Correction of structural and functional disorders of red blood cells by intravenous laser irradiation of peripheral blood in chronic endometritis

Corrección de trastornos estructurales y funcionales de los glóbulos rojos mediante irradiación con láser intravenoso de sangre periférica en la endometritis crónica

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Abstract

The objective of the research is to assess the possibility of correcting the structural and functional disorders of red blood cells in chronic endometritis using low-intensity laser blood irradiation in standard treatment.

Materials and Methods. Forty-two patients of reproductive age with a verified diagnosis of chronic endometritis in incomplete remission were divided into 2 groups: control, with endoscopic surgery for infertility and receiving drug therapy, and the second patient group, receiving combination treatment supplemented with an additional course of intravenous laser blood irradiation (ILBI)) for 7 days, 25 minutes daily,using the Mulat laser therapy apparatus (Russia) with a wavelength of 0.63 microns and an output power of 1.5-2 mW. The structural and functional properties of erythrocytes were determined based on the content of proteins and lipids in the membranes, the sorption parameters of the membrane, and the parameters of intra-erythrocyte metabolism.

Results. Before the start of traditional treatment, 87.9% of indicators of the structural and functional properties of erythrocytes resulted altered when compared with the values of healthy donors, respectively. The traditional combination treatment brought back to normal 10.3%, corrected 55.2% and left unchanged 34.5% of the parameters changed before treatment. The use of ILBI turned out to be much more effective, as it normalized 58.6%, corrected 37.9%, and left unchanged 3.5% of the indicators.

Keywords: intravenous laser blood irradiation, chronic endometritis, correction of immune disorders.

Resumen

El objetivo de la investigación es evaluar la posibilidad de corregir los trastornos estructurales y funcionales de los glóbulos rojos en la endometritis crónica mediante irradiación sanguínea con láser de baja intensidad en el tratamiento estándar.

Materiales y métodos. Cuarenta y dos pacientes en edad reproductiva con diagnóstico verificado de endometritis crónica en remisión incompleta se dividieron en 2 grupos: control, con cirugía endoscópica por infertilidad y en tratamiento farmacológico, y el grupo principal, que recibió tratamiento combinado complementado con irradiación de la sangre con láser intravenoso (ILBI) durante 7 días, 25 minutos diarios, utilizando el aparato de terapia laser Mulat (Rusia) con una longitud de onda de 0,63 micrones, una potencia de salida de 1,5-2 mW. Las propiedades estructurales y funcionales de los eritrocitos se determinaron en función del contenido de proteínas y lípidos en las membranas, los parámetros de sorción de la membrana y los parámetros del metabolismo intraeritrocitario.

Resultados. Antes del inicio del tratamiento tradicional, el 87,9% de los indicadores de las propiedades estructurales y funcionales de los eritrocitos resultaron alterados en comparación con los valores de los donantes sanos, respectivamente. El tratamiento combinado tradicional volvió a la normalidad en un 10,3%, corrigió el 55,2% y dejó sin cambios el 34,5% los parámetros modificados antes del tratamiento. El uso de ILBI resultó ser mucho más efectivo, ya que normalizó el 58,6%, corrigió el 37,9% y dejó inalterado el 3,5% de los indicadores.

Palabras clave: irradiación de sangre con láser intravenoso, endometritis crónica, corrección de trastornos inmunes.

Introduction

Chronic endometritis (CE), despite the numerous studies and information about its significant prevalence (60-65%), still stays in the focus of domestic and foreign specialists. Currently, the urgency of the problem of this disease is acquiring not only medical but also social significance, as it often leads to impaired reproductive function, causing infertility, unsuccessful attempts at in vitro fertilization, not carrying a pregnancy, complicated course of pregnancy and childbirth[1-3].

There are known structural and functional disorders of red blood cells in chronic inflammatory diseases of the female genital area, and the pharmacological correction applied in this case was not effective enough[4,5]. In effect, we have shown changes occurring in red blood cells in patients with CE, among them a decrease in the level of α and αspectrin, ankyrin, anion transport protein (ATB), pallidine, dematin, glyceraldehyde-3-phosphate dehydrogenase (G3PD) was found in the erythrocyte membrane - FD) and glutathione-S-transferase (G-S-T), an increase in the protein content of band 4.1, actin and tropomyosin with a normal level of protein of band 4.5 (Vera A. Ragulina, Olga A. Sunyaikina, Irina I. Kolomoets, Aleksey A. Konoplya, Tatiana M. Grigorieva. Relationship of Immune Indicators and Structural and Functional Properties of Erythrocytes in Chronic Endometritis. J. Med. Chem. Sci. 2021, 4(3) 230-237).

