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Oxidative modifications caused by free radicals in hypertension

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ACE inhibitors are among the main groups of antihypertensive medications. Lysinopril is a synthetic peptide that competitively binds and inhibits the angiotensin converting enzyme. Oxidative stress (OS) is a key factor in the molecular mechanisms associated with cardiovascular and renal diseases associated with hypertension. Moreover, the hypertension, by itself, can also contribute to oxidative stress increasing. The aim of the study is to evaluate the role of OS in vascular pathology and its effect on the antihypertensive effect of ACE inhibitors in moderate-grade AH patients. For this purpose we investigated the ascorbic radicals, ROS products and ·NO radicals as oxidative stress biomarkers. Oxidative stress was determined in 82 people with arterial hypertension receiving regular antihypertensive therapy and 20 healthy volunteers. Patients treated with Lisinopril (n=41) were compared with a combined treatment group (Lisinopril and Bisoprol, n=21) and group treated with a Valsartan (n=20). For this purpose were Electron Paramagnetic Resonance (EPR) methods. Oxidative stress is increased in patients with essential hypertension and its role in the pathophysiology of the disease is possible. Regarding the real time biomarkers of OS, the therapeutic advantage in essential hypertension is use of ACE inhibitor in front of angiotensin-receptor blocker.

Key words: oxidative stress, arterial hypertension, ROS products, NO radicals, Asc radicals

INTRODUCTION

Arterial hypertension (AH) is widespread throughout the world, and degrades the quality of life of the patients and increases cardiovascular morbidity and mortality [1]. Therefore, questions about the prophylaxis and treatment of hypertension are of paramount importance for clinical practice and the scientific world. Among the most successful treatment options for arterial hypertension is the use of an ACE inhibitor, but a large proportion of patients fail to reach the blood pressure norms according to global cardiovascular risk, despite the patient's good affiliation [2]. Reasons for therapeutic tolerance have not been fully elucidated. Among the leading theories are alternative angiotensin II-forming pathways (with a key role of mast cell protease-chymase), genetic polymorphisms, resulting in a different therapeutic effect in patients, conditions that potentiate free-radical initiation of endothelial dysfunction and many others [3]. The exact cause of lack of therapeutic effect in some patients remains unclear. The cardio- and reno-protective properties of ACE inhibitors make them an excellent first choice for pharmacologically influencing hypertension in patients with associated risk factors and direct our efforts in seeking the causes and decreasing therapeutic resistance to this class of medication [4]. Under normal physiological conditions in the human body, there is a delicate balance between ROS/RNS production and elimination from protective antioxidant systems [5]. High levels of ROS/RNS or inadequate removal from cellular defense mechanisms results in oxidative stress that can cause cellular damage to all major components - DNA, proteins, lipids.

The aim of the study is to evaluate the role of oxidative stress in vascular pathology and its effect on the antihypertensive effect of ACE inhibitors in moderate-grade AH patients. For this purpose we investigated the ROS products levels, ascorbate and ·NO radicals as real time oxidative stress biomarkers by using Electron paramagnetic resonance (EPR) spectroscopy.

EXPERIMENTAL

MATERIALS AND METHODS

All chemicals used in this study were of analytically grade and purchased from Sigma-Aldrich Chemie GmbH (Germany). Spin-traps N-*tert*-butyl-α-phenylnitrone (PBN) and 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazole-1-oxyl-3-oxide (Carboxy-PTIO.K), were purchased from Sigma Chemical Co, St. Louis, USA.

In our study were included 82 male patients with arterial hypertension receiving regular antihypertensive therapy with primary arterial hypertension, passed through a preventive surgery of the University Hospital "Prof. Dr. Stoyan

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Kirkovich", ambulatory patients from the Stara Zagora region with arterial hypertension. All studied parameters were compared with 20 healthy male controls.

