PharmacologyOnLine

Archives • 2021 • vol.2 • 819-826

CHANGES IN VASOCONSTRUCTOR-VASODILATION POTENTIAL OF VESSELS IN EXPERIMENTAL ANTIPHOSPHOLIPID SYNDROME

Yakymenko, O. O.[1]; Savytskyi, V. I.[1]; Klochko, V. V.[1]; Savytskyi, I. V.[2]; Badiuk, N.S.[3*]

[1]Odesa National Medical University, Odesa, Ukraine

[2]International European University, Kyiv, Ukraine

[3]State Enterprise Ukrainian Research Institute of Transport Medicine of the "Ministry of Health of Ukraine", Odesa, Ukraine

*badiuk_ns@ukr.net

Abstract

As a result of our study, the development of endothelial dysfunction in experimental antiphospholipid syndrome is confirmed: an increase in the marker of ED (endothelin-1) and a pathological increase in vasoconstrictor potential on the background of weakening of vasodilatation. There was a pathological increase in the vasoconstrictor potential of blood vessels in animals simulated antiphospholipid syndrome, as evidenced by an increase in endothelin-1 levels in the blood of rats ($p < 0,001$). Violation of nitric oxide synthesis was detected against the background of the development of experimental antiphospholipid syndrome, which is confirmed by a marked decrease in the level of S-nitrosothiols. The most pronounced normalization of vasoconstrictor-vasodilatory potential of vessels was found in rats, whose simulated antiphospholipid syndrome was corrected with warfarin, immunoglobulin and L-arginine solution.

Keywords: *antiphospholipid syndrome, L-arginine, endothelin-1, S-nitrosothiols, immunoglobulin, warfarin*

Introduction

Antiphospholipid syndrome (APS) is characterized by recurrent thrombosis associated with the synthesis of antiphospholipid antibodies, in particular to cardiolipin (ACL) IgG or IgM, to β2 - glycoprotein 1, and / or lupus anticoagulant (tAI) [1].

From the very beginning of the studies of the symptom complex, the relationship between APS and SLE (systemic lupus erythematosus) was studied. The common lesions of internal organs, especially the central nervous system in the pathogenesis of both diseases have been proven. In SLE, the presence of ACL in the blood was associated with convulsive syndrome, impaired cerebral blood supply and choreaform hyperkinesias. Ischemic changes on MRI in the presence of ACL and venous thrombosis and reticular livedo have been associated with APS [1, 2].

A number of studies have evaluated the ultrasound of the peripheral vascular system, both arterial and venous, patients with APS and SLE at the expense of its diagnostic value in thrombotic complications. It was proved that in patients with a history of thrombophlebitis and thrombosis, monophasic circulation and failure of physiological functioning of the valvular apparatus of the common femoral vein were recorded, which confirmed the presence of postthrombophlebotic syndrome [1, 11].

Vasculopathy is one of the main etiological factors of thrombotic complications in patients with APS. It represents changes in vessels of all types without specificity of these damages. At the same time, vasculitis is an inflammatory damage to blood vessels. Features of vasculopathy in primary antiphospholipid syndrome, which is characterized by damage to vascular endothelium, the presence of blood clots in the vessels, alteration of the endothelium in the form of its dystrophy, desquamation and necrosis, as well as its proliferation [1]. Against the background of SLE, the peculiarities of vasculopathy in APS are fibrinoid deposition up to necrosis and plasma infiltration of the vascular wall. This indicated primarily alteration in the basement membrane of blood vessels and intracellular and perivascular lymphocytic infiltration - the presence of disease (SLE) [3].

