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CLINICAL RESEARCH

The Nitric Oxide System in Patients with Chronic Heart Failure

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Abstract

The aim of this study was to evaluate the metabolic features of nitric oxide (NO) in patients with chronic heart failure (CHF) of ischemic origin. The study included 303 patients with ischemic CHF who received the standard treatment for CHF. Evaluation of the functional status of the NO system included the detection of arginine concentrations in the peripheral blood, stable metabolites of nitric oxide (NO2/NO3) and endothelial NO synthase (eNOS) activity. After the initial assessment, patients were randomly divided into groups: the T-group who had torasemide (5-10 mg /day) included in the treatment scheme and the F-group with furosemide (20-40 mg/day). The diuretic dose was titrated according to the severity of the edema syndrome. In the randomly formed A-group of patients, L-arginine hydrochloride (Tivortin, URiA-PHARM, Ukraine) was administered in the treatment scheme for three months. The Tivortin dose was 6 g per day (10 mg syrup three times a day). The presence of diuretics in the treatment scheme allowed for normalizing the functioning of the NO system including eNOS activity. The additional use of L-arginine hydrochloride positively affected the NO metabolism.

Keywords**:** *chronic heart failure, torasemide, nitric oxide, arginine.*

Introduction

The current views on the pathogenesis of CHF have significantly expanded which enables us to consider it as a multicomponent metabolic syndrome with hormonal deficit [1] accompanied by an anabolic/catabolic imbalance [2]. Oxidative stress (OS) is regarded as one of the pathogenetic links of chronic heart failure [3,4] characterized by a disparity between the level of free radicals and antioxidant protection, with the accumulation of metabolites such as the fragments of the oxidized nucleotides in combination with the modified lipids and proteins in the cells and tissues [5,6].

NO is an important mediator of intracellular and intercellular interactions [7-12]. NO is a universal modulator of various functions, such as the regulation of respiration, plasticity of the nervous tissue and the release of neurotransmitters, etc [13]. Several researchers consider NO as an independent "stress-limiting NO-system" [14]. It also is known that NO, depending on the number and site of production, induces both protective and damaging effects [15,16].

NO is generated from L-arginine by the action of endothelial NO synthase (eNOS) in the presence of cofactors such as tetrahydrobiopterin [17]. Three isoforms of NOS have been described [18]. NOS1 and NOS3 are constitutively expressed and their activity is calcium dependent. The NOS2 is calcium-independent, and is upregulated in response to pro-inflammatory mediators leading to an increased production of NO [19].

As revealed, NO plays an important role in regulating the stress-recognition reaction of the immuno-neuroendocrine system [13,14]. The stress reaction, initially being adaptive, is included in the pathological process during hormonal changes, which cause a complex of circulatory and metabolic disorders, thus closing the vicious circles of the disease pathogenesis. Thus, a compensatory act develops into a "disease of adaptation" [20].

The aim of this study was to evaluate the metabolic features of Nitric Oxide (NO) in patients with chronic heart failure (CHF) of ischemic origin.

Material and Methods

The study included 303 patients with ischemic CHF who received the standard treatment for CHF: aspirin (75-100 mg/day), olmesartan and bisoprolol (dose titrated according to the hypotensive response and heart rate), atorvastatin (20 mg/day).

The exclusion criteria were disorders of the other organs and systems, including organ failure requiring chronic treatment, acute cardiovascular events (within two months) and CHF associated with valvular and congenital heart disease and respiratory diseases.

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Echocardiographic studies were performed in the left lateral position. LV end-diastolic volume (LVEDV) and end-systolic volume (LVESV) were measured using the modified Simpson's method. The measurements were done in the apical 2 -, 3 -, 4 -, and 5-chamber views. The stroke volume (SV) and ejection fraction (EF) were calculated using the standard formula. LVEF<50% was detected in 108 patients and LVEF≥50% in 195 patients.

Evaluation of the functional status of the NO system included the detection of arginine concentrations in the peripheral blood, stable metabolites of nitric oxide (NO2/NO3) and eNOS activity.