Thus, the objective of the research was to assess the possibility of correcting the structural and functional disorders of red blood cells in chronic endometritis using low-intensity laser blood irradiation in standard treatment.

Materials and Methods

Forty-two patients of reproductive age (18-35 years old) stayed under constant supervision in the gynecological unit of Kursk City Maternity Hospital. The inclusion of patients in the study was carried out based on informed consent. All patients underwent a comprehensive clinical and instrumental examination according to generally accepted standards, while in all cases the diagnosis of CE in incomplete remission was verified. Women were divided into two equal groups, randomized by age, minimal comorbidities in remission, predicted severity of the disease, and underwent endoscopic surgery for infertility. Patients of the first group received traditional treatment (TT) (antibacterial, considering the sensitivity of the pathogen, antimycotic, antiviral, non-steroidal anti-inflammatory drugs, probiotics, and vaginal microflora drugs). The second part of the patients additionally received a course of intravenous laser blood irradiation (ILBI) using a Mulat laser therapy device (Russia) with a wavelength of 0.63 μm, output power at the end of the OS-2 main disposable light guide (KIVL-01) of 2 mW. A laser irradiation session lasted for 25 minutes in a continuous mode; the course of treatment was 7 daily procedures.

Laboratory examination was carried out immediately upon admission to the hospital and upon discharge on the 10th day. Before and after combination treatment, red blood cells were obtained from 10 ml of heparinized blood came down twice in 10 mM sodium phosphate buffer (pH=7.4) containing 0.9% sodium chloride and 3% dextran T-500, within 30 minutes at 37°C. Then, the blood was centrifuged, after the separation of the supernatant, the erythrocyte mass was subjected to additional purification on a chromatographic column through HBS-cellulose, after which the erythrocyte sorption capacity (ESC)[7] and the sorption capacity of their glycocalyx (GSC)[6] were determined. Erythrocyte membranes were isolated according to Dodge[8], membrane lipids were determined by thin-layer chromatography[9]. Protein electrophoresis was carried out in the presence of sodium dodecyl sulfate in vertical plates of polyacrylamide gel according to Laemmli[10]; proteins were stained with Coomassie blue R-250. The intensity of lipid peroxidation (LPO) was assessed based on the content of acyl hydroperoxides (AHP) and malondialdehyde (MDA) in erythrocytes, which is based on the reactivity of an end product of lipid peroxidation, malondialdehyde (MDA) with thiobarbituric acid (TBA) to produce a red adduct. MDA and AHP were measured using TBK-Agat kit (Agat-Med, Russia), with Apel-330 spectrometer (Japan) at wavelengths of 535 nm and 570 nm. To assess the state of the antioxidant system, we used direct/competitive enzyme-linked immunosorbent assay (ELISA) with the detection of reaction products in the wavelength range of 405-630 and ready-made commercial kits to determine the activity of superoxide dismutase (SOD) (Bender Medsystems, Austria) and catalase (Cayman Chemical, USA). Total antioxidant activity (TAA) was determined by a method based on the degree of inhibition of ascorbate and iron-induced oxidation of Tween-80 to MDA. The concentration of stable nitric oxide metabolites (SM_{NO}) was detected in the Griess reaction at a wavelength of 540 nm using an ELISA kit (R&D, England). All ELISA results were recorded using a Sunrise microplate photometer (Tecan, Austria).

Statistical data processing was performed in light of the generally accepted standards of the statistical analysis of variance with the calculation of the mean (M), Standard error of the mean (SEM) using Microsoft Excel, 2010. The significance of the differences was assessed by the Mann-Whitney U-test. Differences with $p < 0.05$ were considered statistically significant.

Results and Discussion

On admission to the hospital, cases with CE were observed to own anion transport protein (ATP), low α and αspectrin, ankyrin, dematin, pallidin, glyceraldehyde-3-phosphate dehydrogenase (G-3-PD), and Glutathione-S-transferase (G-S-T) in the erythrocyte membrane, high band 4.1 protein, actin, and tropomyosin at a standard level of the band 4.5 protein. The conducted surgical and traditional treatment normalized the representativeness of α and αspectrin and tropomyosin in the erythrocyte membrane changed the content of dematin, ankyrin, pallidin, and actin, and didn't alter the ATP level, the band 4.1 protein, G-S-T and G-3-PD. The use of ILBI also, compared to drug therapy, normalized the representativeness of G-3-PD, actin, ATP, G-S-T and, to a greater extent, fixed the level of ankyrin, band 4.1 protein, pallidin, and dematin.

