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# Effect of Xenon on Proinflammatory Activation and Apoptosis of Human Neutrophils Under Ex Vivo Conditions

***O.A. Grebenchikov[1], A.K. Shabanov[1, 2], L.L. Nikolayev[3], A.I. Shpichko[1] ✉, I.V. Bratishchev[2], L.Yu. Marchenko[4], Sh.Zh. Khusainov[1, 2], R.A. Cherpakov[1], N.P. Shpichko[1, 5]***

**Laboratory for Organ Protection in Critical Conditions**
**[1] Federal Scientific and Clinical Center of Resuscitation and Rehabilitation**
**25 building 2 Petrovka St., Moscow 107031, Russian Federation**
**[2] N.V. Sklifosovsky Research Institute for Emergency Medicine of the Moscow Health Department**
**3 B. Sukharevskaya Sq., Moscow 129090, Russian Federation**
**[3] Russian Medical Academy of Continuing Professional Education**
**2/1, building 1 Barrikadnaya St., Moscow 125993, Russian Federation**
**[4] State Scientific Center of the Russian Federation Institute of Biomedical Issues of the Russian Academy of Sciences**
**76A, p. 4 Horoshevskoe Sh., Moscow 123007, Russian Federation**
**[5] Peoples' Friendship University of Russia**
**6 Miklukho-Maklaya St., Moscow 117198, Russian Federation**

✉ **Contacts:** Andrey I. Shpichko, Candidate of Medical Sciences, Senior Researcher of the Laboratory for Organ Protection in Critical Conditions of Federal Scientific and Clinical Center of Resuscitation and Rehabilitation. Email: shpichko.a@yandex.ru

**BACKGROUND** The syndrome of systemic inflammatory response, which underlies the damaging effect of factors of infectious and non-infectious genesis, may cause multiple organ failure. The degree of its severity is determined, among other things, by the activation of neutrophils. The paper highlights new mechanisms of the anti-inflammatory action of the inhalation anesthetic xenon, mediated by a decrease in the ability of neutrophils to pro-inflammatory response.

**AIM OF STUDY** To evaluate the effect of xenon on the activation of human neutrophils under ex vivo conditions.

**MATERIAL AND METHODS** We studied the effect of xenon inhalation on reduction of the ability of neutrophils to be activated proinflammatory by reduced expression of adhesion molecules CD11b and CD66b on the surface of neutrophils and on the phosphorylation of proinflammatory kinases: ERK 1/2 and kinase — p38 in neutrophils of healthy volunteers.

**RESULTS** The use of xenon at a dose of 30 vol. % within 60 minutes in healthy volunteers statistically significantly reduces the ability of neutrophils to pro-inflammatory activation. The addition of lipopolysaccharide (LPS) to the incubation medium of neutrophils causes their pronounced activation, statistically significantly increasing the phosphorylation of key proinflammatory neutrophil kinases ERK1/2 and kinase p38. Inhalation of xenon in volunteers (30% within 60 minutes) has a pronounced anti-inflammatory effect on LPS-stimulated neutrophils, decreasing their activation by inhibiting pro-inflammatory kinase ERK1/2 and pro-inflammatory MAP kinase p38.

**CONCLUSION** The actual study, performed on isolated neutrophils from volunteers who underwent xenon inhalation, revealed the anti-inflammatory properties of the inert gas xenon, which, in our opinion, may have a direct relationship to the identification of the mechanism of its neuroprotective properties. Thus, the research results available today suggest that xenon has a pronounced pleiotropic mechanism of brain protection. This is a partial block of NMDA receptors, and phosphorylation of the enzyme glycogen synthase-3β, and limitation of the inflammatory activation of neutrophils.

**FINDINGS** Inhalation of xenon in volunteers (30% within 60 minutes) has a pronounced anti-inflammatory effect on neutrophils stimulated by lipopolysaccharides, decreasing their activation by inhibiting proinflammatory ERK 1/2 kinase and proinflammatory MAP kinase p38, as well as reducing the expression of markers of activation and degranulation CD11b and CD66b on the surface of neutrophils. Stimulation by lipopolysaccharides statistically significantly reduces spontaneous apoptosis of neutrophils, while xenon increases the ability of neutrophils to apoptosis, which is likely to contribute to the resolution of inflammation.

