Efficacy L-Arginine In Patients With Nonalcoholic Steatohepatitis Associated With Metabolic Syndrome

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Abstract: Background and Purpose: Recent research in the field of hematology indicate that among the many pathogenic mechanisms of development and progression of nonalcoholic steatohepatitis (NASH), which occurs on the background of the metabolic syndrome, an important role is played by endothelial dysfunction and violations of haemocoagulation. The aim of this research was to study the effectiveness of L-arginine as it corrects endothelial dysfunction and disorders of homeostasis haemocoagulation link in patients with NASH associated with the metabolic syndrome. Subjects and Methods: 128 patients with nonalcoholic steatohepatitis associated with metabolic syndrome were examined. Some patients (63 persons) received standard treatment according to national guidelines. To another group (65 patients) on the background of basic therapy L-arginine hydrochloride, followed by transition to oral form of L-arginine aspartate was administered. Blood levels of stable nitrogen monoxide metabolites (nitrites, nitrates), endothelin-1 and plasma recalcification time, prothrombin time, thrombin time, activated partial thromboplastin time, fibrinogen plasma level, activity of antithrombin III and coagulation factor XIII, potential activity of plasminogen, plasma fibrinolytic blood activity were studied. Results: Originally significantly increased levels of endothelin-1 decreased after the therapy in all studied groups, but more noticeable changes in the group with L-arginine appointment were observed ($p<0.05$). In the studied groups normalization of stable nitrogen monoxide metabolites after treatment was also noticed. Significant ($p<0.05$) increase in all haemocoagulation time characteristics and activities of antithrombin-III and factor XIII was found. The positive effect of L-arginine on blood fibrinolytic activity was noted. Discussion and Conclusion: Combined therapy of nonalcoholic steatohepatitis associated with metabolic syndrome, with a differentiated degreeal L-arginine assignment by double increasing the duration of use of parenteral form (L-arginine hydrochloride) and subsequent double increasing of oral form of the drug (L-arginine aspartate) in case of the classic metabolic syndrome and its phenotype (AH + AO + IGT/DM-2) compared with the scheme of his appointment in case of phenotype (AH + DLP + IGT/DM-2) and (AH + DL + AO) is more effective than traditional therapy (essential phospholipids, ursodeoxycholic acid, metformin) for correction of basic clinical and laboratory disease syndromes, restore the functional state of the endothelium and eliminate haemocoagulation violations.

Index Terms: nonalcoholic steatohepatitis, metabolic syndrome, endothelial dysfunction, haemocoagulation, fibrinolysis, L-arginine, blood.

1 INTRODUCTION

Nonalcoholic steatohepatitis (NASH) is considered one of the most topical problems of hepatology [1]. Clinical experience suggests that NASH is often hepatic manifestation of metabolic syndrome (MS) [2]. Typical for modern society sedentary lifestyle, poor diet with increasing amounts of refined products, permanent psychological and emotional stress leads to an increase proportion of people who are overweight among people of all ages, including young people, making MS one of the most difficult medical and social issues of our time [3] Our attention was attracted to the possibility of using the complex pathogenetic treatment of patients with NASH, combined with the MS, with original product of L-arginine [4]. It is proved that nitrogen monoxide (NO) is a unique mediator of intercellular interaction and effectively reduces the production and concentration of free radicals in the plasma and tissues, inhibits the synthesis of asymmetric dimethyl arginine - oxidative stress endogenous stimulator. As a result of increase free radical degradation of NO molecules, its physiological effects decrease, vessels endothelial dysfunction, including vessels of organs developes. Therefore, the use of L-arginine, which is a nitrogen monoxide precursor theoretically and practically is reasonable not only in the treatment of cardiovascular diseases, but also other diseases of internal organs. The aim of the research was to study the effectiveness of L-arginine as it corrects endothelial dysfunction and disorders of homeostasis haemocoagulation link in patients with NASH associated with the metabolic syndrome.

