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The Impact of Interleukin-6 and Hypoxia on the Expression of Brain Injury Marker Proteins in a Cellular Model of the Neurovascular Unit

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Summary

The high incidence of postoperative cognitive dysfunction in children undergoing cardiac surgery underscores the urgent need for effective neuroprotective strategies.

Aim. To examine the effects of hypoxia and interleukin-6 (IL-6) on the expression of claudin-5, occludin-1, and interleukin-1 (IL-1) and IL-6 receptors in neurovascular unit (NVU) cells.

Materials and methods. An *in vitro* NVU model comprising neurons, astrocytes, and endothelial cells was established. The cells were cultured under normoxic and hypoxic conditions with oxygen concentrations of 15%, 10%, 7%, and 5%. The cultures were also treated with patient-derived sera containing high or low levels of IL-6. All incubations were conducted under normothermic conditions for 30 minutes. Injury marker expression was then assessed using fluorescence analysis.

Results. Significant reductions in claudin-5 fluorescence intensity were observed at oxygen levels of 10% and below (15.2 vs. 34.3 in controls, P=0.0105). Hypoxia did not affect occludin-1 expression. IL-1 receptor fluorescence intensity increased under 7% and 5% oxygen conditions (12.2 and 12.9 versus 9.9 in the control group, P=0.0105), while IL-6 receptor expression remained unchanged. In both normoxic and hypoxic conditions, adding patient sera significantly altered marker expression; hypoxia enhanced these effects. Sera with the highest IL-6 levels induced the most pronounced reduction in injury marker fluorescence.

Conclusion. IL-6 had a more significant impact on injury marker expression in NVU cells than hypoxia did. Hypoxic conditions with oxygen concentrations down to 10% did not affect marker expression.

Keywords: neurovascular unit, neurons, astrocytes, endothelial cells, hypoxia, interleukin-6, IL-1 receptor, claudin-5, occludin-1

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

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Introduction

Contemporary advances in cardiac surgery and anesthesiology have advanced to the point that surgical interventions must not only preserve vital functions, but also maintain patients' quality of life. Cognitive function is a critical component of quality of life and should be preserved at or near preoperative levels whenever possible. However, numerous studies have demonstrated that pediatric cardiac surgery is often associated with a high risk of postoperative cognitive dysfunction (POCD). Postoperative delirium (POD) occurs in 40–65% of cases. POD significantly prolongs the duration of stay in intensive care units due to increased requirements for mechanical ventilation, sedative administration, and other supportive measures. Consequently, hospital length of stay and overall mortality increase as well [6].

Moreover, the adverse impact of POD extends beyond its immediate effects. It has been shown to have long-term consequences that reduce cognitive performance in children for months following surgery. These long-term outcomes may result from POD itself or from cerebral injury sustained during the surgical procedure [7, 8]. Studies indicate that children experiencing POD are more likely to exhibit negative behavioral changes, such as difficulties with attention, emotional instability, and impaired social interactions. These disturbances can persist, adversely affecting the child's psychosocial well-being and overall quality of life [9]. These issues can lead to challenges in applying previously acquired

skills and difficulties in learning new information, which is particularly critical for preschool-aged children [10, 11]. Delirium in pediatric patients also leads to increased medical and financial burdens [12]. Given the high incidence of cognitive impairment following surgical correction of congenital heart defects in children, it is important to recognize that cardiac surgery involves numerous pathophysiological factors that can adversely affect the brain and contribute to cerebral injury.

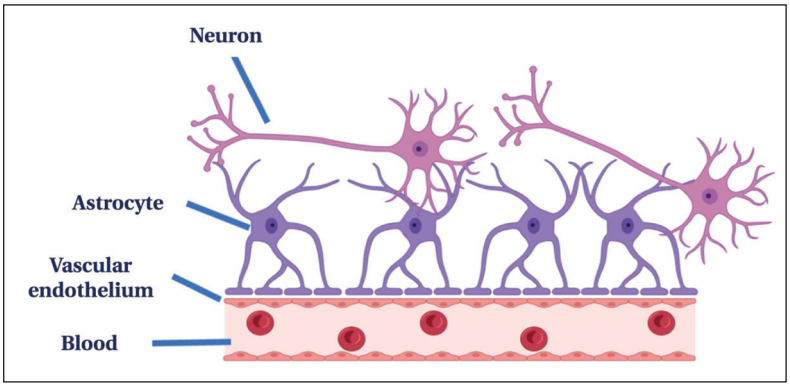

Fig. 1. Schematic representation of the neurovascular unit (illustration by the authors).

