

Effect of Hypothermia on the Expression of Brain Injury Marker Proteins in the Presence of Interleukin-6 and Anoxia *in vitro*

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Summary

Hypothermia has long been used in cardiac surgery for neuroprotection; however, its effectiveness is not clear-cut.

Objective of the study: to investigate the neuroprotective potential of hypothermia under conditions of hypoxia and interleukin-6, which simulates systemic inflammation *in vitro*.

Materials and methods. We established an *in vitro* model of a neurovascular unit (NVU) consisting of neurons, astrocytes, and endothelial cells. The NVU was cultured under anoxic conditions with the addition of serum containing either the maximum or minimum concentration of interleukin-6 (IL-6). Serum samples were obtained from a collection of serum samples from 78 pediatric patients aged 1 month to 6.5 years (13 [9–23] months) with body weights ranging from 3.3 to 21.5 kg (8.7 [6.9–11.0] kg), who underwent surgical correction of congenital septal heart defects under cardiopulmonary bypass. An intact NVU model was used as the control group. Cells were incubated for 15 minutes at different temperatures (20, 24, 28, and 37°C), followed by immunocytochemical assessment of the following NVU damage markers expression: claudin-5, occludin-1, and receptors for interleukin-1 (IL-1) and IL-6.

Results. We found that, during incubation under anoxic conditions, a decrease in the fluorescence intensity of claudin-5 occurred at all temperature conditions, and hypothermia had no protective effect. For occludin-1, a temperature of 24°C counteracted the effect of anoxia only when exposed to serum with the minimum IL-6 concentration ($p=0.442$). Exposure to serum with the maximum IL-6 concentration resulted in a significant decrease in the fluorescence intensity of this marker compared to the control group ($p=0.092$). Hypothermia at 20°C did not restore the expression levels of IL-1 receptors to those of the control group; however, it had a protective effect on the expression of IL-6 receptors and neutralized the effects of anoxia ($p=0.044$), but only in the absence of serum.

Conclusion. Exposure to serum containing varying concentrations of IL-6 results in a more pronounced effect on the expression of NVU damage markers than hypoxia. Hypothermia within the temperature range studied generally did not provide the expected protective effect: the expression of claudin-5, IL-1 and IL-6 receptors was not comparable to that of the control group, even without the addition of serum.

Keywords: hypothermia; cerebral injury; interleukin-6; anoxia; occludin-1; claudin-5; interleukin-1 receptors; neurovascular unit; NVU; septal congenital heart defects in children

Conflict of interest. The authors declare no conflict of interest.

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Introduction

The role of hypothermia in organ protection was first described by W.G. Bigelow et al. in 1950, following an experimental study in dogs: the authors induced cardiac arrest in the animals while cooling them to 20°C for 15 minutes, followed by the restoration of vital functions [1]. In cardiac surgery, hypothermia was first used by F.J. Lewis to correct an atrial septal defect in a child [2]. Thus, hypothermia has been used for organ protection in cardiac surgery for many years, but to date, its efficacy and safety

remain largely under-researched, especially with regard to its neuroprotective effects.

The effects of hypothermia on the neurovascular unit (NVU) — composed of neurons, astrocytes, and endothelial cells — are versatile and include the following:

- Decreased cerebral metabolism: Brain metabolic activity decreases by 5–7% for every 1°C drop in temperature. This leads to a reduction in oxygen and glucose consumption, the conservation of high-energy phosphates (ATP, ADP), and the maintenance

of ion gradients, which allows for an extension of the period during which the brain can tolerate complete ischemia without irreversible damage [3].

- **Suppression of excitotoxicity:** hypothermia directly inhibits the presynaptic release of excitatory neurotransmitters (glutamate, aspartate) and prevents calcium ion overload in neurons via NMDA receptors — a key link in the ischemic cascade — while also modulating postsynaptic receptors, thereby reducing the subsequent activation of lipases and endonucleases [4].

