EXPERIMENTAL WORKS

UDC 616.24:576.385:616-008.6-097.3:543.635.4]-08-092.9 doi: https://doi.org/10.15407/ubj96.05.021

L-ARGININE, AMINOGUANIDINE AND MESENCHYMAL STEM CELLS REDUCE THE LEVEL OF ENDOPLASMIC RETICULUM STRESS MARKERS AND D-DIMER IN THE LUNGS OF MICE WITH ANTIPHOSPHOLIPID SYNDROME

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Received: 22 May 2024; **Revised:** 20 July 2024; **Accepted:** 07 October 2024

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by damage to the intima of the microcirculatory blood vessels as a result of the formation of autoimmune antibodies to phospholipids of cell membranes. Recent data indicate a possible link between the occurrence of autoimmune diseases and endoplasmic reticulum (ER) stress, impaired nitric oxide availability, high plasma D-dimer level. The aim of the study was to estimate the effect of nitric oxide synthesis modulators L-arginine and aminoguanidine, and mesenchymal stem cells (MSCs) on the level of inositol-requiring enzyme-1a (IRE-1a), glucose-regulated protein 78 (GRP-78) as ER stress markers, and the level of D-dimer in the lung tissue of female BALB/c line mice with experimental APS induced with cardiolipin administration. 30 experimental animals were divided into five groups: 1 – control animals; 2 – mice with APS; 3 – mice with APS, injected intraperitoneally with L-arginine hydrochloride (25 mg/kg) and aminoguanidine (10 mg/kg); 4 – mice with APS, injected intraperitoneally with stem cells (5×10^6/kg); 5 – mice with APS, injected with L-arginine hydrochloride, aminoguanidine and stem cells in combination. After 10 days post APS formation animals were removed from the experiment, proteins were extracted from the lung tissue and their level was determined with Western blotting. It was established that in group with APS the levels of IRE-1, GRP-78 and D-dimer were substantially increased as compared to the control group. After separate administration of both arginine with aminoguanidine and MSCs, as well as with their combined use, the level of IRE-1, GRP-78 and D-dimer decreased compared to the indices in animals with induced APS. The obtained data indicated that this effect is probably due to the reduction of ER stress through iNOS inhibition and the anti-inflammatory action of MSCs.

K e y w o r d s: antiphospholipid syndrome, lung, endoplasmic reticulume stress, L-arginine, aminoguanidine, mesenchymal stem cells, IRE-1, GRP-78, D-dimer.

Antiphospholipid syndrome (APS) is a complex autoimmune disease that includes recurrent venous and/or arterial thrombosis and pregnancy complications in the presence of antiphospholipid antibodies (aPL), including anticardiolipin antibodies (aCL), anti-β2-glycoprotein I (anti-β2GPI) antibodies and lupus anticoagulant (LA) [1].

The binding of aPL with β2GPI on cell surfaces activates endothelial cells, monocytes, and platelets, leading to a proinflammatory and prothrombotic response and complement activation. [2].

Endothelial dysfunction is characterized by a violation of the availability of nitric oxide (NO) and a concomitant increase in the formation of reactive oxygen species (ROS). The key mechanism of endothelial dysfunction involves the production of ROS by blood vessels, in particular $O_2^{\bullet-}$, which quickly reacts with NO and inactivates it [3]. It is known that

inflammatory reactions in the endothelium, induced by circulating autoantibodies and other inflammatory mediators, contribute to the pathogenesis of endothelial dysfunction, and numerous studies indicated that the release of cytokines was responsible for the progression of systemic lupus (SLE) erythematosus. It was established that proinflammatory cytokines increase the ROS production in endothelial cells [3, 4].

As in any other autoimmune diseases, APS causes increasing levels of autoantibodies, proinflammatory cytokines, and autoreactive T cells. The triggers for the development of any autoimmune disease include environmental factors, such as microbial infection, exposure to chemicals, and free radicals. All these factors are also known to induce stress on the endoplasmic reticulum (ER) [5], indicating a possible link between the ER stress and the occurrence of autoimmune diseases. ER stress precedes the progression of autoimmune diseases and can lead to the upregulation of many proinflammatory cytokines, including TNFα, IL-1β, IFN-γ, IL-6, and IL-23, which are the hallmarks of the autoimmune diseases. [6].

