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Impact of acute exercise on antioxidant enzymes activity and lipid status in blood of patients with hypertension

Uticaj akutnog izlaganja fizičkom naporu na aktivnost antioksidativnih enzima i lipidni status u krvi bolesnika sa hipertenzijom

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Abstract

Background/Aim. Many studies support the hypothesis that oxidative stress is involved in the pathogenic process of a variety of diseases including hypertension. In humans, hypertension is also considered a state of oxidative stress that can contribute to the development of arteriosclerosis and other hypertension-induced organ damage. The aim of this study was to evaluate an influence of acute physical exercise on antioxidative enzymes activity and lipid status in patients with hypertension. **Methods.** Fourty patients with hypertension and 20 age-matched controls were included in the study. To assess an influence of acute exercise on lipids and antioxidative enzymes activity the following parameters were determined at rest and immediately after the acute cardiopulmonary exercise cycloergometer test: triglycerides (TG), total cholesterol, low density cholesterol (LDL), oxidised LDL cholesterol (OxLDL), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and plasminogen activator inhibitor (PAI). **Results.** In basal condition, hypertensive patients compared to the control group had increased, but not significantly, level of Ox LDL (88.61±14.06 *vs* 79.00±29.26 mmol/L), PAI (3.06±0.56 *vs* 2.6±0.35 U/mL) and activity of GSH-Px (50.22±15.20 *vs* 44.63±13.73 U/g Hb). After acute exercise test, there was significantly greater level of Ox LDL (79.0±29.26 *vs* 89.3±29.07 mmol/L; $p < 0.05$) only in the control group. GSH-Px activity was significantly decreased only in hypertensive patients after acute exercise (50.22±15.2 *vs* 42.82±13.42 U/g Hb; $p < 0.05$), but not in the controls. **Conclusion.** No significantly elevated Ox LDL, GSH-Px and PAI-1 levels were found in hypertensive patients during basal condition in comparison with healthy subjects. Decreased GSH-Px activity was associated with those in acute exercise only in hypertensive patients. It could be an important indicator of deficiency of physiological antioxidative defense mechanism in hypertensive patients during an acute exercise.

Key words:
hypertension; exercise; oxidoreductases; triglycerides; cholesterol; lipoproteins, ldl.

Apstrakt

Uvod/Cilj. Mnoga ispitivanja podupiru hipotezu da je oksidativni stres uključen u patogenezu različitih bolesti uključujući i hipertenziju. S druge strane smatra se da je hipertenzija kod čoveka stanje oksidativnog stresa koje sudeluje u razvoju arterioskleroze i drugih poremećaja izazvanih hipertenzijom. Cilj ove studije bio je da se proceni uticaj akutnog fizičkog napora na aktivnost antioksidativnih enzima i lipidni status kod bolesnika sa hipertenzijom. **Metode.** U ispitivanje bilo je uključeno 40 bolesnika sa hipertenzijom i 20 zdravih osoba iste starosti. Kod njih su u stanju mirovanja, kao i neposredno posle aerobnog treninga (ergometrijskog testa) određivani sledeći parametri: trigliceridi (TG), ukupni holesterol, lipoproteini male gustine (LDL), oksidisani LDL holesterol (Ox LDL), superoksid dismutaza (SOD), glutation peroksidaza (GSH-Px), inhibitor aktivatora plazminogena (PAI). **Rezultati.** U bazalnim uslovima bolesnici sa hipertenzijom u odnosu na kontrolnu grupu imali su viši nivo Ox LDL (88,61±14,06 *vs* 79,00±29,26 mmol/L), PAI (3,06±0,56 *vs* 2,6±0,35 U/mL) i aktivnost GSH-Px (50,22±15,20 *vs* 44,63±13,73 U/g Hb), koji nije dostigao nivo statističke značajnosti ($p > 0,05$). Tokom ergometrijskog testa uočen je statistički značajan porast koncentracije Ox LDL u odnosu na bazalni nivo (79,0±29,26 *vs* 89,3±29,07 mmol/L, $p < 0,05$) samo u kontrolnoj grupi. Aktivnost GSH-Px statistički je značajno opala samo kod bolesnika sa hipertenzijom nakon sprovedenog ergometrijskog testa (50,22±15,2 *vs* 42,82±13,42 U/g Hb; $p < 0,05$), ali ne i kod ispitanika kontrolne grupe. **Zaključak.** Nisu nađeni značajno viši nivoi Ox LDL, PAI-1 i GSH-Px u bazalnim uslovima kod bolesnika sa hipertenzijom u odnosu na ispitanike kontrolne grupe. Nakon fizičke aktivnosti kod bolesnika sa hipertenzijom uočeno je statistički značajno sniženje aktivnosti GSH-Px. Ovo bi mogao biti važan pokazatelj nedostatka fiziološkog mehanizma antioksidativne odbrane kod bolesnika sa hipertenzijom izloženih akutnom fizičkom naporu.