On admission to the hospital, cases with CE were seen to suffer from a low content of phosphatidylinositol (PI), phospha-

tidylcholine (PC), phosphatidylserine (PS), glycerophospholipids (GPL - the sum of PE, LPH, PS, PI, as well as PC), sphingomyelin (SM), phospholipids (PL - the sum of HFL and SM), a rise in the lysophosphatidylcholine (LPC) level, cholesterol (C), triacylglycerols (TAG), and cholesterol esters (CE) with normal content of phosphatidylethanolamine (PE), the amount of mono- and diacylglycerols, DA) and non-esterified fatty acids (NEFA). The given traditional treatment approximated the parameters of LPC, SM, HFL, PS, PI, and PL to those of healthy donors, but did not have an impact on the representativeness of PC, C, EC, and TAG. Supplementing the postoperative drug therapy with ILBI additionally normalized the content of TAG, SM, LPC, PI, EC, and, to a greater extent, modified the PS, HFL, PC, and PL level.

Table 1. Erythrocyte membrane proteins in patients with CE before and after treatment (M±m)

Parameters	1	2	3	4
	Healthy women	Women with CE		
		Before treatment	TT	TT + ILBI
αspectrin	102.4±2.7	92.8±2.3*1	97.4±2.0*2	106.2±4.9*2
αspectrin	120.1±3.6	102.9±2.4*1	119.5±2.5*2	119.2±4.7*2
Ankyrin	94.2±1.6	37.8±1.9*1	46.6±2.4*1.2	76.7±2.9*1-3
ATP	175.4±3.3	191.6±4.3*1	193.5±3.7*1	178.1±3.7*2.3
4.1	42.4±1.6	82.5±1.7*1	78.2±1.9*1	50.4±1.2*1-3
Pallidin	92.4±2.8	47.4±1.4*1	59.4±2.2*1.2	71.7±2.8*1-3
4.5	101.5±2.7	107.4±4.5	102.1±3.9	109.2±6.7
Dematin	68.3±1.9	21.9±1.1*1	31.9±1.8*1.2	50.4±3.7*1-3
Actin	78.5±1.6	90.4±1.9*1	84.4±2.2*1.2	75.2±3.0*1-3
G-3-PD	47.8±2.1	29.3±1.5*1	32.5±1.8*1	45.8±1.9*2.3
Tropomyosin	48.7±1.5	62.9±2.1*1	50.2±1.7*2	50.1±3.1*2
G-S-T	68.6±1.4	47.2±1.3*1	51.6±2.3*1	67.8±1.9*2.3

Note: in this Table and Table 2, an asterisk marks significant differences in arithmetic means ($p < 0.05$); the numbers next to the asterisk indicate the groups of parameters; units of measurement - mg%

Table 2. Erythrocyte membrane lipids in patients with CE before and after treatment (M±m)

Parameters	1	2	3	4
	Healthy women	Women with CE		
		Before treatment	TT	TT + ILBI
PC	28.1±0.8	19.5±0.7*1	20.9±1.1*1	24.6±1.1*1-3
LPC	4.2±0.1	6.4±0.09*1	5.4±0.1*1.2	4.5±0.2*2.3
PE	24.0±1.0	24.2±0.9	23.4±1.4	23.1±2.0
PS	28.4±0.9	17.5±0.7*1	22.1±1.1*1.2	25.2±1.3*1-3
PI	4.5±0.06	3.8±0.04*1	4.1±0.06*1.2	4.5±0.05*2.3
GPL	89.2±2.7	71.4±1.9*1	75.9±1.7*1.2	81.9±1.8*1-3
SM	14.1±0.5	11.9±0.6*1	12.7±0.7*1.2	13.1±0.6*2.3
PL	103.3±3.3	83.3±2.4*1	88.6±1.9*1.2	95.0±2.7*1-3
C	32.4±1.1	40.5±1.4*1	38.9±1.8*1	37.4±2.2*1
EC	28.1±1.2	35.9±1.4*1	32.3±2.2*1	29.9±1.7*2
TAG	13.2±0.6	16.5±0.5*1	17.3±1.1*1	13.9±0.4*2.3
DAG + MAG	11.0±0.4	10.0±0.6	10.5±0.7	10.8±0.6
NEFA	2.9±0.1	2.8±0.04	3.0±0.1	2.8 ±0.1

Before treatment, examining the erythrocyte metabolism parameters discovered the activation of LPO processes, a decrease in antioxidant defense factors (SOD, OAA, and catalase activity). The acquired drug treatment had no effect on the changed indicators of the antioxidant protection of erythrocytes (TAA and SOD). It improved the rest of the investigated parameters of the metabolic activity of erythrocytes towards the healthy donor indicators. ILBI contributed to normalizing the antioxidant defense and sorption parameters and, to a greater extent, improved the LPO parameters and the SMON level. Erythrocyte metabolic parameters in patients with CE are presented in Table 3.