2 MATERIALS AND METHODS

The study involved 128 patients with nonalcoholic steatohepatitis associated with metabolic syndrome. Nutritional care with individual choice of daily dose of protein, fat, carbohydrates, vitamins and trace elements, compulsory 5 meals with the exception of mechanical and chemical irritation were recommended to all the patients. For the purpose of the study and comparison of the effectiveness of the proposed treatment programs randomization of patients into 4 groups was conducted. The first group (group IA) consisted of 39 patients (24 patients with NASH associated with classical MS, and 15 patients with NASH associated with metabolic syndrome without dyslipidemia (DLP) - phenotype of arterial hypertension (AH) + abdominal obesity (AO) + impaired glucose tolerance (IGT) / diabetes mellitus (DM) type 2), which, in addition to the standard basic treatment received L-arginine hydrochloride, 100 ml / iv once per day, 10 days in the first phase of treatment, followed by taking L-arginine aspartate 20 ml 2 times a day. The second group (group comparison I B) included 38 patients (24 patients with NASH associated with classical MS, and 14 patients with NASH associated with metabolic syndrome without dyslipidemia - AH + AO + IGT / DM type 2) who were receiving standard therapy: clinical nutrition; hepatoprotectors (Essentiale Forte N - 10 ml iv once a day № 10, followed by 1800 mg (6 capsules) per day); Metformin (1000 mg / day). Patients third (main) group (group II A), which is made up of 26 people (7 patients with NASH associated with metabolic syndrome phenotype AH + DSP + IGT /DM type 2, and 19 patients with NASH associated with metabolic syndrome without IGT - phenotype H + DSP + AO) on the background of basic therapy administered L-arginine hydrochloride, 100 ml / iv once per day - 5 days followed by transition to the use of L-arginine aspartate 10 ml 2 times a day. Patients of fourth group (comparison group II B), which consisted of 25 people (7 patients with NASH associated with metabolic

syndrome phenotype AH + DSP + IGT / DM type 2, and 18 patients with NASH associated with the metabolic syndrome with impaired glucose tolerance - phenotype of AH + DSP + AO) administered a standard basic therapy clinical nutrition; hepatoprotectors (Ursohol - 15 mg / kg body weight per day); in case of impaired glucose tolerance - Metformin (1000 mg / day). Common clinical, laboratory and instrumental data, results of ultrasound and morphological study of the liver and determination of serum markers of hepatitis viruses B and C were used. The study was performed before treatment and in dynamics (after 4 weeks of treatment). The functional state of the endothelium was studied with a help of blood levels of stable metabolites of NO (nitrites, nitrates) by method of L.C. Green et al. [5], blood levels of endothelin-1 (ET-1) (DRG) by ELISA. Also the plasma recalcification time (PRT), prothrombin time (PT), thrombin time (TT), activated partial thromboplastin time (APTT), the level of fibrinogen (FG) in the plasma activity of antithrombin III (AT III) and coagulation factor XIII (F XIII), the potential activity of plasminogen (PAP), Hageman dependent fibrinolysis (HDF), a state of enzyme and nonenzyme fibrinolysis in plasma were studied. Significance of the statistical tests was defined as a p value of<0.05. The calculations were performed using standard commercial statistical and calculation software packages (STATISTICA, Excel).

3 RESULTS AND ANALYSIS

Under the influence of L-arginine on the 4-5th day of treatment marked improvement in health, reducing the asthenia signs, decrease of pain and dyspeptic symptoms were observed. However, in patients of control groups indicated changes were observed only on 10-12th day. After 2 weeks of therapy asthenic syndrome remained of significantly lower intensity only in 2 people (5.1%) of IA group and 2 patients (7.7%) of IIA group, while in control group it remained in 14 (35.9%) and 7 patients (26.9%) patients, respectively, IB and IIB groups. In 34 (92.1%) patients of IA group and 25 (96.2%) patients of IIA group pain disappeared and a feeling of heaviness in the right subcostal area, hardly bothered dyspepsia (in 32 patients (84.2%) and 23 patients (88.5%), respectively). Up to 15-16th day of treatment in 36 (92.3%) patients of IA group, 25 (96.2%) patients of IIA group jaundice disappeared, in 3 patients (7.7%) of group IA and 1 patient (3.8%) of IIA group jaundice has become of much less intensity. Originally significantly elevated level of ET-1 decreased after the therapy in all studied groups, but noticeable changes were observed after the L-arginine appointment with the basic treatment. In the study groups normalization of stable NO metabolites after treatment was also observed (Table 1).

Table 1: Indicators of the endothelium functional state in patients with nonalcoholic steatohepatitis with metabolic syndrome in the dynamics of L-arginine treatment

DATA	HI n=34	Groups	Before the treatment	In 4 weeks after the treatment
ET-1 fmol/ml	0,38±0,06	IA	1,64±0,10 *	0,59±0,03 */**/***
		IB	1,60±0,12	0,87±0,05 */**
		IIA	1,16±0,08 *	0,48±0,03 */**/***
		IIB	1,08±0,07 *	0,63±0,05 */**
NO, mkmol/ml	23,17±0,94	IA	11,43±0,95 *	21,32 ±1,37 **/***
		IB	10,89±1,30 *	17,95±0,56 */**
		IIA	11,93±1,66 *	23,31±0,75 **/***
		IIB	11,67±1,38 *	15,94±0,70 */**

Note. ** - Differences significant (p<0,05) compared with those HI (healthy individuals); ** - Differences are significant (p<0,05) compared with those before treatment; *** - Differences are significant (p<0,05) compared with those after treatment in patients with IB, IIB groups.*