Contributing factors include the effects of anesthetic agents, which have been shown to induce neuronal apoptosis and disrupt synaptic plasticity — mechanisms that may ultimately lead to cognitive impairment. Additionally, episodes of hypoxia and hemodynamic instability, use of sympathomimetic drugs, and overall surgery duration must be considered — factors that are common, though not unique, to this type of intervention [13–15]. However, a distinctive feature of cardiac surgery is the frequent use of cardiopulmonary bypass (CPB). CPB-related phenomena that increase the risk of cerebral injury include microembolism, non-pulsatile blood flow, and periods of circulatory arrest. Hypothermia, which is routinely used in pediatric cardiac surgery for various reasons, may have both beneficial and harmful effects. The latter include altered blood rheology, microcirculatory disturbances, and the initiation of neuroinflammatory processes via the activation of cold-inducible RNA-binding protein (CIRBP) [16–18]. These factors require close monitoring and strict control to minimize the risk of brain injury.

The function of brain cells relies on the coordinated interaction of components within a structural complex that comprises blood vessels and astrocytes, forming the blood-brain barrier (BBB), as well as neurons (Fig. 1). This integrated system is referred to as the neurovascular unit (NVU). When exposed to direct damaging factors or systemic inflammation, the BBB's integrity is compromised, enabling systemic cytokines to enter the NVU. These cytokines exacerbate neuroinflammation and disrupt NVU function through multiple mechanisms. Notably, interleukin-1β (IL-1β) has been shown to impair glymphatic system function by interacting with astrocytes. This hinders the clearance of neurotoxic substances and amplifies the cycle of neuroinflammation [19–23].

Thus, interleukin-1β can serve as a marker of neuroinflammation. Two additional potential markers are claudin-5 and occludin-1, which are tight junction proteins of the blood–brain barrier. Their expression reflects the structural integrity of this interface. Numerous studies have demonstrated correlations between circulating levels of these proteins and clinical manifestations of brain injury. Furthermore, evidence suggests that claudin-5 and occludin-1 are directly involved in the neuroinflammatory process. This supports their potential utility in assessing the functional state of the NVU [24–26].

Based on the above, it can be concluded that brain injury results from a combination of direct damaging factors and indirect mechanisms mediated by systemic inflammation, which ultimately leads to the destruction of the NVU (see Fig. 2). In this context, strategies aimed at attenuating the systemic inflammatory response (SIR) in children undergoing surgical correction of congenital heart defects (CHDs) are of particular interest. One approach is to minimize the use of donor blood components during the intraoperative and postoperative periods. This concept is especially critical in pediatric cardiac surgery, where transfusions are often given ahead of time to prevent excessive hemodilution. This is due to the difference between the child's circulating blood volume and the volume needed to prime the cardiopulmonary bypass circuit [21].

However, studies have shown that donor blood components themselves may cause SIR [27, 28]. Despite this complexity, the use of donor blood products during CPB is not governed by standardized protocols, but rather depends on the anesthesiologist's or perfusionist's discretion.

Currently, a restrictive transfusion strategy is favored in pediatric practice due to the multitude of associated risks. These risks include infection, acute hemolytic reactions, metabolic complications such as hyperkalemia or hypocalcemia, allergic reactions, transfusion-associated circulatory overload (TACO), and transfusion-related acute lung injury (TRALI). Nevertheless, transfusions remain widely used in cardiac surgery due to concerns about complications from hemodilution during extracorporeal circulation [29–31].

This raises a critical, unresolved question: which has a more damaging effect on the NVU — hypoxia resulting from hemodilution or systemic inflammation triggered by exposure to donor blood components?

This experimental study aimed to determine the effects of hypoxia and interleukin-6 (IL-6) on the expression of claudin-5, occludin-1, and IL-1 and IL-6 receptors in neurovascular unit cells.

