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Activity of Platelets' Aggregation in Patients of The Second Mature Age with Arterial Hypertension of The 3rd degree.

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ABSTRACT

The aim is to determine the activity level of platelets' aggregation and separate mechanisms of its inhibition in patients with arterial hypertension of the 3rd degree. Under observation there were 42 patients with arterial hypertension of the 3rd degree, risk 4, including 22 men and 20 women of the second mature age. The control group was composed of 25 clinically healthy people of the same age. There were applied biochemical, hematological and statistical methods of investigation. The blood of patients was noted to have the increase of thromboxane B₂ by 60.4% at lowering of 6-keto-prostaglandin F_{1α} by 11.9% and quantity depression of nitric oxide summary metabolites by 16.7%. The degree of aggregation and the index of platelets' aggregation with collagen surpassed in patients the control level by 20.0% and by 23.2%, with ristomycin they were higher the control values by 21.6% and by 39.6%, with ADP – by 16.9% and by 25.0%, respectively. The patients were found to have the decrease of platelets-discocytes till 57.8±0.27%. The sum of platelets' active forms in their blood reached 42.2±0.17% (the control value – 17.9±0.09%) at the content of small and large aggregates 14.5±0.09 and 4.1±0.12 (the control values – 2.9±0.06 and 0.2±0.06 on 100 freely lying platelets). Existing in the examined patients surplus platelets' activity has in its basis the rise of adhesive and aggregative activity of platelets at weakening of their capability to disaggregation. Arterial hypertension and strengthening of lipids' peroxidation in plasma should be considered the most significant causal factors of the detected thrombocytopeny.

Keywords: arterial hypertension, aggregation, platelets, thrombophilia, hemostasis.

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INTRODUCTION

After the modern medicine took under control the infectious diseases, cardio-vascular diseases occupied the 1st place among causes of death in the whole civilized world. They still keep lead [1]. Arterial hypertension (AH) and thrombotic complications against its background [2] occupy a large part in their structure. It is explained by firmly proven fatal AH impact on heart and vessels and evident risk increase of thrombotic events while level increasing of arterial pressure [3,4]. The possibility of their coming significantly rises in case of prolonged absence of arterial pressure adequate lowering [5,6] in patients with high AH degree. Increasing readiness of platelets to aggregation [7-10] and the rise of their circulating aggregates' number [11,12] lie in the basis of vascular complications' development in this category of patients. The estimation of the level of platelets' activity and separate mechanisms of its inhibition in patients with AH of the 3rd degree who earlier didn't take regularly hypotensive drugs [13] is very interesting from the scientific and practical points of view. That's why we put the following aim in our research: to determine the activity level of platelets' aggregation and separate mechanisms of its inhibition in patients with arterial hypertension of the 3rd degree.

MATERIALS AND METHODS

The conduction of the research was approved by the local Ethics Committee of the Russian State Social University in May, 25th, 2016 (Record №5). All the examined persons gave written informed consent on participation in the conducted research.

There were observed 42 patients with AH of the 3rd degree, risk 4 [14], including 22 men and 20 women of the second mature age (mean age 45.8 ± 2.7 years). The criteria for the enrollment of the patients into the research were as follows: AH existence in them for not less than 10 years, and it corresponded to the 3rd degree for not less than last 3 years; normal lipid profile and body mass; and also absence of systematic hypotensive treatment because of their personal beliefs.

The criterion of elimination out of the research was the existence of atherosclerosis' clinical manifestations including ischemic heart disease (IHD), oncological and endocrine diseases. 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. 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", acylhydroperoxides (AHP). Determination of antioxidant potential of liquid part of blood was conducted in all the patients [15].

The content of thromboxane A₂ metabolite – thromboxane B₂ and prostacyclin metabolite – 6-keto-prostaglandin F_{1α} was determined in blood plasma of the examined persons 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) ("Renam", Russia) 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.

RESULTS AND DISCUSSION

The level of arterial pressure (AP) in taken under observation patients (systolic – 188.2 ± 4.5 mm of merc. col., diastolic – 115.18 ± 3.0 mm merc. col.) corresponded to AH of the 3rd degree.

Blood of persons composing the group of observation, was noted to have imbalance of arachidonic acid metabolites – thromboxane B₂ turned out to be increased by 60.4%, whereas the level of its functional antagonist's derivative – 6-keto-prostaglandin F_{1α} was lowered by 11.9% (Table). It was accompanied in observed patients quantity lowering of nitric oxide summary metabolites in their blood plasma by 16.7%.