Changes in the overall coagulation blood potential in the dynamics of treatment are shown in Table. 2. Significant (p<0.05) increase in all temporal characteristics of haemocoagulation and ATIII activities and FXIII in the IA group (PRT - 22.5%, PT - 19.0%, TT - by 23.6% , APTT - 18.6%, ATIII - 20.4% FXIII - 35.5%) and group IIA (PRT - 20.1%, PT - 29.1%, TT - 27 3% APTT - 25.9%, ATIII - 22.8% FXIII - 41.8%), which reached the appropriate level in healthy individuals (p>0.05) were found. The changes of PT, APTT, FXIII were most significant when L-arginine was used in treatment and significantly (p<0.05) different from those in IB and IIB groups. ATIII activity and PT after treatment in the control groups were not significantly different from the corresponding parameters in the study group. Changes in the concentration of fibrinogen in the plasma in dynamics of treatment were significantly only in IA (decreased by 26.4%, p<0.05) and IIA (decreased by 26.1%, p <0.05) groups, but at basic treatment it remained significantly higher than in healthy individuals. The positive effect of L-arginine on blood fibrinolytic activity was noted (Table 3). In particular, in all groups studied after treatment there was an increase in TFA 27.7% (p<0.05) - in the IA group, 10.5% (p<0.05) - in the IB group, 17.3% (p<0.05) - in the IIA group, 7% (p <0.05) - in group IIB; NFA reduction - by 18.6% (p> 0.05) - in the IA group, 9.1% (p<0.05) - in the IB group, 20.9% (p<0.05) - in the IIA group by 8.8% (p<0.05) - in group IIB; increase EFA - by 43.0% (p<0.05) - in the IA group, 36.7% (p<0.05) - in the IB group, 51.6% (p <0.05) - in the IIA group by 28.1% (p<0.05) - in group IIB; PAP - by 19.0% (p <0.05) - in the IA group, 13.9% (p<0.05) - in the IB group, 22.7% (p<0.05) - in IIA group by 15.8% (p<0.05) - in group IIB.

Table 2: Data of overall coagulation potential of blood in patients with nonalcoholic steatohepatitis and metabolic syndrome in the dynamics of treatment

DATA	HI n=34	Groups	Before the treatment	In 4 weeks after the treatment
ATPR, sec	96,25±2,22	IA	76,50±3,26 *	93,75±1,59 **
		IB	75,86±3,69 *	94,86±1,32 **
		IIA	78,00±2,65 *	93,70±3,36 **
		IIB	79,58±2,11 *	86,26±1,80 *
PT, sec	23,86±0,89	IA	18,38±1,12 *	21,88±0,40 **
		IB	18,57±1,34 *	20,14±0,34 *
		IIA	18,20±1,31 *	23,50±0,52 **
		IIB	18,42±1,03 *	19,79±0,29 *
TT, sec	18,97±0,48	IA	15,88±1,48 *	18,63±0,68 **
		IB	16,00±1,27 *	17,57±0,69
		IIA	15,00±0,71 *	19,10±0,57 **
		IIB	15,47±0,53 *	17,21±0,47 *
APTT sec	36,46±0,72	IA	29,00±1,90 *	34,38±0,42 **/***
		IB	29,29±1,86 *	31,29±0,42 *
		IIA	29,30±1,42 *	36,90±0,61 **/***
		IIB	28,53±0,94 *	31,68±1,19 *
FG, g/l	3,89±0,16	IA	4,96±0,24 *	3,90±0,15 **
		IB	4,81±0,25 *	4,09±0,22 **
		IIA	5,05±0,22 *	3,73±0,16 **
		IIB	4,86±0,21 *	4,46±0,12 *
ATIII, %	96,67±1,18	IA	79,63±1,51 *	95,88±2,04 **
		IB	80,43±2,37 *	93,29±2,50 **
		IIA	82,10±1,79 *	100,80±2,41 **
		IIB	80,89±2,01 *	90,89±2,43 */**/***
F XIII, %	95,78±1,72	IA	69,00±2,14 *	93,50±2,02 **
		IB	69,57±2,36 *	86,29±2,39 */**/
		IIA	69,20±2,01 *	98,10±1,83 **/***
		IIB	69,16±1,72 *	83,47±2,83 */**
PAP, min	17,14±0,39	IA	23,89±0,54 *	16,53±0,31 **
		IB	24,17±0,49 *	16,14±0,15 **
		IIA	21,98±0,39 *	16,97±0,15 **
		IIB	21,83±0,30 *	16,01±0,24 **
HDF, min	20,58±0,63	IA	35,38±0,78 *	21,63±1,53 **
		IB	34,57±1,49 *	25,14±1,18 */**
		IIA	32,30±1,02 *	20,70±1,85 **/***
		IIB	32,79±0,60 *	25,95±0,73 */**

Note. ** - Differences significant (p<0,05) compared with those HI (healthy individuals); ** - Differences are significant (p<0,05) compared with those before treatment; *** - Differences are significant (p<0,05) compared with those after treatment in patients with IB, IIB groups.*

Thus, the purpose of adjuvant therapy using L-arginine leads to a significant reduction in hemostasis disorders in patients with NASH associated with MS due to the positive impact on the functional status of the endothelium, and the state of the hemostatic system.

