Age-related alterations in arginase-NO-synthase system in patients with coronary heart disease associated with hypertension

Związane z wiekiem zmiany w układzie arginazy-NO-syntazy w grupie pacjentów z chorobą wieńcową współistniejącą z nadciśnieniem tętniczym

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ABSTRACT

INTRODUCTION: Coronary heart disease (CHD) is the most common heart diseases in Europe. The aim of this study was to determine the intensity of L-arginine metabolism by two alternative pathways (oxidative by NOS and nonoxidative by arginase) in the blood plasma of patients with CHD associated with hypertension (HT) of different age groups.

MATERIALS AND METHODS: 50 patients with isolated CHD and 42 patients with CHD associated with HT were enrolled in this study. NOS activity was determined by nitrite anion formed in the reaction. Arginase activity was tested by the formation of urea.

RESULTS: In middle-aged patients with isolated CHD, the total NOS activity statistically significantly increased by 2.2 fold in comparison with healthy subjects of the same age group. In patients with CHD associated with HT, the total NOS activity statistically significantly increased in both middle-aged and older persons 2.3-fold than in healthy subjects of the same age groups. In patients with isolated CHD, the arginase activity increases 1.5-fold in middle-aged patients and 1.7-fold in older patients compared with the healthy participants. In the middle-aged and older patients with CHD associated with HT, the arginase activity statistically significantly increased 1.7- and 1.8-fold than in the healthy subjects of the same age groups.

CONCLUSIONS: In patients with isolated CHD and patients with CHD associated with HT, an increased total NO-synthase and arginase activity in comparison with healthy individuals was found. It was shown that the increase in NO-synthase and arginase activity is more expressed in older patients than middle-aged patients.

KEY WORDS
coronary heart disease, hypertension, nitric oxide, NO-synthase, arginase
Coronary heart disease (CHD) continues to be the main cause of death and a major cause of morbidity and loss of quality of life. CHD is a leading cause of death and disability in Western societies. Moreover, due to its sharply increasing prevalence in non-Western countries, it will inevitably be a major health problem worldwide in the years to come [1,2]. Epidemiological studies have established a strong association between CHD and hypertension (HT). HT is a major independent risk factor for the development of CHD [3].

Endothelial dysfunction plays a fundamental role in the pathogenesis of CHD. There are multiple mechanisms for endothelial dysfunction. However, the common feature is a reduction in the synthesis and amount of bioavailable NO, which is the most powerful endogenous vasodilator known and normally serves to protect the vessel from the molecular events that lead to atherosclerosis [4,5].

NO inhibits vascular smooth muscle cells migration and growth, adhesion and platelet aggregation. NO also inhibits the atherosclerosis process, and an alteration in NO production within the vascular endothelium could contribute to the pathogenesis of CHD.

Under physiological conditions, enzymatic NO formation in humans and animals occurs by nitric oxide synthase (NOS, EC 1.14.13.39), which is a cytochrome P-450 type hemoprotein. NOS has been generally considered to be the primary source of NO in biological systems [6].

NO production is down-regulated by arginase (EC 3.5.3.1). Endothelial cells express arginases that can compete with eNOS for substrate (L-arginine). Arginase exists in 2 isoforms; in human endothelial cells, arginase II seems to be the predominant isoenzyme [7,8].

Evidence for the role of increased enzymatic activity of arginase in endothelial dysfunction also has been provided in animal models of cardiovascular disease such as aging, atherosclerosis, endothelial dysfunction after ischemia-reperfusion and HT induced by aortic coarctation or high salt [7,9,10].

The relationship between the NO-synthase (oxidative) and arginase (nonoxidative) pathways of L-arginine in cells supports the physiological pool of L-arginine and determines the intensity of NO production of its metabolites. According to various pathological conditions, the relation between the oxidative and nonoxidative pathway of L-arginine changes. This may be due to changes in the bioavailability of L-arginine, the development of oxidative stress or hypoxic condition which is typical for CHD.

Despite the large number of papers devoted to NO-synthase and arginase systems in cardiovascular diseases, the age-related changes of the arginase-NO-synthase system and mechanisms maintaining NO homeostasis in patients with isolated CHD and CHD with HT still have not been fully investigated.
The purpose of this article is to determine the intensity of L-arginine metabolism by two alternative pathways (oxidative by NOS and nonoxidative by arginase) in the blood plasma of patients with CHD associated with HT of different age groups.