- **Modulation of apoptosis:** Hypothermia affects intrinsic apoptotic pathways by inhibiting caspase activation, the release of cytochrome C from mitochondria, and the expression of pro-apoptotic proteins, while simultaneously stimulating the synthesis of anti-apoptotic factors [5].

- **Suppression of the inflammatory response:** Hypothermia inhibits microglial activation and the release of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) as well as chemokines, thereby limiting neuroinflammation and damage to the blood-brain barrier (BBB) [6].

- **Reduction of reactive oxygen species (ROS) formation:** massive ROS formation occurs during reperfusion. Hypothermia reduces ROS generation and modulates the activity of antioxidant enzymes, such as superoxide dismutase, thereby reducing oxidative stress [7].

- **In astrocytes,** hypothermia reduces the activity of the astroglial glutamate transporter EAAT2, preventing its reverse transport and thereby inhibiting further glutamate release under excitotoxic conditions [8].

- **Hypothermia stabilizes the BBB** by reducing the expression of adhesion molecules on endothelial cells and inhibiting the activity of matrix metalloproteinases to limit leukocyte infiltration and cerebral edema [5].

Clinical and experimental studies on the efficacy of hypothermia. Studies have shown that deep hypothermia (18°C) significantly preserves high-energy phosphates and intracellular pH, delaying the onset of ischemic damage to the cerebral cortex, basal ganglia, and hippocampus compared to normothermia [9]. According to a systematic review with a meta-analysis, hypothermia not only preserves energy status but also significantly improves functional neurological outcomes and reduces the histopathological extent of brain damage following ischemia [10].

A separate issue is the effect of hypothermia on systemic inflammation. Given its significant role in cerebral injury, it is worth noting a clinical study involving pediatric patients, which found that the use of hypothermia at 27°C initially reduced the concentration of L-selectin, but after 30 minutes, its concentration was comparable to that in the group of subjects undergoing hypothermia at 33°C [11].

In one of the largest and longest-running randomized controlled trials, the Boston Circulatory Arrest Study, strategies involving deep hypothermia with circulatory arrest were compared with deep hypothermia with continuous cardiopulmonary bypass in children undergoing correction of transposition of the great arteries. The results showed that although the circulatory arrest strategy was technically much more convenient for the surgeon, it was associated with a higher risk of postoperative seizures and poorer long-term outcomes in terms of motor development and speech function compared with continuous low-volume perfusion [12]. This suggests that circulatory arrest itself, even under conditions of deep hypothermia, carries significant risks.

Current clinical practices have shifted away from elective hypothermic cardiopulmonary bypass in favor of continuous cerebral perfusion under conditions of moderate (25–32°C) or deep hypothermia. This approach has been shown to be associated with better neurological outcomes, a reduced incidence of postoperative seizures, and lower rates of postoperative cognitive dysfunction [13].

Some researchers even advocate abandoning hypothermia in favor of normothermia with continuous cerebral perfusion. For example, in a study by Caputo M. et al. [14], a comparison was conducted between two groups of children—one undergoing CPB under normothermic conditions and the other under hypothermia at 28°C — and it was found that the groups were comparable in terms of the severity of brain NVU injury, as well as the duration of their subsequent stay in the intensive care unit.

Another study with a similar design showed, however, that the hypothermia group had a significantly higher need for transfusions, as well as longer durations of mechanical ventilation and ICU stay [15].

Researchers at Texas Children's Hospital suggested that moderate hypothermia may be just as effective for neuroprotection during certain types of surgery, and with fewer side effects associated with deep hypothermia [16]. It demonstrates that the optimal temperature range remains a subject of debate.

The aim of this study was to investigate the neuroprotective potential of hypothermia under conditions of hypoxia and interleukin-6, which simulates systemic inflammation *in vitro*.