The patients were with proven primary arterial hypertension and history of at least 6 months with Arterial Pressure to Grade II (systolic Arterial Pressure 160-179 and/or diastolic Arterial Pressure 100-109). The plasma fasting glucose levels were up to 6.9mmol/l. Patients were diagnosed and treated by a general practitioner or cardiologist. Informed consent was obtained from all PD patients and healthy volunteers enrolled in this study, according to the ethical guidelines of the Helsinki Declaration (1964).

The choice of angiotensin-converting enzyme (ACE) inhibitor, angiotensin-receptor blocker and combination therapy is made by the treating physician, in accordance with relevant guidelines of the European Cardiology Society. Patients, according to the type of received therapy, were divided into three groups: patients (n=41) with ACE inhibitor monotherapy (Lisinopril 5-10mg/per day), patients (n=21) with combined antihypertensive treatment (Lisinopril 5-10mg /per day + bisoprolol 5mg/per day) and patients (n=20) on therapy with angiotensin-receptor blocker (ARB-valsartan 160mg). Synthetic antioxidants have been aborted as a dietary supplement. Each participant had normal physical activity and a desire for healthy eating. Fasting samples of venous blood were collected in the morning between 8.00 and 10.00 a.m. Blood for determination of ·NO and ROS products was collected in tubes containing 10% EDTA (ethylene-diamine-tetraacetic acid). All samples from each subject were split and run in triplicate.

Table 1. Age distribution of studied patients for oxidative stress parameters

Gender	N	Mean	Std. Deviation	Std. Error Mean
Patients (male)	82	56.4	± 13.05	± 1.6
Controls (male)	20	49.1	± 6.98	± 2.2

The comparison between the two groups by t-test showed p = 0.088.

Table 2. The mean age of the patients studied for the parameters of the oxidative stress in groups, depending on the type of therapy is:

Therapy	N	Mean	Std. Deviation	Std. Error Mean
ACE inhibitor	41	52.2	± 11.5	± 1.8
Angiotensin-receptor blocker (β blocker)	20	63.7	± 11.6	± 3.7
Combined therapy (ACE+β blocker)	21	67.5	± 12.1	± 4.04

The comparison between the individual groups by t-test shows that ACE inhibitor vs Angiotensin-Receptor Blocker p=0.007, ACE inhibitor vs Combined therapy p=0.001, and Angiotensin-Receptor Blocker vs Combined therapy p=0.489.

Ex vivo electron paramagnetic resonance (EPR) study

EPR measurements were performed at 22ºC temperature on an X-band EMXmicro, spectrometer Bruker, Germany. The experiments were carried out in triplicate and repeated thrice. Spectral processing was performed using Bruker WIN-EPR and Sinfonia software.

Ex vivo evaluation of the levels of ascorbate radicals

The blood plasma from patients and volunteers were prepared according Bailey et al [6]in DMSO in a ratio of 1:3. After centrifugation the supernatants were collected and immediately transferred into a quartz tubes and placed in EPR cavity.

Ex vivo evaluation of the levels of ROS products

The ROS levels were determined according to [7] with modification. To investigate in real time ROS formation in the plasma of patients and controls was used *ex vivo* EPR spectroscopy combined with PBN as a spin-trapping agent.

Ex vivo evaluation of the levels of ·NO radicals

Based on the methods published by Yoshioka et al. [8] and Yokoyama et al. [9], we developed and adapted the EPR method for estimation of the levels of ·NO radicals in serum.

Statistical analysis

Unpaired t-test was used to compare the results of healthy control subjects with the results of patients with asthma. Biochemical parameters were compared in patients with different disease control using one-way ANOVA. The relationship between the various parameters of the study and the degree of airway obstruction was assessed according to Student's t-test. The value of $p \leq 0.05$ was considered statistically significant. To define which groups are different from each other we have used LSD post hoc test.