Materials and Methods

At Krasnoyarsk State Medical University (KrasSMU), a brain injury model associated with cardiac surgery was developed using an NVU cellular model cultured under intraoperative conditions. The experimental study was conducted at KrasSMU in the following steps:

1. Retrieval of primary brain cell cultures *in vitro*.

The procedure for obtaining primary brain cell cultures and forming experimental groups has been described in detail in a previously published study [32]. The concentration of interleukin-6 (IL-6) in patient serum was used as an indicator of systemic inflammation intensity.

2. Experimental Design and Group Allocation.

To evaluate the impact of IL-6 on the NVU cellular model *in vitro*, serum samples containing either minimal or maximal IL-6 concentrations were added to the culture medium. This approach allowed for the simulation of systemic inflammation of varying severity.

The experimental groups were classified based on IL-6 concentration as follows:

1. Control group: intact NVU model without serum exposure.
2. The minimum IL-6 group consisted of cultures treated with serum containing the lowest IL-6 levels.
3. The maximum IL-6 group consisted of cultures treated with serum containing the highest IL-6 levels.

Additional groups were formed according to hypoxic incubation conditions:

1. Control: standard culture conditions: N_2 75%, O_2 20%, CO_2 5%.
2. Hypoxia 1: N_2 80%, O_2 15%, CO_2 5%.
3. Hypoxia 2: N_2 85%, O_2 10%, CO_2 5%.
4. Hypoxia 3: N_2 88%, O_2 7%, CO_2 5%.
5. Hypoxia 4: N_2 90%, O_2 5%, CO_2 5%.

Incubation was performed at 37°C for 30 minutes.

Each experimental condition was replicated five times.

At this stage of the study, we evaluated the effects of sera with high and low IL-6 concentrations, as well as varying oxygen conditions, on the following parameters:

- expression of tight junction proteins (claudin-5 and ZO-1);
- expression of interleukin 1 and 6 receptors;
- cytokine levels (IL-1 and IL-6) in the culture medium.

3. Immunocytochemical Analysis.

A double indirect immunocytochemical staining method was employed to detect target molecular markers, following the manufacturer's protocol for each antibody. The following primary antibodies were used: Claudin-5 (AF0130, Affinity Biosciences); Occludin-1 (ZO1) (AF5145, Affinity Biosciences); Interleukin-1 Receptor Type I (PAA066Ra01, Cloud-Clone Corp.); and Interleukin-6 Receptor (DF2530, Affinity Biosciences). The detailed staining procedure has been previously described in an earlier study of ours [32].

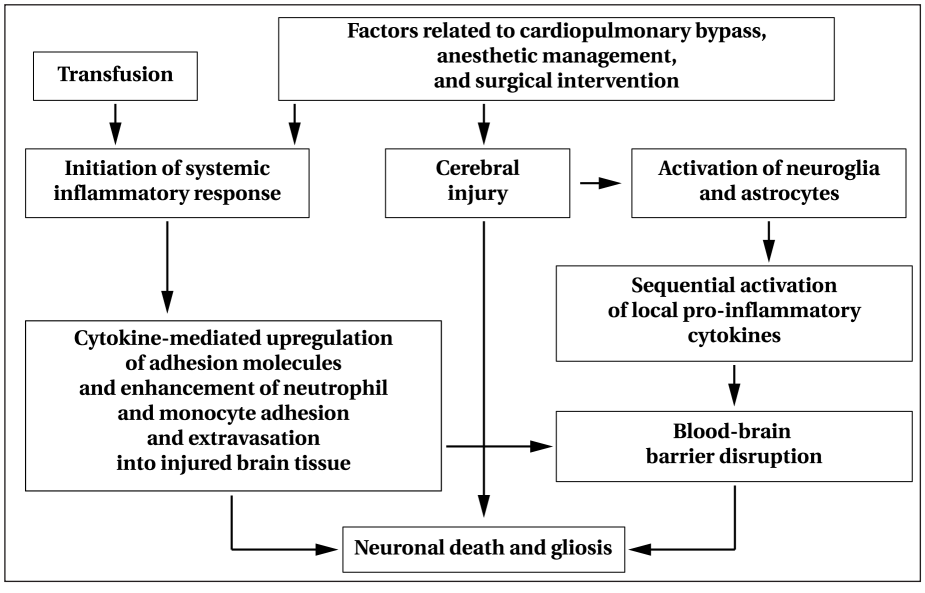

Fig. 2. Schematic representation of the effects of pathological factors on the neurovascular unit during cardiac surgery (illustration by the authors).