Materials and Methods

The clinical part of the study and the collection of patient serum samples were conducted at the Research Institute for Complex Problems of Cardiovascular Diseases (RI CPCD, Kemerovo, Russia) from 2018 to 2023. The experimental part of the study was conducted at the Krasnoyarsk State Medical University named after Professor V. F. Voino-Yasenetsky, Ministry of Health of Russia (KrasSMU). The study

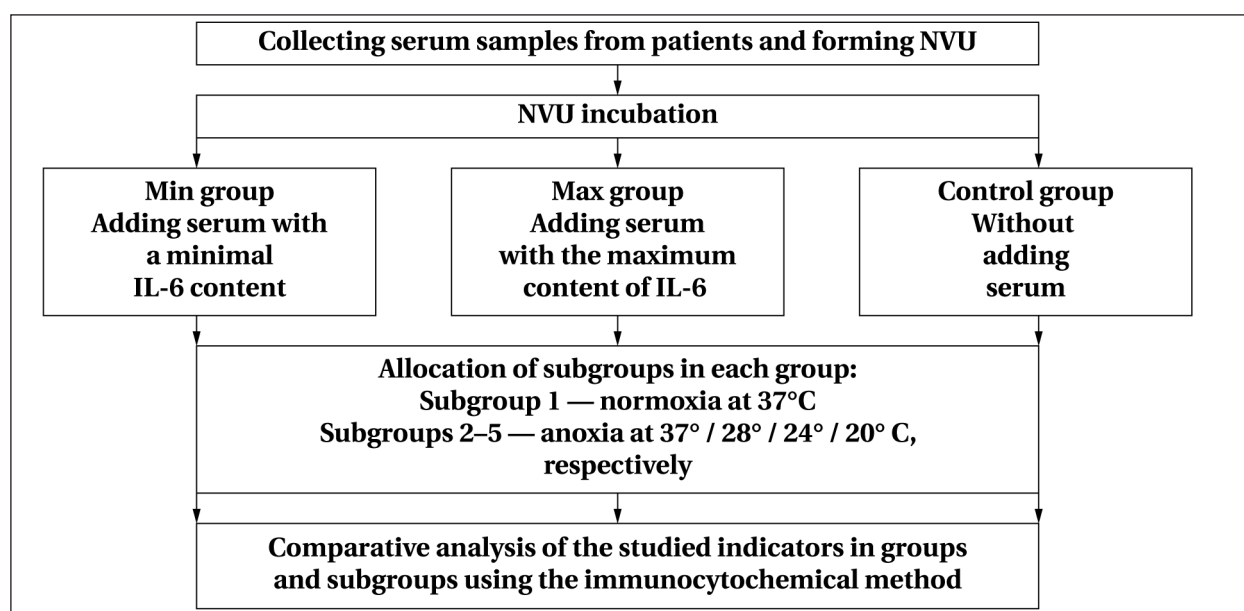


Fig. 1. Study scheme.

was approved by the local ethics committee of the RI CPCD (Protocol No. 20 dated November 20, 2018). The study design is presented in Fig. 1.

A cellular model of the neurovascular unit. A Transwell cellular model of the NVU was used, containing a semipermeable membrane insert 10 μm thick. The insert forms two chambers: an outer upper (luminal) chamber and an inner lower (ablu-minal) chamber, located between the insert and the bottom of the well. This design mimics the lumen of a blood vessel and allows for the study of the mechanisms of blood-brain barrier (BBB) permeability [17, 18]. The inner chamber contains cells that mimic brain tissue — astrocytes and neurons — which are located on the underside of the membrane and at the bottom of the well. A schematic representation of the Transwell model is shown in Fig. 2.

The establishment of the NVU cellular model involved four steps:

1. Isolation of cerebral endothelial cells.
2. Isolation and cultivation of neurospheres.
3. Isolation of astrocytes and neurons from the obtained neurospheres via directed differentiation into astrocytes and neurons.
4. Forming of the NVU model. To do this, a mixture of endothelial cells and neurons was placed at the bottom of the wells of a culture plate, followed by the insertion of culture inserts (Corning-Costar, USA), onto which astrocytes were placed. The cell mixture was cultured a medium consisting of DMEM with FBS, glutamine, and a mixture of antibiotics at 37°C with 5% CO_2 .

