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Functional Peculiarities Of Platelet Activity In Persons With Arterial Hypertension Of The High Degree Developing Against The Background Ofhypodynamia At Metabolic Syndrome.

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ABSTRACT

The aim is to determine the level of platelets' aggregative activity in vitro and in vivo and peculiarities of functional abilities of separate mechanisms controlling them in patients with arterial hypertension of the 3rd degree which was developed against the background of lasting hypodynamia, and then – metabolic syndrome.There were observed 47 patients with arterial hypertension of the 3rd degree, risk 4, including 15 men and 14 women of mature age. Control group was composed of 25 clinically healthy people of the same age. There were applied biochemical, hematological and statistical methods of investigation.Patients' blood was noted to have evident dislipidemia at activation of lipids' peroxidation in it. Patients' plasma was noted to have some increase of thromboxane B_2 by 84.8% at lowering of 6-keto-prostaglandin $F_{1\alpha}$ by 17.9% and quantity depression of nitric oxide summary metabolites by 28.7%. The patients' degree of aggregation and the index of platelets' aggregation with collagen surpassed control values by 25.0% and by 27.5%, with ristomicin they were higher than control values by 25.7% and 46.4%, with ADP – by 25.7% and by 58.4%, respectively. The patients were found to have lowering of platelets-discocytes till 48.6±0.40%. The sum of platelets' active forms in their blood reached 51.4±0.12% (control value – 17.9±0.09%) at the content of little and large aggregates – 18.6±0.08 and 5.4±0.04 (control values – 2.9±0.06 and 0.2±0.06 in 100 freely lying platelets).Existing in examined patients surplus platelets' activity has in its basis the rise of platelets' adhesive and aggregative activity at weakening of their ability to disaggregation. Arterial hypertension, negative changes in plasma lipid composition and strengthening of lipids' peroxidation in it should be considered the most significant causative agents of the found thrombocytopathy.

Keywords: Arterial hypertension; Metabolic syndrome; Aggregation; Platelets; Risk of thrombosis.

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INTRODUCTION

In civilized society the share of physical labour is progressively decreasing and the common level of physical activity of a person is also decreasing. It often leads to hypodynamia. It often creates preconditions for developing of cardio-vascular pathology. In developed countries wide occurrence of cardio-vascular pathologies [1] among causes of death of able-bodied population is mostly connected with it. In the structure of these pathologies a great share belongs to arterial hypertension (AH) and its complications [2]. It is explained by the negative impact of AH on the cardio-vascular system (which was proved in numerous researches) with significant risk increase of thrombotic cases [3]. The possibility of their attack is essentially increased in case of metabolic syndrome's (MS) addition to AH. MS includes dislipidemia, insulin-resistance and abdominal obesity [4,5]. Rising platelets' activity which exceeds essentially the same one in healthy people [6], lies in the basis of these complications' development in the given category of patients, especially with a high degree of AH. Estimation of platelet activity's state and separate mechanisms controlling it in patients with hypodynamia and AH of the 3rd degree at MS is of great scientific and practical interest. That's why, the following aim of research was put in the present work: to determine the level of platelets' aggregative activity in vitro and in vivo and peculiarities of functional abilities of separate mechanisms controlling them in patients with AH of the 3rd degree which was developed against the background of lasting hypodynamia, and then – MS.

MATERIALS AND METHODS

There were observed 47 patients with AH of the 3rd degree, risk 4 [1] including 15 men and 14 women of mature age (48.2±1.6 years). The criteria of patients' involvement into the research were existence of hypodynamia in them for not less than 10 years, AH which was developed against its background not less than 5 years ago, MS which developed not less than a year ago and was diagnosed in strict accordance with its generally accepted criteria [4,7]. In all the patients MS consisted of tolerance' disturbance to glucose, hyperlipidemia of the type II b, abdominal obesity (index of body mass – more than 30 kg/m^2, the ratio of waist capacity to thighs capacity – more than 0.85 in women and more than 1.0 in men). The criterion of elimination out of the research was the existence of atherosclerosis' clinical manifestations including ischemic heart disease (IHD). All the patients had individually selected hypotensive therapy which combined the inhibitor of angiotensin-transforming enzyme or the blocker of angiotensin receptors, prolonged antagonist of calcium (mostly – amlodipin) and diuretic (indapamid). All the patients also received acetylsalicylic acid – not less than 100 mg/a day – and statin (fluvastatin – 80 mg/a day, or simvastatin – 20-40 mg/a day, or lovastatin – 40 mg/a day). The control group was composed of 25 clinically healthy people of the same age. The group of patients and the group of healthy people had normal quantity of platelets in blood. Blood drawing in both groups was conducted after 14-hours' starvation. We determined the content of common cholesterol (CS), CS of high-density lipoproteins (CS HDLP) and triglycerides (TG) by enzymatic colorimetric method with the help of a set of the firm "Vital Diagnostikum"; common lipids (CL) – by a set of the firm "Erba Russ". CS of low-density lipoproteins (LDLP) was calculated according to Friedwald V., CS of very low-density lipoproteins (VLDLP) – according to the formula: TG content/2.2 [6]. The activity of plasma lipids' peroxidation (LPO) was found according to the content of thiobarbituric acid (TBA)-active products by a set of the firm "Agat-Med", acyl hydroperoxides (AHP). Determination of antioxidant potential of liquid part of blood was conducted in all the patients [8].

