against the background of forced diuresis. Nevertheless, the results raise the problem of the advisability of prolonged UUT drainage and underline the necessity of pathogenic indications for the clinical choice of periods of stent use.

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Endogenous Intoxication Syndrome in Patients in the Late Stages of Obliterating Atherosclerosis of the Vessels

A. Yu. Dubikaitis, M. V. Belotserkovskii, S. G. Konyukhova, and V. I. Strashnov

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The role of the main disease in the development of endogenous intoxication syndrome and the relationship of the latter to the stage of atherosclerosis have not yet been clarified. The urgency of this problem stems from the fact that the progression of vessel injuries and the complications sometimes accompanying surgical treatment may provoke the development of acute endotoxicosis [6].

Therefore, the goal of this work was a study of the component contents of the plasma in the context of the pathogenesis of atherosclerosis and endogenous intoxication syndrome in patients with obliterating atherosclerosis of the lower extremities (OALE) at various stages of lower extremity ischemia.

I. P. Pavlov Institute, Center of Efferent Therapy, St.Petersburg (Presented by A. N. Klimov, Member of the Russian Academy of Medical Sciences)

A number of processes, such as lipid peroxidation (LPO), alterations in the peptide composition of the plasma, immunopathogenic reactions, and insufficiency of the detoxication system, are known to play an important role both in the pathogenesis of atherosclerosis [5] and in the development of endogenous intoxication syndrome [9]. These factors were decisive in the choice of the biochemical and immunological methods used in this study.

MATERIALS AND METHODS

One hundred five OALE patients aged 41 to 72 years were examined. The patients were divided into two groups. Group 1 included 61 patients with stage III ischemia of the lower extremities, according to Pokrovskii's classification [11]. The patients had an average age of 47 years, with a 4.9-year mean interval

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from the onset of disease and a mean intensity of intermittent claudication of about 31±14 m. Symptoms of pain at rest were registered in 36 patients. Group 2 included 44 patients with stage IV ischemia of the lower extremities (necrotic involvement of distal areas of the legs), 50 years old on average, with a 5.8-year mean interval from the onset of disease.

LPO activity was assessed by the plasma content of primary and secondary peroxidation metabolites, diene conjugates (DC) [4], and malonic dialdehyde (MDA) [9]. The *in vitro* Fe^{2+}-induced oxidation of DC and MDA (DCo and MDAo), reflecting the integral antioxidant state of the plasma, was recorded after Konyukhova [9]. Some other parameters were also assayed, such as oxidase activity of ceruloplasmin (CP) [16], plasma superoxide dismutase (pSOD) [14], and copper ion concentration [8]. Total plasma protein was measured after Lowry, medium-size peptides after Gabrielyan [3], and 5-30 kD peptides after Dubikaitis [6].

The statistical analysis of the results was performed on a personal computer using registered Statgraaph software.

RESULTS

The data concerning blood component composition of the patients are summarized in Table 1. It can be seen that groups 1 and 2 did not differ from each other and/or from the normal standards in terms of the main, commonly used biochemical indexes, such as bilirubin, creatinine, ALT, blood glucose, and electrolyte levels. However, such metabolic indexes as

TABLE 1. Plasma Component Composition in Patients with Late-Stage Obliterating Atherosclerosis of Lower Extremities ($M \pm m$).