**Keywords:** systemic inflammatory response syndrome (SIRS), xenon, neutrophils

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**Affiliations**

| | |
|---|---|
| Oleg A. Grebenchikov | Doctor of Medical Sciences, Chief Researcher of the Laboratory for Organ Protection in Critical Conditions, Federal Scientific and Clinical Center of Resuscitation and Rehabilitation;<br>http://orcid.org/0000-0001-9045-6017, oleg.grebenchikov@yandex.ru;<br>20%: concept of the article, editing of the primary material, final approval of the text |
| Aslan K. Shabanov | Doctor of Medical Sciences, Chief Researcher of the Laboratory of Clinical Pathophysiology In Critical Conditions Federal Scientific and Clinical Center of Resuscitation and Rehabilitation, Deputy Chief Physicians for Anesthesiology and Resuscitation, N.V. Sklifosovsky Research Institute for Emergency Medicine of the Moscow Health Department;<br>https://orcid.org/0000-0002-3417-2682, aslan_s@mail.ru;<br>15%: editing the primary material |
| Lev L. Nikolayev | Associate Professor of the Department of Anesthesiology and Resuscitation, Russian Medical Academy of Continuing Professional Education;<br>https://orcid.org/0000-0001-7533-7618, llnikolaev@gmail.com;<br>10%: collection of primary material |

| | |
|---|---|
| Andrey I. Shpichko | Candidate of Medical Sciences, Senior Researcher of the Laboratory for Organ Protection in Critical Conditions, Federal Scientific and Clinical Center of Resuscitation and Rehabilitation;<br>https://orcid.org/0000-0002-4652-3259, shpichko.a@yandex.ru;<br>10%: editing of the primary material, final approval of the text, preparation of the text for printing |
| Igor V. Bratishchev | Anaesthesiologist-resuscitator, Lecturer of the Educational Department of N.V. Sklifosovsky Research Institute for Emergency Medicine of the Moscow Health Department;<br>https://orcid.org/0000-0002-1050-1867, bratischev@mail.ru;<br>9%: collecting material, text editing |
| Lilia Yu. Marchenko | Junior Researcher of the State Scientific Center of the Russian Federation Institute of Biomedical Issues of the Russian Academy of Sciences;<br>https://orcid.org/0000-0001-9925-1413, golu bajavoda@mail.ru;<br>9%: collection of primary material |
| Shamil Zh. Khusainov | Anaesthesiologist-resuscitator, N.V. Sklifosovsky Research Institute for Emergency Medicine of the Moscow Health Department, postgraduate student of Federal Scientific and Clinical Center of Resuscitation and Rehabilitation;<br>https://orcid.org/0000-0002-3177-8929, shamilkhusa inov1989@gmail.com;<br>9%: collecting material, text editing |
| Rostislav A. Cherpakov | Researcher of the Laboratory for Organ Protection in Critical Conditions, Federal Scientific and Clinical Center of Resuscitation and Rehabilitation;<br>https://orcid.org/0000-0002-0514-2177, zealot333@mail.ru;<br>9%: collecting material, text editing |
| Nadezhda P. Shpichko | Researcher at the Laboratory of Motor Rehabilitation, Swallowing and Speech Recovery, Federal Scientific and Clinical Center of Resuscitation and Rehabilitation, Assistant of the Department of Anaesthesiology, Resuscitation and Intensive Therapy, Peoples' Friendship University of Russia;<br>https://orcid.org/0000-0003-3289-6107, shpichkonp@yandex.ru;<br>9%: editing of primary material |


APC – anþesthetic preconditioning

CU – chemiluminescence units

FU – fluorescence units

LPS – lipopolysaccharides

ICAM-1 – Inter Cellular Adhesion Molecule

SIRS – systemic inflammatory response syndrome

## INTRODUCTION

The systemic inflammatory response syndrome (SIRS) is the response of the body to the damaging effects of infectious (bacteria, viruses) and non-infectious (burns, severe multisystem trauma, major surgical interventions) origin [1, 2]. The degree of severity of SIRS is determined by the production of proinflammatory cytokines, chemokines, subsequent activation of neutrophils and macrophages, their penetration through histohematic barriers and infiltration of target organ tissues with leukocytes and cytokines [3], which can cause the development of multiple organ failure in the case of a hyperinflammatory response, and this is the leading cause of death in intensive care units today. Neutrophils play an important role in the development, regulation and resolution of inflammation. The immune response to infection and tissue damage originates from the secretion of pro-inflammatory and anti-inflammatory cytokines by neutrophils and macrophages and is aimed at removing the damaging agent and restoring homeostasis [4, 5].