The content of endothelin-1 was determined in blood plasma of the examined persons by radio immunological method with the help of reagents of the firm "DRG" (USA), and also levels of thromboxane A_2 metabolite – thromboxane B_2 and prostacyclin metabolite – 6-keto-prostaglandin $F_{1\alpha}$ by the way of enzyme immunoassay with the help of sets of the firm "Enzo Life science" (USA). Summary content of nitric oxide metabolites in blood of the observed persons was determined according to the method by Metelskaya V.A. and co-authors (2005) [9]. Platelets' aggregation was estimated on two-channel laser analyzer of platelets' aggregation ALAT2-"BIOLA" (model LA230-2, Russia) with application of ADP (0.5×10^{-4} M), collagen (dilution 1:2 of the basic suspension), ristomicin (0.8 mg/ml) (SPS "Renam") as inductors. Platelets' intravascular activity (PIA) was determined by phase contrast [10]. Statistical processing of received results was conducted with the usage of Student's t-criterion.

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RESULTS OF INVESTIGATION AND DISCUSSION

The level of arterial pressure (AP) in taken under observation patients (systolic – 186.1±4.3 mm of merc. col., diastolic – 114.8±3.2 mm merc. col.) corresponded to AH of the 3rd degree.

Blood of patients was noted to have increased levels of CL and common CS prevailing over the control values in 1.67 and 1.30 times, respectively (Table 1). At the same time, blood of persons with AH of the 3rd degree at MS was found to have increase of CS LDLP, CS VLDLP and TG in 1.52, 1.68 and 1.67 times, respectively, combining with lowering of CS HDLP in 1.32 times. Evident LPO activation of plasma was noted against this background in blood of patients – the content of AHP in it (3.70±0.003 D_{233}/1 ml) surpassed control values in 2.28 times, TBA-active products (5.80±0.003 mkmol/l) – in 1.71 times at lowering of the antioxidant potential value of liquid part of blood on 79.5%.

Blood of persons composing the group of observation, was noted to have imbalance of arachidonic acid metabolites – thromboxane B_2 turned out to be increased by 84.8%, whereas the level of its functional antagonist's derivative – 6-keto-prostaglandin $F_{1\alpha}$ was lowered by 17.9% (Table 1). It was accompanied in observed patients by high level of endothelin-1 (21.1±0.27 pg/ml) and quantity lowering of nitric oxide summary metabolites in their blood plasma by 28.7%.

Table 1: Biochemical characteristics of blood plasma in patients with arterial hypertension 3 degrees with metabolic syndrome

Registered parameters	Patients, n=47, M±m	Control, n=25, M±m
Total cholesterol, mmol/l	6.23±0.04	4.79±0.02 p<0.01
HDLcholesterol, mmol/l	1.16±0.006	1.53±0.001 p<0.01
LDL cholesterol, mmol /l	3.89±0.005	2.56±0.03 p<0.01
VLDL, mmol /l	1.18±0.004	0.70±0.002 p<0,01
TG, mmol /l	2.60±0.001	1.56±0.01 p<0.01
totallipids, mmol /l	8.80±0.04	5.26±0.04 p<0.01
AHP, D_{233} /1ml	3.70±0.003	1.62±0.02 p<0.01
TBA-compounds, mcmol / l	5.80±0.003	3.38±0.06 p<0.01
plasmaantioxidantactivity, %	20.5±0.13	36.8±0.03 p<0.01
thromboxaneA_2, pg/ml	289.3±0.61	156.5±0.66 p<0.01
6-keto-prostaglandin $F_{1\alpha}$, pg/ml	69.9±0.46	82.4±0.49 p<0.01
Total metabolites nitrogen oxide, mcmol / l	26.1±0.45	33.6±0.35 p<0.01
endothelin-1, pg/ml	21.1±0.27	8.2±0.15 p<0.01

Symbols: p – reliability of distinctions of indicators between a group of patients and control. In the subsequent table of designation it is similar.