Statistical Analysis

Data were statistically analyzed using BioStat Pro 5.9.8. Since most variables did not follow a normal distribution (as determined by the Shapiro–Wilk test, $P<0.05$), the results are presented as medians *(Me)* with lower *(Q1)* and upper *(Q3)* quartiles. Quantitative variables were compared using the one-tailed Mann–Whitney *U* test. Differences were considered statistically significant at $P<0.05$.

To compare multiple independent groups, the

Kruskal–Wallis test was used. For pairwise group comparisons, the Mann–Whitney *U* test was used with Bonferroni correction. When evaluating the effects of hypoxia on the NVU, four experimental groups were compared to one control group. This resulted in a Bonferroni-adjusted significance threshold of $P<0.0125$. When assessing the effects of IL-6, comparisons were made between the maximum and minimum IL-6 groups with a Bonferroni-adjusted significance level of $P<0.017$.

Results and Discussion

Exposure to hypoxic conditions decreased claudin-5 fluorescence intensity from 34.3 arbitrary units (a. u.) to 27.0 a. u. This reduction was statistically significant in the 10%, 7%, and 5% oxygen groups (Table 1).

Exposure to hypoxia at an oxygen concentration of 15% did not result in a statistically significant reduction in claudin-5 expression. However, the combined effect of hypoxia and IL-6 decreased fluorescence intensity in the subgroup exposed to serum with minimal IL-6 level (P=0.0043). Pairwise comparisons showed that claudin-5 fluorescence intensity was significantly lower in the Hypoxia 2, Hypoxia 3, and Hypoxia 4 groups compared to the normoxic control group (P=0.0105 for each comparison).

The most pronounced decrease in claudin-5 expression was notably observed in the Maximum IL-6 group, even under normoxic conditions (P=0.0105). Further reductions of fluorescence intensity under hypoxic conditions were not observed in this group (P=0.0734). In contrast, under minimal IL-6 exposure, a progressive decrease in fluorescence intensity was noted with increasing hypoxia severity (P=0.043).

An important comparison is the difference in claudin-5 fluorescence intensity between groups exposed to minimal or maximal IL-6 concentrations and a group exposed to 5% oxygen. Statistical analysis revealed no significant difference between the minimal IL-6 group and the 5% hypoxia group (13.0 [11.5–13.6] a. u. vs. 13.5 [11.2–15.8] a. u., P=0.5637). However, the high IL-6 group had significantly lower fluorescence intensity than the hypoxia group (3.6 [3.3–3.8] a. u. vs. 13.0 [11.5–13.6] a. u., P=0.0105).

These findings suggest that severe hypoxia (5% O_2) has a similar effect on claudin-5 expression as minimal systemic inflammation (low IL-6). However, elevated IL-6 levels—and, consequently, a heightened systemic inflammatory response—had a more profound impact on the NVU than hypoxia alone.

Thus, IL-6 had a greater effect on claudin-5 expression than hypoxia. Higher IL-6 concentrations led to maximal suppression of claudin-5 expression, as assessed by fluorescence intensity. Furthermore, hypoxia appeared to potentiate the effects of IL-6, even at low cytokine levels.

Table 1. Claudin-5 fluorescence intensity.

Group	Values in different O_2 levels					*P* value				
	Normoxia (20% O_2)	15% O_2	10% O_2	7% O_2	5% O_2	15% vs 10% vs 7% vs 5%	Control vs 15%	Control vs 10%	Control vs 7%	Control vs 5%
Control	34.3 [30.9–36.9]	27.0 [25.1–28.7]	15.2 [13.5–16.9]	10.6 [8.5–12.5]	13.0 [11.5–13.6]	0.0029	0.0416	0.0105	0.0105	0.0105
Minimum IL-6	13.5 [11.2–15.8]	10.7 [10.0–11.6]	8.6 [8.0–9.0]	7.4 [6.4–8.3]	6.9 [6.4–7.4]	0.0043	0.0956	0.0105	0.0105	0.0105
Maximum IL-6	3.6 [3.3–3.8]	4.7 [3.9–4.9]	2.5 [2.1–2.8]	3.8 [3.7–4.2]	3.6 [3.2–3.7]	0.0734	—	—	—	—
Control vs Minimum vs Maximum, *P*	0.0073	0.0073	0.0073	0.0125	0.0073					
Control vs Minimum, *P*	0.0105	0.0105	0.0105	0.0105	0.0105					
Control vs Maximum, *P*	0.0105	0.0105	0.0105	0.0105	0.0105					
Minimum vs Maximum, *P*	0.0105	0.0105	0.0105	0.0105	0.0105					

Note. Here and in tables 2–4: All values are expressed in arbitrary fluorescence units (a. u.).