Today, it is known that the most sensitive markers of neutrophil activation and degranulation are protein molecules *CD11b* and *CD66b*, located in intracellular granules [6, 7]. Inflammatory stimuli cause the granules to fuse with the cytoplasmic membrane, and *CD11b* and *CD66b molecules are* expressed on the surface of neutrophils (Fig. 1). This process is called degranulation. It is known that *CD11b* interacts with receptors of cell adhesion molecules *ICAM*-1 on the endothelium, thereby ensuring the adhesion of neutrophils to endothelial cells; this moment is the initial stage of their further migration to the focus of inflammation [8]. At the same time, the *CD66b* protein molecule ensures aggregation of neutrophils [6]. In clinical diagnostics, the determination of the expression of *CD11b* and *CD66b molecules* on the surface of

neutrophils has been used for a long time, and it is also known that their level increases with sepsis and severe multisystem trauma [7, 9].

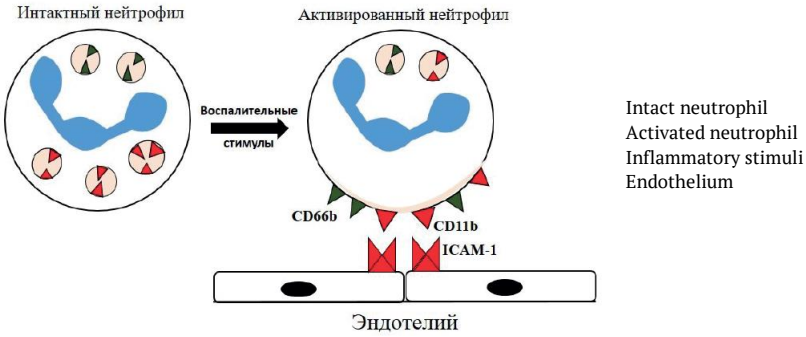


Fig. 1. Activation of neutrophils and their expression of adhesion molecules CD11b (red triangle) and CD66b (green triangle) under the action of an inflammatory stimulus
Note: ICAM-1 is a cell adhesion molecule that is expressed by the endothelium

According to modern concepts, neutrophils are rather short-living leukocytes: after leaving the bone marrow, the average lifespan of neutrophils is 4–5 days [10]. During this time, neutrophils leave the bone marrow, mature and age. Under physiological conditions, neutrophil aging is accompanied by an increase in the expression of the CXCR4 chemokine receptor, which leads to the migration of neutrophils to the bone marrow, liver, and other organs, where they undergo apoptosis and are phagocytosed by macrophages [11]. Apoptosis is an extremely important and difficultly regulated stage in the life cycle of neutrophils, since too rapid and massive death of neutrophils will lead to neutropenia and susceptibility to infections, while an excessively long life, on the contrary, can cause chronic inflammation [12]. Critical states caused by ischemia-reperfusion injury and excessive SIRS are a stimulus for the release of inflammatory mediators and mobilization of leukocytes. It is important to note that activated human neutrophils actively produce glutamate and contain a higher level of *NMDA* receptors [13–15]. In a recent study, inhibition of neutrophil *NMDA* receptors was shown to reduce autocrine glutamate release and oxidative stress mediated by neutrophil activation [16].

To date, it is generally accepted that the anesthetic properties of xenon are due to its ability to inhibit *NMDA* receptors [17, 18]. Recent studies showing that activated human neutrophils actively produce glutamate and contain higher levels of *NMDA* receptors suggest that the inhalational anesthetic xenon may be able to prevent overactivation of neutrophils by inhibiting their *NMDA* receptors.

Thus, the **aim of this work** is to study the effect of xenon on the activation of human neutrophils under *ex vivo conditions* .

## MATERIAL AND METHODS

After obtaining informed consent, 20 blood serum samples from 10 apparently healthy volunteers were used in the study (10 blood serum samples were taken from donors before xenon inhalation and 10 blood serum samples were taken from the same donors after xenon inhalation).

Healthy volunteers, whose average age was 35.6 [28.7; 45.0] years, underwent xenon inhalation in a closed circuit on the KSENA-010 anesthesia machine (LLC PMT, Russia) with the composition of the respiratory gas mixture containing xenon at a concentration of 30 vol.%, oxygen at a concentration of not more than 40 vol, in a hospital. The rest of the mixture is atmospheric nitrogen. Anesthesia monitoring was carried out according to the Harvard standard. The duration of xenon inhalation in all patients was 60 minutes. The course of anesthesia in all cases was smooth, the patients' hemodynamics were stable.