Ključne reči:
hipertenzija; vežbanje; oksidoreduktaze; trigliceridi; holesterol; lipoproteini, ldl.

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Introduction

Many studies support the hypothesis that oxidative stress is involved in the pathogenic process of a variety of diseases including hypertension [1, 2]. In humans, hypertension is also considered a state of oxidative stress that can contribute to the development of arteriosclerosis and other hypertension-induced organ damage [3].

Lipid peroxidation and reduced antioxidant defense mechanisms are important factors affecting oxidation of lipoproteins and thereby the progression of arteriosclerotic disease [1, 3].

An enhanced oxidative stress has been observed in hypertensive patients as indicated by increased free radicals production, lipid peroxidation and diminished antioxidant status [3–5]. Assessment of antioxidant activities and lipid peroxidation byproducts in hypertensive subjects indicates an excessive amount of reactive oxygen species (ROS) and a reduction of antioxidant mechanism activity in both blood and several other cellular systems including not only vascular cells but also those found in circulating blood [3, 6].

Experimental and clinical evidence has demonstrated impairment of endothelium function caused by oxidative products in patients [3].

Antioxidant system includes non-enzymatic components and enzymatic ones such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) [1, 2].

It is known that exercise induces oxidative stress. There are many studies on exercise training and hypertension, but very few on exercise-induced oxidative stress and antioxidant activities in hypertensive patients [5–7]. It has been shown that reduction of superoxide radicals by infusion of superoxide dismutase (SOD) significantly decreases blood pressure in spontaneously hypertensive rats [7].

Exercise, paradoxically, is a well recognized model of oxidative stress and also an important therapeutic tool in hypertensive patients. Reduction of activity of antioxidant system could be a cause of increased oxidative state during exercise. Since physical activity protects against the development of cardiovascular disease (CVD) and modifies risk factors as plasminogen activator inhibitor (PAI), a regular exercise program seems to be desirable. There is a biochemical paradox: considerable amounts of oxygen are necessary to obtain a good performance and a satisfactory cardiopulmonary status, while an excess of oxygen or a defective metabolism of it could be harmful [8, 9] .

In relation to antioxidant enzymes, an increase of SOD and GSH-Px activities has been observed in skeletal muscle, heart and liver during a single bout of acute exercise [10] . It is important that physical training induces enhancement of muscular and liver antioxidant enzymes, mainly GSH-Px, facilitating the removal of reactive oxygen species and the reduction of oxidative stress levels [8].

The aim of this study was to estimate lipid oxidation and antioxidant parameters during basal conditions and after submaximal exercise test in a group of untreated hypertensive subjects, compared with gender- and age-paired healthy controls.

Methods

The study population consisted of consecutive outpatients from the Endocrinology and Cardiology Departments of University Clinical Center "Dr Dragiša Mišović", Belgrade. We evaluated 40 patients with hypertension (20 males and 20 females), aged 51.19±8.37 years and 20 age- and gender-matched controls (healthy, normotensive nonsmokers). Exclusion criteria were secondary arterial hypertension, diabetes, coronary artery disease, rhythm disturbances, cerebrovascular disease, chronic obstructive lung disorder or severe renal failure. Essential hypertension was defined according to the criteria of the VI Joint National Committee WHO grade I – II, non treated for hypertension.

For providing an objective assessment of exercise capacity and impairment we applied cardiopulmonary exercise cycle ergometer test (Jaeger Oxycon Delta ER - 900). All study subjects underwent a symptom-limited incremental test protocol with 25W increments each 3 minutes. Test was designed to be progressive and incremental in order to elicit the important parameters: VO_2 max (mL/min) - maximal O_2 uptake; FAI index (%) - maximal O_2 uptake compared to predictive value; VO_2/Kg (mL/Kg/min) - uptake related to body weight; VE (L/min) - ventilation per minute; RER - respiratory exchange ratio anaerobic threshold; T - time to anaerobic threshold (min). Heart rate and rhythm were continuously monitored using a 12 lead electrocardiogram. Blood pressure was measured before each load change. Gas analyses and flow probes were calibrated before each test. Gas exchange data were collected in a breath by breath manner and averaged into 30-second time period. All parameters were calculated as highest 30-second time period recorded before volitional fatigue was reached. Test lasted from 3 to 12 min depending on physical condition. Blood pressure was measured with a mercury sphygmomanometer, with a patient in the sitting position after a 5-minute rest.

