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L-ARGININE IS AN EFFECTIVE MEDICATION FOR PREVENTION OF ENDOTHELIAL DYSFUNCTION, A PREDICTOR OF ANTHRACYCLINE CARDIOTOXICITY IN PATIENTS WITH ACUTE LEUKEMIA

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Aim: **To evaluate the effectiveness of L-arginine in the prevention of endothelial dysfunction, which may be a predictor of anthracycline-induced myocardial injury, in patients with acute leukemia (AL) on the background of anthracycline antibiotics low cumulative doses from 100 to 200 mg/m^2.** ***Materials and Methods:*** **A total of 81 adult AL patients (38 males and 43 females with the age of 16–59 years) were studied. The patients were divided into two groups: group I (n = 34), AL patients treated with chemotherapy (CT) and L-arginine hydrochloride; group II (n = 47) – AL patients treated with CT only. Cardiac evaluation and endothelial function assessment were performed at baseline and after second CT. Electrocardiography (ECG) parameters, lipid peroxidation activity, antioxidant protection and NO system state were evaluated.** ***Results:*** **The bioelectric activity abnormalities of the myocardium were observed in studied patients with low cardiac risk after induction CT. In case of L-arginine administration, only minimal daily ECG changes were recorded. A significant difference in the lipid peroxidation and antioxidant defense system activity in patients of groups I and II was determined. We noticed deepening of endothelial dysfunction on the background of cytostatic therapy with anthracycline antibiotics compared with baseline values in patients of group II. It was found that prophylactic L-arginine increases superoxide dismutase level and reduces the total NOS activity due to its inducible isoform.** ***Conclusion:*** **The leading factor of anthracycline-induced cardiotoxicity is the imbalance between free radical generation and their inactivation that leads to endothelial dysfunction development. L-arginine eliminates the prooxidant-antioxidant imbalance and improves the endothelial function.**
Key Words**: cardiotoxicity, anthracyclines, acute leukemia, L-arginine, endothelial dysfunction.**

Chemotherapy (CT) with the inclusion of anthracycline antibiotics (AA) is accompanied by the formation of systemic endothelial dysfunction, which potentiates the risk of cardiovascular complications, even in patients with low cardiac risk. There is a systemic endothelial dysfunction at the onset of acute leukemia (AL), which primarily creates conditions for the occurrence of cytostatic-induced cardiotoxic effects. The heart tissue injury significantly limits the effectiveness of CT, taking into account the risk of acute coronary events development, by increasing morbidity and/or mortality [1].

Anthracycline-induced toxic effect on vascular endothelium is realized by increased oxidative stress. Simultaneously, tumor cells also require adequate functioning of the endothelium for their own growth and proliferation. It is interesting to note, some authors [2, 3] consider AA to be more toxic towards the endothelial than to blast cells. Thus, doxorubicin and other quinones cause inhibition of topoisomerase II, leading to chemical and oxidative DNA damage and therefore the myocardium injury. Doxorubicin induces production of hydrogen peroxide (H_2O_2), causing toxicity in relation to both endothelial cells and cardiomyocytes [4]. In the presence of oxygen the anthracycline semiquinones can directly transfer their unpaired electrons to oxygen, generating superoxide anion radical. If it is not balanced with antioxidants the lipid peroxidation (LPO) activates, followed by cardiomyocytes' injury [5, 6].

Endothelial cells are of great importance in the heart tissue functioning. The cardiomyocytes generate contractile force, the fibroblasts secrete extracellular matrix components and paracrine factors, and the endothelial cells are lining the coronary vessels and provide through the blood flow the delivery of free fatty acids and oxygen needed to support the high metabolic needs of contractile myocytes. Furthermore, the endothelial cells produce glycoproteins and neuregulin 1, which binds to a receptor tyrosine kinase ErbB-4, which activates intracellular signaling extracellular kinase 1/2 (ERK 1/2) and phosphatidylinositol-3-kinase (PI-3 K), involved in ensuring the contractile function of cardiomyocytes and their proliferation. Healthy endothelium is very important for the homeostasis of the whole cardiovascular system. Mature endothelial cells and their predecessors are involved in maintaining physiological homeostasis of cardiac tissue, including the regulation of vascular tone and permeability and thickness of the intima, vascular remodeling and angiogenesis processes, coagulation and fibrinolysis [7].