ER stress is a consequence of the mismatch between the load of unfolded and misfolded proteins in the ER and the ability of the cellular machinery to cope with this load. Misfolded proteins are stored in ER lumen for proper folding or degradation [7]. Accumulation of unfolded/misfolded proteins leads to the ER stress protein response, also known as unfolded protein response, (UPR), through three main approaches: increasing the ER ability to fold and modify proteins; decreasing global mRNA translation; and activated ER-associated degradation (ERAD) and autophagy. When the UPR cannot resolve the problem, it becomes chronic and consistent until cell death [8-10].

RNA-like ER kinase (PERK) - protein kinase, which activates transcription factor 6 (ATF6), and inositol-requiring enzyme-1a (IRE-1a) are major components of the UPR [11].

IRE-1 is an indicator of ER stress. When sufficient protein folding capacity is present in the ER, IRE-1 is in its inactive state. The enzyme is bound to the ER by the chaperone binding immunoglobulin protein (BiP). During ER stress, BiP dissociates to allow IRE-1 to fold into large oligomers, but some evidence suggests a possible direct interaction between IRE-1 and misfolding proteins in the ER lumen [10-12]

Another multifunctional stress and heat shock protein (HSP) of the ER that plays an important role in the UPR is a glucose-regulated protein 78 (GRP-78). GRP-78 production in the lungs is increased during various stresses, and high levels of this protein in the lungs are expressed during chronic obstructive pulmonary disease. In addition to its intracellular localization and functions, GRP-78 can also be exported to extracellular spaces, whereupon it acts as a cytokine through affinity binding to specific cell surface receptors, which transmit danger signals [13].

A variety of pathological conditions can increase protein misfolding, leading to ER stress, where GRP-78 initiates signaling cascades that regulate the UPR. GRP-78 binds hydrophobic surfaces on newly synthesized polypeptides and is first in line for protein folding. This function is enhanced by the misfolding of polypeptides that accumulate in the ER as a result of cellular stress. GRP-78 binds to unfolded proteins in its ATP-bound form and mediates their folding by ATP [14]. When GRP-78 functions as a chaperone, it dissociates from the ER transmembrane stress sensor proteins IRE-1, PERK and ATF6, which triggers the UPR [15].

The development of APS is accompanied by increased coagulation ability. Tissue injury increases the expression of activated tissue factor (aTF) in the endothelium. This activates FVII, which in turn activates FX, causing the aTFF-VIIa-FXa complex formation. This complex with the thrombin can send proinflammatory signals to the cells. On the other hand, the protein C complex, which contains thrombin, thrombomodulin, and activated protein C, deactivates FVIIIa and FVa (acceleration factors) and slows the clotting process. This anticoagulant pathway (protein C complex) requires an intact vascular endothelium expressing the endothelial cell protein C receptor (EPCR). Damaged endothelium releases EPCR (soluble EPCR), which actively binds to free activated protein C complex, and its loss of the anticoagulant moiety leads to hypercoagulation [16]

It was shown previously that such corrective action substances as L-arginine and aminoguanidine, also known as nitric oxide synthesis modulators, have had a positive impact on cytokine profile [17], as well as affected levels of immunoglobulins and circulating immune complexes [18], in experimental artificially induced APS in mice.

Research data on the study of the ER stress in APS, as well as possible corrective action by L-argi-

nine, aminoguanidine, and mesenchymal stem cells. and its mechanisms are insufficient or even lacking, so this topic is relevant for further research.

We aimed to investigate the effect of modulators of nitric oxide synthesis (L-arginine, aminoguanidine) and mesenchymal stem cells on the level of endoplasmic reticulum stress and the level of D-dimer in the lungs of female BALB/c line mice with experimental antiphospholipid syndrome.

