CELLULAR & MOLECULAR BIOLOGY LETTERS
Volume 9, (2004) pp 635 – 641
http://www.cmbl.org.pl

Received 27 April 2004
Accepted 9 August 2004

Short Communication

THE MARKERS OF OXIDATIVE STRESS AND ACTIVITY OF THE ANTIOXIDANT SYSTEM IN THE BLOOD OF ELDERLY PATIENTS WITH ESSENTIAL ARTERIAL HYPERTENSION

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Abstract: We estimated the nitrate/nitrite, carbonyl groups, reduced glutathione (GSH) and malondialdehyde (MDA) concentrations and Cu,Zn superoxide dismutase (SOD-1), catalase (CAT), glutathione peroxidase (cGSH-Px) and glutathione S-transferase (GST) activities in the blood of 17 normotensive young subjects (mean age 39±7.0 years), 21 normotensive elderly subjects (mean age 82±8.2 years) and 38 patients with essential arterial hypertension (mean age 73±8.0 years). Our examinations showed that hypertension in the elderly is associated with greater than normal levels of protein and lipid oxidation, decreased nitric oxide concentration and an imbalance in antioxidant status (decreased GSH concentration and SOD-1 activity). The increased activity of GST compensated the decreased activity of cGSH-Px in the blood of hypertensive patients. Our study confirms that the degree of oxidative stress in elderly patients intensifies, especially if said patients have associated essential arterial hypertension.

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Abbreviations used: ROS – reactive oxygen species; MDA – malondialdehyde; NO – nitric oxide; GSH – reduced glutathione; SOD-1 – cellular Cu-Zn superoxide dismutase; CAT – catalase; cGSH-Px – cellular glutathione peroxidase; GST – glutathione S-transferase.

Key Words: Aging, Hypertension, Markers of Oxidative Stress, Nitric Oxide, Reduced Glutathione, Antioxidants Enzymes

INTRODUCTION

According to Harman's theory, oxidative stress is intensified with the process of aging, and in the elderly, this is accompanied by a more common occurance of primary hypertension [1, 2]. An excessive ROS concentration, especially hydroxyl radical, has been found in patients with essential arterial hypertension [2]. ROS induce lipid peroxidation, increased disulfide/sulfhydryl ratios and modification of amino acid residues to carbonyl derivatives [2]. Changes in the ratios of oxidative modifications of proteins are positively correlated with age and with the intensity of oxidative stress, which may be associated with an increased risk of several pathologies [3]. Essential arterial hypertension is also associated with impaired nitric oxide (NO) production/degradation. Under pathological conditions and during aging, an accelerated inactivation of nitric oxide caused by the superoxide anion ($O_2^{\bullet -}$) may be related to hypertension [2, 4]. The most important source of the superoxide anion in the vessel wall is membrane-bound NAD(P)H oxidase [2, 4]. NO can be scavenged by $O_2^{\bullet -}$ to form $ONOO^-$, which can be transformed to a highly reactive oxygen species – peroxynitrous acid. $ONOO^-$ may also induce oxidation reactions of endogenous compounds. Both $ONOO^-$ and peroxynitrous acid may be involved in numerous pathophysiological processes. The pathomechanism of hypertension has close relevance with an impaired bioavailability of NO and a large amount of $ONOO^-$ [2, 4].

In mammalian cells, there are several mechanisms by which an organism defends itself against oxidative stress. Among them, there are small molecular antioxidants such as reduced glutathione (GSH) and antioxidant scavenging enzymes such as cellular Cu,Zn superoxide dismutase (SOD-1), catalase (CAT), cellular glutathione peroxidase (cGSH-Px) and glutathione S-transferase (GST) [3, 4]. Sato *et al.* [5] postulated that SOD-1 is a key enzyme in protecting the vessel wall against oxidative injury. Measurement of the activity of both glutathione-dependent enzymes may serve as an estimation of functioning of antioxidant system. GSH is a cosubstrate of cGSH-Px and GST, and in reactions with peroxides, it is converted to glutathione disulfide. GSH is responsible for protecting cellular thiol against oxidation. The levels of GSH fluctuate under various physiological conditions, including aging and hypertension, which are accompanied by increased lipid peroxidation [6]. The aim of our study was to evaluate the concentrations of markers of oxidative stress and to examine the functioning of the antioxidative system in the blood of elderly patients with primary hypertension.