Platelets' aggregation in persons with AH of the 3rd degree at MS turned out to be strengthened (Table 2). Their platelets reacted most actively on collagen. At the same time, the degree of aggregation with

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this inductor surpassed the control value by25.0%, and the index of aggregation – by 27.5%. Platelets' aggregation of the patients on ristomicin was a bit weaker. At the same time, the degree of patients' aggregation with it was higher than control value by 25.7%, and the index of aggregation surpassed it in healthy persons by 46.4%. Platelets' aggregation in response to ADP addition was still less active in the examined patients. At the same time, the values of aggregation degree and aggregation index surpassed control values by 25.7% and by 58.4%, respectively.

Table 2: The functional activity of platelets in patients with arterial hypertension 3 degrees with metabolic syndrome

Параметры		Patients, n=47, M±m	Control, n=25, M±m
platelet aggregation with collagen, s	the degree of aggregation, relative units	10.0±0.29	8.0±0.32 p<0.01
	rate of aggregation, relative units	8.8±0.29	6.9±0.27 p<0.01
platelet aggregation with ADP, s	the degree of aggregation, relative units	9.2±0.35	7.1±0.24 p<0.01
	rate of aggregation, relative units	8.2±0.34	5.6±0.16 p<0.01
platelet aggregation with ristomicin, s	the degree of aggregation, relative units	9.3±0.31	7.4±0.15 p<0.01
	rate of aggregation, relative units	8.4±0.33	5.3±0.22 p<0.01
Number of platelets in aggregates, %		15.1±0.05	6.7±0.08 p<0.01
Number of little aggregates (in 100 free platelets)		18.6±0.08	2.9±0.06 p<0.01
Number of medium and large aggregates (in 100 free platelets)		5.4±0.04	0.2±0.06 p<0.01
Platelets-discocytes, %		48.6±0.40	82.1±0.11 p<0.01
disco-echinocytes, %		27.8±0.09	13.5±0.04 p<0.01
spherecytes, %		14.4±0.08	2.1 ±0.12 p<0.01
sphere-echinocytes, %		7.0±0.06	1.5±0.08 p<0,01
platelets' bipolar forms, %		2.2±0.07	0.8±0.04 p<0.01
Sum of platelets' active forms, %		51.4±0.12	17.9±0.09 p<0.01

While studying platelets' intravascular activity (Table 2) the patients with AH of the 3rd degree at MS were found to have lowering of discocytes till 48.6±0.40% (the control value – 82.1±0.10%). The quantity of disco-echinocytes in their blood turned out to be twice increased. The content of spherecytes, sphere-echinocytes and platelets' bipolar forms also significantly surpassed the control values and reached in patients 14.4±0.08%, 7.0±0.06% and 2.2±0.07%, respectively. The sum of platelets' active forms in patients was equal to 51.4±0.12% (the control value – 17.9±0.09%). The content of little and large aggregates in blood of persons from the observation group was equal to 18.6±0.08 and 5.4±0.04, against the control values – 2.9±0.06 and

0.2±0.06 in 100 freely lying platelets, respectively. At the same time, the quantity of platelets in patients' aggregates prevailed over the level of comparison group in 2.2 times what pointed at evident PIA strengthening in them.

Blood of all the observed patients had concentrations' rise of TG, CL, common CS, CS VLDLP, CS LDLP at evident lowering of CS HDLP. The presence of dislipidemia, lowering of plasma antioxidant protection and concentration increase of primary and secondary LPO products created the threat of fast atherosclerosis progression in the given category of patients. LPO strengthening in blood mostly lies in the basis of atherogenic danger formation as it causes plasma lipids' peroxidation and damage of endotheliocytes. It lightens CS penetration into vascular wall and forms conditions for thrombosis development in succeeding time [11].

In conducted earlier researches it was found out that AH, especially in combination with MS, actively disturbs the functions of vascular wall and regular blood elements thus promoting the formation of some thrombotic phenomena [12]. At the same time, the activity of platelet hemostasis in these patients is still studied rather poorly and needs additional estimation. At the same time, the disturbance degree of basic mechanisms' deviation from the norm of platelets' activity in thrombocytopathy formation as the basis of thrombophilia [13] is far from being fully studied in the given category of patients.