Materials and Methods

The study was conducted on 30 BALB/c mice (females), which were kept on a standard vivarium diet. The experiments were performed in compliance with the principles of bioethics in accordance with the General Ethical Principles of Animal Experiments, adopted at the First National Congress on Bioethics (Kyiv, 2000) and consistent with the provisions of the European Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986), and European Union Directive 2010/10/63 EU on animal experiments. APS was modeled using cardiolipin (Sigma, USA), which was injected intramuscularly four times (30 μg per one injection), with 14-day intervals between injections [19]. To increase the efficiency of the immune response, cardiolipin was emulsified in 75 μl of complete Freund's adjuvant (first injection); subsequent injections were carried out with incomplete Freund's adjuvant. APS developed two weeks after the last cardiolipin injection. Reaction of microprecipitation with cardiolipin antigen was performed to confirm the development of APS. In groups of BALB/c mice with APS, the presence of anticardiolipin antibodies was established [19]. All experimental animals were divided into five groups: group 1 – control animals; group 2 – mice with APS; group 3 – animals with APS, which were injected with L-arginine hydrochloride (Sigma, USA, 25 mg/kg) and aminoguanidine (Khimlaborreaktiv, Ukraine, 10 mg/kg); group 4 – mice with APS, which were injected with stem cells; group 5 – animals with APS, which were given a combined injection of L-arginine hydrochloride with aminoguanidine and stem cells. L-arginine hydrochloride and aminoguanidine were injected intraperitoneally once a day for ten consecutive days from the moment of APS formation, as described previously [20]. The stem cells were injected into mice once intraperitoneally at a dose of 5×10^6 cells/kg, and 4 group of animals was removed from the experiment on the 7th day after correction with sodium thiopental anesthesia. Control group animals were injected with identical volumes of 0.9% sodium chloride. All animals from other group 1, 2, 3, 5 were removed from the experiment using sodium thiopental anesthesia after 10 days after correction (intraperitoneal administration of 1% solution at the rate of 50 mg/kg of animal weight).

Cryopreserved human umbilical cord mesenchymal stem cells (MSCs) were kindly provided by H. Lavrenchuk (Institute of Experimental Radiology of the National Scientific Center of Radiation Medicine of the National Academy of Sciences of Ukraine). Umbilical cord tissue had been acquired from a single donor after a standard delivery process with donor's informed consent on record. The umbilical cord tissue had been aseptically dissected into smaller fragments and enzymatically processed with 0.1% collagenase I (Sigma-Aldrich, USA), diluted in DMEM/F12 Advanced culture medium (Gibco, USA). Isolated cells were seeded into culture flasks and cultivated in controlled conditions at 37°C and 5% CO_2. MSC passaging was performed before cells reached 90-100% confluence using TrypLE Express Enzyme (Gibco, USA). Cryopreservation of MSCs was done at passage 4 after achieving 80-90% cell monolayer. Frozen samples were stored in liquid nitrogen at -196°C.

The obtained MSCs underwent immunophenotyping using flow cytometry (Accuri™ C6 Plus Personal Flow Cytometer, Becton Dickinson, USA) and mouse anti-human monoclonal antibodies targeting CD73, CD90, CD105, CD34, and CD45 (Invitrogen, USA). Therefore, the MSCs were identified as CD73+, CD90+, CD105+, CD34-, CD45- cells. Following thawing, the cells were cultured using DMEM/F12 Advanced medium, supplemented with 2% FBS, 1% L-Glutamine–Penicillin–Streptomycin solution (Sigma, USA), and heparin (240 μg/l) (Sigma, USA) in a controlled environment (37°C, 5% CO_2). MSCs in passage 5 were used for experimental procedures. The mice receiving MSCs injections were not simultaneously subjected to immunosuppression, as these stem cells typically evoke minimal or no immune response [21, 22].

Protein extraction from the lung tissue. Lungs were ground up in liquid nitrogen, preparing aliquots of the same weight, which were later homogenized in tissue lysis buffer with the following composition: 0.05 M Tris-HCl (pH 7.4), 0.135 M NaCl, 0.1% SDS, 1% Triton X-100, 0.5% sodium deoxychalate, 0.03%

NaN_3, with the addition of a cocktail of protease and phosphatase inhibitors (Pierce Protease and Phosphatase Inhibitor, cat. No. A32959, ThermoFisher Scientific, USA). The tissue to buffer ratio was 1:10 (w/v). After additional ultrasound treatment using a Sartorius disintegrator (Germany), the homogenates were centrifuged at 16,000 g for 60 min at 4°C. Supernatants were collected in Eppendorf tubes and total protein content was determined. Samples were prepared for electrophoresis using **2x** Laemmli buffer with the following composition: 62.5 mM Tris-HCl (pH 6.8), 0.1% SDS, 10% β-mercaptoethanol, 10% glycerol, 0.001% bromophenol blue. The samples were boiled in a water bath for 5 min for solubilization and denaturation of proteins.

