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PROTEIN FACTORS OF OXIDATIVE STRESS AND ATHEROGENESIS IN PATIENTS WITH ARTERIAL HYPERTENSION

The aim of this study was to evaluate the protein components of HDL in patients with arterial hypertension (AH). The study included 30 patients (mean age of 47.4 ± 3.3 years) with AH 2 grade. The accumulation of carbonyl oxidation protein products in blood, HDL and LDL eventually results in oxidative modification of HDL and LDL, and loss of its functional properties. The activity of HDL-associated enzymes (PON1 and MPO) is the most informative indicator of functional state of HDL, and not the level of HDL-Cholesterol. Changes of MPO and PON1 activity may serve as a useful marker of dysfunctional HDL. Our evaluation showed a significant decrease of PON1 activity and increase of MPO activity that may contribute to the HDL oxidation, irrespective of HDL-Cholesterol levels. Demonstrated changes in the functional state of HDL, in our opinion, create a predisposition to development and progression of atherosclerosis in patients with AH.

Key words: arterial hypertension; atherogenesis; oxidative stress; paraoxonase-1; myeloperoxidase

INTRODUCTION

Arterial hypertension (AH) remains the one of the most significant problem due to its wide prevalence in the world. Over 40 % of the adult population of Ukraine has increased arterial pressure. On the other hand, AH is an important risk factor for major cardiovascular diseases and especially atherosclerosis. It is associated with high mortality and disability due to myocardium infarction, stroke and heart failure [1, 9]. It is known that systemic inflammation with the formation of oxidative stress and endothelial dysfunction are important in the development of AH [1, 11, 17, 18]. These factors are also known to be in the basis of development of atherosclerosis [3, 11, 18]. Numerous experimental and clinical studies have shown that free radical modification of LDL and VLDL on a background of inflammation and oxidative stress underlie increased blood atherogenic potential; and potentiate the mechanisms of initiation and progression of atherogenesis.

In the assessment of lipid metabolism in patients with AH the quantitative indexes of lipoproteins and level of lipoprotein cholesterol are usually taken into account. These indexes are used in clinical practice to assess the treatment efficiency, especially in order to employ the lipid-lowering drugs in the complex treatment. According to modern studies the qualitative status of lipoproteins and their atherogenic or antiatherogenic properties are determined by corresponding proteins – apoproteins and

enzymes. The activity of these enzymes is more important than indicators of their blood levels or lipoprotein cholesterol content [3, 9, 12, 13]. Atheroprotective activities of HDL are thought to be mediated in part by both ApoA1 within the core lipoprotein particle and an assortment of HDL-associated proteins. HDL particle enriched in paraoxonase-1 (PON1) (EC 3.1.8.1), an atheroprotective protein, have been linked to the antioxidant, anti-inflammatory, and lipid cargo-carrying functions of HDL. PON1 has been shown to help HDL prevent the accumulation of lipid peroxides in oxidized LDL (low-density lipoproteins), inactivate bioactive oxidized phospholipids, stimulate HDL-mediated eNOS-dependent NO production, and enhance cholesterol efflux from cholesterol-laden macrophages [3, 9, 14, 16]. Myeloperoxidase (MPO) (EC 1.11.1.7), like PON1, both binds to HDL and is mechanically linked to oxidative stress and atherosclerosis. MPO is released from activated leukocytes in the activation of the inflammatory reaction. Excessive activation of MPO can lead to oxidative modification of LDL and HDL, inactivation of PON1, and oxidative modification of different macromolecules, contributing to the development of atherogenesis. MPO plays an important role in the vascular damage mediated by leukocytes [4, 5, 12, 16]. In this regard, according to many studies, MPO can act as a marker of determination of character of the inflammation, the intensity of oxidative stress and development of endothelial dysfunction [5, 12]. The aim of this study was to evaluate the qualitative status of the main classes of lipoproteins together with the activity of corresponding enzymes in patients with AH.

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Table 1

CONTENT OF CARBONYL PRODUCTS OF PROTEIN OXIDATION, FREE RADICAL OXIDATION LIPID PRODUCTS, AND ACTIVITY OF PON1, CATALASE, SUPEROXIDEDISMUTASE AND MPO IN PATIENTS WITH AH (M ± m)

Indexes	Control group (healthy persons), n = 15	Patients with AH, n = 30
PON1 activity, kU/l	5.66 ± 0.93	2.02 ± 0.34*
TBA-positive products, U/l	9.11 ± 0.21	11.06 ± 0.14*
Catalase activity, U/l	9.11 ± 0.44	7.78 ± 0.51*
Superoxide dismutase activity, U/l	1281 ± 121	1708 ± 140*
Carbonyl products of protein oxidation in serum, conv.u./ml	4.13 ± 0.16	5.55 ± 0.22*
Carbonyl products of protein oxidation in LDL + VLDL fraction, conv.u. × mg ⁻¹ of lipids	0.57 ± 0.05	0.81 ± 0.06*
Carbonyl products of protein oxidation in HDL fraction, conv.u./ml	1.82 ± 0.07	2.04 ± 0.09*
Degree of oxidative modification of LDL+VLDL, conv.u./mg of lipids	2.41 ± 1.10	3.49 ± 0.30*
MPO activity, conv.u./min	0.00242 ± 0.0005	0.0031 ± 0.0004*

Note: * – The difference with Control group is statistically significant; P ≤ 0.05.