Existing in the observed patients increased AP already in itself influences evidently and negatively the vascular wall causing damage of endothelium and exposing subendothelial fibers which can contact activate platelets. Existing in patients hypercholesterolemia, in its turn, significantly aggravates the given process accelerating the development of angiopathy. The synthesis of biologically active substances which can limit platelets' adhesion and aggregation lowers in such conditions in the vascular wall. The synthesis of pro-aggregants strengthens at that in platelets [14]. So, noted in the observed patients intensification of thromboxane formation and production weakening of its functional antagonist – prostacyclin – form imbalance of arachidonic acid metabolites. It, evidently, has activation of platelet thromboxane-synthetase and depression of vascular prostacyclin-synthetase activity in its basis. Given disturbances are aggravated by developing increase of endothelin-1 production in vascular wall and weakening of NO production in it. Probably, it happens in the result of endothelial NO-synthase suppression by surplus LPO and hereditary predisposition to dislipidemia [15].

Forming biochemical changes of blood plasma are inevitably accompanied by AP (aggregation of platelets) strengthening what was noted in response to all the applied inductors. Forming on their membranes surplus plasmatic thromboplastin accelerates thrombin-formation leading to the growth of platelets' aggregates and acceleration of fibrin fibers' formation on them with the appearance of platelet-fibrin clots. They can embolize little vessels [16].

In these conditions the patients inevitably had increased sensitivity of platelets to inductors of aggregation which was realized through some mechanisms. So, platelets' surface of the observed patients could be stated to have significant density increase of glycoproteidsIa – IIa and VI participating in platelets' adhesion[17]. It could be judged by AP intensification in response to collagen [18]. Intensification of platelets' adhesion in the observed patients is also connected with surplus expression of receptors to von Willebrand's Factor on their surface [19,20]. Given mechanism of strengthening of platelets' adhesive activity in patients was managed to register according to AP intensification with ristomicin which influences platelets similarly to subendothelial structures of vessels [21]. So, von Willebrand's Factor is necessary for the coming of ristomicin AP as it fixes the molecules to ristomicin (as to collagen) by one side, and by the other side – to platelets through their receptor Ib [22]. That's why, the given category of patients can be stated to have strengthening of "adhesion axis" formation: ristomicin (collagen) – WF – GPIb. At the same time, the very significant quantity increase of binding places of von Willebrand's Factor on platelets' membranes is the important mechanism of coming of their surplus adhesive ability [23].

At the same time, the fixation of strong inductor – collagen – to its receptors inevitably increases against the background of synthesis' deficiency of physiological antiaggregants in vessels of patients with AH of the 3rd degree at MS on platelets' surface. It's accompanied by phospholipase C activation, synthesis stimulation of diacylglycerol and proteinkinase C with consequent evident proteins' phospholiration of the contractile system. In these conditions inositol-triphosphate actively stimulates Ca^{2+} inflow out of platelets' depo promoting very fast decrease of actomyosin [24].

ADP referring to weak inductors of platelets' aggregation, in conditions of formation deficiency of nitric oxide and prostacyclin in vessels also actively interacts with receptors of their membranes causing mighty expression of fibrinogenic receptors on them with activation of phospholipase A_2 which provides precipitation of arachidonic acid out of membrane phospholipids [25].

Surplus quantity of platelets' active forms in patients' blood has in its basis, from one side, deficiency of nitric oxide and prostacyclin formation in vascular walls, and, from the other side, activity rise of platelets themselves. Besides, high PIA speaks about excessive availability of vascular wall's collagen for platelets because of its endothelium's damage against the background of constant presence of surplus diluted aggregation inductors, great amount of lipids and active LPO in patients' blood leading to chemical damage of endothelium [26]. Constantly high AP leads to mechanicalmicrotraumas of vascular walls what also inevitably leads to PIA rise in the examined patients [27]. Developing content increase of platelets' active forms inevitably rises the quantity of moving in blood aggregates of different sizes which are also able to damage endotheliocytes. It additionally exposes sub-endothelial structures. Given disturbances close "vicious circle" causing significant weakening of vascular hemostasis' activity and risk increase of thrombosis' coming [28]. Circulating aggregates also generally block vasa vasorum what leads to weakening of vessels' functions and progression of atherosclerosis against the background of existing metabolic and rheological blood disturbances of persons with AH at MS.

CONCLUSION

Surplus platelets' activity which exists in the examined patients and can be found in vitro and in vivo has in its basis some rise of adhesive and aggregative platelets' activity, and also – weakening of their ability to disaggregation mostly on behalf of imbalance of pro- and antiaggregative compounds in their blood. Hypodynamia and developing against its background arterial hypertension, negative changes in plasma lipid composition and strengthening of LPO in it should be considered the most significant causes of the given imbalance. Forming in blood of patients with AH of the 3rd degree at MS conditions for support of platelets' high activity develop serious danger of thrombosis development in them.

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