**MATERIALS AND METHODS**

Fifty patients with isolated CHD without concomitant diseases (32 M, 18 F) aged 45–75-years old (mean ± s.d.: 56.8 ± 4.7) and forty-two patients with CHD associated with HT (22 M, 20 F) aged 45–75-years old (mean ± s.d.: 54.4 ± 4.6) were enrolled in this study. Inpatients were only included in the study if CHD was verified by instrumental data (ECG, including daily monitoring ECG), echocardiography, bicycle ergometry test). Patients who have not received treatment by nitro medication, but occasionally used nitroglycerin for angina pectoris were included in the study. The patients of each group were divided into two subgroups with respect to their age: middle-aged patients (45–60 years) and older patients (61–75 years). The patients of both subgroups were matched for sex, disease duration and number of pain attacks.

Twenty healthy volunteers (divided into two age groups) with no clinical symptoms of cardiovascular disease, matched by age and sex were enrolled in this study. Written informed consent was obtained after full explanation of the study procedure. The protocol was approved by the Ethical Committee of Danylo Halytsky Lviv National Medical University.

The activity of the arginase-NO-synthase system was tested in blood plasma. The intensity of L-arginine metabolism by the oxidative pathway was evaluated by NOS activity. The total NO-synthase activity was determined by nitrite anion formed in the reaction. The incubation medium for determining the total NOS activity contained: 0.1 M Tris-HCl (pH 7.4), 5 mM MgCl₂, 1.0 mM NADPH («Sigma», USA), 1 mM L-arginine and 10 mM CaCl₂. The reaction was initiated by adding 0.2 ml of blood to the incubation mixture (final volume 2.0 ml). The samples were kept for 20 min in a water bath (37°C) under constant shaking. The enzymatic reaction was stopped by additions of 1.25 mL of 85 mM NaOH and 1.25 mL of 75 mM ZnSO₄. The control samples were prepared similarly, but without the substrate in the incubation medium. After stopping the enzymatic reaction, the samples were centrifuged at 3000 g for 15 min. The NO₃⁻ concentration was determined in an aliquot of the supernatant using the Griess reaction [11]. The total NOS activity was expressed as nmol NO₂/min per 1 ml.

As a result of the studies it was found that the total NOS activity in middle-aged and older healthy individuals is not statistically significantly different. The total NOS activity in the middle-aged healthy subjects is 0.27 ± 0.05 nmol NO₂/min per 1 ml and in the elderly healthy individuals it is 0.32 ± 0.02 nmol NO₂/min per 1 ml (Fig. 1). In the middle-aged patients with isolated CHD the total NOS activity statistically significantly increased 2.2 fold in comparison with healthy participants of the same age group and is 0.59 ± 0.03 nmol NO₂/min per 1 ml. In the elderly patients with isolated CHD the total NOS activity statistically significantly increased 2.6-fold compared to elderly healthy subjects and is 0.84 ± 0.04 nmol NO₂/min per 1 ml.

In patients with CHD associated with HT the total NOS activity statistically significantly increased in both the middle-aged and older patients 2.3-fold in comparison to healthy subjects of the same age groups and is 0.62 ± 0.04 and 0.73 ± 0.05 nmol NO₂/min per 1 ml respectively.

It was shown that arginase activity in the middle-aged and elderly healthy subjects is 1.3 ± 0.1 and 1.4 ± 0.2 micromol urea/min per 1 liter, respectively. In patients with isolated CHD the arginase activity increases 1.5-fold in the middle-aged patients and 1.7-fold in the older patients compared with the healthy participants. The arginase activity in the middle-aged and older
patients with isolated CHD is 1.9 ± 0.2 and 2.35 ± 0.17 micromol urea/min per 1 liter, respectively. In the middle-aged and older patients with CHD associated with HT the arginase activity statistically significantly increased 1.7- and 1.8-fold in contrast to healthy subjects of the same age groups and is 2.2 ± 0.2 and 2.5 ± 0.2 micromol urea/min per 1 liter, respectively. The arginase activity in the older patients with isolated CHD and patients with CHD associated with HT was significantly different from this value in the middle-aged patients.