Determination of total protein content. The total protein content in lung tissue lysates was determined spectrophotometrically by measuring the absorbance of the sample at 260 nm and 280 nm wavelengths. To calculate the protein content, the following equation was used [23].

Electrophoresis in a SDS-PAGE was performed according to the standard methods [24]. A vertical gel electrophoresis chamber manufactured by Bio-Rad (USA) was used. TEMED and 10% ammonium persulfate solution were used for the acrylamide polymerization in gels for concentration (pH 6.8) and separation (pH 8.8) of samples. The amount of total protein was 100 μg per well/track. A buffer solution containing 25 mM Tris-HCl (pH 8.3), 0.19 M glycine and 0.1% SDS was used for gel immersion. The concentration of samples was carried out at a voltage of 30-35 V (15-18 mA), while separation - at 70-110 V (30-35 mA).

Gels, which were later used for immunoblotting (Western blot analysis), were carefully removed from the chamber after electrophoresis and washed in a transfer buffer containing 20% methanol.

Western blot analysis. Western blot (immunoblotting) was used to determine the levels of marker proteins in lung tissue. Immunoblotting was performed according to the standard methods [25] with minor modifications. After electrophoretic separation, proteins from the gel were transferred to a nitrocellulose membrane by electroblotting in a buffer solution containing 25 mM Tris-HCl (pH 8.3), 0.19 M glycine, and 20% methanol. The voltage during the protein transfer was 42-48 V with a current of 220-240 mA, the duration of the transfer was 120 min. After the transfer, the nonspecific binding sites of the antibodies on the membrane were blocked with a 5% solution of nonfat dry milk proteins dissolved in buffered saline containing 0.05% Triton X-100 (PBST) for 90 min at 37°C. After blocking, the blots were incubated with primary antibodies against the target proteins, diluted according to the manufacturer's recommendations, in a 3% solution of BSA for 16 h at 4°C. Following this, the membranes were washed six times for 5 min in PBST to remove non-specifically bound antibodies and incubated with the appropriate anti-species horseradish peroxidase (HRP)-conjugated secondary antibodies for 120 min at 37°C. After incubation with antibody-conjugates, the membranes were washed with PBST according to the previously described scheme.

Development of immunostaining was recorded by enhanced chemiluminescence (ECL) using 0.25 M luminol in DMSO, 0.09 M coumaric acid in DMSO, 0.1 M Tris-HCl (pH 8.4), and 0.0035% hydrogen peroxide solution, during autoradiography on X-ray films, as described earlier [26]. Depending on the intensity of the chemiluminescence signal, the exposure time of the membrane on the film fluctuated between 2 s and 15 min. To develop blots, X-ray films were treated with commercial developer and fixative solutions. The molecular weights of proteins in the samples were determined by comparing their migration with the location of PageRuler™ Prestained Protein Ladder markers in the range of 10-250 kDa (cat. no. 26619, ThermoFisher Scientific, USA). After developing, the film was scanned, and semi-quantitative protein level analysis was performed using TotalLab software (TL120, Nonlinear Inc, USA). The level of the immunoreactive zone of the target protein was expressed in arbitrary units (a.u.) of optical density and normalized by the level of tubulin in each sample.

Results and Discussion

It was established that in the APS group, the IRE-1 indicator increased almost 5 times compared to the indicators in the control group (Fig. 1). A dramatic increase in IRE-1α expression was found in B cells from patients with active systemic lupus erythematosus (SLE) compared to those from healthy controls [27]. An increase in IRE-1α activity was also detected in neutrophils isolated from the blood of SLE patients. In several mouse models of SLE, inhibition of IRE-1α reduced the release of neutrophil extracellular traps (NET) and slowed disease progression [28].