Table 2. Occludin-1 fluorescence intensit.

Group	Values in different O_2 levels					*P* value			
	Normoxia (20% O_2)	15% O_2	10% O_2	7% O_2	5% O_2	15% vs 10% vs 7% vs 5%	Control vs 15%	Control vs 10%	Control vs 7%	Control vs 5%
Control	53.8 [51.6–56.9]	58.7 [56.4–60.4]	54.7 [53.2–58.2]	60.6 [58.8–62.0]	62.2 [61.0–64.0]	0.2092	—	—	—	—
Minimum IL-6	26.3 [25.0–28.1]	26.2 [24.9–26.8]	22.1 [21.6–23.4]	19.8 [18.9–20.4]	20.4 [19.4–21.2]	0.0106	0.3864	0.0416	0.0105	0.0105
Maximum IL-6	8.2 [7.8–8.6]	9.1 [8.3–9.3]	8.0 [7.7–8.5]	11.0 [10.3–11.6]	12.3 [11.3–12.9]	0.0065	0.1932	0.4423	0.0105	0.0105
Control vs Minimum vs Maximum, *P*	0.0073	0.0073	0.0073	0.0073	0.0073					
Control vs Minimum, *P*	0.0105	0.0105	0.0105	0.0105	0.0105					
Control vs Maximum, *P*	0.0105	0.0105	0.0105	0.0072	0.0105					
Minimum vs Maximum, *P*	0.0105	0.0105	0.0105	0.0105	0.0105					

The second protein involved in maintaining the functional activity of the blood-brain barrier is the tight junction protein occludin-1.

Hypoxic exposure did not cause a statistically significant change in the fluorescence intensity of occludin-1, which ranged from 58.7 to 62.2 arbitrary units (*P*=0.2092). Only a slight trend toward increased fluorescence intensity was observed (Table 2).

The presence of patient serum in the culture medium led to a significant decrease in occludin-1 fluorescence intensity in both the low IL-6 (*P*=0.0151) and high IL-6 (*P*=0.0105) groups.

Under hypoxic conditions, a further reduction in fluorescence intensity was observed in only two groups: the Hypoxia 3 group (*P*=0.0105) and the Hypoxia 4 group, which contained serum with both low and high IL-6 levels (*P*=0.0105).

These findings suggest that IL-6 significantly affects occludin-1 at all tested concentrations. Furthermore, the effect of IL-6 was concentration-dependent; higher IL-6 levels were associated with lower occludin-1 fluorescence intensity.

In contrast, hypoxia alone did not have a statistically significant effect on occludin-1 expression.

The next set of experiments focused on assessing the expression of the interleukin-1 receptor (IL-1R).

Under hypoxic conditions and in the absence of IL-6 in the culture medium, changes in IL-1 receptor (IL-1R) fluorescence intensity were observed in the Hypoxia 3 and Hypoxia 4 groups (oxygen concentrations of 7 and 5%, respectively). Increases were 23% (*P*=0.0105) and 30% (*P*=0.0105), respectively (Table 3).

Under normoxic conditions, low IL-6 concentrations led to a more than twofold increase in IL-1R fluorescence intensity (from 9.9 to 22.7 a. u.; *P*=0.0105), whereas high IL-6 concentrations caused a 2.8-fold increase, up to 27.6 a. u. compared to 9.9 a. u. in the control group (*P*=0.0105).

As hypoxia deepened, IL-1R fluorescence intensity increased progressively. The most pronounced changes were observed in the Maximum IL-6 subgroup under severe hypoxia (Hypoxia 4), with a fourfold increase compared to intact cells cultured in normoxia (*P*=0.0105). In the Minimum subgroup, a threefold increase in IL-1R fluorescence intensity was observed (*P*=0.0105).

In the group with minimal IL-6, hypoxia at 15% oxygen did not yet have a statistically significant effect on IL-1R fluorescence. Significant differences only emerged in groups with oxygen concentrations of 10% or lower.

