammation and oxidative stress (OS) in the pathogenesis of cardiovascular diseases, including hypertension, has been actively studied (Bautista et al., 2005; Mahmud & Feel, 2005). The term "oxidative stress" (OS) is understood as a condition in which the amount of free radicals formed in the body is significantly higher than the activity of endogenous antioxidant systems that ensure their elimination (Kovaljova et al., 2005; Allison 2016). It has been suggested that OS and immune inflammatory changes participate in the pathogenesis of cardiovascular dysfunction, are interrelated and can induce each other forming a vicious circle (Kovaljova et al., 2015).

Among the proinflammatory cytokines, the tumor necrosis factor-α (TNF-α) deserves special attention in the context of hypertension. Firstly, this is due to the fact that, as shown by experimental and insignificant number of clinical studies, hemodynamic stress caused by increased blood pressure (BP) is one of the stimuli to increase production and release of pro-inflammatory cytokines, including TNF-α, into the bloodstream (Goldhaber et al., 1996; [Grainger](javascript:;), 2007). Secondly, due to existing data on the ability of this cytokine to modulate the structure and function of the cardiovascular system through a number of mechanisms. As an example, TNF-α is able to suppress myocardial contractility. This may be due to the blocking of β-adrenergic signals, reduction of the content of nitric oxide in the heart, or changes in intracellular calcium homeostasis (Goldhaber et al., 1996). TNF-α can also induce structural changes in the myocardium in patients with hypertension and chronic heart failure, such as cardiomyocyte hypertrophy and interstitial fibrosis (Kovalyova & Ashcheulova, 2003). In addition, TNF-α promotes apoptosis of cardiomocytes, and also activates metalloproteinases and depresses the expression of their inhibitors, thus promoting cardiac remodeling (Li et al., 2000; Haider et al., 2002) and eventually leading to cardiac dysfunction.

In healthy people, TNF-α is barely detectable in the serum. Its level increases with infection, under the influence of bacterial endotoxins (Vasan, 2006). There are two types of active receptors for TNF-α on the surface of almost all nuclear cell types that can virtually be cytokine targets. Soluble forms of receptors, which are considered endogenous inhibitors of TNF-α, are formed by separating the extracellular fragments of active receptors (Simbirtsev, 2013). TNF-α type I receptor is the main mediator of cytokine’s biological activity, therefore it was this type of soluble receptor (sTNF-α RI) that we selected for our study.

C-reactive protein (CRP) is a recognized marker of the acute phase of inflammation. With the advent of new highly sensitive techniques for its quantitative determination, it attracts increasing attention of cardiologists. This is due to the data on elevated CRP levels having possible predictive role for the development of the set of cardio- and cerebrovascular complications such as congestive heart failure, myocardial infarction, stroke, sudden cardiac death and peripheral vascular disease (Sproston & Ashworth, 2018). The prognostic value of a high CRP level in patients with stable angina pectoris, acute coronary syndrome and myocardial infarction has been shown (Melnikov et al., 2019; Melnikov et al., 2020). However, it should be noted that there were fewer reports on the diagnostic value of this systemic inflammation marker in essential hypertension, and there is insufficient evidence of the relationship between the CRP, TNF-α and 8-iso-PgF2α (8-isoprostane) as the main OS marker in patients with hypertension from the clinical studies.

According to current data detection of 8-isoprostane in the blood or urine is a sensitive method for evaluating the intensity of the OS and one of the most reliable and specific markers that allows to assess the level of free radicals production in the human organism in a wide spectrum of completely different diseases. 8-Isoprostane is a product of metabolism in reactions of peroxidation of arachidonic acid that is isomeric to prostaglandin F2α. Its level is proportional to the amount of free radicals formed. This substance belongs to the family of eicosanoids, that are a product of the non-enzymatic (free radical) oxidation of phospholipids of cellular biomembranes (Lawson et al., 1999; Cracowski et al., 2000). There is evidence (Greco et al., 2000; Czerska et al., 2016) of an increase in the level of 8-isoprostane in neurodegenerative diseases, coronary heart disease and hypertension. A significant increase in the level of 8-isoprostane has been observed in a number of conditions characterized by increased oxidative activity, particularly in tobacco smoking (Morrow et al., 1995), diabetes mellitus (Davi et al., 1999), hypercholesterolemia (Davi et al., 1997). Evaluatation of the OS activity based on the levels of 8-isoprostane, TNF-α and its soluble type I receptor and C-reactive protein might allow to reveal the correlations between the level of OS, immune activation and nonspecific inflammation in the human organism, which is relevant for further understanding of pathogenesis in hypertension and improvement of the early diagnostics of heart failure.

