

IMPROVEMENT OF BLOOD-BRAIN BARRIER PROPERTIES BY A COMBINATION OF SMALL MOLECULES HAS A PROTECTIVE EFFECT IN A CELL CULTURE MODEL OF ISCHEMIC STROKE



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HUN-REN
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Introduction

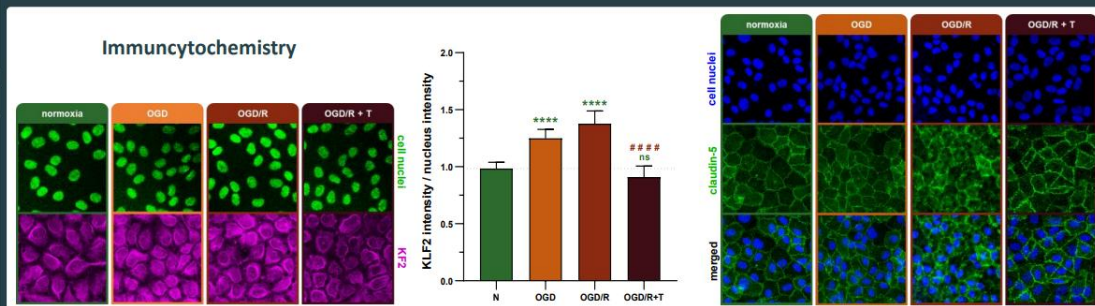
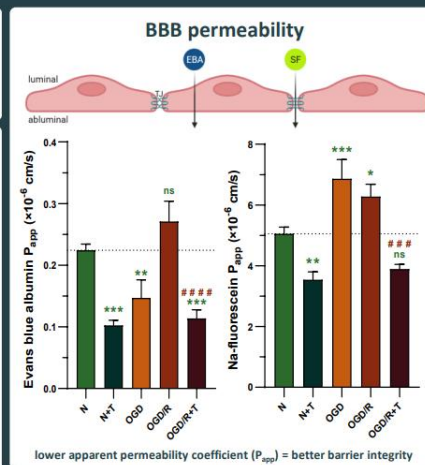
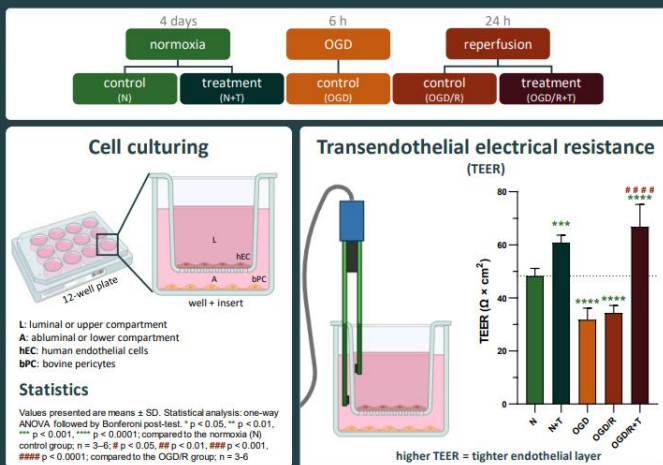
During an ischemic event, blood flow interruption and oxygen-glucose deprivation (OGD) cause blood-brain barrier (BBB) disruption and neuronal death. Our aim was to protect BBB by targeting three signalling pathways with a combination of small molecules improving barrier functions and increasing the expression of Krüppel-like factor 2 (KLF2), the main transcriptional regulator of blood flow response. We cultured human endothelial cells under different conditions: normoxia, OGD and reperfusion after OGD (OGD/R) and then tested the effect of our small molecule combination against BBB dysfunctions.

Methods

- transendothelial electrical resistance (TEER) values reflect the tightness of the endothelial cell layer
- permeability reflects the passage of transcellular (Evans blue albumin) and paracellular (Na-fluorescein) markers through the endothelial cell layer, measured by spectrofluorimeter
- immunostaining of KLF2 and tight junction protein claudin-5

Results and conclusion

OGD disrupts BBB integrity by decreasing TEER and increasing fluorescein permeability, but treatment with small molecules has the opposite, protective effect both in normoxia and OGD/R. However, immunostaining showed that these protective effects were not linked to KLF2, but tightening the BBB by increasing claudin-5 levels with a combination of small molecules may have a protective effect in ischemic stroke.