Due to their secretory function, immune cells have a larger ER with higher protein folding activity

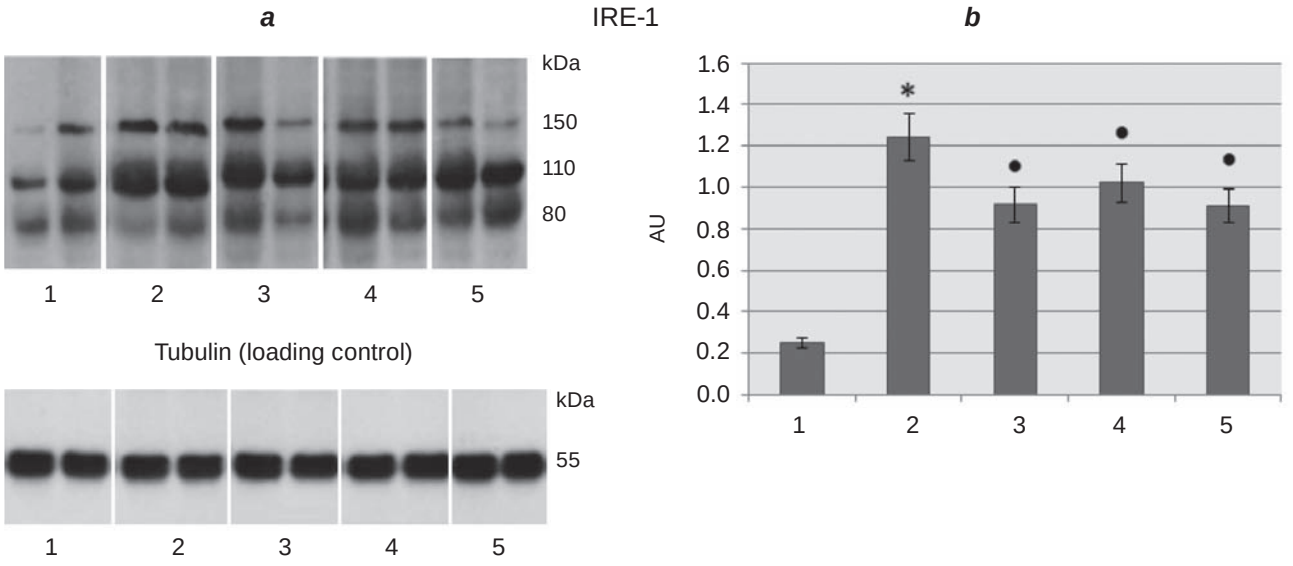

*Fig. 1. Western blot analysis (**a**) and tubulin (loading control), densitometry (**b**) analysis of endoplasmic reticulum stress marker protein IRE-1 in the lung tissue of female mice with experimental APS and under corrective action substances. Group 1 – control, intact animals; Group 2 – mice with APS, Group 3 – APS + L-arginine hydrochloride + aminoguanidine; Group 4 – APS + stem cells; Group 5 – APS + L-arginine hydrochloride + aminoguanidine + stem cells (M ± m, n = 6). *The difference is significant relative to the control group; •the difference is significant relative to the APS alone group, (P < 0.005)*

and therefore, they are more susceptible to agents that cause ER stress during autoimmune diseases. This may require the presence of IRE-1α in immune cells. In addition to pathophysiological effects, IRE-1α activation plays an important function in the normal physiology of immunologically important cell types. The role of IRE-1α in immune functions is gradually being identified as a possible mechanism for many complex immune-related diseases [6].

The level of GRP-78 increased by 2.7 times compared to the control group (Fig. 2). ER stress plays an important role in the progression of an autoimmune disease such as rheumatoid arthritis (RA). Some studies have shown that the expression levels of the ER stress markers, such as GRP-78 and IRE-1, were increased in RA. ER-stressed fibroblasts in RA showed increased expression of GRP-78, which promoted cell survival and proliferation and finally promoted synovial proliferation. Indeed, an increase in GRP-78 in the ER lumen induced by the ER stress promotes the translocation of GRP-78 from the ER to the cell surface. Under these circumstances, cell surface GRP-78 can be used as an anti-cyclic citrullinated peptide antibody and an autoantigen for T and B cells, promoting the development of self-reactive T cells [29].

The ER lumen is an ideal environment for the proper synthesis and folding of proteins destined for secretion or display on the cell surface. Homeostasis in the ER is maintained through the coordination of the UPR and ER-associated degradation; however, a variety of perturbations increase protein misfolding, leading to the ER stress, where GRP-78 initiates signaling cascades that are regulated by the UPR [15, 30].

The D-dimer level in the APS alone group increased by 5.3 times compared to the control group (Fig. 3). Weinberg et al. reported that patients with autoimmune status had higher plasma D-dimer levels than patients without autoimmune disease. It was also found that the synovial membrane of patients with RA had higher levels of D-dimer compared to patients with osteoarthritis and traumatic joint injury. According to the study, plasma D-dimer levels were found to be significantly associated with the severity of disease in RA patients [31].