**The purpose** of thisresearch was to assess the activity of proinflammatory cytokines and C-reactive protein serum levels (as independent markers of systemic inflammation) in the context of oxidative stress development, depending on the severity and duration of hypertension, and their correction under the influence of combined antihypertensive therapy.

**Materials and methods**

The study was conducted in accordance with the current ethical requirements. The protocol of the study was approved at the meeting of the Committee of Bioethics of the Kharkiv National Medical University, Department of Propedeutics of Internal Medicine No. 2 and Nursing Care. The informed consent was obtained from all participants of the study. 202 subjects have been examined at the in-hospital setting, including 186 patients with essential hypetension of grades 1 to 3 who had asymptomatic target organs lesions (left ventricular hypertrophy/generalized retinal angiopathy/carotid intima-media thichening or presence of atherosclerotic plaques/microalbuminuria), aged 30 to 65 mean 54.7 ± 5.8 years, who previously have not been receiving regular antihypertensive therapy. The control group included 16 practically healthy individuals (8 male and 8 female), aged 27 to 55 mean 43.7 ± 4.2 years, without cardiovascular, renal and endocrine anamnesis. Study participants included 149 female and 37 male patients, with the mean duration of disease of 10 years.

European Society of Cardiology (ESC) / European Society of Hypertension (ESH) (2018) were used for verification of the diagnosis and estimation of the hypertension grade. Exclusion criteria were: secondary hypertension, associated inflammatory and endocrine disorders (including diabetes mellitus) as well as other conditions that could have an impact on the activity of oxidative processes, including smoking anamnesis. Patients receiving statin therapy were not included into study; statin-naïve patients who had indications for lipid-lowering therapy had been prescribed such after completion of the 2-month course of treatment. Of the patients enrolled into study, 33 have been diagnosed with essential hypertension of grade 1, 58 – of grade 2, and 95 – of grade 3.The level of 8-isoprostane as a main marker of OS have been assessed in 34 patients, including 27 females and 7 males. Of this sub-group, 8 patients have been diagnosed grade 1 hypertension, 8 – of grade 2, and 18 – of grade 3.

The contents of serum 8-isoprostane, TNF-alpha and its type I soluble receptor (sTNF-αRI) were determined in all subjects using the 8-isoprostane ELISA ("US Biological", USA), ProCon TNFα ("Protein contour", Russian Federation) and sTNF-αRI EASIA ("BioSource Europe S.A", Belgium) ELISA kits, respectively. The serum level C-reactive protein (CRP) was determined using the test-system for quantification of CRP ("Ukrmedservice Ltd.", Ukraine).

Hypertensive patients enrolled into study had been randomized into 3 groups that received different protocols of combined anti-hypertensive therapy. The aforementioned parameters were reassessed after 2 months of treatment. I clinical group, n = 102: a combination of a β-adrenoblocker (BAB) and diuretic (D) (bisoprolol 2.5–10.0 mg/day, and indapamid 1.5–2.5 mg/day). The daily dose of bisoprolol was administered by continuous slow titration, starting with low doses of 1.25 mg/day. Gradually the dose was increased to the maximum tolerated or target under the control of clinical parameters, especially blood pressure and heart rate (HR). II clinical group, n = 30: a combination of a calcium channel blocker (CCB) and angiotensin receptor blocker (ARB) (lacidipine 2 mg, 4 mg and candesartan 4 mg, 8 mg, 16 mg). III clinical group, n = 54: a combination of an angiotensin-converting enzyme (ACE) inhibitor and diuretic (D) (fosinopril sodium 20 mg/day and hydrochlorothiazide 12.5 mg/day). The level of 8-isoprostane was assessed in 10, 14, and 10 patients of the three clinical groups, respectively. The dose of medications was individually up-titrated in cases of need during the course of treatment. As one can see (Table 1) different clinical groups were comparable in terms of age, gender structure and clinical course of hypertension.