MATERIALS AND METHODS

Our investigation was carried out on a group of 17 normotensive young subjects aged from 23-48 years (mean age 39±7.0 years), 21 normotensive elderly subjects aged from 64-97 (mean age 82±8.2 years) and 38 elderly patients with essential arterial hypertension aged from 64-91 (mean age 73±8.0 years). The patients came from the Geriatric Clinic of the Medical University in Bydgoszcz, Poland. The normotensive subjects were clinically examined and had lab tests done so that the influence of illness or other conditions on the oxidative state of their organism could be excluded. The patients with hypertension were subjected to monotherapy with thiazyd or thiazide-derived diuretics, although they did not receive any antihypertensive drugs in the 48 hour period before their participation in research. Patients were under medical care in the Geriatric Clinic. The examination excluded patients addicted to alcohol and/or tobacco, and patients with diabetes, ischaemic heart disease, a history of stroke, kidney disorder or other conditions of known free radical etiology. The criterion for dividing subjects into normal blood pressure and hypertension groups has been set at a blood-pressure of 140/90 mmHg. Routine diagnostic investigations excluded patients with secondary hypertension diseases.

Venous blood taken from the cubital vein on a blood clot or heparin was used as the material for marking. The concentration of GSH was assayed by the colorimetric method in a sample of whole blood with 5',5' dithio-bis-2-(nitrobenzoic acid), according to Beutler [7]. In the obtained serum, the concentration of carbonyls in the protein formed by reaction with diphenylohydrazine was determined in accordance with the method of Levine *et al.* [8]. The carbonyl groups reacted with 2,4-dinitrophenyl hydrazine, and the concentration of the products of this reaction was measured colorimetrically at 370 nm. Protein content was measured using the method of Gornall *et al.* [9]. The concentration of nitrate/nitrite products in the serum was analyzed via a Griess reaction in accordance with Marletta *et al.* [10]. Nitric oxide decomposed rapidly in aerated solutions to form stable nitrate/nitrite products. MDA concentration, expressed as the concentration of thiobarbituric acid reactive substances in the erythrocytes was determined as per the method of Placer *et al.* [11]. SOD-1 (E.C.1.15.1.1.) activity was determined in accordance with the method of Misra and Fridovich [12] and CAT (E.C.1.11.1.6.) activity in accordance with the method of Beers and Sizer [13]. The cGSH-Px (E.C.1.11.1.9.) activity was determined in erythrocytes according to Paglia and Valentine [14] with tert-butyl hydroperoxide as the substrate, and the GST (E.C.3.1.2.7.) activity according to Habig *et al.* [15] with 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate. Haemoglobin concentration was determined in the haemolisate in the cyanmethaemoglobin form using a commercial reagent (Biomed, Poland). The statistical significance of differences was estimated using the Student's t-test. The significance level was set at $p \leq 0.05$ level.

