Prooxidant-antioxidant balance in blood during the surgical treatment of obliterating arterial atherosclerosis in the lower extremities

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Abstract

The chronic disorder of arterial circulation in the lower extremities due to the atherosclerotic injuries of the femoral-popliteal-tibial arteries was accompanied by the shift of the blood prooxidant-antioxidant balance towards more active free radical oxidation and by a depletion of antioxidant system. After restoring arterial blood flow in the ischemic lower extremities this balance was shifted towards more prominent lipid peroxidation processes. Such pattern of prooxidant-antioxidant balance changes implicates the development of strategies for its correction targeted on the reduction of lipid peroxidation activity and therefore on a tissue defense against the reperfusion injury.

Key words: obliterating atherosclerosis, blood prooxidant-antioxidant balance, reperfusion-reoxygenation injury.

Introduction

The recovery of the blood flow in the ischemic lower extremities causes the complex pathophysiological, biochemical and morphological processes [1,2] induced by 2 mutually dependent, but different pathophysiological mechanisms: "no-reflow phenomenon" and reflow-associated injuries called a "reflow-paradox" [3-5]. Reactive oxygen species (ROS) are the mediators of reflow-paradox in ischemia-reperfusion [2]. Lipid peroxidation (LPO) products cause both direct and indirect toxic effects on the vascular endothelium [6,7]. However, healthy people have a relatively low content of LPO products due to the presence of the regulatory systems that efficiently maintain the level of LPO products and inhibit LPO reactions when the oxidative processes are enhanced, thereby forming the body prooxidant-antioxidant balance [8,9]. The antioxidant system (AOS) that includes the enzymatic and non-enzymatic mechanisms for LPO product inactivation is a part of such protection [10].

The pathogenetic mechanisms of prooxidant-antioxidant state disorder with the reperfusion-reoxygenation syndrome (RRS) in reconstructive surgery of acute arterial failure in the lower extremities are not completely understood [7,11]. Acute muscular ischemia in the lower extremities was shown to be associated with tissue accumulation of LPO products [5]. LPO activation increases with the duration of ischemia and depends on the ischemic sensitivity of the organ [12]. The significant rise of malondialdehyde (MDA) and conjugated dienes (CD) in venous outflow from the ischemic leg was observed since 1-2 hrs after the start of reperfusion and was accompanied by increased edema [13,14]. In contrast, Yokayama K. et al. [15] showed somewhat later LPO activation: within the first 24-72 hrs after the operation.

Experiments in animals and clinical practice have shown that acute muscular ischemia in the lower extremities was associated with the reduction of plasma antioxidant activity [2]. The recovery of arterial circulation in the ischemized lower extremities caused further disorder of the AOS [10]. However, other investigators [12] did not find the differences in antioxidant enzyme activities (superoxide dismutase – SOD, glutathione peroxidase) in m.gastrocnemius between control and ischemized extremities after the reconstructive surgery of the arterial femoral-popliteal-tibial segment.

Therefore, the present investigation is aimed to study the role of the main parameters of prooxidant-antioxidant state in the development of the RRS during the surgical treatment of

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chronic leg ischemia caused by the atherosclerotic injuries of the femoral-popliteal-tibial arteries.

Material and methods

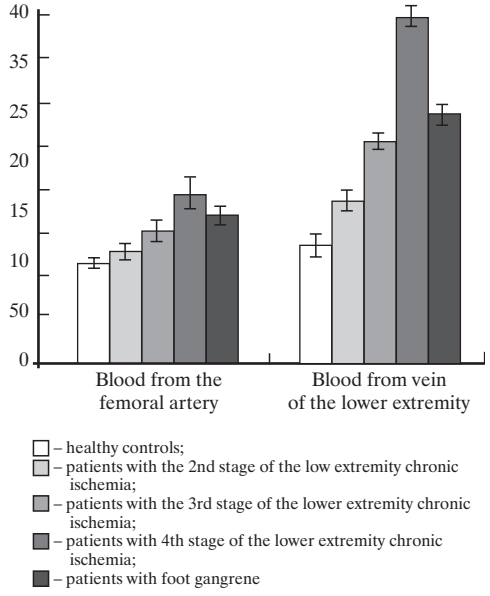
The study was carried out in 145 patients with atherosclerotic injury of the arteries of femoral-popliteal-tibial segment. According to the rules of the Grodno State Medical University Ethical Commission, the patients signed the informed consent for the blood sampling. 45 patients had chronic leg ischemia (CLI) in the lower extremities, stage 2 (by Fontaine), 61 patients had stage 3, and 28 patients – stage 4. Foot gangrene was diagnosed in 11 patients. Femoropopliteal autovenous bypass was performed in 122 patients. Profundoplastics was carried out in 12 cases. The reconstructive arterial operations in the lower extremities were performed under peridural anesthesia. The control group consisted of 34 patients without the signs of atherosclerotic arterial injuries in the lower extremities. 134 patients received usual drugs before and after the operations: vasodilator (xanthinol nicotinate) and disaggregant (pentoxyfilin).