**Table 1**

**Comparative characteristic of clinical groups of patients with hypertension**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **I clinical group**  **(n = 102)** | **II clinical group**  **(n = 30)** | **III clinical group**  **(n = 54)** |
| Sex  Male, n  Female, n | 20  82 | 6  24 | 11  43 |
| Age, years | 54.5 ± 5.4 | 54.7 ± 5.8 | 54.5 ±5.4 |
| Hypertension history, years | 9.6 ± 0.7 | 10.7 ± 1.4 | 9.7 ± 1.4 |
| Hypertension grade, %  1  2  3 | 17.6  28.4  54.0 | 23.3  26.7  50.0 | 14.8  38.8  46.4 |
| New York Heart Association class, %  0  I  II  III | 11.9  19.6  65.6  2.9 | 16.7  20  60  3.3 | 16.6  22.2  59.3  1.9 |

The results were statistically processed using Statistica 6.0 and 7.0 (StatSoft Inc., USA). Statistically average values and their standard deviation are shown in the text and tables (х ± SD). The intergroup analysis was performed using one-way analysis of variance (ANOVA) with the calculation of the Fisher test (F); Wilcoxon T-test was used for paired intragroup analysis, considered significant at P < 0.05.

**Results**

Analysis of the proinflammatory cytokines activity has shown a significant increase in the level of TNF-α in patients with hypertension compared to control: 187 ± 18.1 and 13.2 ± 3.40 pg/mL, respectively (P < 0.001). A similar tendency was observed with respect to soluble fractions of the receptors for TNF-α. The value of sTNF-αRI for hypertension also exceeded that of the control group: 2.14 ± 0.28 and 1.20 ± 0.60 ng/mL, respectively (P < 0.001). An increase in the serum 8-isoprostane was also found in patients with hypertension compared to healthy controls: 17.2 ± 3.12 and 1.41 ± 0.25 pg/mL, respectively (P < 0.05). The level of 8-isoprostane in the presence of hypertension was 12.2 times compared to control group. Considering the available data on the importance of CRP as a marker of systemic inflammation in cardiovascular pathology and a predictor of the development of heart failure (HF) and hypertension, we have assessed the levels of serum CRP in patients with hypertension and revealed the values exceeding control group mean 6.23 ± 0.33 mg/L.

In order to elucidate the influence of not only the presence, but also the grade of increase in blood pressure on the expression of TNF-α, sTNF-αRI, CRP, and also to evaluate the presence of oxidative stress and the degree of its manifestation, all patients were divided into groups depending on the grade of hypertension (Table 2).

**Table 2**

**Levels of TNF-α, sTNF-αRI, CRP, and 8-isoprostane in patients with varying the grade of hypertension (x ± SD)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Сontrol group | Patients with hypertension | | |
| 1 grade | 2 grade | 3 grade |
| TNF-α, pg/mL | 13.2 ± 3.4 | |  |  | | --- | --- | | 112 ± 26.1\* |  | | |  | | --- | | 142 ± 27.2\*\*♦ | | 117 ± 18.5\* |
| sTNF-αRI, ng/mL | 1.20 ± 0.6 | 2.02 ± 0.22\* | 2.13 ± 0.16\* | 2.19 ± 0.08\* |
| 8-isoprostane, ng/mL | 1.41 ± 0.25 | 4.48 ± 0.55\*\*\* | 10.0 ± 0.99\*\*\*♦♦♦ | 25.9 ± 2.87\*\*\*♦♦♦ ●● |
| CRP mg/L | 6.23 ± 0.33 | 6.25 ± 0,57 | 6.01 ± 0.77 | 6.36 ± 0.45 |

Note: \* – P < 0.05, \*\* – P < 0.01, \*\*\* – P < 0.001 vs control group; ♦ – P < 0.05, ♦♦ – P < 0.01, ♦♦♦ – P < 0.001 vs patients with the 1 grade hypertension; ● – P < 0.05, ●●  – P < 0.01, ●●●– P < 0.001 vs patients with the 2 grade hypertension.