The eNOS activity was determined by the increase in the production of NO from L-arginine in the presence of NADPH, and was expressed in μmol/L. NADPH consumption by eNOS was determined spectrophotometrically at 340 nm. Nitrite/nitrate detection in the serum was performed employing the spectrophotometric assay based on Griess reagent.

After the initial assessment, patients were randomly divided into groups (Table 1) : the T-group who had torasemide (5-10 mg /day) included in the treatment scheme and the F-group with furosemide (20-40 mg/day). The diuretic dose was titrated according to the severity of the edema syndrome. In the randomly formed A-group of patients, L-arginine hydrochloride (Tivortin, URiA-PHARM, Ukraine) was administered in the treatment scheme for three months. The Tivortin dose was 6 g per day (10 mg syrup three times a day).

A follow-up examination was performed after three months of therapy. The control group (CG) consisted of 20 healthy volunteers without cardiovascular diseases, including any of functional nature.

Table 1.

Composition of research groups

Groups	LVEF<50%				LVEF≥50%			
	n=108				n=195			
	T		F		T		F	
	57		51		93		102	
	A+	A-	A+	A-	A+	A-	A+	A-
	19	38	17	34	31	62	34	68

Abbreviations: **T**,Torasemide; **F**, Furosemide; **A,** Arginine

All the data was processed employing the variation statistical methods using the software Statistica for Windows 6.0. For data with normal distribution, inter-group comparisons were performed using student's t-test. The mean (M) and standard error (SE) of the mean were calculated. The difference was considered reliable when *P<0.05*.

Results and Discussion

The NYHA class II was diagnosed in 210 (69.3%) patients and class III in 93 (30.7%) patients. By the end of the third month of treatment, the number of NYHA class III patients decreased from 27 (47%) to 21 (37%) at the initial LVEF<50% and from 21 (23%) to 18 (19%) at the initial LVEF≥50% in the T-group. The number of NYHA class III patients decreased from 27 (53%) to 25 (49%) at the initial LVEF<50% and from 18 (18%) to 17 (17%) at the initial LVEF≥50% in the F-group. The total score by the 'Minnesota Living with Heart Failure Questionnaire' dropped by 25.0% in the whole group. The score reduction in the T-group was 32.2% versus 18.1% in the F-group (*P<0.001*). Side effects requiring discontinuation of therapy were not observed.

CHF patients had initially reduced their arginine concentration in the peripheral blood, which, however, was more pronounced at LVEF<50% (Table 2). By the end of three months of therapy, the arginine concentration in the peripheral blood significantly increased by 61.9% in patients with LVEF<50% and by 37.5% in patients with LVEF≥50% (*P<0.001*). However, it remained significantly lower in patients with LVEF <50% than in those patients with LVEF≥50% (*P<0.001*). At the end of the three of months therapy, the increased arginine concentration in the peripheral blood was noted in both groups (+66.2% for T-group and +26.6% for F-group), although the torasemide effect was seen to be more pronounced than the furosemide effect among the patients as with EF<50% (+81.5% vs +40.0%; *P<0.001*), and patients with EF≥50 %.(+56.8% vs +19.9%; *P<0.001*).

The frequency of Tivortin inclusion in the treatment scheme was comparable in all the clinical subgroups. Its additional positive effect on arginine concentration in the peripheral blood was noted (+51.63% in the A-group and +43.53% in the group without arginine; *P<0.01*). The distribution of patients based on the LVEF showed that the positive effect of Tivortin was visible only in patients with LVEF<50% (+69.63% in the A-group and +58.32% in the group without arginine; *P<0.01)*. In patients with LVEF≥50%, the following increase in the arginine concentration was observed: +41.66% in the A-group ("A+") and +35.37% in the group without arginine ("A-"); *P>0.05*.