The indices of prooxidant-antioxidant state were studied in plasma from the subcutaneous dorsal foot vein and from the common femoral artery of the ischemic leg. Blood was sampled before and on the 6th and 11th days after the reconstructive operations in the lower extremity arteries, and much later (1-5 years after the surgical intervention). LPO activity was estimated by CD and Schiff base (SB) concentrations. The CD content was measured by intensity of the UV absorbance at 232-234 nm (typical of lipid hydroperoxides with conjugated double bonds) [16]. SB level was determined by the fluorescence intensity of chloroform extract at excitation and emission wavelengths of 344 and 440 nm [16] measured with spectrofluorimeter "F-4010" (Hitachi). AOS state was evaluated by plasma content of α-tocopherol (α-TP) [17], β-carotene, coenzyme Q [18]. The data were processed by the routine methods of variation statistics using the software packages EXCEL and STATISTIC.

Results

The obtained results have shown that the development of CLI in the lower extremities because of the atherosclerotic occlusive or stenotic injury in the femoral-popliteal-tibial segment of the major arteries is associated with a significant ($p<0.01$) increase of CD and SB content vs the control group – both in arterial blood from the femoral arteries and in venous blood of the ischemic leg (*Fig. 1*). In addition, the progressing ischemic disorder in the lower extremities was accompanied by directly related activation of the free radical processes ($r=0.79$, $p<0.01$). The higher CLI stage was accompanied by more prominent accumulation of primary LPO products (CD vs SB). However, the persons with foot gangrene did not have further activation of the free radical processes comparing with patients with the 4th stage of the femoral lower extremity CLI; their CD and SB contents in the arterial and venous blood were even lower ($p<0.01$).

***Figure 1*. Schiff base (U/ml) in plasma from different vascular regions in patients with atherosclerotic injury of the femoral-popliteal-tibial arteries**



Simultaneously, the reduction ($p<0.01$) of both enzymatic and non-enzymatic AOS components was observed during the CLI progression (*Fig. 2*). The data of *Fig. 2* suggest that the degree of AOS reduction in our groups of patients also was directly related to the CLI stage ($r=0.86$, $p<0.01$). The lowest content of antioxidant was observed in patients with foot gangrene.

The recovery of the major artery blood flow through the femoral-popliteal-tibial segment of the patients that received routine therapy before and after the operation was accompanied by considerable LPO activation and AOS inhibition. Thus, *Fig. 1* shows that the highest CD and SB contents in the venous outflow from the reperfused-reoxygenated leg was observed on the 6th day after the operation; thereafter the levels of LPO products decreased, however, even 11 days after the operation it was significantly higher than before one ($p<0.01$). The activation of the free radical processes in the early postsurgical period directly depended on the initial CLI stage in the lower extremities ($r=0.88$, $p<0.01$). The relationship between the degree of LPO activation and the type of a reconstructive arterial operation (i.e. the volume of revascularized muscles) was observed. Thus, in the patients with the 3rd CLI stage in the lower extremities CD and SB content after profundoplastics was less than after bypass ($p<0.01$). Their highest CD and SB values were registered on the 11th day of the postsurgical observation.

The content of antioxidant continuously decreased within the first 6 days after successive bypass in the arterial femoral-popliteal-tibial segment (*Fig. 2*). Later the AOS state somewhat ameliorated with significant ($p<0.01$) increase in both enzymatic and non-enzymatic antioxidant levels comparing with their values on the 6th day. However, on the 11th day after the operation these levels were lower than before it ($p<0.01$).