Unlike in TNF-α and 8-isoprostane, the CRP levels did not significantly differ between groups. As can be seen from Table 2, in patients with hypertension, a 3.2–fold, 7.1–fold and 18.4–fold increase (grades 1, 2, and 3, respectively) in the 8-isoprostane serum content was observed compared to the control group. When comparing the levels of 8-isoprostane in patients with hypertension, depending on the level of blood pressure, it was revealed that the concentration of 8-isoprostane in the blood serum increased as the disease progressed: in patients with grade 3 hypertension, it exceeded 5.8 times the same index in patients with grade 1 and 2.6 times – in those with grade 2.

The levels of the studied parameters, namely of TNF-α, sTNF-αRI, 8-isoprostane and CRP in the examined patients change not only depending from the grade hypertension, but on the duration of hypertension (Table 3).

**Table 3**

**Levels of TNF-α, TNF-αRI, CRP, and 8-isoprostane in patients with different duration of hypertension, yrs (x ± SD)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Сontrol group | Patients with duration of hypertension, yrs | | |
| < 5 | 5-10 | > 10 |
| TNF-α, pg/mL | 13.2 ± 3.4 | |  |  | | --- | --- | | 108 ± 23.0\* |  | | |  | | --- | | 128 ± 25.8\*♦ | | 145 ± 28.4\*\*♦ ● |
| sTNF-αRI, ng/mL | 1.20 ± 0.60 | 2.08 ± 0.21\* | 2.14 ± 0.18\* | 2.20 ± 0.19\* |
| 8-isoprostane, ng/mL | 1.41 ± 0.25 | 9.17 ± 1.72\*\*\* | 19.3 ±4.09\*\*\*♦♦♦ | 14.9 ± 3.79\*\*\*♦ |
| CRP mg/L | 6.23 ± 0.33 | 6.08 ± 0.65 | 6.28 ± 0.51 | 6.38 ± 0.59 |

Note: \* – P < 0.05, \*\* – P < 0.01,\*\*\* – P < 0.001, vs control group; ♦ – P < 0.05, ♦♦ – P < 0.01, ♦♦♦ – P < 0.001 vs patients with duration of hypertension < 5 years; ● – P < 0.05, ●● – P < 0.01, ●●● – P < 0.001 vs patients with duration of hypertension 5-10 years.

Rank correlation analysis has been performed to determine the relationship between blood pressure and levels of TNF-α, CRP and 8-isoprostane. The relationship between the grade of hypertension and the change in the levels of the studied cytokines was as follows: the correlation coefficient (r) for the TNF-α blood level was 0.204 (P = 0.25), for sTNF-αRI, r = - 0.01 (P = 0.97), for 8-isoprostane r = 0.11 (P = 0.94), for CRP r = 0.02 (Р = 0.44). Correlation coefficients with the duration of hypertension were: for TNF-α r = 0.24 (P = 0.17), for sTNF-αRI r = 0.17 (P = 0.53), for 8-isoprostane r = 0.01 (P = 0.95), for CRP r = 0.32 (Р = 0.74). Taking into account the insignificance of the correlations revealed, a one-factor dispersion analysis was applied which allows to determine with certainty the influence of the grade and duration of hypertension on the dynamics of the studied indices levels. It was found that the grade of hypertension is related to the serum levels of TNF-α and 8-isoprostane, (F = 9.58, Р = 0.002 and F = 8.34, Р = 0.004 respectively), but not sTNF-αRI and CRP, and the duration of hypertension is related to the levels of TNF-α, sTNF-αRI and CRP (F = 6.72, Р = 0.003; F = 2.34, Р = 0.006; F = 9.96, P = 0.002 respectively), with no effect on the level of 8-isoprostane. Analysis of the relationships between the levels of 8-isoprostane, TNF-α and sTNF-αRI has shown no significant correlations.

In the first group of patients on the background of treatment (Table 4) with bisoprolol and indapamide, the mean levels of TNF-α significantly decreased to 70.2 pg/mL compared to baseline before treatment, showing a 61.0% reduction. The mean levels of soluble sTNF-αRI, which is a natural inhibitor of TNF-α, increased to 0.24 ng/mL (11.1%) as a result of treatment. A significant decrease in the ratio of TNF-α sTNF-αRI at 34.4 (64.9%) indicated a predominant increase in the level of sTNF-αRI along with a decrease in TNF-α. Since soluble forms of receptors act as natural antagonists of TNF-α, a decrease in this ratio reflects suppression of autoimmune and apoptotic activity in patients as, a result of treatment.