The reduced concentration of nitric oxide (NO) in the endothelium of coronary vessels is the basis of endothelial dysfunction, due to the deterioration

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Abbreviations used: AA – anthracycline antibiotics; AL – acute leukemia; ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia; CT – chemotherapy; ECG – electrocardiography; ECOG – Eastern Cooperative Oncology Group scale; LPO – lipid peroxidation; NO – nitric oxide; NOS – nitric oxide synthase; SEs – supraventricular extrasystoles; SOD – superoxide dismutase; TBARS – thiobarbituric acid reactive substances.

of L-arginine bioavailability, a decrease of NO synthesis or its accelerated catalysis [8]. In the present aspect, the NO-donor — L-arginine — is a promising drug for prevention of anthracycline-induced myocardial injury. L-arginine is a conditionally essential amino acid, which is an active and multifaceted cellular regulator of many essential functions in the organism, plays antihypoxic, membrane stabilizing, antioxidant and detoxification actions. In the human body, it acts as a substrate for NO-synthase in the NO synthesis reaction. Also, L-arginine is able to control the release of NO and restore endothelial function.

Non-enzymatic pathway of L-arginine is of great interest. In conditions of high oxidative stress that occurs during AA CT, L-arginine by the reaction with H_2O_2 leads to the NO formation. Thus, L-arginine makes more antioxidant effect that is characterized by free radicals inactivation with simultaneous formation of NO, which significantly reduces the LPO activity and leads to endothelium dependent vasodilatation [9, 10].

Considering the mechanisms of cardiotoxic AA injury and L-arginine cardioprotective effect, in our view, the evaluation of its prophylactic effectiveness deserves special attention in patients with AL during induction courses of CT.

The aim of our study was to evaluate the effectiveness of L-arginine in the prevention of endothelial dysfunction, a predictor of anthracycline-induced myocardial injury in patients with AL on the background of AA low cumulative doses from 100 to 200 mg/m^2 in the CT dynamics.

MATERIALS AND METHODS

A total of 81 patients with newly diagnosed AL without any comorbid cardiovascular diseases were included in the study. The cohort consisted of 38 (46.9%) males and 43 (53.1%) females with the age of 16–59 years. All patients were hospitalized in the Hematology Department of M.V. Sklifosovsky Poltava Regional Clinical Hospital. 29 (35.8%) patients were with acute lymphoblastic leukemia (ALL) and 52 (64.2%) patients — with acute myeloid leukemia (AML), by ECOG I–II. The ALL patients were treated with cytostatic therapy according to Hoelzer protocol, and AML patients — with "7+3" or "5+2" courses that included AA.

Depending on the treatment patients were divided into two groups: I (n = 34) — AL patients treated with CT and for anthracycline cardiotoxicity prevention received L-arginine hydrochloride 4.2% 100 ml intravenously the day before and in the days of AA infusion, followed by oral L-arginine aspartate 5 ml three times a day during one month; II (n = 47) — AL patients treated with CT without prevention of AA cardiotoxic action. The control group consisted of 18 healthy individuals (10 men and 8 women, mean age 22.5 ± 2.1 years). The study was approved by the local ethical committee and all patients had given a written consent before they were included in the study.

The patients' condition assessment was performed twice: before CT and after two induction of remission courses in achieving AA cumulative dose of 100–200 mg/m^2. In all patients, resting 12-lead electrocardiography (ECG) was performed with dynamic assessment of ECG parameters: heart rate, *QRS* voltage, *Q–T* interval, *ST* segment and presence of repolarization changes, arrhythmias or other abnormalities. Daily ECG-monitoring was performed using ECG Holter monitoring system "CardioSens K" (KAI Medica, Ukraine, 2014), assessed the general characteristics of heart rate, types of cardiac arrhythmias, changes in *ST* segment and *Q–T* interval. The results were processed and evaluated using a computer program for statistical analysis "CardioSens K".

Simultaneously, the LPO activity was examined according to thiobarbituric acid reactive substances (TBARS) concentration. The state of antioxidant system was assessed by superoxide dismutase (SOD) activity in the blood serum. NO system assessment was carried out by determining the stable metabolites of nitric oxide — nitrite anions $[NO_2]^-$ in serum through the formation of diazo compounds in reactions with sulfanilic acid, and total nitric oxide synthase (NOS) activity. Blood for the study was taken in the morning from cubital vein, patients were fasting.