Thus, it was found that hypoxia only modestly affected IL-1R fluorescence intensity, with changes remaining within a 20% range. In contrast, the presence of IL-6 in the culture medium resulted in more significant alterations than hypoxic exposure

Table 3. Expression levels of interleukin-1 receptors (IL-1R).

Group	Values in different O_2 levels					*P* value			
	Normoxia (20% O_2)	15% O_2	10% O_2	7% O_2	5% O_2	15% vs 10% vs 7% vs 5%	Control vs 15%	Control vs 10%	Control vs 7%	Control vs 5%
Control	9,9 [9,8–10,1]	10,1 [9,8–10,4]	10,6 [10,3–10,9]	12,2 [11,7–12,7]	12,9 [12,1–13,3]	0,0117	0,2819	0,0956	0,0105	0,0217
Minimum IL-6	22,7 [21,7–23,7]	22,0 [21,3–22,4]	28,8 [27,7–29,7]	32,6 [31,3–34,1]	33,1 [32,3–33,8]	0,0027	0,2339	0,0105	0,0105	0,0105
Maximum IL-6	27,6 [26,6–29,0]	32,2 [31,0–34,4]	36,0 [33,6–37,8]	38,3 [34,9–41,0]	39,8 [38,5–40,7]	0,0218	0,0416	0,0217	0,0217	0,0105
Control vs Minimum vs Maximum, *P*	0,0097	0,0073	0,0073	0,0073	0,0073					
Control vs Minimum, *P*	0,0105	0,0105	0,0105	0,0105	0,0105					
Control vs Maximum, *P*	0,0105	0,0105	0,0105	0,0105	0,0105					
Minimum vs Maximum, *P*	0,0217	0,0105	0,0105	0,0745	0,0105					

Table 4. Expression levels of interleukin-6 receptors (IL-6R).

Group	Values in different O_2 levels					*P* value			
	Normoxia (20% O_2)	15% O_2	10% O_2	7% O_2	5% O_2	15% vs 10% vs 7% vs 5%	Control vs 15%	Control vs 10%	Control vs 7%	Control vs 5%
Control	8.6 [7.9–9.0]	8.0 [7.5–8.5]	8.5 [8.3–8.6]	8.6 [8.4–8.8]	8.9 [8.6–9.2]	0.0552	—	—	—	—
Minimum IL-6	8.8 [8.2–9.4]	10.9 [10.3–12.3]	16.0 [14.1–17.8]	21.1 [17.7–24.1]	20.7 [19.7–22.2]	0.0034	0.0217	0.0105	0.0105	0.0105
Maximum IL-6	8.9 [8.3–10.1]	16.2 [15.0–17.0]	19.4 [17.1–22.3]	33.6 [30.6–36.0]	41.3 [39.2–44.4]	0.0014	0.0105	0.0105	0.0105	0.0105
Control vs Minimum vs Maximum, *P*	0.6678	0.0097	0.0169	0.0073	0.0073					
Control vs Minimum, *P*	—	0.0105	0.0105	0.0105	0.0105					
Control vs Maximum, *P*	—	0.0105	0.0105	0.0105	0.0105					
Minimum vs Maximum, *P*	—	0.0217	0.0956	0.0105	0.0105					

alone. At the same time, the study observed a potentiating effect of hypoxia on IL-6-mediated IL-1R responses.

Under hypoxic conditions without IL-6 in the culture medium, no statistically significant changes in IL-6 receptor (IL-6R) fluorescence intensity were detected (*P*=0.5152) (Table 4).

Even under intensified hypoxic conditions, with an ambient oxygen concentration as low as 5%, no statistically significant changes in fluorescence intensity were observed compared to normoxic conditions.

When patient-derived serum containing IL-6 was added to the culture under normoxia, no statistically significant changes in fluorescence intensity were observed, either in the group with minimal IL-6 (*P*=0.2819) or in the group with maximal IL-6 levels (*P*=0.1933).

In the group with minimal IL-6, exposure to increasing hypoxia led to significant elevations in IL-6R fluorescence intensity at 10% (*P*=0.0105), 7% (*P*=0.0105), and 5% (*P*=0.0105) oxygen, relative to normoxic conditions.