**Table 4**

**Dynamics of the cytokines, CRP and 8-isoprostane in the course of combined antihypertensive therapy in the observed patients(x ± SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | I group | II group | III group |
| TNF-α, pg/mL, prior to treatment | 115 ± 19.6 | 133 ± 22.6 | 78.2 ± 23.7 |
| TNF-α, pg/mL, after 2 months treatment | 44.8 ± 8.21\*\*\* | 45 ± 5.63\*\*\* | 40.6 ± 15.8\*\*\* |
| sTNF-αRI, ng/mL, prior to treatment | 2.17 ± 0.12 | 2.10 ± 0.16 | 2.25 ± 0.21 |
| sTNF-αRI, ng/mL, after 2 months treatment | 2.41 ± 0.03\* | 2.63 ± 0.53\* | 2.38 ± 0.19 |
| TNF-α/ sTNF-αRI, prior to treatment | 53 ± 17.4 | 63.3 ± 19.3 | 34.8 ± 12.2 |
| TNF-α/ sTNF-αRI, after 2 months treatment | 18.6 ± 7.8\*\* | 17.1 ± 6.4\*\* | 17.1 ± 6.6\*\* |
| 8-isoprostane, ng/mL, prior to treatment | 20.5 ± 17.4 | 12.7 ± 9.63 | 20.2 ± 11.9 |
| 8-isoprostane, ng/mL, after 2 months treatment | 12.3 ± 7.27\* | 2.42 ± 1.49\* | 10.2 ± 7.61\* |
| CRP mg/L, prior to treatment | 5.86 ± 0.48 | 6.81 ± 0.59 | 6.3 ± 0.49 |
| CRP mg/L, after 2 months treatment | 3.85 ± 0.36 | 3.68 ± 0.38\* | 5.14 ± 0.42 |

Note: \* – p < 0.05, \*\* – p < 0.01, \*\*\* – p < 0.001 vs levels prior to treatment

In the second group (Table 4) of patients, on the background of treatment with a combination of lacidipine and candesartan, the mean TNF-α decreased by 88 pg/mL (66.2%) compared to the baseline before treatment. With respect to sTNF-αRI, the reverse trend was observed, that is, an increase in its average level by 0.53 ng/mL (25.2%) as, a result of treatment. A 73.0% decrease in TNF-α/sTNF-αRI ratio reflected a significant decrease in the level of autoimmune activation under the influence of 10-week ARB + CCB therapy. In the third group of patients, the combination of fosinopril sodium with hydrochlorothiazide significantly reduced the mean TNF-α by 37.6 pg/mL (48.1%). With respect to sTNF-αRI an insignificant increase in the mean level of sTNF-αRI as, a result of treatment, by 0.13 ng/mL (5.8%). The value of TNF-α/sTNF-αRI ratio decreased by 17.7, or approximately twice, by 50.9%, compared to baseline before treatment, which also reflected a decrease in the level of autoimmune activation under the influence of therapy.

Analyzing the obtained data with respect to the level of 8-isoprostan after 2 months from the start of therapy on the background of treatment in the third group patients, its level decreased by 50% (2-fold) from the initial level. In the second group of drugs, the reduction in serum levels of 8-isoprostane by 80.9% (5.2-fold) was observed after 2 month of treatment compared with the baseline. In the first group of patients, the level of 8-isoprostane also decreased by 40% (1.7-fold) from the initial level after 2 month of therapy. Thus, different protocols of combined therapy with beta-adrenoblocker plus diuretic (bisoprolol and indapamide) and ACE inhibitor plus diuretic (fosinopril and hydrochlorothiazide) expressed similar effect on the dynamics of 8-isoprostane levels during 2 months of treatment. Analyzing the dynamics of CRP on the background of combined treatment, we obtained the following data: the mean values of CRP in blood plasma decreased by 2.01 mg/L (34.3%), by 3.13 mg/L (46.0%) by 1.16 mg/L (18.4%) as, a result of therapy in the I, II and III clinical groups, respectively.

Under the influence of combined antihypertensive therapy with all the three schemes, there was an improvement in clinical status, presented by a decrease in the intensity and frequency of headache, dizziness, pain in the heart area, fatigue, and an increase in exercise tolerance as a result significant reduction in blood pressure (Table 5). All patients who received treatment with one of the combined anti-hypertensive therapy regimens were discharged from the hospital (on the 10th -14th day of treatment) in a satisfactory condition.