Isolation of primary brain cell cultures. We used primary cultures of brain endothelial cells, astrocytes, and neurons isolated from Wistar rats. The animals were housed in cages with free access

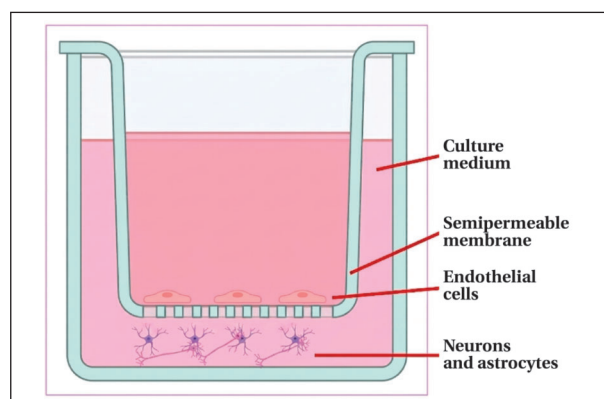


Fig. 2. Cellular model of the neurovascular unit (figure by the authors).

to food and water. The temperature was maintained at a constant $21 \pm 1^\circ\text{C}$. The light-dark cycle was 12 hours light/12 hours dark. Animal experiments were conducted in accordance with the principles of animal welfare set forth in the European Union Directive (2010/63/EU) [19]. The total number of animals was $n = 10$.

At the Krasnoyarsk State Medical University laboratory, a model of brain injury during cardiac surgery was developed based on the NVU cell model; this model can be cultured under various conditions that mimic factors of the intraoperative period.

Serum samples. The authors have a collection of serum samples from 78 pediatric patients — ranging in age from 1 month to 6.5 years (13 [9–23] months) with body weights ranging from 3.3 to 21.5 kg (8.7 [6.9–11.0] kg), who underwent surgical correction of congenital septal heart defects under cardiopulmonary bypass (CPB) at the RI CPCD.

The inclusion criteria for patients in the study were as follows: elective surgery for correction of a congenital heart defect (atrial or ventricular septal defect) under CPB, the presence of informed consent for participation in the study signed by the child's legal representative, and the child's age ranging from 1 month to 7 years; body weight ranging from 3 to 25 kg (the «Serum Samples» subsection provides actual data on the patients included in the study).

Exclusion criteria: emergency and urgent surgical procedures; the presence of clinically significant anemia, hypothermic CPB, or episodes of desaturation during the perioperative period; the presence of congenital heart defects other than atrial or ventricular septal defects, as well as combinations thereof; a history of central nervous system disorders; an implanted pacemaker; hemodynamic instability requiring preoperative pharmacological and/or mechanical support; any history of cerebrovascular events or such events in the perioperative period; the presence of severe comorbidities that impair mental and physical status; acute infection or exacerbation of a chronic infection in the perioperative period; concomitant autoimmune diseases; presence of malignant neoplasms; and surgical complications in the postoperative period.

The concentration of interleukin-6 (IL-6), one of the main markers of the systemic inflammatory response (SIR), was evaluated in each serum sample [20].

For the *in vitro* study, three serum samples each with the highest and lowest IL-6 concentrations were selected from the available collection, as they reflected the highest and lowest levels of SIR, respectively. The frozen serum was transported to KrasSMU under controlled temperature conditions.

Study groups. To determine the effects of IL-6 on the cellular model of NVU, serum mixed from three samples with the lowest IL-6 concentration or mixed from three samples with the highest IL-6 concentration was added to the culture medium.

Groups were formed based on the concentration of IL-6 in the serum.

1. «Control» group — intact NVU model.
2. «Minimum» (Min) group — addition of a mixture of serum samples with the lowest IL-6 content to the culture.
3. «Maximum» (Max) group — addition of a mixture of serum samples with the highest IL-6 content to the culture.