Table 3: Indicators of fibrinolytic plasma activity in patients with nonalcoholic steatohepatitis and metabolic syndrome in the dynamics of treatment

DATA	HI n=34	Groups	Before the treatment	In 4 weeks after the treatment
TFA, E440/ml/hour	1,44±0,07	IA	1,19±0,03 *	1,42±0,05 **
		IB	1,24±0,02 *	1,31±0,07 **
		IIA	1,27±0,11 *	1,50±0,04 **
		IIB	1,28±0,06 *	1,37±0,06 **
NFA, E440/ml/hour	0,52±0,03	IA	0,70±0,06 *	0,57±0,02 **
		IB	0,66±0,07 *	0,60±0,04
		IIA	0,67±0,06 *	0,53±0,04 **
		IIB	0,68±0,04 *	0,62±0,04 *
EFA, E440/ml/hour	0,92±0,04	IA	0,49±0,03 *	0,85±0,05 **
		IB	0,58±0,12 *	0,71±0,06 */**
		IIA	0,60±0,11 *	0,97±0,05 **
		IIB	0,60±0,07 *	0,75±0,07 */**

Note. ** - Differences significant (p<0,05) compared with those HI (healthy individuals); ** - Differences are significant (p<0,05) compared with those before treatment; *** - Differences are significant (p<0,05) compared with those after treatment in patients with IB, IIB groups.*

4 DISCUSSION AND CONCLUSION

It is known that the pathogenesis of NASH violations of metabolic homeostasis at the molecular level. Therefore, the treatment of the comorbidity should include complex therapeutic drugs of hepatoprotective and antioxidant actions that would improve the rheological properties of red blood cells and reversed the phenomenon of endothelial dysfunction. The main objective of the work was to develop an improved method of pathogenetic reasonable treatment of NASH associated with metabolic syndrome (depending on the type of metabolic syndrome). Experimental studies have found that L-arginine does not show toxic effect during parenteral and enteral administration. The drug does not have cumulative properties, it does not cause allergic, carcinogenic and teratogenic effects. Therefore the drug is characterized by antihypoxic, membrane stabilizing, cytoprotective, antioxidant, antiradical effects. Detoxification activity manifests itself as an active regulator of energy processes, plays a role in maintaining hormonal balance in the body. It is known that arginine increases the blood levels of insulin, glucagon, growth hormone, prolactin and is involved in the synthesis of proline, included in the process fibrinolysis, spermatogenesis, has membrane depolarizing

action. Arginine is one of the main substrates in the cycle of urea synthesis in the liver. Hipoammoniemic effect of the drug is sold by activating the conversion of ammonia to urea. It provides hepatoprotective effect through antioxidant, anti-hypoxic and membrane stabilizing activity, positively affects energy processes in hepatocytes. L-Arginine ® is a substrate for NO synthase - the enzyme that catalyzes the synthesis of nitric oxide in endothelial cells. The drug activates guanylate cyclase and increases cyclic guanidinemonophosphate in the vascular endothelium, reduces the activation and adhesion of leukocytes and platelets to vascular endothelium, inhibits protein synthesis adhesion of VCAM-1 and MCP-1, preventing thus the formation and development of atherosclerotic plaques, inhibits synthesis of endothelin-1, which is a potent vasoconstrictor and stimulator of proliferation and migration of smooth muscle cells of the vascular wall. L-Arginine ® inhibits also the synthesis of asymmetric dimethyl arginine - a powerful stimulator of endogenous oxidative stress. The drug stimulates the thymus gland, which produces T-cells that regulates glucose levels during exercises. Thus, combined therapy of nonalcoholic steatohepatitis associated with metabolic syndrome, with a differentiated degreeal L-arginine assignment by double increasing the duration of use of parenteral form (L-arginine hydrochloride) and subsequent double increasing of oral form of the drug (L-arginine aspartate) in case of the classic metabolic syndrome and its phenotype (AH + AO + IGT/DM-2) compared with the scheme of his appointment in case of phenotype (AH + DLP + IGT/DM-2) and (AH + DL + AO) is more effective than traditional therapy (essential phospholipids, ursodeoxycholic acid, metformin) for correction of basic clinical and laboratory disease syndromes, restore the functional state of the endothelium and eliminate haemocoagulation violations.

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