**Neutrophils of healthy donors** after inhalation with a gas mixture containing xenon for 60 minutes were isolated according to the following method: heparinized venous blood was used, which was mixed with a solution of *T -500 dextran* (*Pharmacosmos*, Denmark) to a final dextran concentration of 1% and left at room temperature for 30 minutes for erythrocyte sedimentation. The upper plasma layer (rich in leukocytes and devoid of erythrocytes) was layered on Ficoll (PanEco, Russia) with a density of 1.077 g/ml and centrifuged at room temperature at 300 g for 30 minutes in a centrifuge with the brake off. The supernatant was then removed and all further procedures were performed on ice using chilled solutions. Removal of erythrocytes was carried out by resuspension of the sediment in 2 ml of deionized sterile water for 45 seconds, and then 2 ml of 2-fold PBS (phosphate buffered saline) was added to restore

tonicity and centrifuged at 200 g at a temperature of +4ºC for for 10 minutes. Precipitated neutrophils were washed with PBS and resuspended in culture medium *RPMI* -1640 (PanEco, Russia), 10% *FBS* with a low content of endotoxins).

**Activation (degranulation) of neutrophils** was measured using antibodies conjugated with fluorescent dyes *CD11b - FITC* and *CD66b-AlexaFluor* 647 (*BD Biosciences,* USA) according to the manufacturer's protocol.

In order to determine the effect of xenon on neutrophil degranulation, lipopolysaccharides (LPS) at a dose of 200 ng/ml were added to a cell concentrate of 4 million/ml from healthy donors and incubated for 30 minutes at a temperature of +37°C. Then, antibodies were added and incubated for 30 minutes on ice, after which the fluorescence level (fluorescence units, FU) was measured on a *Beckman-Coulter FC* 500 flow cytometer.

**Apoptosis of neutrophils.** In order to determine the apoptosis of neutrophils from 6 healthy donors, they were incubated with LPS at a dose of 200 ng/ml for 22 hours at a temperature of +37°C in a humidified $CO_2$ incubator. The cells were then centrifuged at 400 g for 5 minutes and the pellet was resuspended in 70 µl of buffer (10 mM *HEPES*, 120 mM sodium chloride, 2.5 mM calcium chloride, pH=7.4). Annexin V (2.5 µl) conjugated with fluorescent dye *FITC* (*ThermoFisher, USA) was* added to each sample and left for 25 minutes at +37°C. Next, propidium iodide was added to a final concentration of 5 µg/ml, incubated for another 5 minutes, after which at least 50,000 cells were analyzed using a *Beckman Coulter CytoFLEX flow cytometer*. Annexin *V* -positive and propidium iodide-negative cells were considered apoptotic.