To determine the effects of hypothermia under anoxic conditions, the following subgroups were formed for each group:

1. Normoxia 37°: standard culture conditions, N₂ 75%, O₂ 20%, CO₂ 5%, t = 37°C.
2. Anoxia 37°: N₂ 95%, O₂ 0%, CO₂ 5%, t = 37°C.
3. Anoxia 28°: N₂ 95%, O₂ 0%, CO₂ 5%, t = 28°C.
4. Anoxia 24°: N₂ 95%, O₂ 0%, CO₂ 5%, t = 24°C.
5. Anoxia 20°: N₂ 95%, O₂ 0%, CO₂ 5%, t = 20°C.

Anoxia and hypothermia conditions were established in the HypoxyLab hypoxic chamber (Oxford Optronix, UK). Plates containing cell cultures were placed in the chamber, which had been preset to specific temperature and oxygen levels. The duration of exposure to anoxia and hypothermia was 15 minutes. Immediately after exposure, the cells were fixed with a 4% formaldehyde solution and subsequently analyzed by immunocytochemistry for marker molecules. The number of observations per group was 5 wells per group. At least 5 fields of view were analyzed in each well.

Immunocytochemical studies. To detect target marker molecules, we used a double indirect immunocytochemical staining method in accordance with the antibody manufacturer's protocol. Cells were fixed with 4% formaldehyde solution in PBS for 15 minutes. Permeabilization was performed with a 0.25% solution of Triton X-100 in PBS for 5 minutes at room temperature. Blocking of non-specific binding was performed in a 10% solution of BSA in PBS for 30 minutes at 37°C. Primary antibodies used included those against GFAP (ab4648, Abcam), NeuN (ABN78, Merck Millipore), VWF (LS-B4034, LSBio), Claudin-5 (AF0130, Affinity Biosciences), ZO1 (AF5145, Affinity Biosciences), Interleukin-1 Receptor Type I (PAA066Ra01, Cloud-Clone Corp), Interleukin-6 Receptor (DF2530, Affinity Biosciences), TNF Receptor I (AF0282, Affinity Biosciences), and TNF Receptor II (AF0364, Affinity Biosciences). The primary antibodies were used at a working dilution of 1:200. The incubation time with the primary antibodies was 18 hours at 4°C. FITC-labeled secondary antibodies (SAA544Rb58, Cloud-Clone Corp.) were used at a dilution of 1:400, with an incubation time of 2 hours at 37°C.

Cell microscopy was performed using a ZOE fluorescence microscope (Bio-Rad, USA). The fluorescence intensity of the markers under study was quantified using ImageJ software.

Statistical analysis. Data were analyzed statistically using BioStat Pro 5.9.8. Data in the text and tables are presented as the median (*Me*), lower (*Q1*), and upper (*Q3*) quartiles, since most of the data followed a distribution other than normal (Shapiro-Wilk test, $p < 0.05$). Quantitative variables were compared using the two-sided Mann-Whitney *U* test. Differences were considered statistically significant at $p < 0.05$. The Kruskal-Wallis test was used to identify significant differences among multiple independent groups. The Bonferroni correction was applied when comparing multiple groups or subgroups. The effect of different IL-6 concentrations on NVU — in two groups with serum added and the first control group without serum added — showed a *p*-value of 0.017. The effect of NVU incubation temperature for each group was assessed in four subgroups with anoxia and one subgroup with normoxia; the significance of the differences was 0.0125.

Results

Claudin-5 expression (Table 1). In the Control and Min groups under anoxic conditions, there was a statistically significant decrease in claudin-5 expression in all subgroups compared to normoxia ($p=0.003$). At temperatures below 37°C, a trend toward stabilization and an increase in claudin-5 expression was observed in these groups. However, the values did not reach those observed under normoxia ($p=0.011$).

In the Max group, temperature and oxygen concentration did not affect claudin-5 expression ($p=0.130$).

The decrease in claudin-5 expression compared to the control was more pronounced in the Max group than in the Min group, both under normoxia and anoxia ($p=0.011$). Thus, under normoxic conditions, the presence of serum in the culture medium led to a decrease in claudin-5 expression compared to the control: 2.5-fold in the Min group ($p=0.011$) and 9.6-fold in the Max group ($p=0.011$).