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CERTIFICATE OF ATTENDANCE

This certificate is presented to

Koppány Párdi

István Hernádi
President of the Hungarian
Neuroscience Society

Szilárd Szócs
Organizer

Barbara Fülöp
Organizer

Gergely Szarka
Organizer



For attending the 7th Hungarian
Neuroscience Doctoral Conference
for Undergraduate Students,
Graduate Students and Junior Post-
Docs (HUNDOC)

24th of January 2024

International Conference

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Certificate id 4503568997

Introduction

Our research focuses on the protection of the blood-brain barrier (BBB), as a therapeutic target after ischemia. The major transcriptional regulator of blood flow responses of microvascular endothelial cells is Krüppel-like factor 2 (KLF2). Laminar shear stress has been shown to promote endothelial survival and to have antithrombotic and anti-inflammatory effects in cardiovascular diseases. SAHA, a clinically used drug is able to enhance KLF2 expression and has good BBB permeability. The aim of the study was to investigate whether SAHA has a protective effect on a human cell culture model of the BBB against dysfunctions caused by oxygen-glucose deprivation and reperfusion (OGD/R).

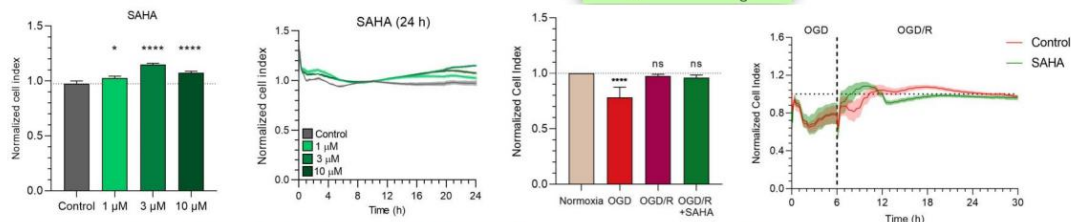
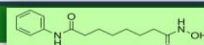
Methods

- Cellular toxicity: impedance measurement
- BBB integrity: monitored by measuring transendothelial electrical resistance (TEER)
- Permeability: penetration of BBB marker molecules measured by fluorescent spectrophotometry
- Immunocytochemistry of KLF2: visualized by confocal microscope

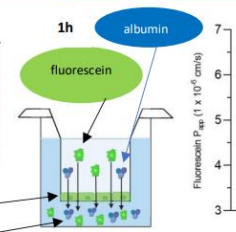
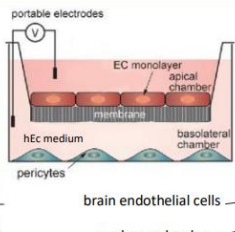
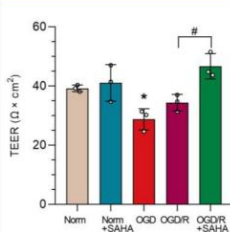
Conclusion

In conclusion, SAHA was able to prevent the BBB damage after ischemic stroke but the cellular mechanism underlying the protective effect does not seem to be related to KLF2.

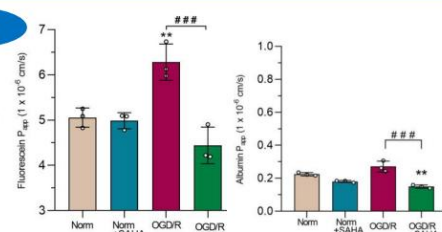
Results: effect of SAHA on the viability of endothelial cells



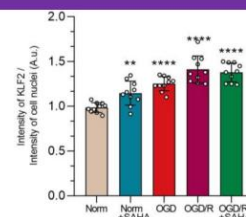
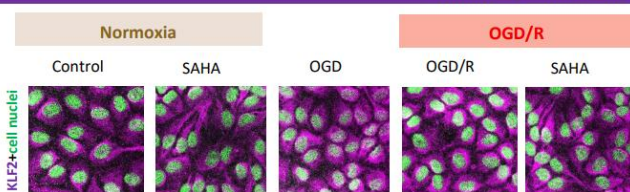
Transendothelial electrical resistance (TEER)



BBB permeability



Immunocytochemistry of KLF2



This work was funded by OTKA Young Researcher Excellence Program (OTKA-FK 143233) by National Research, Development and Innovation Office of Hungary.

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STRAUB- NAPOK

2024. MÁJUS 30-31.



A HUN-REN Szegedi Biológiai Kutatóközpont "Straub-Napok" rendezvénye 2024-ben május 30-31-én kerül megtartásra a HUN-REN SZBK Temesvári körüli épületében. A rendezők minden érdeklődőt szeretettel várnak. A HUN-REN SZBK "Straub-Napok 2024" rendezvénye – immár a hagyományoknak megfelelően – a HUN-REN Szegedi Biológiai Kutatóközpont és a Hungarian Centre of Excellence of Molecular Medicine (HCEMM) szervezésében kerül megrendezésre.