The key pathogenetic mechanism of thrombotic non-inflammatory vasculopathy, which is the basis of vascular pathology in APS is β2-GP1-dependent activation of AFL platelets, endothelial cells and monocytes. It should be emphasized that AFL induces the expression of such cellular adhesion molecules (CMA) as intercellular adhesion molecule, vascular adhesion cell molecule, E-selectin, P-selectin on the surface of endothelial cells and enhances the adhesion of leukocytes to the vascular endothelium [1]. It was proved that in patients with SLE, regardless of the presence of APS, the level of soluble vascular adhesion molecules increases. The presence of a vascular cell adhesion molecule was associated with glomerulonephritis and atherosclerosis in patients with SLE and APS. And in patients with primary APS, these changes were associated with thrombosis. The presence of elevated levels of E-selectin in patients with SLE with APS and primary APS confirmed the activation of the vascular endothelium.

In the study of homocysteine levels in the blood of patients with APS and SLE, a high concentration of homocysteine was recorded in comparison with healthy donors [4]. The association between hyperhomocysteinemia (GHC) and thrombosis is indicative of GHC as an additional risk factor in the pathogenesis of vascular complications in systemic autoimmune diseases, in particular as a key factor in thrombus formation in APS and SLE.

Organ and tissue damage in APS, in particular intestinal lesions, have been studied in sufficient detail [5].

Given the above, it is necessary to further study the functional state of blood vessels and the effectiveness of correction of its disorders in antiphospholipid syndrome.

Objective: to increase the effectiveness of treatment by administering warfarin, immunoglobulin and L-arginine in experimental antiphospholipid syndrome based on the study of disorders of vasoconstrictor-vasodilating potential of blood vessels.

Methods

The study was conducted on 100 outbred male rats weighing 180-220 grams. Animals were divided into the following groups:

1st group - control - intact animals that were on

the standard diet of the vivarium (n = 20).

Group 2 - rats, which were simulated antiphospholipid syndrome (n = 20).

3rd group (n = 20) - animals that on the background of the simulated pathology received correction by intraperitoneal administration of human immunoglobulin (CJSC "Biopharma") at a dose of 0.5 g / kg body weight and intragastric administration of a solution of L-arginine in 0.9% sodium chloride solution at a dose of 500 mg / kg.

4th group (n = 20) - animals that on the background of the simulated pathology received correction with warfarin in doses calculated by the coefficient of compliance and intraperitoneal immunoglobulin of a person (CJSC "Biopharma") at a dose of 0.5 g / kg body weight.

Group 5 (n = 26) - animals that on the background of the simulated pathology received correction with warfarin, intraperitoneal administration of human immunoglobulin (CJSC "Biopharma") at a dose of 0.5 g / kg body weight and intragastric administration of a solution of L-arginine in 0.9% sodium chloride solution at a dose of 500 mg / kg.

Antiphospholipid syndrome was simulated by subcutaneous administration of cardiolipin antigen in a total dose of 0.2-0.4 mg per rat every other day for three weeks [6] The daily dose of Warfarin for rats weighing 200 g - 0.2 mg.

Administration of nitric oxide donor - L-arginine solution (SIMESTA, PRC, USP32 quality standard) was carried out by intragastric administration of L-arginine solution in 0.9% sodium chloride solution at a dose of 500 mg / kg through a syringe with an intragastric zone. The volume of the solution depended on the weight of the animal and did not exceed 1 ml. The drug was administered once a day before morning feeding, daily for 10 days [7].

The study lasted 8 weeks, during which the animals were monitored and / or treated.

Rats were removed from the experiment under light ether anesthesia in accordance with the "Rules for performing work using experimental animals", approved by the Order of the Ministry of Health of Ukraine № 249 from 01.03.2012 and the Law of Ukraine № 3447-IV "On protection of animals from cruelty" .2009 and from 16.10.2012).

After euthanasia, blood was taken from rats to study endothelial function (endothelin-1 and S-nitrosothiol content) in the blood of laboratory animals.

The content of S-nitrosothiols (which are known to be stable metabolites of NO) was determined by spectrofluorimetric method [8]. The principle of the method is based on the following: there is a conversion of the group of S-nitrosothiols into nitrites (using mercury chloride) and subsequent acid-catalyzing nitrosotation of 2,4-diaminonaphthalene. Before that, NO_2 is completely removed with ammonium sulfate (reduces NO_2 to N_2 in acidic conditions).