**Immunoblotting.** Protein electrophoresis was performed in 12.5% polyacrylamide gel under denaturing conditions. The samples were dissolved in a buffer containing 0.125 M Tris-HCl (pH 6.8), 4% sodium dodecyl sulfate (*Sigma Chemical Co.*, USA), 20% glycerol, 0.005% bromine phenol blue (*Sigma Chemical Co.,* USA) and 10 % 2 β□-mercaptoethanol (*Merck,* Germany). The samples were boiled for 2 min in a water bath and added to the gel wells. The separation gel was prepared using a 30% mixture of acrylamide (*Sigma Chemical Co.*, USA) and bis-acrylamide (*Sigma Chemical Co.*, USA) (37.5:1), which was diluted to 12.5% with 1.5 M Tris- HCl buffer (pH=8.8) and water to a final Tris-HCl concentration of 375 mM. Sodium dodecyl sulfate up to 0.1%, ammonium persulfate (*Sigma Chemical Co.*, USA) up to 0.1%, and TEMED (tetramethylethylenediamine, *Acros,* Belgium) up to 0.1% were also added to the mixture. To prepare a concentration gel, a 30% mixture of acrylamide and bis-acrylamide was diluted to 5% with 1 M Tris-HCl buffer (pH=6.8) and water to a final Tris-HCl concentration of 125 mM. 0.1% sodium dodecyl sulfate, 0.1% ammonium persulfate and 0.1% TEMED were also added to the mixture. Glasses 8x10 cm with spacers 1 mm thick were used in the work. For electrophoresis used Tris-glycine electrode buffer containing 25 mm Tris-HCl, 192 mm glycine, 0.1% sodium dodecyl sulfate, pH=8.3. Electrophoresis was carried out at a constant current of 10 mA in the concentration mode and 15 mA in the separation mode. At the end of electrophoresis, the proteins were transferred to a *PVDF* membrane (*Amersham Pharmacia Biotech,* UK). The transfer was carried out by the semi-dry method for 2 hours at 200 mA, 20 V. The transfer quality was assessed by staining part of the gel and staining the membrane with 2% *Ponceau solution* (*Sigma Chemical Co.,* USA). The membranes were blocked for 12 hours at +40°C in Tris buffer medium (*Sigma Chemical Co.,* USA) containing 5% skimmed dry milk. Then the membranes were washed with Tris-buffered medium 3 times for 10 minutes and incubated for 2 hours at room temperature with primary antibodies (against GSK-3β or the phosphorylated form of GSK-3β) at a dilution of 1:1000 in Tris-buffered medium in a solution containing 0.5% bovine serum albumin *(Calbiochem)* and 0.01% *Tween* -20 *(Sigma Chemical Co.)*. The membranes were washed three times for 15 minutes in *TBS* containing 0.01% *Tween* -20 and incubated for 1 hour with secondary antibodies conjugated with horseradish peroxidase at a dilution of 1:10,000 in Tris buffer medium, in a solution containing 0.01% *Tween* -20. After the final washing to remove unbound antibodies, the bands were detected using the chemiluminescent substrate of horseradish peroxidase *ECL* (*Enhanced chemiluminescence system, Amersham Pharmacia Biotech,* Belgium). Chemiluminescence was detected on photographic film *Kodak Professional T-MAX P* 3200 *TMZ* 135-36 (*Kodak,* USA). The images were digitized on an *Epson Perfection V* 750 *Pro scanner* (*Seiko Epson Corp.,* Japan) and analyzed using the *ImageJ program*.

The content of the phosphorylated form of *ERK* 1/2 and *p* -38 was expressed in chemiluminescence units (CU).

*Statistica* 10.0 (*StatSoft, Inc.*) and *MedCalc* 12.5.0.0 (*MedCalc Software bvba*) programs were used for statistical analysis . The results are presented by the median with an interquartile interval. Intergroup differences in indicators were assessed using the Wilcoxon *W test for linked samples and were considered statistically significant at p* <0.05.

## RESULTS

As can be seen from Table. 1, the expression level of *CD11b* on the surface of intact neutrophils (healthy volunteers) was 3577.50 [3389.0–3889.0] fluorescence units (FU). Pro-inflammatory stimulation of isolated neutrophils by LPS at a dose of 200 ng/ml is statistically significant on average 4.5-fold - up to 16124.5 [15035.0–17343.0] FU ($p$ =0.005) increases the expression of *CD11b* on their surface.

*Table 1*

**The level of expression of CD11b on the surface of neutrophils upon exposure to lipopolysaccharides and their combination with xenon**

| Group | Expression of *CD11b* , FU, median [interquartile interval] | *p*, statistical significance relative to data on intact neutrophils (1st row) | *p*, statistical significance relative to data on incubation of neutrophils with LPS (2nd row) |
|---|---|---|---|
| Intact neutrophils | 3577.50 [3389.0 - 3889.0] | | |
| Incubation of neutrophils with LPS (200 ng/ml) | 16,124.5 _ [ 15,035.0–17,343.0 ] | 0.005 | |
| Xenon + LPS 200 ng/ml | 6475.0 [5388.0–7778.0] | 0.005 | 0.005 |

Note: LPS — lipopolysaccharides

Immediately after the end of xenon inhalation in healthy volunteers (30 vol.% for 60 minutes), neutrophils were isolated, in which pro-inflammatory stimulation (LPS - 200 ng/ml) was performed. The results of the studies showed that LPS stimulation statistically significantly increased the expression of *CD11b* on the surface of neutrophils by 1.8 times — 6475.0 [5388.0–7778.0] FU ($p$ = 0.005).

Thus, xenon inhalation at a dose of 30 vol.% for 60 minutes statistically significantly reduces the ability of neutrophils to pro-inflammatory activation by 2.5 times ($p = 0.005$).

As can be seen from Table. 2, the expression level of *CD66b* on the surface of intact neutrophils (healthy volunteers) was 10778.0 [10088.0–11555.0] FU. Pro-inflammatory stimulation of LPS at a dose of 200 ng/ml by 2 times, up to 21,475.0 [18,115.0–24,156.0] FU ($p$ =0.005), increases the expression of *CD66b* on the surface of neutrophils.