Parameters, measurement units	Stage III ischemia	Stage IV ischemia	Normal level
DC, nM/ml	112.7±11.4*	111.1±11.5*	75.4±3.2
MDA, nM/ml	10.25±0.85*	2.39±1.02*	7.30±0.44
DCo, nM/ml	439.5±55.3*	445.3±50.6*	292.1±14.1
MDAo, nM/ml	300.8±42.8	266.7±30.5	260.2±9.7
pSOD, conventional units	2.92±0.49*	3.46±0.50*	1.58±0.24
CP, mM/h×liter	24.77±1.05*	27.72±1.94*	18.80±0.90
Copper, μM/liter	22.4±0.5*	24.0±0.5*	14.1±0.7
Copper/CP	0.91±0.06*	0.87±0.06	0.75±0.06
Total lipids, g/liter	7.17±0.62	5.19±0.41**	6.4±0.7
Total protein, g/liter	73.9±3.7	64.9±3.1**	71.2±4.7
Total proteolytic activity, conventional units	9.01±0.88	7.15±0.64**	4.7-9.1
5-30 kD peptides, conventional units	0.643±0.068*	0.853±0.097*,**	0.287±0.07
300-5000 D peptides, conventional units	0.204±0.011*	0.183±0.018	0.161±0.015
Creatinine, μM/liter	79±4	84±5	70-130
Bilirubin, mM/liter	8.70±0.43	8.25±0.43	8-18.8
Cholesterol, mM/liter	6.41±0.41	5.22±0.43	3.2-6.7
AST, mM/h×liter	0.250±0.018	0.473±0.112**	0.10-0.78
ALT, mM/h×liter	0.287±0.029	0.429±0.058	0.10-1.14
Glucose, mM/liter	5.39±0.38	4.93±0.15	4.2-5.7
Potassium, mM/liter	4.54±0.09	4.41±0.09	4.0-5.5
Erythrocytes, 10^{12}/liter	4.56±0.07	4.28±0.12**	4.1-5.0
Leukocytes, 10^9/liter	6.94±0.29	8.60±0.45*,**	5.0-7.0
Leukocyte intoxication index, units	1.60±0.10	2.11±0.24*,**	0.6±1.6
Platelets, 10^9/liter	262.1±11.7	292.8±19.5	250-350
ESR, mm/h	14.8±2.0*	26.7±4.0*,**	3-11
Lysozyme, IU	35.5±1.3	41.7±2.1*,**	31.4±3.1
C3, IU	17.23±0.84	21.90±1.40*,**	16.7±9.9
IgG, g/liter	9.65±0.76	10.32±0.61	6.8-10.8
IgM, g/liter	2.32±0.30*	2.05±0.28*	0.5-1.08
IgA, g/liter	5.14±0.31*	3.99±0.47*	1.0-1.8
Circulating immune complexes, conventional units	0.283±0.055*	0.182±0.058*	0.06-0.08

Note: * – value reliably differs from normal level ($p<0.05$); ** – values in stage III and in stage IV patients reliably differ from each other ($p<0.05$).

protein and lipid content varied reliably between the groups, although they remained within the normal range. For instance, the total lipid level was 7.17±0.62 g/liter in group 1 and 5.15±0.41 g/liter in group 2. The groups also differed from each other in the total protein content, although to a lesser degree. It is significant that such a typically atherosclerosis-associated parameter as cholesterol concentration was lower in group 2 than in group 1 (i.e., patients with stage III ischemia of the lower extremities) and was, respectively, 5.22±0.43 mM and 6.41±0.41 mM. Thus, the blood cholesterol level in group 2 patients was on average within the normal range.

Analysis of parameters reflecting cell and tissue metabolism revealed a significant increase of 10-30 kD peptides in both groups of patients, twice above the normal value in group 1 and three times above it in group 2. At the same time, medium-weight (500-10,000 D) oligopeptides (the most common markers of intoxication) were within normal limits in both groups, though slightly increased in the patients with stage III ischemia of the lower extremities (group 1). The plasma proteolytic activity was at the upper border of the normal range in group 1 and 21% lower ($p<0.05$) yet still within normal limits in group 2.

The LPO indicators were markedly increased in both groups, both in absolute values and per gram of total lipids or total protein (Table 2). The revealed selective accumulation of LPO primary products (DC) reflected either significant activation of free radical reactions or a slowing down of further stages of LPO primary oxidation products. Significantly, DCo was also increased. A rise of the MDA level was observed only in the patients with stage IV disease (group 2). The MDAo values were moderate in both groups. This was probably due to sufficient functional activity of the antioxidant defense system. CP and pSOD activities were significantly increased, especially in group 2.