**Table 5**

**Blood pressure and heart rate dynamics during the course of treatment (x ± SD)**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | I group | II group | III group |
| Systolic blood pressure prior to treatment | 168.3 ± 26.6 | 176.0 ± 27.2 | 180.2 ± 28.6 |
| Systolic blood pressure after 2 weeks of treatment | 129.5 ± 11.9\*\*\* | 131.6 ± 11.0\*\*\* | 132.6 ± 9.7\*\*\* |
| Diastolic blood pressure prior to treatment | 100.8 ± 12.7 | 105.2 ± 14.1 | 107.1 ± 11.3 |
| Diastolic blood pressure after 2 weeks of treatment | 75.5 ± 6.3\*\*\* | 75.3 ± 5.0\*\*\* | 76.5 ± 5.8\*\*\* |
| Average BP blood pressure prior to treatment | 123.3 ± 16.0 | 128.8 ± 17.6 | 131.3 ± 15.7 |
| Average BP blood pressure after 2 weeks of treatment | 93.4 ± 6.5\*\*\* | 93.9 ± 6.2\*\*\* | 95.0 ± 5.7\*\*\* |
| Pulse blood pressure prior to treatment | 67.5 ± 19.9 | 71.2 ± 18.0 | 73.1 ± 22.2 |
| Pulse blood pressure after 2 weeks of treatment | 54.0 ± 11.8\*\*\* | 55.9 ± 10.6\*\*\* | 55.6 ± 10.1\*\*\* |
| HR prior to treatment | 83.4 ± 11.9 | 74.2 ± 9.2 | 78.4 ± 12.1 |
| HR after 2 weeks of treatment | 75.3 ± 6.2\* | 76.7 ± 4.8 | 73.1 ± 6.6\* |

Note: \* – p < 0.05; \*\* – p < 0.01; \*\*\* – p < 0.001; vs levels prior to treatment

In patients treated with bisoprolol and indapamide, a reduction of the mean values of office systolic blood pressure (SBP) by 38.8 mm Hg, office diastolic blood pressure (DBP) by 25.3 mm Hg, average BP by 29.8 mm Hg, pulse BP by 13.5 mm Hg, during the period of inpatient treatment has been observed (23.1%, 25.1%, 24.2%, and 20.0% respectively, compared to the prior to treatment) (Table 5). In the group of patients who took candesartan with lacidipine, there were similar changes during the inpatient treatment in the mean values of the office SBP by 44.5 mm Hg, office DBP 29.9 mm Hg, mean BP by 34.8 mm Hg, pulse BP by 15.2 mm Hg (25.3%, 28.4%, 27.1% and 21,4% respectively, compared to the prior to treatment). In the third group, treated by fosinopril sodium with hydrochlorothiazide during the period of inpatient treatment, there was a decrease in the average values of office SBP by 47.6 mm Hg, office DBP by 30.6 mm Hg, mean BP by 36.3 mm Hg, pulse BP by 17.5 mm Hg (26.4%, 28.6%, 27.6% and 24.0% respectively, compared to the prior to treatment). Thus, there was a significant decrease in both the office SBP and DBP and, accordingly, mean BP and pulse BP in patients, regardless of the group of drugs they took. The level of blood pressure reduction reached the target for both SBP and DBP.

There was also a change found in the average heart rate during the inpatient treatment period: a decrease in the heart rate in patients taking bisoprolol with indapamide and fosinopril with hydrochlorothiazide by 8 bpm (9.6%) and by 5 bpm (6.7%), respectively. Conversely, patients of the II group tended to increase HR by 3 bpm (3.4%).

**Discussion**

The mean value of TNF-α was 14.2 times higher than the control values, suggesting the possible role of hemodynamic stress as one of the stimuli for increasing synthesis and release of pro-inflammatory cytokines, in particular TNF-α, into the circulation (Grander, 2004; Azra & Feel, 2005; Bautista et al., 2005). The mean value of sTNF-αRI in patients with increased TNF-α activity, likely, due to increased blood pressure was 1.78 times, or 78.3% higher, compared to normal subjects.