MATERIALS AND METHODS

The study included 30 patients (mean age of 47.4 ± 3.3 years) with AH 2 grade, who received basic therapy according to the Recommendation of the European Society of Cardiology and the Association of Cardiologists of Ukraine. All patients were subjected to a set of necessary general and functional diagnostic methods. As control group we enrolled 15 healthy persons (mean age of 49.2 ± 2.1 years).

Blood biochemistry assays were performed in all subjects. Level of carbonyl oxidation protein products in serum, HDL and LDL + VLDL fractions was evaluated spectrophotometrically after [2]. Level of TBA-positive products in serum was assayed after [19]. The degree of oxidative modification of LDL + VLDL was determined spectrophotometrically after [7]. Activity of catalase and superoxide dismutase in serum were assayed spectrophotometrically and spectrofluorometrically after [8] and [15], correspondingly. Activities of PON1 in serum and MPO in plasma were assayed after [14] and [5] correspondingly. Serum lipid parameters were measured, such as total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol. The differences were considered to be statistically significant at P < 0.05.

RESULTS AND DISCUSSION

We found that arylesterase activity of PON1 decreases by 65 % in patients with AH compared with the control group (Tab. 1). PON1 hydrolyses lipid peroxides and promotes elimination of oxidized LDL, cholesterol biosynthesis inhibition and stimulation of HDL-mediated cholesterol efflux from macrophages [3, 9]. PON1 protects HDL from oxidation and together with other HDL-associated proteins and enzymes determines antioxidant, anti-inflammatory and antiatherogenic properties of HDL [9]. Decrease of arylesterase activity of PON1 correlates with increase of content of lipid peroxidation products, including the TBA-positive products in serum by 21 % if compared with control (Tab. 1). Activation of free radical pro-

tein oxidation is higher in patients with AH. This is evidenced by increase of carbonyl products of protein oxidation in serum by 35 %. The increase of carbonyl products of protein oxidation in HDL and LDL + VLDL fractions by 20 % and 41 %, correspondingly, is noticeable in comparison with healthy persons (Tab. 1). This may indicate oxidative modification of these particles. These changes, together with accumulation of TBA-positive products in HDL and LDL + VLDL fractions are the basis for formation of high blood atherogenic potential. These changes are taking place against a background of reduced enzyme activity level of antioxidant protection system. Thus, the activity of superoxide dismutase and catalase in serum of patients with AH decreases by 33 % and 15 %, correspondingly (Tab. 1). These changes reflect the general reaction of the organism and indicate the formation of oxidative stress involving both lipid and protein factors, and inhibition of antioxidant defense mechanisms aimed against increase in content of ROS and free radical oxidation products of macromolecules.

MPO activity increases in plasma of patients with AH by 27 % compared with healthy persons (Tab. 1). Today there is much evidence of the important role of leukocytes in the vascular lesions. In particular, the activation of leukocytes can serve as an alternative risk factor for atherosclerosis. MPO contained in azurophilic granules of neutrophils and released into the extracellular space in the activation of these cells [10, 11, 12]. The demonstrated increased MPO activity in patients with AH indicates the presence of inflammatory response.

In circulation MPO forms a complex with HDL-associated enzyme PON1. PON1 partially inhibits the activity of MPO, while MPO is able to inactivate PON1, oxidizing the tyrosine-71 residue, which leads to disruption of interaction between the enzyme molecule and HDL. As a result of the leukocytes activation MPO produces a number of reactive oxygen species, which can lead to damage to lipoprotein particles. MPO can bind to endothelium and aggravate the local inflammation of blood vessels [6, 16].

Table 2

THE CONTENT OF LIPIDS IN BLOOD OF PATIENTS WITH AH (M ± m)

Parameter	Control group (healthy persons), n = 15	Patients with AH, n = 30
Cholesterol, μmol/l	4.45 ± 0.15	4.93 ± 0.36
Triglycerides, μmol/l	1.45 ± 0.17	1.88 ± 0.13*
Cholesterol-HDL, μmol/l	1.12 ± 0.09	1.22 ± 0.08
Cholesterol-LDL, μmol/l	2.10 ± 0.19	2.70 ± 0.21*
Coefficient of atherogeneity	2.70 ± 0.35	2.88 ± 0.29

Note: * - The difference with Control group is statistically significant; P ≤ 0.05.