Table: Hematologic characteristics of patients with arterial hypertension 3 degrees

Registered parameters		Patients, n=42, M±m	Control, n=25, M±m
AHP, D ₂₃₃ /1ml		3.41±0.011	1.62±0.02 p<0.01
TBA-compounds, mcmol / l		5.34±0.009	3.38±0.06 p<0.01
plasma antioxidant activity, %		24.6±0.16	36.8±0.03 p<0.01
thromboxane A ₂ , pg/ml		251.0±0.52	156.5±0.66 p<0.01
6-keto-prostaglandin F _{1α} , pg/ml		73.6±0.40	82.4±0.49 p<0.01
Total metabolites nitrogen oxide, mcmol / l		28.8±0.43	33.6±0.35 p<0.01
platelet aggregation with collagen, s	the degree of aggregation, relative units	9.6±0.23	8.0±0.32 p<0.01
	rate of aggregation, relative units	8.2±0.26	6.9±0.27 p<0.01
platelet aggregation with ADP, s	the degree of aggregation, relative units	8.3±0.32	7.1±0.24 p<0.01
	rate of aggregation, relative units	7.0±0.29	5.6±0.16 p<0.01
platelet aggregation with ristomicin, s	the degree of aggregation, relative units	9.0±0.34	7.4±0.15 p<0.01
	rate of aggregation, relative units	7.4±0.20	5.3±0.22 p<0.01
Number of platelets in aggregates, %		13.6±0.10	6.7±0.08 p<0.01
Number of little aggregates (in 100 free platelets)		14.5±0.09	2.9±0.06 p<0.01
Number of medium and large aggregates (in 100 free platelets)		4.1±0.12	0.2±0.06 p<0.01
Platelets-discocytes, %		57.8±0.27	82.1±0.11 p<0.01
Sum of platelets' active forms, %		42.2±0.17	17.9±0.09 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 turned out to be strengthened (Table). Their platelets reacted most actively on collagen. At the same time, the degree of aggregation with this inductor surpassed the control value by 20.0%, and the index of aggregation – by 23.2%. 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 21.6%, and the index of aggregation surpassed it in healthy persons by 39.6%. 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 16.9% and by 25.0%, respectively.

In blood the patients with AH of the 3rd degree were found to have lowering of plateletsdiscocytes till 57.8±0.27% (the control value – 82.1±0.10%). The sum of platelets' active forms in patients was equal to 42.2±0.17% (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 14.5±0.09 and 4.1±0.12, 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 2.0 times what pointed at evident PIA strengthening in them.

In the blood of all observed patients a decrease in the antioxidant protection of plasma and an increase in the concentration of primary and secondary products of LPO were found to create conditions for damaging the membranes of platelets and endotheliocytes [17,18].

Previously found out that AH actively disturbs the functions of vascular wall and regular blood elements thus promoting the formation of some thrombotic phenomena [19, 20]. 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 thrombocytopeny formation as the basis of thrombophilia [21] is far from being fully studied in the given category of patients [22].

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 [23]. 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 [24]. 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 [25,26].

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 [24].

Platelets' surface of the observed patients could be stated to have significant density increase of glycoproteids Ia – IIa and VI participating in platelets' adhesion. It could be judged by AP intensification in response to collagen. Intensification of platelets' adhesion in the observed patients is also connected with surplus expression of receptors to von Willebrand's Factor on their surface. Given mechanism of strengthening of platelets' adhesive activity in patients was managed to register according to AP intensification with ristocetin which influences platelets similarly to subendothelial structures of vessels. So, von Willebrand's Factor is necessary for the coming of ristocetin AP as it fixes the molecules to ristocetin (as to collagen) by one side, and by the other side – to platelets through their receptor Ib. That's why, the given category of patients can be stated to have strengthening of "adhesion axis" formation: ristocetin (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 [27].

Of patients with AH of the 3rd degree on platelets' surface. It's accompanied by phospholipase C activation, synthesis stimulation of diacylglycerol and protein kinase C with consequent evident proteins' phospholiration of the contractile system [28]. In these conditions inositol-triphosphate actively stimulates Ca²⁺ inflow out of platelets' depo promoting very fast decrease of actomyosin [29]. 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 A₂ which provides precipitation of arachidonic acid out of membrane phospholipids [30].

Large 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 and active LPO in patients' blood leading to chemical damage of endothelium [27]. Constantly high AP leads to mechanical microtraumas of vascular walls what also inevitably leads to PIA rise in the examined patients. 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 [31]. Circulating aggregates also generally block vasa vasorum what leads to weakening of vessels’ functions 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 with AH of the 3rd degree 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 persistently high level arterial hypertension and strengthening of LPO in it should be considered the most significant causes of the given imbalance. Available in blood of patients with AH of the 3rd degree conditions for support of platelets’ high activity develop serious danger of thrombosis any localization development in them.

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