*Table 2*

**The level of expression of CD66b on the surface of neutrophils upon exposure to lipopolysaccharides and their combination with xenon**

| Group | Expression of *CD66b*, FU median [interquartile interval] | *p*, statistical significance of differences relative to data on intact neutrophils (1st raw) | *p*, statistical significance of differences relative to data on incubation of neutrophils with LPS (2nd raw) |
|---|---|---|---|
| Intact neutrophils (control) | 10778.0 [10088.0 - 11555.0] | | |
| Incubation of neutrophils with LPS (200 ng/ml) | 21,475.0 [18,115.0–24,156.0] | 0.005 | |
| Xenon + LPS 200 ng/ml | 14,030.0 [11,988.0–14,976.0] | 0.028 | 0.013 |

Note: LPS — lipopolysaccharides

After inhalation of xenon 30 vol.% for 60 minutes of stimulation (LPS) at a dose of 200 ng/ml statistically significantly increases the expression of *CD66b* on the surface of neutrophils by 1.3 times ($p$ = 0.028) in comparison with the control.

Thus, xenon inhalation (30 vol.% for 60 minutes) statistically significantly more than 1.5 times ($p$ = 0.013) reduces the expression of *CD66b* on the surface of neutrophils in comparison with the group where intact neutrophils were incubated with LPS (200 ng/ml).

Table 3 shows the results of the effect of LPS 200 ng/ml on the level of apoptosis of human neutrophils 22 hours after isolation in the compared groups, quantitative assessment was carried out using annexin *V* and propidium iodide on a flow cytometer. The results of the study showed that spontaneous apoptosis of neutrophils 22 hours after isolation is 56.3%. The addition of LPS (200 ng/ml) to the neutrophil incubation medium statistically significantly reduces the number of neutrophils that underwent spontaneous apoptosis by 60% to 22.6% ($p$ = 0.028).

*Table 3*

**The level of apoptosis of neutrophils as a result of the action of lipopolysaccharides and their combination with xenon**

| Indicators | Volunteers | | | | | | Median [interquartile range] | *p*, statistical significance of differences in relation to control data (1st row) | *p*, statistical significance relative to data on incubation of neutrophils with LPS (2nd row) |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | | | |
| Intact neutrophils (control) | 55.8 | 59.0 | 54.9 | 55.3 | 56.8 | 57.1 | 56.3 [55.3–57.0] | | |
| Incubation of neutrophils with LPS, 200 ng/ml | 20.7 | 24.0 | 22.4 | 23.3 | 22.8 | 21.9 | 22.6 [21.9–23.3] | 0.028 | |
| Xenon+LPS 200 ng/ml | 38.4 | 41.5 | 42.3 | 40.7 | 41.2 | 42.1 | 41.35 [40.70–42.10] | 0.028 | 0.028 |

Notes: the statistical significance of the between-group differences is p=0.028. LPS — lipopolysaccharides

Neutrophils isolated immediately after xenon inhalation (30 vol.% for 60 minutes) almost 2-fold - up to 41.35% statistically significantly increase the ability of neutrophils to spontaneous apoptosis ($p$ = 0.028).

The results of the study showed that the addition of LPS to the neutrophil incubation medium significantly reduces spontaneous apoptosis of neutrophils and contributes to the formation of conditions for maintaining inflammation, while the use of xenon increases the ability of neutrophils to spontaneous apoptosis and, probably, will contribute to the resolution of inflammation.

Today, it is known that neutrophil activation is regulated by the so-called MAP kinase cascade, the essence of which is the phosphorylation of *ERK* 1/2 kinase and MAP kinase *p38,* which are rapidly activated in response to pro-inflammatory signals [19]. In the present study, the effect of LPS at a dose of 200 ng/ml and xenon (30% for 60 minutes) on the phosphorylation of kinases: *ERK* 1/2 and kinase *p* 38 in neutrophils of healthy volunteers was studied.

The results of the study showed that *ERK* 1/2 kinase in neutrophils is rapidly and statistically significantly activated (phosphorylated) in response to LPS stimulation 2-fold, up to 1,098,976 [ 998,767–1,196,343 ] CU. in comparison with the control 545 789 [515 878–587 977] CU ($p$ <0.05), xenon exposure is statistically significant 3-fold, up to 183,925 [ 155,937–225,521 ] CU ($p$ <0.05), reduces kinase phosphorylation — *ERK* 1/2 compared to control (incubation with volunteer serum), pro-inflammatory stimulation of LPS after exposure to xenon statistically insignificantly increases ERK1/2 phosphorylation 1.4-fold compared to control up to 780 905 [652 554–897 348] CU ($p$ >0.05). The data are presented in Fig. 2 and in Table 4.