RESULTS AND DISCUSSION

The results obtained from clinical studies on the antioxidant vitamins use in cardiovascular diseases

have not met expectations despite positive experimental studies [10]. Therefore, their use is not recommended for prophylaxis and treatment from the American Cardiology Association and the Canadian Society of Hypertension. The results are similar in the administration of Se containing supplements, plus a number of adverse effects of the latter [11]. For this reason, Touyz et al [12] suggest antioxidant effects of classical antihypertensive agents, such as B-blockers (carvedilol), ACE inhibitors, angiotensin receptor blockers and Ca-antagonists, thus assigning them to direct NADPH oxidase inhibitors. In the treatment course, drugs and their metabolites can act as plasma pro-oxidants or anti-oxidants. In the behavior recommendations in the AH, the European Cardiological Association 2013, reaffirmed five major groups of antihypertensive medicaments. Moreover, it focuses on the desired blood pressure lowering score regardless of the chosen regimen. In the present study, we highlight the drug's response to Renin–angiotensin system RAS and the oxidative stress indicators [13]. The evidence for the benefit of the angiotensin converting enzyme (ACE), angiotensin receptor blockers (ARB) and beta blockers used in reducing cardiovascular risk is unquestionable. Regarding the blood pressure values optimization in each of these therapeutic strategies, resistance of some patients to the selected treatment was observed [14, 15, 16]. In order to clarify the reasons for the lack of therapeutic effect, we conducted our own study on the antioxidant effect of classical antihypertensive agents in view of the possibility of influencing oxidative toxicity in antihypertensive treatment. We compared the levels of real time indicators in patients with arterial hypertension divided by the type of antihypertensive therapy and a group of normotensive volunteers. The results obtained show a significantly higher level of oxidative stress ROS products and •NO radicals in patients with diagnosed arterial hypertension compared to controls and confirm the authors' finding of higher radical formation in hypertensive [17,18].

The ROS products (Fig.1) in the plasma of AH patients treated with ACE inhibitor (Lisinopril) was statistically significant higher compared to controls (mean 2.54±0.1vs mean 0.73±0.05, p=0.000, t-test). A similar statistically significant increase was observed in the other two patient groups: Angiotensin-receptor blocker (Valsartan) vs controls (mean 2.41±0.1vs mean 0.73±0.05, p=0.000, t-test) and combined therapy vs controls (mean 2.26±0.1vs mean 0.73±0.05, p=0.000, t-test). Moreover, there was not statistically significant difference between HA patients groups: ACE inhibitor vs Angiotensin-receptor blocker p=0.48, ACE inhibitor vs combined therapy p=0.73, Angiotensin-receptor blocker vs combined therapy p= 0.64.

Statistically significant higher NO• levels (Fig. 2) were measured in all HA patient's groups compared to controls: ACE inhibitor vs controls (mean 32.7± 1.5vs mean 11.07± 0.3, p=0.000, t-test), Angiotensin-receptor blocker (ARB) (Valsartan) vs controls (mean 32.7± 1.5vs mean 11.07± 0.3, p=0.000, t-test) and combined therapy vs controls (mean 33.37±1.4vs mean 11.07± 0.3, p=0.000, t-test). Also there was statistically significant difference ACE inhibitor vs Angiotensin-receptor blocker p=0.03, and Angiotensin-receptor blocker vs combined therapy p=0.01.

After correlation analysys: NO radicals vs ROS products r=0.4773; p= 0.000

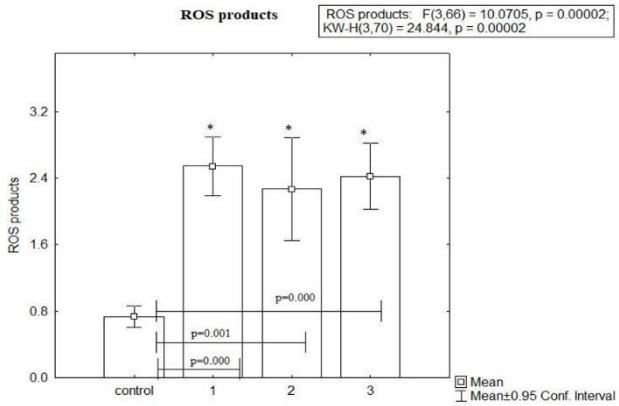

Fig. 1. ROS products levels expressed in arbitrary units in plasma of controls, AH patients with ACE inhibitor therapy (1), Angiotensin-receptor blocker (2), and combined therapy (3). (*) $p \leq 0.05$ – AH groups vs controls; To define which groups are different from each other we have used LSD post hoc test.