The results of quantitative determination of component content of lipids in patients with AH are shown in Tab. 2. Our studies demonstrate no significant differences in lipid status in patients with AH in comparison to control group, with the exception of triglycerides and LDL cholesterol, which are respectively 29 % and 28 % above the reference value.

It should be noted that despite the relative normalization of content of lipids, activation of free radical oxidation reactions, as indicated by significantly higher levels of lipid and protein oxidation product in serum remains in patients with AH.

Catalase and superoxide dismutase activities are decreased in patients with AH in comparison to control. These changes indicate the presence of imbalance between pro- and antioxidant systems. Noteworthy, the degree of oxidative modification of LDL + VLDL is increased by 45 % in patients with AH (Tab. 1). Content of carbonyl products of protein oxidation in HDL fraction also increases by 20 % compared with the control group. This may be due to inhibition of PON-1 activity, which is responsible for protecting HDL, and activation of MPO activity, which is aimed at peroxidation of lipoprotein particles and other macromolecules that participate in the processes of atherogenesis and progression of the pathological process in atherogenesis.

CONCLUSIONS

1. The accumulation of carbonyl products of protein oxidation in blood, HDL and LDL eventually results in oxidative modification of HDL and LDL, and loss of their functional properties.
2. Our evaluation showed a significant increase of MPO activity and decrease of PON1, catalase and superoxide dismutase activity in patients with AH.
3. Changes of MPO and PON1 activity may serve as a useful marker of dysfunctional HDL. The ratio "MPO/PON1" can be used as a predictor of inflammatory response and atherosclerotic process and to evaluate the effectiveness of treatment.
4. The activity of HDL-associated enzymes (PON1 and MPO) is the most informative indicator of functional state of HDL, and not the level of HDL-cholesterol.

5. Demonstrated changes in the functional state of HDL, in our opinion, create a predisposition to development and progression of atherosclerosis in patients with AH.

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БІЛКОВІ ФАКТОРИ ОКСИДАТИВНОГО СТРЕСУ І АТЕРОГЕНЕЗУ У ПАЦІЄНТІВ З АРТЕРІАЛЬНОЮ ГІПЕРТЕНЗІЄЮ

Метою даної роботи було вивчення якісного стану основних класів ліпопротеїнів та активності асоційованих з ними білків-ферментів у крові пацієнтів з артеріальною гіпертензією (АГ). В дослідження були включені 30 пацієнтів з АГ II стадії (середній вік – $47,4 \pm 3,3$ років). У пацієнтів з АГ зберігається інтенсифікація процесів вільнорадикального окиснення білків, що супроводжується переокисненням ЛПНГ, ЛПДНГ і ЛПВГ із збільшенням їх індексу перекисної модифікації і атерогенного потенціалу крові. У пацієнтів з АГ встановлено зростання активності МПО на фоні зниження активності антиоксидантних ферментів – ПОН-1, каталази і супероксиддисмутази. Зміна активності МПО і ПОН-1 може бути використана в якості предиктора активності запальної реакції за участі нейтрофільних лейкоцитів і прогресування атеросклеротичного процесу, а також для оцінки ефективності лікування. Для оцінки якісного стану ліпопротеїнів крові і ступеня їх атерогенності інформативними показниками слід вважати активність ліпопротеїн-асоційованих білків-ферментів (ПОН-1 і МПО), а не тільки рівень холестеролу в них.

Ключові слова: артеріальна гіпертензія; атерогенез; оксидативний стрес; параоксоназа-1; міелопероксидаза

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БЕЛКОВЫЕ ФАКТОРЫ ОКСИДАТИВНОГО СТРЕССА И АТЕРОГЕНЕЗА У ПАЦИЕНТОВ С АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ

Целью данной работы было изучение качественного состояния основных классов липопротеинов и активность ассоциированных с ними белков-ферментов в крови пациентов с артериальной гипертензией (АГ). В исследование были включены 30 пациентов с АГ II стадии (средний возраст – $47,4 \pm 3,3$ лет). У пациентов с АГ сохраняется интенсификация процессов свободнорадикального окисления белков, что сопровождается переокислением ЛПНП, ЛПОНП и ЛПВП с увеличением индекса их перекисной модификации и атерогенного потенциала крови. У пациентов с АГ установлено возрастание активности МПО на фоне снижения активности антиоксидантных ферментов – ПОН-1, каталазы и супероксиддисмутази. Изменение активности МПО и ПОН-1 может быть использовано в качестве предиктора активности воспалительной реакции с участием нейтрофильных лейкоцитов и прогрессирования атеросклеротического процесса, а также для оценки эффективности лечения. Для оценки качественного состояния липопротеинов крови и степени их атерогенности информативными показателями следует считать активность липопротеин-ассоциированных белков-ферментов (ПОН-1 и МПО), а не только уровень холестерина в них.

Ключевые слова: артериальная гипертензия; атерогенез; оксидативный стресс; параоксоназа-1; миелопероксидаза

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