D-dimer is the end product of cross-linked fibrin degradation, formed by plasmin hydrolysis. This is the only ideal indicator that directly reflects the level of thrombin, and is also a specific marker of secondary hyperfibrinolysis. An increased D-dimer level can be considered as a sign of hypercoagulation and fibrinolysis [32].

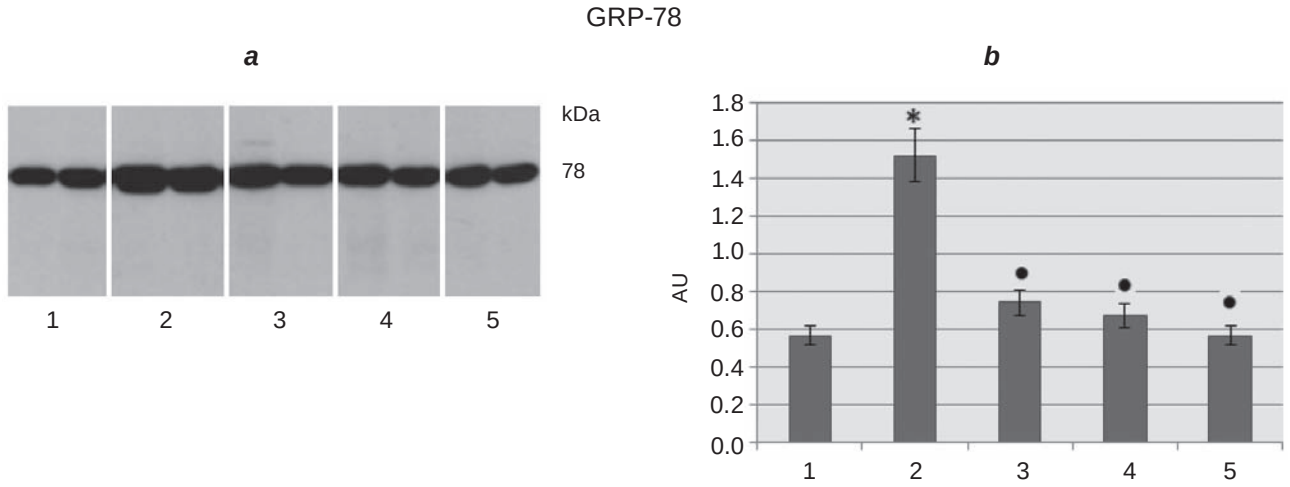

*Fig. 2. Western blot (**a**) and densitometry (**b**) analysis of the endoplasmic reticulum stress marker protein GRP-78 in the lung tissue of female mice with experimental APS and under corrective action substances. Group 1 – control, intact animals; Group 2 – mice with APS, Group 3 – APS + L-arginine hydrochloride + aminoguanidine; Group 4 – APS + stem cells; Group 5 – APS + L-arginine hydrochloride + aminoguanidine + stem cells (M ± m, n = 6). *The difference is significant relative to the control group; •the difference is significant relative to the APS alone group, (P < 0.005)*