Numerous epidemiological studies have shown a relationship between tachycardia and hypertension. Increasing heart rate is associated with many risk factors, including dyslipidemia, hyperinsulinemia, obesity, and elevated hematocrit. Tachycardia is a marker of increased activity of the sympathetic nervous system. It is known that the decrease in heart rate correlates with longer life in mammals. Moreover, ß-blockers affect the renin-angiotensin-aldosterone system (RAAS), inhibiting the release of renin by blockade of ß 1 -receptors in juxtaglomerular apparatus of the kidneys. Bisoprolol is a β-blocker with a high degree of cardioselectivity, from which this value is 1:75. Bisoprolol does not effect the level of atherogenic fractions of lipids (triglycerides, cholesterol, low-density lipoproteins) in the long reception (Ostroumova & Maksimov, 2012; Knjaz’kova, 2013). The combination of bisoprolol with indapamid is able to improve endothelial function due to the presence of its antioxidant properties. An important role in this combination is played by indapamid, which, unlike other diuretics has a direct vasodilator action. (London, 2004)

Lacidipine occupies a special place among the CCB, showing the most pronounced antiatherosclerotic properties. Its antioxidant activity is comparable to one of vitamin E and exceeds that of all other CCB (Svischenko, 2002). Lacidipine forms a stronger bond to the cell membrane compared to the other CCB. This determines its high local concentration in the blood vessels tissues (to which it is highly selective), which is particularly important in conditions of hypercholesterolemia, when the cell membrane’s availability to CCB is decreased due to high cholesterol load. Lacidipine’s binding to the cell membrane remains strong even in described conditions, being significantly higher than other CCB’s (Herbette, 1995). The tight bond with the membrane defines a longer duration of lacidipine action and its ability to intervene in the majority of intracellular processes that take place in the early stages of atherogenesis. The main role in the action of the drug on various links of the atherosclerotic process is given to its high vascular selectivity and the ability to tightly bind to the cell membrane in conjunction with an intense antioxidant effect and the ability to optimize the calcium homeostasis. Importantly, the anti-atherogenic effect of lacidipine is not determined by its antihypertensive effect: it is observed even when using lacidipine in low doses that do not affect blood pressure (Svischenko, 2002).

Fosinopril is the only angiotensin-converting enzyme (ACE) inhibitor that retains the phosphinic acid residue in its chemical formula. Fosinopril - an ester that is hydrolyzed in the body by the action of esterases in the active communication of fosinoprilat (Ageev & Mareev, 2000; Sirenko & Rekovec, 2005). Fosinopril due to its specific connection phosphinate group of ACE prevents conversion of angiotensin I to angiotensin II, resulting in vasopressor activity and aldosterone secretion reduced (Preobrazhenskij & Savchenko, 2000; Dzjak et al., 2005;). Fosinopril is de-esterified by the liver or gastrointestinal mucosa and is converted to its active form, fosinoprilat. Fosinoprilat competitively binds to ACE, preventing ACE from binding to and converting angiotensin I to angiotensin II. Inhibiting the production of AII lowers peripheral vascular resistance, decreases afterload, and decreases blood pressure, thus helping to alleviate the negative effects of AII on cardiac performance. The second mechanism of action of ACE-inhibitors on endothelial function is their ability to prevent the splitting of bradykinin (Greene, 2000). The ACE is identical to the kinase II enzyme, which causes the conversion of bradykinin to an inactive state. Bradykinin is a potent stimulator for the release of endothelium-dependent relaxing factors such as nitric oxide, endothelium hyperpolarization factor and prostacyclin (PGI2).

The advantage of fosinopril is the balanced double way of isolation – slightly more than half of the total volume of the drug (54.0%) is excreted from the body by renal excretion with urine, the rest (46.0%) by hepatic degradation of active metabolites with their sequential release through the gastrointestinal tract. It is important to note that the decrease in renal filtration increases proportionally hepatic route of excretion of the drug, and on the contrary, in liver disease - increase the contribution of renal excretion. Actually, this pharmacokinetic feature of fosinopril is the basis of the important clinical recommendations: as in renal and hepatic insufficiency additional correction doses of fosinopril is not usually required (Ageev & Mareev, 2000; Karpov, 2001). This characteristic of fosinopril makes the drug a safer choice than other ACE inhibitors for heart failure patients with impaired kidney function resulting from poor perfusion as fosinopril can still be eliminated by the liver, preventing accumulation of the drug in the body.