The eNOS activity was seen to significantly reduce in the CHF patients compared with the CG patients (8.82±0.06 μmol/L vs 12.96±0.02 μmol/L; *P<0.001)*. Reduction in the eNOS activity was more pronounced in patients with LVEF<50% (7.59±0.03 μmol/L vs 9.50±0.02 μmol/L in patients with LVEF≥50%; p<0.001). At the end of three months of therapy, the eNOS activity was significantly increased by 7.0% in the whole patient group. At the same time, the increase in the eNOS activity was 14.6% in patients with LVEF<50% versus 2.8% in patients with LVEF ≥ 50% (*P<0.001)*. However, the absolute values of the eNOS activity in patients with an LVEF≥50% were significantly higher than in those patients with an LVEF<50% (9.76±0.05 μmol/L vs 8.70±0.06 μmol/L; *P<0.001*). We found a more pronounced effect of the torasemide compared with the furosemide, regardless of the LVEF, on the eNOS activity (+12.09% vs +2.04%; *P<0.001*). L-arginine hydrochloride contributed significantly to the greater increase in the eNOS activity (+11.53% for "A+" and +4.76% for "A-"; *P<0.001*). The positive effect of L-arginine hydrochloride on eNOS activity was not dependent upon the initial LVEF.

The concentration of the stable NO metabolites in patients with lLVE <50% was significantly higher when compared with the CG patients (15.52±0.14 μmol/L vs 13.28±0.63 μmol/L; *P<0.001*), which may have been caused by the activation of the NOS activated macrophages. A reflection of this process is the phenomenon of peripheral vasodilation observed in the CHF patients. The blood concentrations of NO2/NO3 in patients with LVEF≥50% was lower than in the CG patients and patients with LVEF<50%. At the end of the 3rd month of therapy, the blood concentrations of NO2/NO3 in patients with LVEF≥50% significantly increased in group "A+" (+75.4% for "A+" and +62.05% for "A-"; *P<0.001*) and T-group compared with F-group

Table 2.

NO system in patients with ischemic CHF under standard therapy with loop diuretics and L-arginine

Parameters		**Control**	**CHF**			
Baseline	L-arginine, μmol/L	170.85±4.73	110.74±3.68”			
	NO2/NO3, mmol/L	13.73±0.64	9.73±0.25”			
	NOS, mmol/L	12.49±0.03	8.8±0.06”			
After 3 months	L-arginine, μmol/L		155.52±1.21			
	NO2/NO3, mmol/L		10.50±0.05			
	NOS, mmol/L		9.38±0.05			
LVEF			***LVEF<50%***		***LVEF≥50%***	
Baseline	L-arginine, μmol/L		93.01±0.14		120.55±5.60ˆ	
	NO2/NO3, mmol/L		15.52±0.14		6.52±0.02ˆ	
	NOS, mmol/L		7.59±0.03		9.50±0.02ˆ	
After 3 months	L-arginine, μmol/L		150.59±1.96		158.25±1.50ˆ	
	NO2/NO3, mmol/L		9.89±0.07		10.85±0.06ˆ	
	NOS, mmol/L		8.70±0.06		9.76±0.05ˆ	
Diuretic			***T***		***F***	
Baseline	L-arginine, μmol/L		106.68±0.96		114.71±7.23	
	NO2/NO3, mmol/L		10.00±0.36		9.47±0.35	
	NOS, mmol/L		8.81±0.08		8.84±0.08	
After 3 months	L-arginine, μmol/L		175.65±0.55		135.77±0.50**	
	NO2/NO3, mmol/L		10.99±0.06		10.03±0.06**	
	NOS, mmol/L		9.81±0.06		8.96±0.05**	
Diuretic and LVEF			***LVEF<50%***	***LVEF≥50%***	***LVEF<50%***	***LVEF≥50%***
Baseline	L-arginine, μmol/L		93.12±0.20	115.00±0.64ˆ	92.20±0.21	125.62±10.69ˆ
	NO2/NO3, mmol/L		15.64±0.03	6.54±0.3ˆ	15.39±0.30	6.51±0.03ˆˆˆ
	NOS, mmol/L		7.62±0.04	9.54±0.03ˆ	7.56±0.04	9.47±0.03ˆ
After 3 months	L-arginine, μmol/L		168.97±0.63	179.75±0.40ˆ	130.04±0.95**	138.64±0.32ˆ**
	NO2/NO3, mmol/L		10.33±0.08	14.40±0.06ˆ	9.39±0.08**	10.34±0.05ˆ**
	NOS, mmol/L		9.12±0.06	10.24±0.05ˆ	8.22±0.06**	9.32±0.04ˆ**
L-arginine			***A+***		***A-***	
Baseline	L-arginine, μmol/L		107.25±1.16		112.48±5.49	
	NO2/NO3, mmol/L		9.79±0.44		9.70±0.31	
	NOS, mmol/L		8.83±0.10		8.82±0.07	
After 3 months	L-arginine, μmol/L		160.88±2.0		152.91±1.47$^{\theta}$	
	NO2/NO3, mmol/L		11.11±0.08		10.21±0.05$^{\theta\theta}$	
	NOS, mmol/L		9.79±0.08		9.18±0.05$^{\theta\theta}$	
L-arginine and LVEF			***LVEF<50%***	***LVEF≥50%***	***LVEF<50%***	***LVEF≥50%***
Baseline	L-arginine, μmol/L		93.08±0.26	115.10±0.75**	93.04±0.17	123.28±8.39**
	NO2/NO3, mmol/L		15.66±0.04	6.54±0.03**	15.33±0.24	6.51±0.03**
	NOS, mmol/L		7.60±0.05	9.50±0.4**	7.61±0.04	9.50±0.03**
After 3 months	L-arginine, μmol/L		157.89±2.99	162.53±2.62	147.31±2.43$^{\theta}$	156.10±1.80*$^{\theta\theta}$
	NO2/NO3, mmol/L		10.47±0.10	11.47±0.09**	9.63±0.08$^{\theta\theta}$	10.54±0.05**$^{\theta\theta}$
	NOS, mmol/L		9.10±0.11	10.16±0.07**	8.52±0.06$^{\theta\theta}$	9.56±0.05**$^{\theta\theta}$