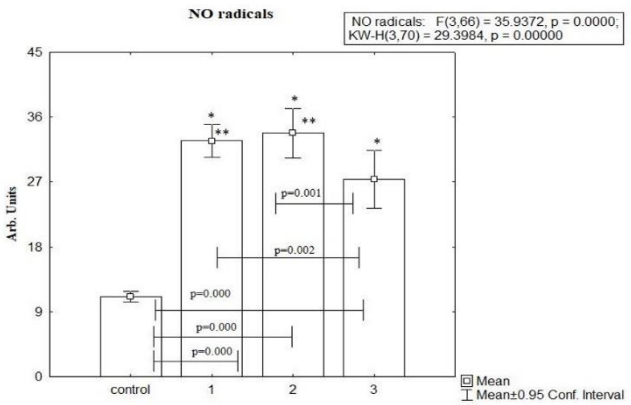

Fig. 2. Levels of NO radicals expressed in arb. units in plasma of controls, AH patients with ACE inhibitor therapy (1), Angiotensin-receptor blocker (2), and combined therapy (3). (*) $p \leq 0.05$ – AH groups vs controls; (**) $p \leq 0.05$ – ACE vs ARB; (#) $p \leq 0.05$ combined therapy vs ARB; To define which groups are different from each other we have used LSD post hoc test.

Montezano and co-authors [19] believe that increased radical formation is associated with many diseases of the cardiovascular system, including hypertension, atherosclerosis, heart failure, and others. Fortuno et al., [20] suggests that oxidative stress is associated with the pathogenesis of hypertension and its complications through changes in the NO metabolism. Based on the study of 51 hypertensives and 43 normotensive controls, they concluded that hypertension is associated with a decrease in the bioavailability of NO and an increase in oxidative stress [21, 22].

As is seen on Fig. 3 the levels of ascorbate radicals are statistically significant higher in all HA patients compared to controls: ACE inhibitor vs controls (mean 0.26± 0.1 vs mean 0.11± 0.04, p=0.001, t-test), Angiotensin-receptor blocker (ARB) (Valsartan) vs controls (mean 0.27± 0.1 vs mean 0.11± 0.04, p=0.000, t-test) and combined therapy vs controls (mean 0.24±0.1 vs mean 0.11± 0.04, p=0.000, t-test). There was no difference between the AH group.

The correlation analyze have shown that Ascorbate radicals vs ROS products has positive correlation r=0.694; p= 0.000; The same positive correlation has been seen between Ascorbate radicals and NO radicals r=0.312; p= 0.009.

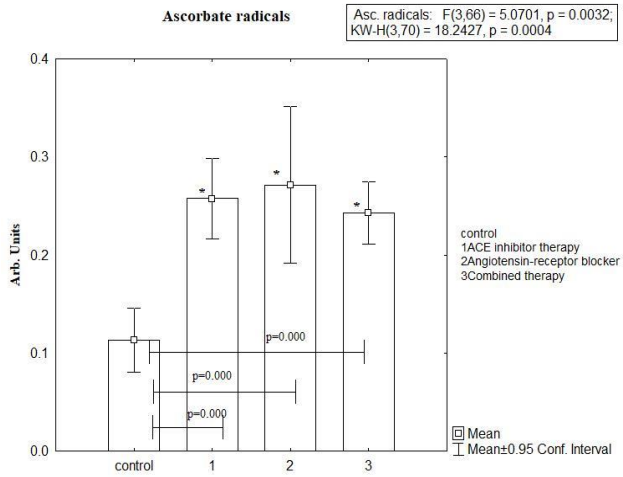

Fig. 3. Levels of Ascorbate radicals expressed in arb. units in plasma of controls, AH patients with ACE inhibitor therapy (1), Angiotensin-receptor blocker (2), and combined therapy (3). (*) $p \leq 0.05$ – AH groups vs controls. To define which groups are different from each other we have used LSD post hoc test.