This investigation was approved by the Ethical Committee of University Clinical Centre "Dr Dragiša Mišović", Belgrade.

Lipid parameters triglyceride, total cholesterol, HDL and LDL cholesterol, oxidized LDL cholesterol (mmol/L) were measured from serum.

Triglycerides (TG) were measured by an enzymatic colorimetric method (Elitech).

HDL-cholesterol was measured after precipitation of LDL and VLDL by phosphotungstic acid (Serbolab).

LDL-cholesterol was calculated according to the Friedwald formula.

Oxidised LDL-cholesterol was measured by the Elisa method (Mercodia) from serum.

Plasminogen activator inhibitor-type 1 (PAI-1) (U/mL) was measured by the spectrophotometer method using a commercial kit (Behring).

Superoxide dismutase (SOD) (U/g Hb) was measured by the enzymatic colorimetric method from erytrocyte after centrifugation of 0.5 mL of whole blood for ten minutes at 3 000 rpm and then aspiration off the plasma. Erytrocytes were washed 4 times with 3 mL of 0.9% Na Cl solution and centrifuged for 10 minutes at 3 000 rpm after each wash. The

washed centrifuged erytrocytes should then be made up to 2.0 mL with cold redistilled water, mixed and left to stand at +4 C° for 15 minutes. The lysat was diluted with Ransot sample diluent, so that the % of inhibition fell between 30% and 60%.

The activity of SOD was measured at 500 nm with a commercially available kit (Randox Laboratories, kit Ransod superoxide dismutase) by testing the inhibition degree of a tetrazolium salt oxidation reaction. The coefficient of variability between assays was 4.2%.

The activity of GSH-Px (U/g Hb) was evaluated by a commercial kit (Ransel glutathione peroxidase, Randox Laboratories) in erythrocytes at 340 nm by measuring the decrease of NADPH absorbancy. The coefficient of variability between assays was 4%.

All data were expressed as mean ± standard deviations (SD).

Statistical analysis was done by a Statistical Package for the Social Sciences Program (SPSS). Comparisons of all measurements were made with the paired Student's *t*-test and Mann-Whitney *U* test. Simple and multiple linear regression analysis determined all correlations.

Differences between groups were considered significant at $p < 0.05$.

Results

All demographics and biochemical parameters are shown in Tables 1 and 2. Table 2 shows that during basal condition, oxidised LDL cholesterol (Ox LDL), GSH-Px and PAI-1 were increased, but not significantly, in the hypertensive patients as compared to those of the controls.

There was no change in SOD activity between hypertensive and healthy subjects, before and after the exercise (Table 2). The patients with hypertension had significantly decreased levels of GSH-Px after exercise ($p < 0.05$). Ox LDL cholesterol was significantly increased during acute exercise only in the healthy subjects ($p < 0.05$) (Table 2).

Peak oxygen uptake (VO_2) was significantly greater in the healthy group ($p < 0.01$). There were no significant differences between hypertensive patients and healthy subjects for exercise time and ventilation per minute (VE). There was a negative correlation between VO_2 peak and Ox LDL before exercise ($r = -0.65$, $p < 0.05$) (Table 3).

Systolic and diastolic blood pressure were significantly higher during exercise in the hypertensive group ($p < 0,01$) (Table 4). There was no correlation between blood pressure (BP) and antioxidant enzymes (GSH-Px and SOD) activity.

Table 1

Clinical and biochemical parameters in the hypertensive patients and the control group

Parameters	Control group (healthy subjects) (n = 20)	Hypertensive group (n = 40)
Female/male	10/10	20/20
Body mass index (kg/m^2)	25.01 ± 1.90	26.82 ± 3.14
Glycemia (mmol/L)	5.08 ± 0.68 – rest	5.41 ± 0.95 – rest
	4.92 ± 0.74 – exercise	5.39 ± 0.87 – exercise
Tryglycerides (mmol/L)	1.74 ± 0.9 – rest	2.08 ± 1.35 – rest
	1.98 ± 0.8 – exercise	2.22 ± 1.09 – exercise
Total cholesterol (mmol/L)	5.57 ± 1.67 – rest	6.62 ± 0.78 – rest
	5.9 ± 1.88 – exercise	6.78 ± 0.69 – exercise
LDL cholesterol (mmo/L)	3.61 ± 1.56 – rest	4.42 ± 0.82 – rest
	3.55 ± 1.55 – exercise	4.65 ± 0.64 – exercise
PAI-1 (U/mL)	2.6 ± 0.35 – rest	3.06 ± 0.56 – rest
	2.22 ± 0.65 – exercise	2.87 ± 0.93 – exercise