In the cases of early shunt thrombosis within the first 11 days after the operation (10 cases) we did not note the previously

Figure 2. **Indices of antioxidant blood system from different vascular regions in patients with atherosclerotic injury of the arterial femoral-popliteal-tibial segment**

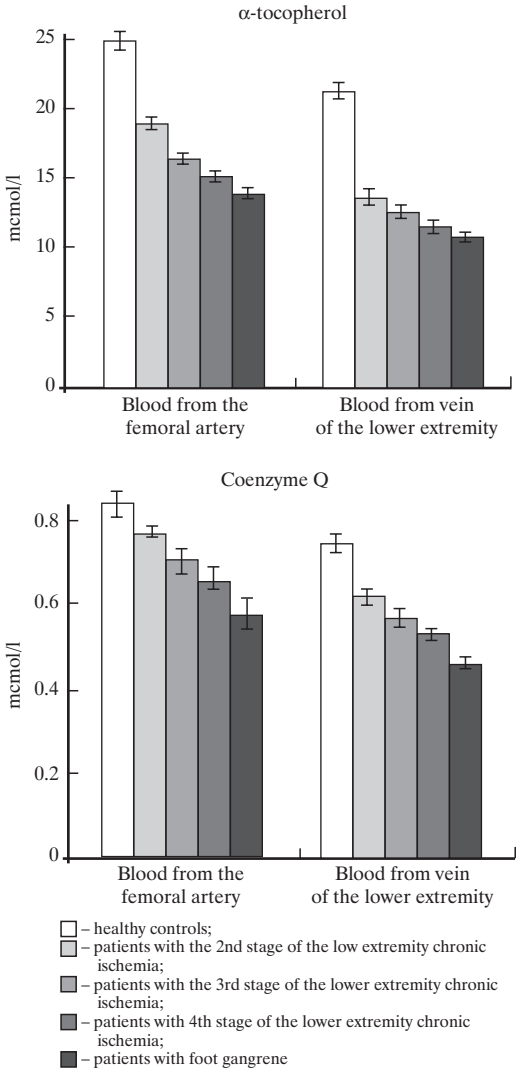
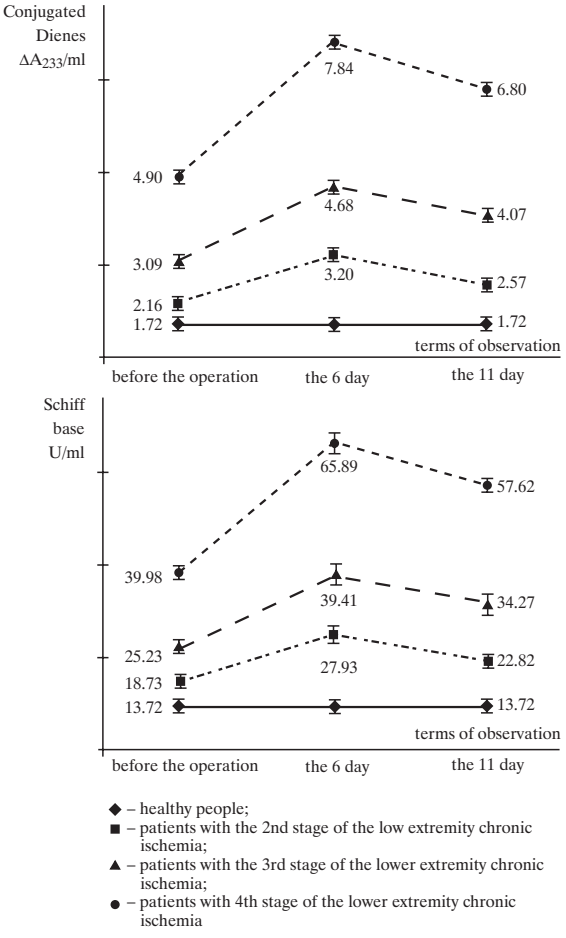


Figure 3. **Indices of lipid peroxidation in venous blood plasma from reperfused-reoxygenated the lower extremity in patients with atherosclerotic injury of the arterial femoral-popliteal--tibial segment**