These clinical and biochemical investigations were processed by variation statistics of Student and Fisher. Statistical analysis of parameters was performed on a personal computer using Microsoft Office Excel 2010. Differences were considered as statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

At baseline dyspnea during physical activity disturbed 16 (47.1%) patients of group I and 25 (53.2%) — of group II. Palpitation, feeling disruptions in heart work noted 7 (20.6%) patients of group I and 9 (19.1%) — of group II. The above complaints and their severity at the onset of AL, primarily, may be associated with different intensity of intoxication and anemic syndromes.

During standard resting 12-lead ECG before starting CT in 7 (20.6%) patients of group I cardiac arrhythmias in the form of sinus tachycardia were noted, which also were recorded in 11 (24.4%) patients of group II. According to the results of Holter daily ECG-monitoring signs of tachycardia were recorded in 17 (50.0%) patients and single supraventricular extrasystoles (SEs) in 14 (41.2%) patients of group I, that met the increase of their physical activity. Similar changes were observed among patients of group II: tachycardia — in 25 (53.2%) patients, SEs — in 19 (40.4%) patients. The changes of ST segment and *Q–T* interval in patients of both groups were not registered.

After standard induction CT cumulative AA dose reached (179.2 ± 19.24 mg/m^2) and (174.07 ± 22.13 mg/m^2) in patients of groups I and II, respectively ($p > 0.05$). Patients of group I treated with L-arginine to prevent anthracycline myocardial injury, did not show any cardiac complaints. Increasing AA cumulative doses

in patients of group II was accompanied by short-term (during 4.12 ± 1.2 days) complaints on shortness of breath during physical activity and palpitation in 12 (25.5%) patients. Reducing the intensity and duration of cardiac complaints in the accumulation of doxorubicin within 100–200 mg/m^2 compared with the baseline data in patients who did not receive prophylaxis of AA cardiotoxic action can be explained by decrease of intoxication and anemic syndromes under adequate specific and symptomatic treatment.

At standard ECG registration in patients of group I after induction phase with prophylactic L-arginine administration the following changes were found: sinus tachycardia — in 6 (17.6%) patients, reduced repolarization processes — in 2 (5.9%) and reduced *QRS* voltage — in 1 (2.9%) patient. According to daily ECG-monitoring in 9 (26.5%) patients isolated SEs during episodes of tachycardia were detected.

According to the data of standard ECG in 16 (34.0%) patients in group II the presence of sinus tachycardia was recorded, which was accompanied by repolarization abnormalities in 9 (19.1%) patients and total *QRS* decrease in 5 (10.6%) patients. However, during the daily ECG-monitoring in 23 (49.0%) patients with minimal physical activity on the background of tachycardia periods the SE episodes were detected. The *ST*-segment depression and *Q–T* prolongation in patients the both groups were not observed.

ECG changes registered in our study in patients who received low cumulative dose of AA as a part of induction CT without prophylactic L-arginine administration, namely, sinus tachycardia, decreased repolarization processes and reduced total *QRS* voltage, are consistent with those obtained previously in Horacek *et al.* study [11].

Thus, after AL induction CT in the background of AA low cumulative doses the bioelectric activity abnormalities of the myocardium were observed in studied patients with low cardiac risk. During CT without prevention of anthracycline cardiotoxicity the ECG changes of daily monitoring were detected in 49% of patients. At the same time, 26.5% of group I patients, who received L-arginine, only minimal daily ECG changes were recorded. So, we can assume that AA cause progressive changes in the myocardium of young and middle age patients without comorbid cardiovascular diseases. The L-arginine administration can significantly improve the bioelectric activity parameters of the myocardium and minimize cardiomyocytes' injury with anthracyclines.

Evaluating the LPO activity in patients of groups I and II before the CT revealed increased TBARS concentration in the blood serum in 1.6 times compared to almost healthy ($p < 0.01$), which indicates a high level production of aggressive free radicals in the AL manifesto. At the same time, the reduced in 2.3 times SOD activity was noted in serum compared to normal values ($p < 0.001$) (Table). Reducing the potential antioxidant defense system, namely SOD activity, may be due to its depletion in the reaction with superoxide anion-radical. Simultaneously with oxidative stress activation the increased total NOS activity in 3.9 times ($p < 0.001$) was noted, which can be explained by massive production of inducible NOS under the influence of pro-inflammatory cytokines. The concentration of nitrite anions that reflect the level of endothelial NO, in patients without cardiac risk factors was not significantly decreased, indicating the absence of clinically significant endothelial dysfunction at the baseline.