A tendency toward leukocytosis was recorded in both groups, the leukocyte number rising to 8.6±0.4× $\times 10^9$ cells/ml in the patients with stage IV ischemia. The patients of group 2 also showed an increased leukocyte index of intoxication (2.1±0.2× $\times 10^9$) as compared to the patients with stage III ischemia, this value slightly exceeding the upper normal limit. All patients examined had accelerated erythrocyte sedimentation rates.

All tested indexes of humoral immunity reliably exceeded normal limits (except the IgG concentration). Levels of lysozyme and C3 complement component activity positively correlated with the stage of ischemia. Meanwhile, the IgA and IgM concentrations as well as the increased level of circulating immune complexes were associated with less advanced stages of ischemia.

The increased LPO rate at the late OALE stages is in accordance with the reported increase in the free radical processes observed in ischemia [2] and in various atherosclerotic vessel injuries. The simultaneous rise of antioxidant defense system enzymatic activity (CP and pSOD) is also confirmed by other reports [12]. The increase in the DC and DCo levels, despite a high pSOD, is possibly the result of the dyslipidemia typical for atherosclerosis patients. The high plasma content of low-density and very-low-density lipoproteins (products with prooxidant activity [10]) in OALE patients, the lower high-density lipoprotein concentration (products with antioxidant properties [7]), and the SOD-like activity of certain peptides produced from pathological protein degradation and from immune complexes, may determine the imbalance of the antioxidant defense system and, consequently, its reduced efficiency. In our opinion, this is corroborated by the reported lack of correlation between CP and pSOD activity in atherosclerosis and other pathological states [12].

Enhanced CP activity probably accounts for the relatively weak increase in MDA and MDAo along

TABLE 2. Specific Concentration of LPO Metabolites and Peptides and Specific Activity of Antioxidant Defense Enzymes in Patients with Late–Stage Obliterating Atherosclerosis of the Vessels of the Lower Extremities ($M \pm m$)

Parameters	Stage IIIi schemia	Stage IV ischemia	Normal level
DC/TL, nM/g TL	15.72±0.71*	19.15±1.11*,**	10.30±0.36
MDA/TL, nM/g TL	1.43±0.11	2.14±0.13*,**	1.45±0.05
DCo/TL, nM/g TL	61.31±3.85*	76.78±4.57*,**	45.90±0.88
MDAo/TL, nM/g TL	46.0±3.2	46.1±2.7	47.6±1.2
CP/TP, U/g TP	3.35±0.16*	4.27±0.26*,**	2.63±0.20
pSOD/TP, conventional U/g TP	3.94±0.51*	5.33±0.50*,**	2.32±0.30
Cu/TP, μM/g TP	3.03±0.11*	3.76±0.14*,**	1.98±0.07
300–500 D peptides per g TP, conventional U/g TP$\times 10^{-4}$	27.9±0.5*	28.2±0.7**	22.7±0.4
5–30 kD peptidesper g TP, conventional U/g TP$\times 10^{-3}$	3.7±0.9*	13.1±1.0*,**	4.0±0.9

Note: TL – total lipids, TP – total protein. For other details see note to Table 1.

with advanced stages of ischemia of the lower extremities, because CP prevents formation of MDA from DC [15].

Thus, one may state that a definite balance exists between the free radical reactions and the antioxidant defense system in patients with late-stage OALE. In stage III ischemia the balance is compensated on the whole due to the strength of the antioxidant system, while in stage IV ischemia the antioxidant factors are unable to fully neutralize the peroxidation processes in the blood. The relationship of the stage of ischemia and the peroxidation rate is still more striking when the specific amounts of LPO products are analyzed.

The regular increase in the plasma 5-30 kD peptides along with the progression of ischemia is evidence of increased intensity of the membrane-destructive processes in OALE patients with stage IV ischemia. The latter trend is also confirmed by the simultaneous increase in the rate of LPO reactions. The reduced protease activity in the patients

with stage IV ischemia is apparently indicative of the progressive deterioration of the plasma fibrinolytic potential in OALE patients [1].