Abbreviations as in Table 1.

*”P<0.001 vs control; ^P<0.001 between LVEF<50% and LVEF≥50%; *P<0.01, **P<0.001 between T and F;*
$^{\theta}$P<0.01, $^{\theta\theta}$P<0.001 between A+ and A-.

(+74.59% vs +59.13%; *P<0.001*). Patients with LVEF<50% showed a significant reduction in the blood concentration of NO2/NO3 in all the therapeutic subgroups. However, the absolute values of NO2/NO3 from the T-group were significantly higher than those from the F-group, regardless of the LVEF level. The blood NO2/NO3 concentration was significantly higher in group "A+" than in group "A-", regardless of the LVEF level (Table 2).

The presence of diuretics in the treatment plan helped to improve the hemodynamic and functional status in the CHF patient group and improved their quality of life; therefore, the effect of the torasemide is significantly higher than the effect of the furosemide. The addition of the L-arginine hydrochloride to the basic treatment of the CHF can improve the patient's quality of life, as shown in our study, and other studies [21].

Our results indicate significant impairments in the NO synthesis at ischemic CHF. In CHF patients with LVEF≥50%, a significant reduction in the arginine and NO2\NO3 concentrations in the peripheral blood was revealed, as well as a reduction in the eNOS activity. Against a background of therapy with diuretics, the arginine level in the peripheral blood increased, simultaneously, while the eNOS activity and the blood NO2\NO3 concentration rose. The L-arginine hydrochloride had potentiated the effect of the therapy [22].

The blood concentrations of NO2/NO3 in CHF patients with LVEF<50% increased against the background of the reduced arginine concentration and eNOS activity. The correction of the hemodynamic disturbances using diuretics had contributed to the normalization of the endothelial function and restoration of the eNOS activity.

Conclusion

The presence of diuretics in the treatment scheme allowed for normalizing the functioning of the NO system including eNOS activity. The additional use of L-arginine hydrochloride positively affected the NO metabolism.

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