Occludin-1 Expression (Table 2). The second protein under investigation, which plays a role in maintaining the functional activity of the blood-

brain barrier, was the tight junction protein occludin-1. In the control group and the Max group, following anoxia exposure at all temperature conditions, no statistically significant change in occludin-1 expression was detected compared with normoxia ($p>0.0125$). Under normoxia, occludin-1 expression in the Min group decreased by a factor of 2.05 ($p=0.011$), and in the Max group by a factor of 6.6 ($p=0.011$). However, in the Min group, starting with hypothermia at 24°C, no statistically significant difference was observed between the normoxia and anoxia conditions ($p=0.442$).

Expression of interleukin-1 receptors (IL-1R) (Table 3). It was found that, in the control group, incubating cells under anoxic conditions at 37°C led to a statistically significant increase in IL-1R fluorescence intensity from 9.85 to 21.20 RU ($p=0.011$). As the temperature decreased, a trend toward a reduction in the negative effect of anoxia was observed. Starting at 24°C, this reduction was statistically significant ($p=0.011$).

Nevertheless, even at 20°C, the fluorescence intensity of IL-1R expression differed statistically significantly from that of the control group ($p=0.011$).

Table 1. Fluorescence intensity of claudin-5 expression.

Groups	Indicator values in subgroups					<i>p</i>				
	Normoxia 37°C	Anoxia				Normoxia vs Anoxia				
		37°C	28°C	24°C	20°C	vs 37°C vs 28°C vs 24°C vs 20°C	vs 37°C	vs 28°C	vs 24°C	vs 20°C
Control	34.25 [30.90–36.88]	9.20 [8.23–9.80]	9.20 [8.43–9.75]	11.20 [10.28–11.93]	17.55 [16.75–18.23]	0.003	0.011	0.011	0.011	0.011
Min	13.50 [11.18–15.80]	4.15 [3.95–4.58]	4.95 [4.68–5.45]	6.35 [5.68–7.10]	7.40 [7.20–7.75]	0.003	0.011	0.011	0.011	0.011
Max	3.55 [3.28–3.83]	2.30 [2.18–2.33]	2.15 [2.08–2.53]	2.25 [1.98–2.78]	2.50 [2.25–2.80]	0.130	—	—	—	—
Control vs Min vs Max	0.007	0.007	0.007	0.007	0.007					
Control vs Min	0.011	0.011	0.011	0.011	0.011					
Control vs Max	0.011	0.011	0.011	0.011	0.011					
Min vs Max	0.011	0.011	0.011	0.011	0.011					

Note. For Tables 1–4: All values are expressed in relative units (RU) of fluorescence; the significance level for differences within groups is 0.017; for the normoxia vs. anoxia subgroups, it is 0.0125.

Table 2. Fluorescence intensity of occludin-1 expression.

Groups	Indicator values in subgroups					<i>p</i>				
	Normoxia 37°C	Anoxia				Normoxia vs Anoxia				
		37°C	28°C	24°C	20°C	vs 37°C vs 28°C vs 24°C vs 20°C	vs 37°C	vs 28°C	vs 24°C	vs 20°C
Control	53.75 [51.60–56.85]	43.90 [40.98–48.38]	43.35 [42.98–45.05]	57.40 [56.70–58.38]	62.0 [59.28–64.48]	0.003	0.042	0.022	0.124	0.042
Min	26.25 [25.0–28.10]	16.65 [15.43–17.70]	18.05 [16.95–18.88]	26.90 [25.68–27.68]	29.15 [27.35–30.65]	0.007	0.011	0.011	0.442	0.193
Max	8.15 [7.83–8.58]	8.40 [7.98–8.83]	8.30 [8.13–8.78]	10.05 [9.28–10.83]	12.0 [11.68–12.58]	0.092	—	—	—	—
Control vs Min vs Max	0.007	0.018	0.018	0.018	0.018					
Control vs Min	0.011	0.011	0.011	0.011	0.011					
Control vs Max	0.011	0.011	0.011	0.011	0.011					
Min vs Max	0.011	0.011	0.011	0.011	0.011					

Table 3. Fluorescence intensity of interleukin-1 receptor expression.