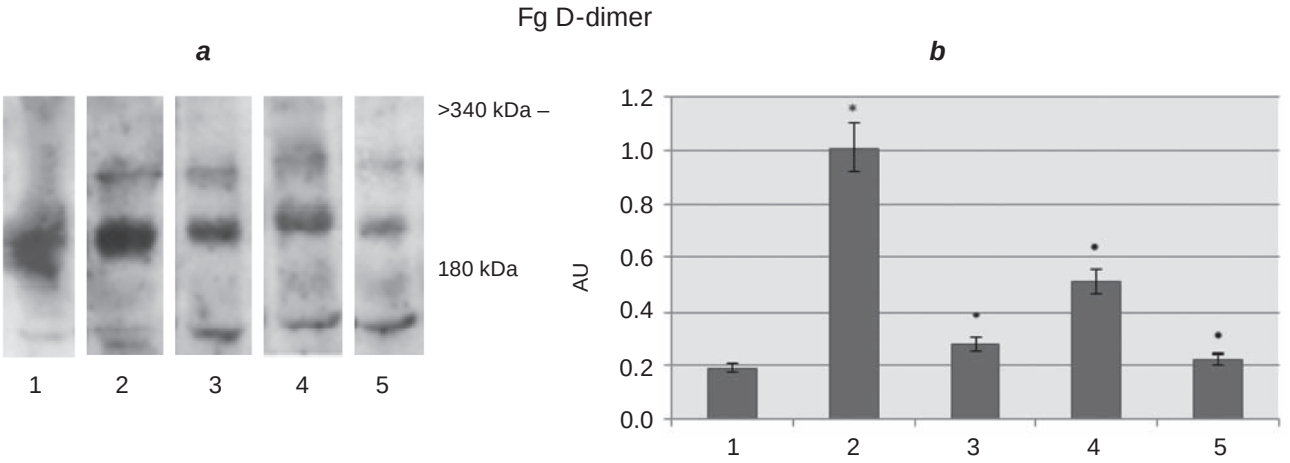

*Fig. 3. Western blot (**a**) and densitometry (**b**) analysis of D-dimer in lung tissue of female mice with experimental APS and under corrective action substances. Group 1 – control, intact animals; Group 2 – mice with APS, Group 3 – APS + L-arginine hydrochloride + aminoguanidine; Group 4 – APS + stem cells; Group 5 – APS + L-arginine hydrochloride + aminoguanidine + stem cells (M ± m, n = 6). *The difference is significant relative to the control group; •the difference is significant relative to the APS alone group, (P < 0.005)*

The level of IRE-1 was 1.4 times lower in the group of APS animals with the combined use of aminoguanidine and L-arginine for the correction of APS in BALB/c female mice, compared to the group of animals with experimental APS and no corrective action (Fig. 1). In the group, where corrective substances were used, the level of GRP-78 was lower by 2 times compared to the indicator in the group of animals with experimental APS alone (Fig. 2); and the level of D-dimer decreased by 4 times (Fig. 3).

It was shown that enhanced NO generation by iNOS may contribute to acute lung injury. The use of aminoguanidine (AG) as an iNOS inhibitor promotes the elimination of excess nitric oxide, reduces cell damage and reduces the production of proinflammatory mediators [33]. AG can reduce the accumulation

of glycosylation products, which can lead to endoplasmic reticulum stress-induced cell apoptosis [34].

Under the influence of proinflammatory factors, oxidative stress and L-arginine deficiency, the dimeric structure of eNOS is disrupted. Suppression of NO production leads to increased inflammation and thrombus formation in blood vessels. A high concentration of ROS has a cytotoxic and mutagenic effect and causes aging and apoptosis of endothelial cells [35]. That is, by using the nitrogen oxide donor L-arginine, we activate the antioxidant system, which is important for protecting the vascular endothelium and lung tissue as a whole in APS.

The combined use of L-arginine and aminoguanidine caused an increase in the content of eNOS and a decrease in the content of iNOS in blood serum and liver of animals with experimental APS [36]. Such data confirm the expediency of using these corrective substances because they exhibit high antioxidant and anti-inflammatory effects.

NO-induced apoptosis is mediated by mitochondrial damage [35]. Bagchi A.K. et al. have previously shown that iNOS is a critical factor that can cause disseminated intravascular coagulation (DIC) syndrome. DIC may also be characterized by aberrant UPR signaling, leading to inhibition of many adaptive responses through increased levels of NO and iNOS. These data indicate that iNOS/NO are required to promote inflammation after ER stress-induced apoptosis [37].

In this study, mesenchymal stem cells (MSCs) were used in the 4th group of animals to correct APS. Against the background of the introduction of MSCs, the content of IRE-1 decreased by 17%, GRP-78 decreased by 56%, and D-dimer decreased by 49% (Fig. 1-3), compared to the parameters of animals with pathology alone.

In studies which used MSCs to treat COVID-19 pneumonia, reductions in leukocyte counts, D-dimer levels, and CRP levels, which are predictive indicators of the severity of COVID-19, were observed [38]. Due to their properties, MSCs reduce ER stress and regulate D-dimer level through immunosuppressive effect in experimental APS. Data on whether APAs really cause thrombotic complications in patients with COVID-19 are controversial [39, 40], as hypercoagulability was observed both in APA-positive, as well as in APA-negative COVID-19 patients.