Table 3. Erythrocyte metabolic parameters in patients with CE before and after treatment (M±m)

Parameters	Units of measurement	1	2	3	4
		Healthy women	Women with CE		
			Before treatment	TT	TT + ILBI
MDA	μmol/L	0.39±0.03	1.1±0.07*1	0.7±0.04*1.2	0.5±0.02*1-3
AHP	a.u.	0.22±0.02	0.89±0.04*1	0.76±0.02*1.2	0.36±0.08*1-3
TAA	%	35.1±3.0	25.6±2.8*1	27.0±1.9*1	36.1±3.0*2.3
SOD	a.u.	18.9±0.9	14.1±1.1*1	15.2±1.7*1	18.4±2.1*2.3
Catalase	μkat/L	24.1±1.1	17.6±2.2*1	20.1±2.3*1.2	24.7±2.1*2.3
SM_{NO}	μmol/L	2.4±0.2	4.9±0.1*1	4.1±0.2*1.2	2.9±0.2*1-4
GSC	10^{-12} g/RBC	1.8±0.06	1.3±0.04*1	1.5±0.05*1.2	1.7±0.04*2.3
ESC	%	33.1±1.8	18.5±1.9*1	22.7±1.4*1.2	32.2±1.1*2.3

Therefore, it can be stated that prior to surgical and traditional treatment, nearly 87.9% of indicators of erythrocytes' structural and functional properties in cases with CE worked out to be distinct from those of healthy donors, in turn. The combining treatment taken back to an average of 10.3%, improved 55.2%, and left unchanged 34.5% of the parameters altered prior to treatment (Tables 1 and 2).

Our data indicate significant changes in the proteins responsible for the structure formation and stabilization of the erythrocyte membrane (α and αspectrin, dematin are the main proteins of the cytoskeleton, ankyrin, band 4.1 protein, pallidin), the formation and flexibility of the membrane (actin, tropomyosin), and intracellular metabolism (G-3-PD, G-S-T).

The revealed changes in the content and ratio of the lipid composition of the membrane, primarily, low content of membrane HPL and SM, which form the basis of the double lipid framework of the cell membrane and play a major role in the ordering of protein macromolecules and normal metabolism of erythrocytes, along with a change in the architectonics of proteins leads to serious disorders in the functional properties of peripheral blood erythrocytes in CE even in remission, as evidenced by an increase in LPO processes and SM_{NO} content, which are an indirect indicator of the level of NO. Also, a significant decrease in the activity of key antioxidant defense enzymes (SOD and catalase) in red blood cells indicates the development of oxidative stress.

Mature erythrocytes cannot synthesize proteins and lipids; the maintenance and changes in their content and ratio are due to the microenvironment of erythrocytes, namely, the composition of blood plasma, which changes significantly in pathology, which in turn has a significant effect on cell morphology, lipid-protein interactions in the erythrocyte membrane and the activity of its enzyme systems caused by the latter[11]. Considering our data and literature, CE provokes both systemic and local severe immune and oxidative disorders, which significantly changes the qualitative and quantitative composition of blood plasma[12,13]. ILBI corrects immune-metabolic disorders[14], which is associated with the mechanism of low-intensity laser radiation: an increase in life expectancy and stimulation of intracellular metabolism of immunocompetent cells, normalization of the imbalance in the synthesis of cytokines and components of the complement system, increased bactericidal activity of blood serum, normalization of lactoferrin content in blood serum[15-17], which changes the composition of the microenvironment of red blood cells and explains the positive effect of ILBI on the erythrocyte membrane. At the same time, a direct influence of ILBI on erythrocyte membranes is entirely possible[18-20].

Conclusion

The results indicate that the traditional postoperative drug therapy does not have an adequate corrective effect on the structural and functional disorders of red blood cells in patients with CE. The use of a course of intravenous laser blood irradiation in this pathology can significantly increase the success of treatment.

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