Groups	Indicator values in subgroups					<i>p</i>				
	Normoxia 37°C	Anoxia				Normoxia vs Anoxia				
		37°C	28°C	24°C	20°C	vs 37°C vs 28°C vs 24°C vs 20°C	vs 37°C	vs 28°C	vs 24°C	vs 20°C
Control	9.85 [9.78–10.05]	21.20 [19.98–22.58]	18.25 [16.05–21.00]	15.70 [14.55–16.68]	13.95 [13.30–14.90]	0.004	0.011	0.011	0.011	0.011
Min	22.65 [21.65–23.68]	43.25 [42.20–44.33]	40.80 [38.45–43.18]	33.30 [38.45–43.18]	31.25 [29.60–33.73]	0.003	0.011	0.011	0.011	0.011
Max	27.55 [49.35–50.25]	49.35 [48.15–50.25]	44.25 [41.80–47.50]	40.70 [39.45–42.48]	38.65 [35.25–40.60]	0.006	0.011	0.011	0.011	0.022
Control vs Min vs Max	0.010	0.007	0.017	0.018	0.018					
Control vs Min	0.011	0.011	0.011	0.011	0.011					
Control vs Max	0.011	0.011	0.011	0.011	0.011					
Min vs Max	0.022	0.011	0.095	0.011	0.124					

Table 3. Fluorescence intensity of interleukin-1 receptor expression.

Groups	Indicator values in subgroups					<i>p</i>				
	Normoxia 37°C	Anoxia				Normoxia vs Anoxia				
		37°C	28°C	24°C	20°C	vs 37°C vs 28°C vs 24°C vs 20°C	vs 37°C	vs 28°C	vs 24°C	vs 20°C
Control	8.55 [7.85–8.98]	22.90 [22.33–24.68]	22.70 [20.68–24.15]	15.20 [13.98–16.75]	8.35 [7.33–9.15]	0.003	0.011	0.011	0.011	0.044
Min	8.80 [8.20–9.40]	30.85 [28.93–32.78]	33.95 [30.95–36.13]	27.15 [26.13–28.53]	20.85 [19.05–22.55]	0.003	0.011	0.011	0.011	0.011
Max	8.90 [8.25–10.10]	49.45 [45.55–51.78]	43.40 [41.63–46.00]	39.15 [36.05–41.65]	34.25 [32.60–35.63]	0.005	0.011	0.011	0.011	0.011
Control vs Min vs Max	0.668	0.013	0.007	0.010	0.007					
Control vs Min	—	0.042	0.011	0.011	0.011					
Control vs Max	—	0.011	0.011	0.011	0.011					
Min vs Max	—	0.011	0.011	0.022	0.011					

We also found that under normoxic conditions, the presence of blood serum in the culture medium led to a statistically significant increase in IL-1R expression ($p=0.011$), regardless of the concentration of IL-6 in the serum. Under hypothermic conditions at 20°C in the Max group, the differences in IL-1R expression between normoxic and hypoxic conditions were negated ($p=0.022$).

Expression of interleukin-6 receptors (IL-6R) (Table 4). It was found that in the Control group, under anoxic conditions at 37°C, 28°C, and 24°C, there was a significant increase in IL-6R expression compared with normoxia ($p=0.011$). At the same time, a trend toward decreased expression was observed as the temperature decreased. At 20°C, IL-6R expression did not differ from that under normoxia ($p=0.044$).

In the Min and Max groups under anoxic conditions, IL-6R expression was higher than under normoxia in all subgroups (37°C, 28°C, 24°C, and 20°C; $p=0.011$). Lowering the temperature to 20°C in both groups did not restore IL-6R expression levels to those observed under normoxic conditions ($p=0.011$).