The results of this study allow to suggest that in individuals with grade 1 hypertension, the detachment of extracellular parts of sTNF-αRI (2.02 ± 0.22 ng/mL) at high levels of the circulating cytokine (112 ± 26.11 ng/mL) can possibly be regarded, as an adaptive mechanism that, on one hand, reduces the number of active receptors on the surface of target cells, and on the other hand, might neutralize the bioactivity of TNF-α through the soluble receptor forms (sTNF-αRI) binding with the latter. In patients with grade 2 hypertension, a parallel increase in sTNF-αRI (2.13 ± 0.16 ng/mL) was probably not enough to neutralize the negative effect of TNF-α (142 ± 27.2 ng/mL), as could be evidenced by a further increase in the TNF-α/sTNF-αRI ratio (66.7 vs 55.4 in grade 1 hypertension and 11.0 in normotensive individuals). At the maximal levels of blood pressure the levels of TNF-α were decreasing (117 ± 18.5 ng/mL), which possibly could be due to its binding to sTNF-αRI. On the other hand, we can suggest the possibility of TNF-α triggering an apoptotic cascade, which leads to the death of cells producing the cytokine. The further increase in sTNF-αRI on the background of a decrease in TNF-α has a positive significance. The fact that the TNF-α/sTNF-αRI value was 53.4, with the TNF-α levels also still exceeding those in grade 1 hypertension and healthy individuals, could indicate that such a level of sTNF-αRI was unable to inactivate TNF-α cytotoxicity.

Thus, according to single-factor dispersion analysis, there was an increase in the level of CRP in the blood serum of patients with hypertension. This increase depended only on the duration of hypertension, but not on the grade of increase in blood pressure; there was also no significant correlation between CRP and BP, possibly being consistent with existing data on the definition of CRP as an independent risk factor for hypertension. In particular, an increased level of CRP has been shown in normotensive individuals who have developed essential hypertension in the future. The increased level of CRP is related to the deterioration of endothelium-dependent relaxation, posing the potential risk of developing hypertension. In the hypertensive population, it is established that CRP is an independent predictor of atherosclerosis progression, more significant than pulsatile and systolic blood pressure. Our findings suggest that elevated CRP levels can be considered as a marker of systemic inflammation in hypertensive patients (Bautista, 2001; Onat et al., 2008).

Taking into account the correlations revealed, this may indicate the role of OS in the pathogenesis of hypertension as a damaging mechanism that promotes the activation of immune mechanisms, nonspecific inflammation and the further progression of the disease. OS causes damage to the vascular endothelium, and the main disorders in the vascular wall that are characteristic of hypertension are endothelial dysfunction and hypertrophy of the smooth muscle cells.

**Conclusions**

The obtained data allows to suggest the possible role of oxidative stress as one of the important pathogenetic mechanisms in hypertension, manifested in this study as an increase in the content of serum 8-isoprostane compared to practically healthy individuals. Thus, oxidative stress possibly promotes the activation of potentially damaging immune mechanisms mediated by pro-inflammatory cytokines, nonspecific inflammation and drives the further progression of the target organs lesions.

An increase in circulating TNF-α, sTNF-αRI, CRP and 8-isoprostanelevels supports data on involvement of immune activation and oxidative stress in hypertension. It was found that the grade of hypertension influences the increase in the blood levels of TNF-α and 8-iso-PgF2α, but not on of sTNF-αRI, and the duration of hypertension affects the activity of the C-reactive protein, TNF-α and the content of sTNF-αRI, with no effect on the level of 8-iso-PgF2α.

The data obtained in the course of 2-months treatment testifies to anti-inflammatory and anti-apoptotic effects of the combined antihypertensive therapy. The most pronounced result was observed in individuals receiving combined therapy with lacidipine plus candesartan and bisoprolol plus indapamide.

It is important that further studies are made to assess the activity of proinflammatory cytokines, namely tumor necrosis factor-α and its type I soluble receptor, the level of C-reactive protein, as an independent marker of systemic inflammation in relation to the development of oxidative stress, by the content of 8-isoprostane, involved large study populations with long follow-up times. The results of advanced research will help in the formation of pathogenetically caused and prognostically significant antihypertensive therapy regimens.

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