In plasma, the ascorbate radicals are really an extremely effective peroxide trap, more than any other endogenous antioxidant. Ascorbate has only a protective role and does not act as a pro-oxidant, thereby providing increased benefits with increasing concentration. The presented results show that in human blood plasma ascorbate is only an endogenous antioxidant that can completely protect lipids from peroxide damage induced by peroxide radicals [21,22]. In this type of oxidative stress, ascorbate is a much more effective antioxidant than protein thiols, α-tocopherol, and bilirubin. The ascorbate catches almost all the peroxide radicals in the aqueous phase before they can diffuse into the plasma lipids. Once the ascorbate is completely consumed, the other water-soluble antioxidants, urate, bilirubin and protein thiols can only capture some of the peroxide radicals [23]. In plasma, ascorbate retains antioxidant activity even at very high concentrations. The oxidative effect of the ascorbate was not observed at a concentration of 5 mM. This confirms that plasma metal ions are strongly bound in plasma and are not available for free radicals reactions [24]. The results also show that the higher the ascorbate concentration, the better, or the longer the protection against the aqueous oxidants (provided that the free metal catalysts are not available). Ascorbate is an antioxidant due to the high reduction potential of its carbon-carbon double bond, which easily donates one or two H^+ and electrons to various oxidants, including ROS and RNS [25-27]. Each step of the oxidation of the ascorbate is reversible and this allows its recycling. The partially oxidized form of ascorbate, called (mono) ascorbate radical can serve as an electronic acceptor or donor

Higashi et al. [27] experimentally support the claim that the increase in oxidative stress in patients with hypertension is due to activation of the renin-angiotensin system and renal arterial angioplasty would improve endothelial dysfunction in these patients by reducing oxidative stress. The idea of secondary increased radical formation as a result of the renin-angiotensin-aldosterone system activation and in particular the direct stimulation of vascular NADPH oxidase in patients with essential hypertension has been developed by other authors [12]. The relationship between various parameters of ocular stress and essential hypertension is suggested and theoretically justified by Baradaran, A. and co-authors, but the team works towards antioxidant treatment options without experimental evidence of the hypothesis [28]. Our study also suggests a link between oxidative stress and elevated blood pressure, and allows the reactive oxygen species participation in the etiopathogenesis of hypertonic disease. It is logical to assume that NO detected in plasma is a major product of NOS. The observed multiple elevations in NO, as well as the ROS products compared to controls, indicate that oxidative processes have occurred at the time of the study. Increased GSH and GPx_1 activity in the 2nd

and 3rd trimester is consistent with the findings of other investigators [12, 22, 23, 27], who reported that GSH and GPx_1 levels increased during late stages of pregnancy. These higher enzymatic levels in the IIIrd trimester protected normotensive pregnant woman against hydrogen peroxide and free-radical toxins or protect fetus against highly reactive stress compounds [12, 24]. Additionally, our results suggest that increased SOD, CAT, GSH and GPx_1 activities suppressed the lipid peroxidation levels in the late pregnancy. Moreover, antioxidant peptides prevent direct inactivation of ROS/RNS molecules, leading to normalization of the intracellular redox status, provides the antioxidant- prooxidant balance, promoting normal fetal development [5, 12, 23] and reduced the OS- detoxification processes.

CONCLUSIONS

The finding presents a certain advantage when using ACE and renin inhibitors to ARB with respect to the real time oxidative stress indicators. This fact alone is not a basis for the benefit of ACE inhibitor therapy to ARB, even with accompanying diseases in which etiopathogenesis is discussed increased oxidative stress. The choice of antihypertensive agents should be made individually according to the relevant European guidelines.

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