Under normoxic conditions, the Control, Min, and Max groups did not differ significantly in terms of IL-6R expression levels ($p=0.668$). However, under

anoxic conditions, a statistically significant increase in IL-6R expression was observed compared to the Control group: in the Min group — starting at 28°C ($p=0.011$), and in the Max group — starting at 37°C ($p=0.011$). Starting at 28°C and below, the increase in IL-6R expression compared to normoxia was more pronounced in the Min and Max groups than in the Control group ($p\leq 0.010$).

Discussion

The observed decrease in the expression of claudin-5 and occludin-1 under anoxic conditions is fully consistent with existing data indicating that ischemia/hypoxia is a potent destabilizing factor for BBB integrity. It has been shown that, within just a few minutes after middle cerebral artery occlusion in rodents, there is a rapid decrease in the expression and redistribution of tight-junction proteins, leading to increased BBB permeability [21,22]. Similar findings have been obtained *in vitro*: oxygen-glucose deprivation in cultures of brain endothelial cells leads to the dissociation of occludin-1 and claudin-5 complexes from the cytoskeleton and their internalization [23]. Our results, demonstrating a decrease in the expression of these proteins during anoxia in the serum-free group and in the group with low IL-6 levels, confirm this widely ac-

cepted pathophysiological concept. It appears that, even despite the possible protective effects of hypothermia on stabilizing the BBB, these effects are insufficient to preserve it. However, this requires further study in more complex NVU models that simulate blood flow.

Of particular interest are the findings regarding the effect of patient serum on the NVU model. It was found that even serum with minimal IL-6 concentrations significantly reduced the expression of claudin-5 and occludin-1, while serum with high IL-6 levels had the most damaging effect, virtually eliminating the difference between the effects of anoxia and normoxia. This indicates that systemic inflammation, mediated by humoral factors in the blood, is an independent and potent damaging agent for the blood-brain barrier. This conclusion is supported by studies demonstrating that pro-inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, are capable of directly modulating the expression and organization of tight junction proteins. In particular, it has been shown that IL-6 can activate the JAK-STAT signaling pathway, leading to the suppression of claudin-5 gene transcription [24].

As our data show, the neuroprotective effect of hypothermia depends to a large extent on concomitant factors, in particular, the presence of concomitant systemic inflammation. The most pronounced protective effect of hypothermia in the experiment was observed in groups without added serum or with serum containing low levels of IL-6. An increase in claudin-5 expression was observed when the temperature was lowered to 20°C compared to anoxia at 37°C, but it was statistically significantly lower than the values in the control group; consequently, the effects of hypothermia proved insufficient for neuroprotection. With regard to IL-1R, hypothermia also demonstrated an insufficient protective effect, failing to reduce their anoxia-induced overexpression. Hypothermia significantly reduced IL-6R expression only at a temperature of 20°C.

However, a key limitation to the effectiveness of hypothermia, identified within the temperature range studied, is its inability to fully counteract the damaging effects of high concentrations of pro-inflammatory factors in serum. In the Max group, based on IL-6 levels, hypothermia had no significant effect on either claudin-5 expression or IL-6R expression. This suggests that the signaling cascades triggered by high cytokine concentrations (e. g., JAK-STAT, NF- κ B) are highly resistant to the modulatory effects of temperature reduction. Overcoming this resistance may require deeper or longer-lasting hypothermia, or combination therapy that includes, in addition to hypothermia, pharmacological inhibitors of pro-inflammatory pathways.

The limitations of the study are primarily related to the NVU model itself, which does not simulate blood flow. In addition, the limited level of hypothermia (20°C) may not have been sufficient to produce protective effects. However, in cardiac surgery practice, it is rare to cool a patient to lower temperatures [25, 26]. Furthermore, it is important to note that to simulate the effects of SIR, the researchers used not IL-6 itself, but blood serum from patients with known IL-6 levels. This may skew the results, as serum also contains many other factors that trigger systemic inflammation.

Conclusion

Exposure to serum containing various concentrations of IL-6 had a more pronounced effect on the expression of NVU damage markers than anoxia.

Hypothermia within the temperature range studied did not provide the expected protective effect: the expression levels of claudin-5 and receptors for IL-1 and IL-6 were not comparable to that of the control group, even without the addition of serum.

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