Determination of endothelin-1 content, which is a marker of vasoconstriction [9], was performed by enzyme-linked immunosorbent assay in blood serum.

Results

An increase in the level of endothelin-1 (E-1), which is a marker of ED. These changes indicate an increase in vasoconstrictor potential of vascular tone against the background of simulated pathology (Table 1).

Endothelin is a broad-spectrum bicyclic polypeptide and plays a key role in the regulation of vascular endothelial function. It is produced by endotheliocytes in the form of preendothelin, which is converted into Big-endothelin by cleavage of oligopeptide fragments. The latter consists of 39 amino acid residues and with the participation of endothelin-converting enzyme is converted into endothelin-1. The activity of endothelin-1 increases 140 times, and its half-life is reduced. The vast majority of endothelin - about 80% is inactivated in the pulmonary vessels. Endothelin-1 is the most pronounced vasoconstrictor, which is 10 times more potent than angiotensin II, and 100 times more potent than norepinephrine. This vasoconstrictor is formed in endothelial cells, but does not accumulate there. Its formation is facilitated by angiotensin-II, adrenaline, vasopressin, cytokines and thrombin, as well as mechanical effects. Vasoconstriction and vasodilation of blood vessels depend on the concentration of endothelin-1. At low concentrations, endothelin affects endothelial cells by activating relaxation factors, while increasing its level activates receptors on smooth muscle cells, causing vascular spasm. An important modulator of endothelin formation is transforming growth factor (TGF) β, which increases the formation of

preproendothelin. There are direct and indirect effects of endothelin. The first is the effect on vascular smooth muscle. This causes vasoconstriction, increased mitosis, cell proliferation and intimal fibrosis with increased vascular stiffness. During the indirect effect, vasoactive factors such as nitric oxide, prostacyclin and atrial natriuretic peptide are released from the endothelium, which lead to vascular relaxation. Endothelin-1 is important as a marker and predictor of the severity of cardiovascular disease. It also plays an important role in the pathogenesis of atherosclerosis, pulmonary hypertension, postpartum vascular injury, ischemic brain injury, glomerulonephritis, diabetes and its complications.

In the second group in the conditions of our experiment a significant increase in the level of endothelin-1 in blood of animals with simulated antiphospholipid syndrome without correction compared with the results of intact animals (p <0,001). In 3rd group the level of the studied marker of vasoconstriction at the time of determination was also statistically significantly increased relative to the norm (at the level of significance p <0.001), but less pronounced than in 2nd group (p_{3-2}<0.001). The analysis of data of animals of the fourth group revealed the following: the level of endothelin-1 in the blood of rats of this group is elevated in comparison with data from intact animals (p <0.001) and with the results of 3rd group. But its pathological increase is less pronounced than in the blood of rats of the 2nd group. The most pronounced positive effect of corrective action was found in 5th group: the level of endothelin-1 is statistically very high and significantly lower compared to the results of the 2nd, 3rd and 4th groups (p <0.001). In comparison with the data of intact animals, no differences were found, which indicates the normalization of this marker of ED and vasoconstriction (Fig. 1).

The study of S-nitrosothiols in animals with simulated antiphospholipid syndrome (Table 2).

The content of S-nitrosothiols (S-NO) in the blood indicates the state of production of nitric oxide and vasodilatory potential. In 2nd group there was a very significant decrease in the level of S-NO in the blood of animals with simulated APS without further correction (p <0.001). The above indicates a violation of the physiological synthesis of NO and the weakening of the vasodilating potential of blood vessels caused by the development of antiphospholipid syndrome. In 3rd group the effect of corrective agents is manifested in the form of increased pathologically reduced levels of S-nitrosothiols: differences compared with the group without correction were found at the level of p <0,001, and compared with intact animals - less pronounced - at the level of significance p <0, 01. In 4th group, the normalization of the studied marker is less pronounced: the level of S-nitrosothiols is lower compared to the data of intact animals is very high (p <0.001), and established significant differences compared with the results of 3rd group (p <0.001). It is noteworthy that no differences were found between the results of this group and the group without correction (2nd) in the analysis of the marker of nitric oxide production. The most positive dynamics was found in the study of S-nitrosothiol content in the 5th group, which received a comprehensive correction: the recovery of S-NO content was found to be very high compared to the data of groups 2nd, 3rd and 4th groups (at the level of significance p <0.001). Also, when comparing this marker in the fifth group and in intact animals, no differences were found, which indicates the restoration of physiological synthesis of nitric oxide and improving the vasodilatory potential of blood vessels (Fig. 2).