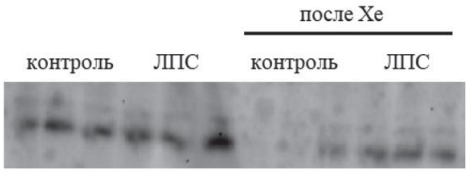


Fig. 2. Western blotting for phospho — ERK 1/2 content in isolated neutrophils after exposure to LPS at a dose of 200 ng/ml and after inhalation of xenon in healthy volunteers (30 vol.%) and exposure to LPS at a dose of 200 ng/ml on neutrophils after xenon inhalation (30%) (control — serum of healthy volunteers; LPS — lipopolysaccharide; after Xe-exposure (30%) for 60 minutes)

*Table 4*

**Densitometric analysis of Western blots for the content of phospho-ERK1/2 kinase in neutrophils after exposure to lipopolisharides, exposure to xenon and exposure to lipopolisharides after exposure to xenon, Me [LQ–HQ]**

| Group | The content of phosphorylated kinase *ERK* 1/2 (CU), median [interquartile interval] | % |
|---|---|---|
| Control (neutrophils when exposed to volunteer serum) | 545 789 [515 878–587 977] | one hundred |
| Volunteer neutrophils after exposure to LPS (200 ng/ml) | 1,098,976* [998 767–1 196 343] | 203 |
| Neutrophils of volunteers after inhalation of xenon 30 vol.% | 183 925 [155 937–225 521]* | 31 |
| Neutrophils of volunteers after inhalation of xenon 30 vol.% and exposure to LPS (200 ng/ml) | 780 905 [652 554–897 348] | 139 |

Notes: * — p<0.05 relative to control (exposure to serum from healthy donors). LPS — lipopolisharides

The study showed that the content of kinase — *p* 38 in intact neutrophils was 407 754 [400 343–476 699] c.u., and stimulation of intact neutrophils with LPS statistically significantly increased phosphorylation of kinase — *p* 38 by 5 times to 2 025 323 [ 1 999 765–2 300 332] CU ($p$ <0.05), xenon inhalation reduces kinase phosphorylation — *p38* in neutrophils isolated after anesthesia compared with control ($p$ <0.05). Pro-inflammatory stimulation of LPS after xenon does not statistically significantly increase phosphorylation — *p* 38 ($p$ >0.05). The data are presented in Fig. 3 and in Table 5.

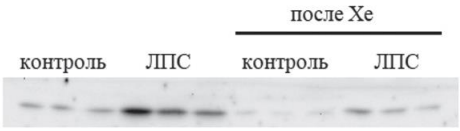


Fig. 3. Western immunoblotting of the content of phospho-p38 kinase in neutrophils after exposure to LPS at a dose of 200 ng/ml and exposure to xenon (30%) against the background of exposure to LPS at a dose of 200 ng/ml (control — serum of volunteers; LPS — lipopolysaccharide; after Xe-exposure (30%) for 60 minutes)

*Table 5*

**Densitometric analysis of Western blots for the content of phospho-p38 kinase in neutrophils after exposure to lipopolysaccharides, inhalation of xenon and exposure to lipopolysaccharides after xenon, Me [LQ–HQ]**

| Group | Content of phosphorylated kinase phosphorus- *p* 38 (CU), median [interquartile interval] | % |
|---|---|---|
| Control (neutrophils when exposed to volunteer serum) | 407 754 [400 343–476 699] | 100 |
| Neutrophils upon exposure to LPS (200 ng/ml) | 2025323* [1999 765–2,300,332] | 496 |
| Neutrophils after exposure to xenon 30 vol.% | 133 925 [121 954–171 452]* | 33 |
| Neutrophils after inhalation of xenon 30 vol.% and exposure to LPS (200 ng/ml) | 477 898 [421 222–499 543] | 117 |

Notes: * — p <0.05 relative to control (exposure to serum from healthy donors). LPS — lipopolysaccharides

Thus, the addition of LPS to the incubation medium for neutrophils LPS causes their pronounced activation, statistically significantly increasing the phosphorylation of the key pro-inflammatory neutrophil kinases *ERK* 1/2 and kinase *p* 38. Xenon inhalation in volunteers (30% for 60 minutes) has a pronounced anti-inflammatory effect on neutrophils stimulated by LPS, reducing their activation by inhibiting the pro-inflammatory kinases *ERK* 1/2 and MAP kinase *p* 38.