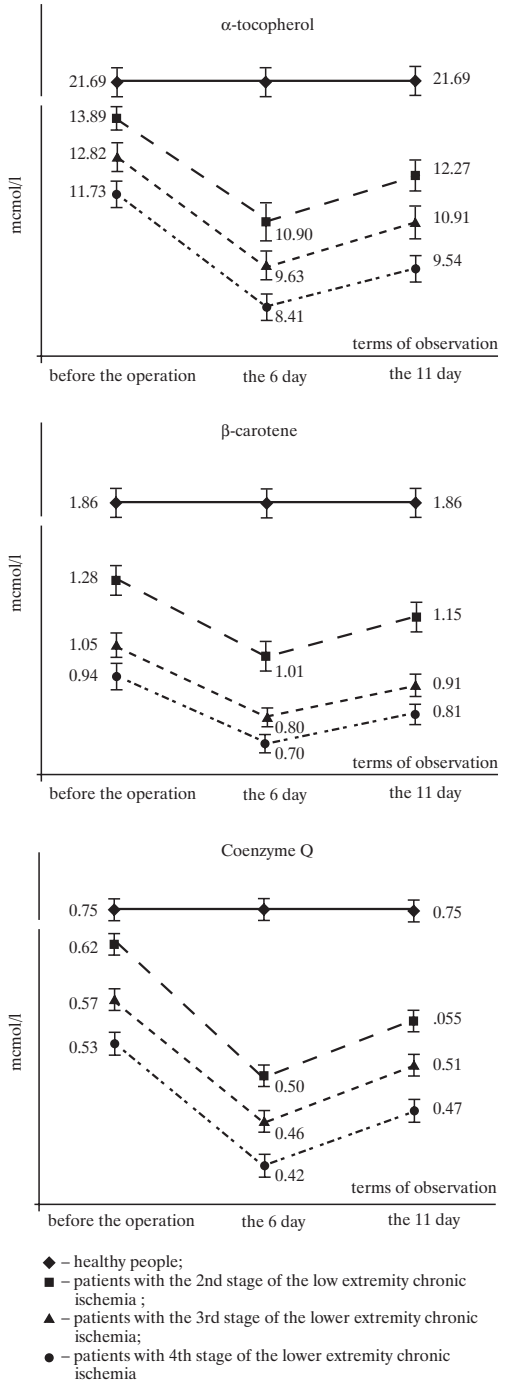
described dynamics of LPO activity and AOS state in blood obtained from the subcutaneous vein of the treated leg. The preoperative levels of LPO products and AOS indices in such patients were not changed after the operation. In persons with functional shunts the levels of CD and SB in the venous blood from the treated leg during the remote postsurgical period were essentially the same as in healthy controls, such as the values of AOS ($p>0.5$). In cases of later shunt thrombosis (12 observations) the values of investigated LPO and AOS parameters were similar to observed those in the patients with the 2nd (5), 3rd (4) or 4th (3) stage of the lower extremity CLI.

Discussion

The present investigation of LPO activity and AOS state during the atherosclerotic injury of the arterial femoral-popliteal-tibial segment indicated that chronic tissue hypoxia of the lower extremities was accompanied by the disorder of prooxidant-antioxidant balance. Such disorder was related to the activation of the free radical oxidative processes and AOS depletion and was directly dependent on the stage of CLI in the lower extremities. However, the development of irreversible trophic disorders in the ischemic legs diminished such dependence.

The return of the blood flow to the ischemized lower extremities of the patients with an atherosclerotic arterial injury in the femoral-popliteal-tibial segment is associated with an "oxygen burst" (dramatic increase of oxygen inflow) after the operation. Such burst initiates an active LPO process, expressed as a considerable rise of LPO products in the venous blood of the reperfused-reoxygenated leg. The degree of LPO activation is directly dependent on the CLI stage in the lower extremities, type and result of the arterial reconstruction performed. The observed increase in the lipoperoxide content is undoubtfully due to the beginning disparity between their generation and

***Figure 4*. Indices of antioxidant blood system from reperfused-reoxygenated the lower extremity in patients with atherosclerotic injury of the arterial femoral-popliteal-tibial segment**



insufficient physiological mechanisms limiting the LPO and AOS in the first turn.

The absence of excessive increase in venous LPO products in the reperfused leg after profundoplastics may be explained by lower blood flow to this leg. The multistep return of the blood flow to the ischemic tissues [19,20] may also have an important role in the slower peroxidative reactions in such patients. Ischemia is obviously associated with the imbalance between oxygen supply and velocity, efficiency of its cellular use. Furthermore, the efficiency of the oxygen consumption mechanisms, i.e. the relationship between the amount of generated free radicals and AOS activity is more important for LPO activation than absolute tissue oxygen content [9]. The close functional links between hemoglobin-oxygen affinity and as well activity of tissue free radical lipid oxidation were observed during hypoxia [9,21]. The direction and degree of changes in LPO processes and AOS activity in such conditions was shown to be dependent on the blood oxygen-binding properties [22]. These data about OA-induced LPO processes during the CLI in the lower extremities reveal a necessity to search antioxidant drugs for OA treatment.

Thus, the CLI development and progress in the lower extremities due to the atherosclerotic injuries of the arterial femoral-popliteal-tibial segment were accompanied by a disordered prooxidant-antioxidant balance. In patients with obliterating atherosclerosis such balance is shifted to the activation of free radical oxidation and inhibition of AOS. The recovery of arterial circulation in the ischemic lower extremities initiates the LPO activation, that depends on the blood flow to reperfused-reoxygenated leg, efficiency of oxygen use mechanisms and relationship between the amount of the generated radicals and AOS activity.

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