It is also necessary to dwell on the properties of L-arginine as a major component of normalization of the functional state of the endothelium in pathological conditions. Endothelial dysfunction, which occurs due to impaired synthesis of nitric oxide, is an important modulator of a range of biological effects in blood vessels . NO regulates apoptosis and vascular condition, and in the endothelium maintains angioprotective properties - we can say that it is a classic vasodilator. Arginine is the main substrate for nitric oxide production. Although this amino acid is involved in many metabolic processes, its main role is to regulate the functional state of the endothelium and improve blood supply. Its protective universality is explained by the fact that it is a precursor of L-citrulline, L-ornithine, L-glutathione, gamma-aminobutyric acid, spermitin, and other compounds. L-arginine was first isolated by E. Schulze and E. Steiger in 1886, and the structure and properties of E. Schulze and E. Winterstein in 1897 were described in detail [10].

There are two alternative ways of conversion of L-arginine: the first - with the participation of NO-synthase, oxidative, with the formation of NO and L-citrulline; the second - when L-ornithine and urea (non-oxidized) are formed with the help of arginase I.

Polyamines, anabolic hormones, and nitric oxide are secreted by L-arginine. It regulates vascular endothelial function, causing potent vasodilation by insulin activation and growth hormone secretion. Due to the stimulation of endothelial NO-synthase activity, L-arginine enhances NO synthesis, breaks the NO-S complex with oxidized LDL. Due to its antioxidant activity, L-arginine enhances the bioactivity of NO, and on the other hand blocks the activity of norepinephrine, releases histamine from basophils, resulting in the development of vasodilating effect. L-arginine significantly reduces the concentration of NO-S-mediated superoxide, reduces the level of endothelin-1 and stimulates the expression of eNO-S. The L-arginine-NO complex regulates the functional state of the cardiovascular system. normalizing the state of the endothelium and optimizing its function during the development of hypercholesterolemia. Nitric oxide and its derivatives play an important role in the formation of the inflammatory response, reducing the negative effects of oxidative stress and during apoptosis.

L-arginine improves microcirculation and systemic circulation, cardiomyocyte function, thereby increasing the chances of survival of animals during experimental hemorrhagic shock. A positive therapeutic effect in the correction of endothelial dysfunctions by L-arginine was observed in patients with obliterating atherosclerosis.

Conclusions

1. The development of endothelial dysfunction in experimental antiphospholipid syndrome is confirmed: an increase in the marker of ED (endothelin-1) and a pathological increase in vasoconstrictor potential against the background of weakening of vasodilation.

2. The established pathological increase in vasoconstrictor potential of vessels in animals, which were simulated antiphospholipid syndrome, as evidenced by an increase in the level of endothelin-1 in the blood of rats ($p < 0,001$).

3. The violation of nitric oxide synthesis against the background of the development of experimental antiphospholipid syndrome, which is confirmed by a marked decrease in the level of S-nitrosothiols.

4. The use of immunoglobulin and L-arginine solution in the treatment of rats, in a simulated antiphospholipid syndrome, corrects the functions of the vascular endothelium and leads to the normalization of vasoconstrictor-vasodilatory potential.

5. The use of immunoglobulin and warfarin in the treatment of rats, in a simulated antiphospholipid syndrome, has a therapeutic effect, but is less pronounced.

6. The most significant effect on the vasoconstrictor-vasodilatory potential of blood vessels was found in rats, with the complex use of warfarin, immunoglobulin and L-arginine solution.