**DISCUSSION**

In this study, we showed that the total NOS activity increased in both patients with CHD and patients with CHD associated with HT. The increase in total NOS activity indicates NO overproduction and may be due to the overexpression of the inducible isoform of NOS (iNOS), which ensures the formation of additional amounts of NO in the cell during various pathological conditions. While eNOS and nNOS are normal constituents of healthy cells, iNOS is not usually expressed in undiseased vascular tissue and its expression is seen mainly in pathological conditions [12]. It was shown that the activation of iNOS leads to the synthesis of NO in amounts that greatly exceed its synthesis by eNOS [13]. Our findings are consistent with report [14] where it was demonstrated that stimulated expression of the iNOS gene and higher iNOS serum levels are associated with CAD. Moreover, the expression of iNOS in active atherosclerotic plaques was detected. It is possible that iNOS contributes to tissue damage or other features of plaque development or stability [15]. It was found that iNOS has a role in the pathophysiology of vascular aging [16]. Impaired endothelial dependant vasodilatation associated with abnormal iNOS may be an important factor in the development and progression of atherosclerosis and hypertension. In addition, endothelial
dysfunction in hypertensive patients may initiate vascular inflammation that leads to cytokine-induced activation of inducible NOS which favors the formation of peroxynitrite contributing to cytotoxicity and tissue injury [17].

NO overproduction by iNOS may be a compensatory mechanism improving tissue perfusion. On the other hand, excessive NO generation is more dangerous than NO deficit. iNOS causes the production of "harmful" NO and free radical products, which are involved in the activation of lipid peroxidation processes, resulting in cell damage and superoxide anion-radical (O₂⁻) accumulation. High NO concentrations activate oxidative and nitrosative stress which promote diverse pathologic reactions including atherosclerosis. This causes violation of the prooxidant-antioxidant balance. In high concentrations, NO is profoundly toxic and is a factor of endogenous intoxication that determines its cytotoxic effect and causes cell death by apoptosis and necrosis. As a result of this, the activation of apoptotic mechanisms and initiation of destructive processes occur in cardiomyocytes, endothelial cells and other cells, which leads to the progression of cardiovascular system dysfunction [18,19,20].

Since the overproduction of NO is toxic to macrophages and neighboring cells, a mechanism to prevent the overproduction of NO may exist. Both NOS and arginase use arginine as a common substrate, and arginase may down-regulate NO production by competing with NOS for arginine [21,22]. Competitive relationships occur between NOS and arginase. Arginase is a limiting factor for NO synthesis. Reducing the concentration of L-arginine arginase directly inhibits NO synthesis [23,24]. In the present study, there was a significant increase in arginase activities both in patients with CHD and those with CHD associated with HT. Li et al. [25] prepared endothelial cells expressing arginase I or arginase II and demonstrated that both isoforms can down-regulate NO production. Increased arginase activity indicates less NO production [26].

The up-regulation of arginase activity and expression has been reported to play a role in aging. Berkowitz et al. [9] reported that arginase decreases NO production in aortic rings in rats and that arginase up-regulation contributes to the endothelial dysfunction of aging blood vessels. Therefore, arginase down-regulates NO production and may have important implications for cardiovascular function. An increase in arginase expression and activity has been shown to contribute to vascular dysfunction of extracranial blood vessels in aged mice and rats, as well as in atherosclerotic mice [27,28]. It was shown that the inhibition of arginase increases endothelium-derived NO formation and improves endothelium-dependent vasodilatation in animal models and patients with coronary artery disease [29,30,31].

Activation of the non-oxidative metabolic pathways of L-arginine is a compensatory mechanism which limits the bioavailability of L-arginine as a substrate for the synthesis of "harmful" NO under conditions of pathology. Our results suggest that changes in the functional state of the arginase-NOS-synthase system are determining mechanisms that lead to the disruption of regulatory properties and NO homeostasis under endothelial dysfunction.

The present study demonstrated that disturbance of the endothelial function is characterized by increased activity of total NO-synthase, which leads to the overproduction of "harmful" NO. An increase in arginase activity is more expressed in the elderly and is the compensatory mechanism to limit L-arginine bioavailability. It is assumed that dysfunction of the arginase-NOS-synthase system plays an important role in the disturbance of NO regulatory properties and vascular tone homeostasis.

Certain limitations of the present study deserve comment. The populations used in the present study have a small number of participants that necessitate careful interpretation of the results. Future studies should incorporate the investigation of gender, smoking status and other demographic characteristics of the examined patient group that might represent underling influences on NOS and arginase activity besides age.

**CONCLUSIONS**

In patients with isolated CHD and patients with CHD associated with HT, increased total NO-synthase and arginase activity in comparison with healthy individuals was found. It was shown that an increase in NO-synthase and arginase activity is more expressed in older patients than middle-aged patients.

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