## DISCUSSION

Intractable inflammation accompanied by multiple cell death and multiple organ failure, imperfect immune responses, and increased susceptibility to infectious agents are the main causes underlying the high risk of death associated with critical illness. This leads to the search for new approaches for the pathogenetic impact on these causes. Today, there are no sufficiently effective methods for managing the hyperactivated state of the immune system. Therefore, one of the most important tasks of modern resuscitation is the search for drugs for the prevention and treatment of SIRS in order to prevent subsequent complications. Studies of the last decade have revealed that xenon, in addition to anesthetic properties, also has good organ-protective properties [18, 20, 21].

It is well known that inhalation halogen-containing anesthetics not only have the ability to favorably influence the oxygen balance of organs and tissues, but also have organ-protective properties [22]. We are talking about the specific effect of inhalation anesthetics on vital organs, increasing their resistance to ischemia, which is called anesthetic preconditioning (APC) [23, 24]. Discussed in the scientific literature and their anti-inflammatory properties [25]. At the same time, the molecular mechanisms of realization of the organ-protective properties of xenon remain unstudied, and their search in model experiments *in vivo* and *in vitro* seems quite justified. A recent *in vivo* study showed that a possible mechanism of the neuroprotective action of xenon is phosphorylation of the enzyme glycogen synthase-3β in the brain of experimental animals, which prevents the opening of the mitochondrial pore, inhibiting the apoptosis of neurons mediated by the death of mitochondria and increasing the level of antioxidant protection in them [26]. It is important to note that it was previously shown that inhaled halogen-containing anesthetics also affect the phosphorylation of glycogen synthase kinase- 3β, a key enzyme in the anti-ischemic defense of brain cells [27].

## CONCLUSION

The present study, performed on the isolated neutrophils of volunteers who underwent xenon inhalation, revealed its anti-inflammatory properties, which, in our opinion, may be directly related to the identification of the mechanism of its neuroprotective properties. It is well known that a brain lesion of ischemic or traumatic origin is characterized by a pronounced zone of perifocal inflammation, and limiting excessive inflammation in this zone may have a significant neuroprotective potential [28, 29]. Thus, the research results available to date suggest that xenon has a pronounced pleiotropic brain protection mechanism. This is a partial block of *NMDA* receptors, and phosphorylation of the enzyme glycogen synthase-3β, and limiting the inflammatory activation of neutrophils.

## FINDING

1. Xenon inhalation in volunteers (30% within 60 minutes) has a pronounced anti-inflammatory effect on neutrophils. Thus, the exposure of xenon is statistically significant 3-fold, up to 183,925 CU ($p$ <0.05), reduces kinase phosphorylation — ERK1/2 in comparison with the control — 545,789 CU (incubation with the serum of volunteers), pro-inflammatory stimulation of LPS after exposure to xenon statistically insignificantly increases ERK1/2 phosphorylation 1.4-fold in comparison with the control to 780 905 [652 554–897 348] CU ($p$ >0.05). Xenon inhalation statistically significantly reduces kinase phosphorylation — p38 in neutrophils isolated after anesthesia 3-fold compared to the control — from *407,754* CU to 133,925 ($p$ <0.05), and pro-inflammatory stimulation of neutrophils by LPS after xenon inhalation statistically insignificantly increases kinase phosphorylation — $p$ 38 ($p$ > 0.05). In addition, a decrease in the expression of activation and degranulation markers 2.5-fold was noted on the surface of neutrophils (group of incubation with LPS 16 124.5 FU, group xenon + lipopolysaccharides 6475.0 FU, $p$ = 0.005) and *CD66b* more than 1.5-fold (incubation group with LPS 21 475.0 FU, group xenon + lipopolysaccharides 14 030.0 FU, $p$ = 0.015).

2. Stimulation with lipopolysaccharides is accompanied by a statistically significant decrease in spontaneous neutrophil apoptosis by 60% (from 56.3% to 22.6%, $p$ = 0.028), while the use of xenon (30 vol.% for 60 minutes) leads to a statistically a significant increase in the ability of neutrophils to apoptosis 2-fold($p$ = 0.028), which, probably, can contribute to the resolution of inflammation.

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