Table 2

Indicators of oxidative stress and antioxidative defense before and after the acute exercise in the hypertensive and the control group

Indicators	Hypertensive group (n = 40)		Control group (n = 20)	
	rest	exercise	rest	exercise
Ox LDL (mmol/l)	88.61 ± 24.06	95.3 ± 22.51	79.0 ± 29.26	89.3 ± 29.07*
SOD (U/g Hb)	904.7 ± 99.66	928.08 ± 73.66	877.14 ± 153.18	895.0 ± 193.49
GSH-Px (U/g Hb)	50.22 ± 15.2	42.82 ± 13.42*	44.63 ± 13.73	43.97 ± 25.97*

* $p < 0.05$ *vs* the basal value (at the rest)

Table 3

Respiratory and cardiovascular post-exercise data in the hypertensive and the control group

Variable	Hypertensive group	Control group
VO_2 peak (mL/min)	20.55 ± 6.73	26.35 ± 10.53*
VE (L/min)	53.72 ± 14.07	52.00 ± 9.89
Exercise time (min)	13.58 ± 4.78	11.05 ± 1.48

* $p < 0.01$ vs the hypertensive group; VO2 oxygen uptake; VE = ventilation per minute

Table 4

Blood pressure before and during the exercise in the hypertensive and the control group

Blood pressure – BP (mmHg)	Hypertensive group		Control group	
	Rest	During exercise	Rest	During exercise
Systolic BP	139.65 ± 22.96	206.8 ± 22.9*	122.37 ± 12.09	128.0 ± 30.64
Diastolic BP	90.3 ± 12.04	103.75 ± 11.89*	80.0 ± 9.36	73.6 ± 16.56

*$p < 0.01$ vs the basal value (at the rest)

Discussion

There are some contradictory results in the literature regarding oxidative stress parameters and lipids in hypertensive patients during acute exercise. The oxidation of LDL cholesterol is considered the key event in initiation of arteriosclerosis [9, 10]. In our study OxLDL was increased but not significantly in hypertensive patients in basal condition, compared to the control group. On the other hand, we found increased Ox-LDL after the exercise test only in the healthy subjects. Our results are consistent with those of other studies on oxidative stress [10, 11]. It has been proposed that oxidative stress may be associated with the pathogenesis of hypertension and its complications [11, 12].

In this study we observed an increase in GSH-Px activity in the hypertensive patients compared with the controls during basal condition. In relation to acute exercise, a decrease in GSH-Px was observed only in the hypertensive patients. This finding is in accordance with that of Redon et al [3]. There was no significant change in SOD activity in basal condition and after exercise, in both groups (hypertensive patients and controls). A possible explanation for these findings is that the rise in some enzyme activities in the patients with hypertension could be a compensatory mechanism of the body to prevent tissue damage [13, 14].

Our results suggest that there seems to be an imbalance between erytrocyte oxidant and antioxidant systems in patients with hypertension. This disturbance of the oxidative metabolism may affect endothelial cell functions and contribute to the development and maintenance of cardiovascular complications during hypertension. Reduced levels of GSH-Px after acute bouts of exercise are related to an extensive number of metabolic and gene expression disturbances [15–17]. Whether a lower GSH-Px activity is a cause or a consequence of an increased oxidative status, needs further evaluation [3].

The absence of a relationship between the BP values and antioxidant parameters in the group of hypertensive subjects may indicate that factors other than BP values alone, such as an enhanced activity of angiotensin II or hyperinsulinemia, may be responsible for altered oxidative state in blood, which is in accordance with the previous studies [18, 19].

In this study we found elevated concentrations in plasma of PAI-1 only in patients with hypertension, in basal condition. An acute exercise decreased the level of PAI-1 only in the healthy subjects, but not significantly. It has been shown that an increased PAI-1 may contribute to acceleration of atherosclerosis in condition characterized by insulin resistance [20, 21].

The evidence of a high oxidative profile during exercise in hypertension is not directly related to an increase risk of CVD [22]. An increase of antioxidant enzyme activity (GSH-Px), related to the intensity of exercise after different levels of training has also been described [23]. Accordingly, after a regular physical training program an improvement in the counterbalance of the oxidative stress could be expected.

Conclusion

It can be concluded that acute exercise induces an effect well counterbalanced only in the healthy subject, but not in the hypertensive patients.

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