MSCs have a noticeable immunoregulatory effect against autoimmune disorders. For example, MSCs are able to inhibit not only the proliferation and activity of natural killer (NK) cells, but also the proliferation of T/B cells and the maturation of dendritic cells (DC). MSC-based therapy for many diseases, including autoimmune disorders, can be linked to the production of a wide range of biomolecules, such as proteins, mRNAs and miRNAs, through the release of secretory growth factors or extracellular vesicles (EVs) [41].

To summarize, with the combined use of arginine, aminoguanidine and MSC as corrective action substances in APS, the following results were obtained: a 26% decrease in IRE-1 content, a 63% decrease in GRP-78, and a 78% decrease in D-dimer levels (Fig. 1-3). The most effective interference for reducing the D-dimer level was the combined use of MSC with L-arginine and aminoguanidine. This effect is probably due to the anti-inflammatory effect of MSCs[38] and the reduction of ER stress through iNOS inhibition [37].

Conclusion. During experimental APS in female BALB/c line mice, clear signs of endoplasmic reticulum stress of the lung tissue and an increase in D-dimer levels were observed. With the combined administration of arginine and aminoguanidine, separately MSCs, and their simultaneous use, the investigated indicators of IRE-1, GRP-78 and D-dimer decreased compared to the indicators in animals with induced APS. This indicates that corrective substances reduce autoimmune proinflammatory, prothrombotic processes and reduce signs of ER stress in experimental APS.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. This research was conducted without any external funding.

Acknowledgments. The authors thank Dr Artem Tykhomyrov for his valuable advice, discussion, and critical reading of the manuscript.

L-АРГІНІН, АМІНОГУАНІДИН ТА МЕЗЕНХІМАЛЬНІ СТОВБУРОВІ КЛІТИНИ ЗНИЖУЮТЬ РІВЕНЬ МАРКЕРІВ СТРЕСУ ЕНДОПЛАЗМАТИЧНОГО РЕТИКУЛУМА ТА D-ДИМЕРУ У ЛЕГЕНЯХ МИШЕЙ З АНТИФОСФОЛІПІДНИМ СИНДРОМОМ

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Антифосфоліпідний синдром (АФС) – аутоімунне захворювання, що характеризується ураженням інтими мікроциркуляторного русла внаслідок утворення аутоімунних антитіл до фосфоліпідів клітинних мембран. Попередні дослідження вказують на можливий зв'язок між виникненням аутоімунних захворювань і стресом ендоплазматичного ретикулума (ЕР), порушенням доступності оксиду азоту та високим рівнем D-димеру в плазмі крові. Метою роботи було дослідження впливу модуляторів синтезу оксиду азоту (L-аргініну, аміногуанідину) та мезенхімальних стовбурових клітин (МСК) на рівень інозитол-залежного ензиму 1a (IRE-1a) та глюкозо-залежного протеїну 78 (GRP-78), як маркерів стресу ендоплазматичного ретикулума, на рівень D-димеру в легенях самок мишей лінії BALB/c із антифосфоліпідним синдромом, спровокованим кардіоліпіном. Тридцять піддослідних тварин розділили на п'ять груп: 1 – контрольні тварини; 2 – миші з АФС; 3 – миші з АФС, яким внутрішньочеревно вводили L-аргініну гідрохлорид (25 мг/кг) і аміногуанідин (10 мг/кг); 4 – миші з АФС, яким внутрішньочеревно вводили МСК (5×10^6/кг); 5 – миші з АФС, яким вводили L-аргініну гідрохлорид, аміногуанідин та МСК. Усіх тварин виводили з досліду на десятий день з подальшим екстрагуванням протеїнів легеневої тканини. Рівень окремих протеїнів визначали за допомогою Вестерн-блоту. Встановлено, що в групі з АФС рівні IRE-1, GRP-78 і D-димеру були значно підвищені порівняно з контрольною групою. Після застосування аргініну з аміногуанідином, а також МСК, як окремо, так і при їх спільному застосуванні, рівень IPE-1, GRP-78 та D-димеру знижувався порівняно з показниками у тварин із індукованим АФС. Отримані дані свідчать про те, що цей ефект, ймовірно, зумовлений зниженням стресу ЕР через інгібування iNOS і протизапальну дію МСК.

Ключові слова: антифосфоліпідний синдром, легені, стрес ендоплазматичного ретикулума, L-аргінін, аміногуанідин, мезенхімальні стовбурові клітини, IRE-1, GRP-78, D-димер.

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