RESULTS AND DISCUSSION

In the case of hypertensive patients, the mean systolic and diastolic blood pressures (mmHg) were found to be statistically significantly higher than those of the control groups ($p<0.05$). No statistically significant differences were observed between the remaining parameters measured (glucose, creatinine, total cholesterol, low-density lipoproteins and high-density lipoproteins and triglycerides concentrations). Examinations have shown that the nitrate/nitrite level in the plasma of hypertensive erderly patients and normotensive erderly subjects was statistically significantly lower ($p<0.001$) in comparison to that for normotensive young subjects. The carbonyl groups concentration in the serum of hypertensive patients was statistically significantly higher ($p<0.001$) than that found for normotensive young and elderly subjects. The MDA level in the erythrocytes of hypertensive patients was significantly higher ($p<0.01$) than that for the control young and elderly groups ($p<0.001$). The GSH concentration in the whole blood of hypertenisve patients was significantly lower ($p<0.01$) than that for the normotensive young and elderly subjects. The hypertensive group had a statistically lower ($p<0.05$) mean erythrocyte SOD-1 activity than the young control group and a nonstatistically lower activity of this enzyme than the normotensive elderly control group. In the hypertensive subjects the activities of CAT and cGSH-Px were not statistically different to the activities of these enzymes in normotensive elderly people. However, the activity of CAT in the young subjects was statistically lower ($p<0.001$) than that of normotensive elderly and hypertensive patients. The activity of cGSH-Px in the normotensive young subjects was nonstatistically higher than that of the normotensive elderly and of the hypertensive patients. The increase in the mean activity of GST was statistically evident ($p<0.01$) in the erythrocytes of hypertensive patients when compared with the activity found for normotensive young and elderly subjects. The comparison of the chosen examined parameters is shown in Tab. 1. The presented data shows that patients with hypertension have an increased concentration of carbonyl groups and a decreased content of nitric oxide in comparison to the subjects in the normotensive groups. Agarval and Sohal [16] proved that carbonyl groups are among the more important markers of oxidative stress, which can be observed in the diseases of old age, for example in hypertension. The latter was confirmed by other investigators, too [17]. A lowered concentration of nitrate/nitrite indicates damage to the endothelium in hypertension. The self-study not only showed significantly lower nitrate/nitrite serum levels in patients with essential arterial hypertension than in younger and older controls, but also significantly lower nitrate/nitrite serum levels in older normotensive subjects than in younger ones. Other researchers came to the similar conclusion [18]. Ideas involving the activity of antioxidative enzymes in elderly people are diverse. According to some authors, the activity of antioxidative enzymes in erythrocytes negatively correlates with age in the case of SOD-1 but positively in the case of CAT. This was also confirmed by our

own research. No correlation has been found between age and the activity of CAT [3, 18]. Some researchers have shown that age does not have an influence on the activity of SOD-1 [19], while others observed a distinct decrease in the enzyme's activity along with age both in a group of healthy people and in a group with essential hypertension [20]. Our research has revealed that in the erythrocytes of patients with essential hypertension, there is a significantly higher mean MDA level and a significantly lower mean GSH level than in those of subjects from the normotensive groups. Decreased SOD, GSH-Px and CAT activity and increased MDA level in the blood of arterial hypertension patients were observed by Redon ***et al.*** [21]. It is worth mentioning that the research conducted by these authors did not include older arterial hypertension patients.

Tab. 1. The comparison of chosen parameters for normotensive young and elderly subjects with those for hypertensive patients (mean ± SD).

Parameter	Normotensive young (n=16)	Normotensive elderly (n=21)	Hypertensive patients (n=38)
Plasma nitrate/nitrite (μmol/l)	2.96±1.49	1.91±1.26*	1.47±0.56*
Serum carbonyl groups (nmol/mg protein)	0.075±0.04	0.085±0.024	0.245±0.212*
Blood MDA concentration (μmol/gHb)	0.230±0.115	0.186±0.03	0.307±0.116*.**
Blood GSH concentration (mmol/l)	2.61±0.23	2.77±0.33	2.48±0.35**
Blood SOD-1 activity (U/gHb)	3218±793	2976±404	2835±469***
Blood CAT activity (BU/gHb)	17.04±2.89	23.5±3.9*	23.4±4.8*
Blood GST activity (nmol/CDNB -GSH/mgHb/min)	2.78±0.5	2.92±0.7	4.7±2.8**

*differences considered statistically significant between normotensive subjects and hypertensive patients and between normotensive young and elderly; *$p<0.001$, **$p<0.01$, ***$p<0.05$.

Our research did not confirm changes in cGSH-Px activity, but we did observe an increase in GST activity. GST substitutes for cGSH-Px in conditions when cGSH-Px activity is insufficient for the reduction of organic peroxides [22]. GST is said to be a nonselenium glutathione peroxidase [22]. The activity of cGSH-Px in erythrocytes is rigidly correlated with the concentration of selenium (Se). Moreover, other authors showed decreased selenium levels, both in

erythrocytes and in the serum of aged patients, which was connected with decreased glutathione peroxidase activity [23]. Therefore, the increased activity of Se-independent GST in conditions of increased MDA concentration may be a factor restraining the intensified process of lipid peroxidation.

Summing up, this study confirms the intensification of oxidative stress in elderly patients, especially with associated essential arterial hypertension. Moreover, this study indicates the possible participation of reactive oxygen species, not only in the physiological aging process, but also, most importantly, in the pathogenesis of old age diseases, including arterial hypertension, which may accelerate the aging process.

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