Table. Prooxidant-antioxidant status and NO system changes in patients with AL in CT dynamics

Study groups		TBARS, μmol/l	SOD, U/ml	Nitrites, μmol/l	NOS, μmol/l · min
Practically healthy (n = 18)		8.16 ± 0.51	0.94 ± 0.034	3.2 ± 0.36	0.61 ± 0.08
Group I (n = 34)	before treatment	13.0 ± 1.44*	0.44 ± 0.031*	2.61 ± 0.43	2.33 ± 0.12*
	after CT	10.7 ± 1.25	0.55 ± 0.021*$^{\surd}$	2.9 ± 0.35	1.4 ± 0.11*$^{\surd}$
Group II (n = 47)	before treatment	13.1 ± 1.42*	0.41 ± 0.028*	2.7 ± 0.41	2.4 ± 0.11*
	after CT	17.0 ± 1.09*$^{\surd\S}$	0.46 ± 0.031*§	2.2 ± 0.26$^{\surd}$	2.42 ± 0.1*§

Notes: significant differences ($p < 0.05$): *between healthy donors and in the groups; $^{\surd}$between indicators before and after induction CT in every group; §between indicators after induction CT in patients of groups I and II.

The TBARS level during induction CT in group I patients' blood serum, what underwent prevention of anthracycline cardiotoxicity, has decreased the relative baseline level in 1.2 times ($p > 0.05$). In addition, the decrease in LPO activity was accompanied by a moderate antioxidant protection recovery. After the second CT course on the background of L-arginine administration a significant increase in 1.2 times of SOD activity in serum ($p < 0.02$) was noted compared with those before treatment, but remained significantly lower level of its activity compared to almost healthy ($p < 0.001$). In the context of the anthracycline-induced myocardial injury prevention with L-arginine in patients of the group I during cytostatic treatment a significant decrease in total NOS activity in 1.7 times was observed compared with the baseline value ($p < 0.001$), indicating a decrease in production of inducible NO-synthase. At the same time, the nitrite anion concentration tended to increase in 1.1 times in the serum of patients of this group after induction CT ($p > 0.05$). L-arginine administration to prevent the development of early anthracycline cardiotoxicity leads to endothelial function improvement and consequently reduces the risk of anthracycline-induced myocardial injury on the background of CT induction courses.

In comparison with the baseline values, TBARS level significantly increased in 1.4 times ($p < 0.02$) in patients of group II, who did not receive L-arginine, after the second CT course. In parallel we observed the moderate growth of SOD activity in the blood serum (0.46 ± 0.031 U/ml *vs* 0.41 ± 0.028 U/ml; $p > 0.05$), indicating the preservation of low antioxidant system activity and therefore a high risk of multiple organ complications including heart tissue injury. Patients of group II after induction CT kept consistently high activity of total NOS (2.42 mmol/l/min), which was accompanied by a significant decrease of nitrites level in 1.45 times ($p < 0.05$) compared

with practically healthy and in 1.2 times ($p > 0.05$) compared with baseline level (see Table). Based on the previous experimental studies of Weinstein *et al.* [12], it is conceivable that the injuring effects on the endothelium are caused by the inducible isoform of NOS. Therefore, the above changes in total NOS activity with simultaneous progressive reduction of nitrites, which we obtained in this study, can be considered as deepening of endothelial dysfunction on the background of cytostatic therapy with AA.

Thus, in evaluating the TBARS concentration and SOD activity rates after induction CT in patients of the groups I and II, a significant difference in the LPO and antioxidant defense system activity was determined. It was found that prophylactic L-arginine administration improves antioxidant protection by increasing the SOD activity, which leads to more adequate inactivation of aggressive free radicals and effectively corrects signs of endothelial dysfunction, reducing the total NOS activity due to its inducible isoform.

CONCLUSION

The leading factor of anthracycline-induced cardiotoxicity is the imbalance between free radical generation and their level of inactivation that leads to endothelial dysfunction development. At reaching AA cumulative dose of 100–200 mg/m^2 the progressive LPO activation is observed, accompanied by a pronounced exhaustion of antioxidant protection.

L-arginine administration during the induction CT courses in patients with AL achieves tendency to eliminate prooxidant-antioxidant imbalance by increasing the activity of antioxidant defense system with simultaneous improvement of endothelial function.

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