Acknowledgments

The authors declare that there are no conflicts of interest.

References

1. Lattice TM. Studies on antiphospholipid syndrome in the Federal State Budget Scientific Institution "Research Institute of Rheumatology. V.A. Nasonova "- the main achievements (to the 40th anniversary of the Dissertation Council). Scientific and practical rheumatology. 2016 (54) 6: 614–627
2. Travkina IV, Ivanova MM, Nasonov EL, etc. Clinical and immunological characteristics of the lesion of the central nervous system in systemic lupus erythematosus: binding to antibodies to cardiolipin. Therapeutic archive. 1992; (5): 10-4
3. Radenska-Lopovok CT, Reshetnyak TM. Vascular pathology in antiphospholipid syndrome. Archive of pathology. 2002; 64 (1): 54-7.
4. Shirokova IE, Reshetnyak TM. Hyperhomocysteinemia as an additional risk factor for thrombosis in systemic lupus erythematosus and antiphospholipid syndrome. Scientific and practical rheumatology. 2003; 41 (4): 39-43.
5. Doroshkevich IA, Radenska-Lopovok SG, Karateev AE, etc. Clinical and endoscopic condition of the

gastric mucosa in systemic lupus erythematosus and antiphospholipid syndrome. Scientific and practical rheumatology. 2004; 42 (3): 23-6.
6. Urakova, MA and Bryndina, I.G. (2013) Metabolic activity and water balance of the lungs in the simulation of autoimmune pathology in rats. Bulletin of TVSU. Series: Biology and Ecology (29). Pp. 272-276.
7. Pokrovsky MV, Pokrovskaya TG, Korchakov VI etc. Endothelioprotective effects of L-arginine in modeling nitric oxide deficiency. Experiment. and wedge. pharmacol. 2008; 71 (2): 29–31.
8. Kovaleva OM, Demidenko GV, Gorbach TV Diagnosis of endothelial function - assessment of vasoactive nitric oxide pool // Ministry of Health of Ukraine. Ukrainian Center for Scientific Medical Information and Patent and License Work. Guidelines. Kyiv, publishing house SPD FO Tarasenko VP- 2007.-16 p.
9. Reutov VP, Sorokina EG, Okhotin BE, Kositsyn NS. Cyclic transformations of nitric oxide in mammals. M .: Nauka, 1997.156 s.
10. Levitsky IM Experimental substantiation of methods of pathogenetic correction of rhegmatogenous retinal detachment: dissertation of the candidate of medical sciences: 14.03.04 / Chernivtsi, VDNZ «Bukovynian state medical university», 2019.170 p.
11. Yakymenko O. O., Savytskyi V. I., Klochko V. V., Savytskyi I. V., Mykhailiuk M. M., Gerasymenko T. V., Badiuk N. S. Dynamics of markers of endothelial dysfunction in experimental antiphospholipid syndrome / PharmacologyOnLine; Archives - 2021 - vol.1 P. 74-81.

Table 1. The results of the study of the level of endothelin-1 in the blood of experimental rats with simulated antiphospholipid syndrome and its correction (M ± m)

	1st group	2nd group	3rd group	4th group	5th group
E-1	3.02 ± 0.17	7.03 ± 0.16	4.25 ± 0.13	6.01 ± 0.14	3.11 ± 0.17