An increase in the blood leukocyte count and leukocyte intoxication index in the late stages of OALE along with advanced ischemia stages proceeds in parallel with LPO activation and increase in the 5-10 kD peptide level, and correlates with total impairment in the course of atherosclerosis progression. Taken as a whole, all the alterations are related to the presence of ischemic tissues that release into the bloodstream numerous toxic products of hypoxia-altered metabolism. Therefore, the increased leukocytosis and leukocyte intoxication index should be regarded as a reflection of intensified blood detoxication function [13].

The accelerated erythrocyte sedimentation rate also indicates the appearance of inflammatory factors and toxic substances, specifically peptides, in the patients' blood. These products may cause reduction of the erythrocyte zeta-potential and therefore induce further impairment of blood rheology, this leading to aggravated tissue hypoxia in the areas with affected blood flow. In this way one of the vicious circles determining the severity of the atherosclerotic process is closed.

The activation of lysozyme and complement in severe ischemia is probably related to the enhancement of nonspecific immunity due to necrotic damage in the extremities and the release of a large mass of antigens into the circulation. Patients with atherosclerosis show an increased level of modified lipoproteins, these apparently being responsible for the rise of circulating immune complexes. The lower concentration of immune complexes in the patients with stage IV ischemia may be related to the decrease in plasma cholesterol and total lipid levels as compared to patients with stage III ischemia [6].

Correlation analysis revealed a connection between immune complexes and IgG levels in the patients with stage III and stage IV ischemia (respectively, r=+0,38 and r=+0,43). This observation leads to the assumption that the increased level of immune complexes in the main is not the result of insufficient clearance, as if this were so, there would be no positive correlation. Rather, it is the consequence of a massive antigen release from the tissues and/or it results from the modification of the plasma lipoproteins due to LPO reactions.

The normal values of blood creatinine and potassium are evidence of efficient renal function, despite the increased load due to the need for the elimination of ischemia-altered metabolic products. Normal levels of bilirubin, transaminases, and glucose provide indirect evidence of efficient liver function.

The examined patients exhibited enhanced LPO rate (despite the stepped-up activity of antioxidant factors) and increased blood peptide content (especially 5-30 kD peptides) when compared to normal values. The patients also manifested a rise of the leukocyte intoxication index and erythrocyte sedimentation rate, together with enhanced parameters of humoral immunity, as well as a stepwise increase of these values along with the advancement of atherosclerosis. These data point to the development of chronic endotoxicosis at the late stages of OALE. Normal values of the common biochemical indexes of liver and renal functions confirm the chronic, compensatory nature of endotoxicosis processes. At the same time, the hematological and immunological findings indicate marked compensatory enhancement of the detoxication function of the blood along with the progression of lower extremity ischemia.

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BIOPHYSICS AND BIOCHEMISTRY

Influence of Activating Agents on the Membrane Potential of Lymphocytes in Patients with Hypertensive Disease

L. T. Malaya, S. N. Koval', V. M. Brovkovich, and N. B. Kurilo

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There are few data concerning the membrane conception of hypertensive disease (HD). HD has been found to induce numerous changes in cell membranes, including alterations in ion permeability, activity of membrane-associated enzymes, physicochemical state, and in the structure of the membrane protein-lipid matrix [1-3,9]. Membrane defects, which have been demonstrated in a variety of blood cells, among them lymphocytes [3,5,8], are known to cause changes in the functional activity of immunocompetent cells and to promote the development of immune disorders in HD patients, as has been confirmed by previous studies [4,7].

Kharkov Research Institute of Therapy

In view of this, the purpose of the present work was to study the changes in the membrane potential (MP) of the peripheral blood lymphocytes in patients with HD under the influence of activating agents.

MATERIALS AND METHODS

Twenty-five patients (males) with HD and 8 healthy person (males) aged 25 to 35 years were examined. Among the patients examined, stage I HD was diagnosed in 9 persons (36%) and stage II in 16 persons (64%). An HD duration of up to one year was detected in 4 patients (16%), from one year to 5 years in 12 patients (48%), and from 6 to 10 years in 9 patients (36%). In-depth clinico-instrumental and biochemical examination in a specialized cardiological department