In groups with high IL-6 levels, a progressive increase in IL-6R fluorescence intensity was also observed with greater degrees of hypoxia. This effect became statistically significant at 15% oxygen (*P*=0.0105) and continued to increase proportionally as oxygen levels decreased.

Similar to the IL-1R response, the highest fluorescence values were detected in the group with maximal IL-6 under 5% oxygen, where IL-6R expression was 4.8 times higher than in the control group (*P*=0.0105).

These findings demonstrate that the presence of IL-6 in the culture medium promotes increased IL-6R expression as measured by fluorescence intensity and that the effects of IL-6 are amplified under hypoxic conditions. The observed changes closely mirrored the combined effects of hypoxia and IL-6 on IL-6R.

All experiments demonstrated that the cellular NVU model was less impacted by hypoxia than by IL-6. However, increasing degrees of hypoxia progressively decreased fluorescence intensity across all investigated markers.

These results imply that strategies to avoid the use of donor blood components during pediatric cardiac surgery could be effective. Although restrictive transfusion strategy carries the risk of hemic hypoxia,

transfusion itself significantly enhances the SIRS and subsequent neuroinflammation, as previously described by our group [27, 28].

These results are consistent with those of other studies examining the impact of transfusion on the NVU in children. These studies have shown an association between transfusion and an increased risk of postoperative delirium and cognitive dysfunction [33, 34].

Furthermore, a previous clinical study demonstrated that surgical repair of septal CHD using cardiopulmonary bypass can be safely performed without transfusion in children weighing over 8 kg without preoperative anemia [35]. The study also found that children who did not receive donor blood components had lower levels of SIRS and cerebral injury.

Several limitations of the present study must be acknowledged.

First, the evaluation of only a limited number of experimental groups with varying oxygen concentrations may have reduced the statistical power to detect the robust effects of hypoxia on the NVU model.

Second, the study used an imperfect in vitro model. Several types of NVU models are currently available. The simplest consists of a monoculture of endothelial cells [36]. A more advanced approach is the Transwell model, which uses a semipermeable membrane insert to simulate the vascular lumen. This enables the study of BBB permeability and modeling of brain diseases [37]. This model is currently the most widely used and was applied in our study.

More complex systems include three-dimensional (3D) matrix-based BBB models, which incorporate extracellular matrix scaffolds [38]. These models represent an early step toward spatially accurate models that better simulate cellular interactions. However, these models face challenges, including limited nutrient delivery to the matrix, the absence of blood flow, and the poor reproducibility of matrix composition. These issues restrict their widespread use.

A more recent innovation involves spheroid-based models [39]. These models are formed via co-culture of endothelial cells, astrocytes, and pericytes. This results in self-organizing spheroids in which endothelial cells form the outer layer, astrocytes form the core, and pericytes reside in the intermediate zone. Spheroid models exhibit higher expression of tight and adherens junction proteins (ZO-1, occludin, and claudin-5). They also offer ease of handling, reproducibility, and long viability — up to 21 days. Their limitations include an insufficient nutrient supply to the core of the spheroid and an inability to measure transendothelial electrical resistance (TEER).

The most advanced and promising approach is the use of microfluidic models [40]. These models have a 3D architecture and a defining feature: continuous laminar flow through microchannels. This allows for automated nutrient delivery, reduces the risk of contamination, and induces shear stress that promotes cell morphology resembling in vivo conditions. However, these systems have limitations, such as the difficulty of measuring TEER and the need for expensive, specialized equipment.

Using spheroid or microfluidic models may have provided more detailed insights into the effects of hypoxia and systemic inflammation on the NVU. However, the current lack of materials and technical infrastructure prevents their wide implementation.

Conclusion

Using an in vitro NVU model, this study demonstrated that hypoxia had a smaller impact on the expression of cerebral injury markers than IL-6 concentration.

Claudin-5 expression increased significantly only under hypoxic conditions with 10% oxygen or less. Occludin-1 expression did not respond to changes in oxygen levels, similar to IL-1 receptor expression. In contrast, IL-6 receptor expression significantly changed under more severe hypoxia, specifically at 7% and 5% oxygen.

Adding patient serum to the NVU culture induced greater changes in fluorescence intensity than hypoxia alone and potentiated its effects. Notably, the negative impact increased proportionally with higher IL-6 concentrations in the added serum.

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