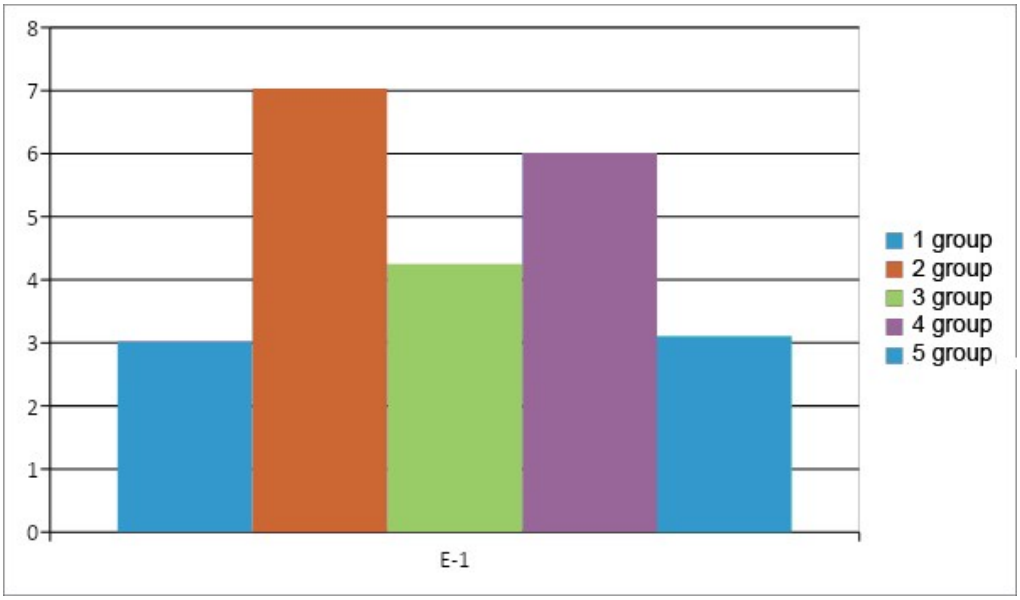

Figure 1. The results of the study of the level of endothelin-1 in the blood of experimental rats with simulated antiphospholipid syndrome and its correction

Table 2. The results of the study of S-nitrosothiols blood of experimental rats with simulated antiphospholipid syndrome and its correction (M ± m)

	1st group	2nd group	3rd group	4th group	5th group
S-NO	0.38 ± 0.02	0.16 ± 0.02	0.3 ± 0.02	0.19 ± 0.01	0.38 ± 0.01

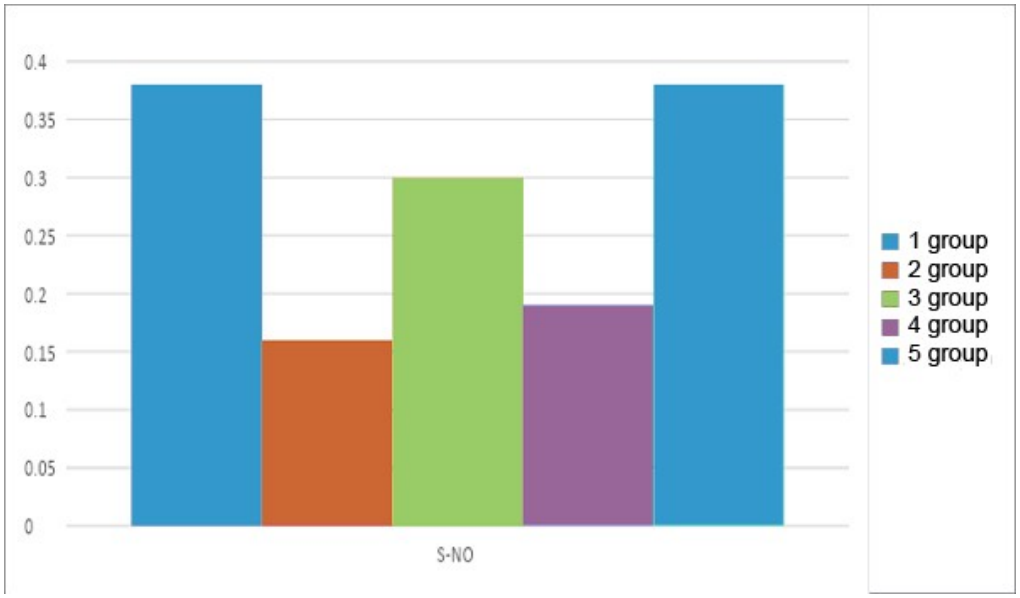

Figure 2. The results of the study of the level of endothelin-1 in the blood of experimental rats with simulated antiphospholipid syndrome and its correction