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ALANINE AND GLUTATHIONE TARGETING OF DOPAMINE- OR IBUPROFEN-COUPLED POLYPEPTIDE NANOCARRIERS ELEVATES CROSSING ACROSS THE BLOOD-BRAIN BARRIER AND PROTECTIVE EFFECTS

Mária Mészáros¹, Thi Ha My Phan², Judit P. Vigh^{1,3}, Gergő Porkoláb^{1,2}, Anna Kocsis¹, Anikó Szecskó^{1,2}, Emese K. Páli¹, Tamás F. Polgár^{1,4}, Fruzsina R. Walter¹, Jeng-Shiung Jan², Tamás Janáky⁵, Szilvia Veszelka¹ and Mária A. Deli¹

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IMPROVEMENT OF THE BLOOD-BRAIN BARRIER PROPERTIES BY A COMBINATION OF SMALL MOLECULES HAS A PROTECTIVE EFFECT IN A CELL CULTURE MODEL OF ISCHEMIC STROKE

Koppány Párdi¹, Anikó Szecskó^{1,2}, Janet Adegbite¹, Gergő Porkoláb^{1,2} and Mária A. Deli¹, Szilvia Veszelka¹

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BBB 2024

Szeged, Hungary

2024, September 11-13



PROGRAM AND ABSTRACT BOOKLET

**26th International Symposium on
„Signal Transduction at the Blood-Brain Barriers”**

Improvement of blood-brain barrier properties by a combination of small molecules has a protective effect in a cell culture model of ischemic stroke

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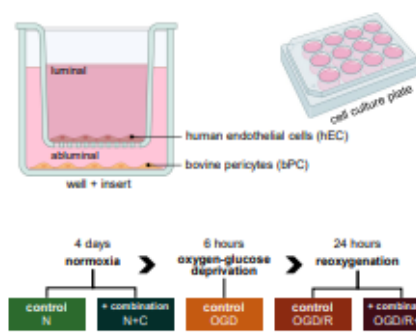
²Doctoral School of Biology, University of Szeged, Szeged, Hungary

Introduction

During **ischemic stroke**, blood flow interruption, **oxygen-glucose deprivation (OGD)** and the following **reoxygenation (OGD/R)** cause **blood-brain barrier (BBB)** disruption and neuronal death. Our aim was to **protect** the endothelial cells of BBB by improving barrier functions with a **combination** of molecules (combination-1). We cultured human endothelial cells under different conditions: **normoxia**, **OGD**, and **OGD/R** and then tested the effect of combination-1 against BBB dysfunctions. We also investigated whether the effects of combination-1 were exerted via the induction of Krüppel-like factor 2 (KLF2), the main transcriptional regulator of blood flow response, or **claudin-5**, an important tight-junction protein.

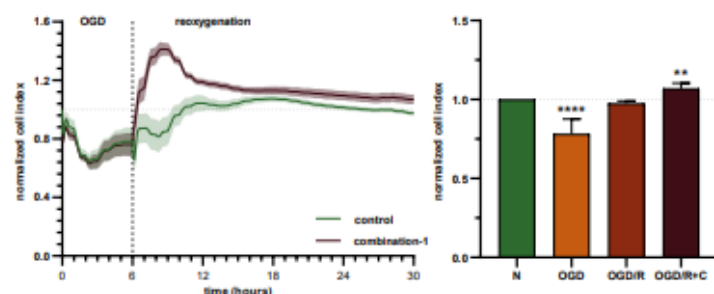
Methods

- BBB model:** human endothelial cells co-cultured with bovine pericytes
- cellular viability:** impedance-based, non-invasive, real time cell analysis
- transendothelial electrical resistance measurements:** reflects the tightness of the endothelial cell layer
- permeability measurements:** penetration of transcellular (Evans blue albumin) and paracellular (Na-fluorescein) markers through BBB model measured by fluorescent spectrophotometry
- immunocytochemistry:** localization and intensity of the staining of KLF2 and claudin-5 proteins

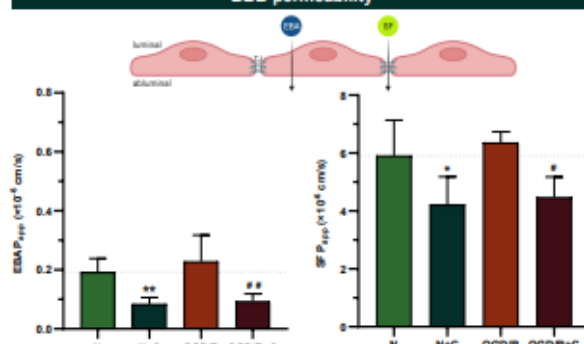


Results

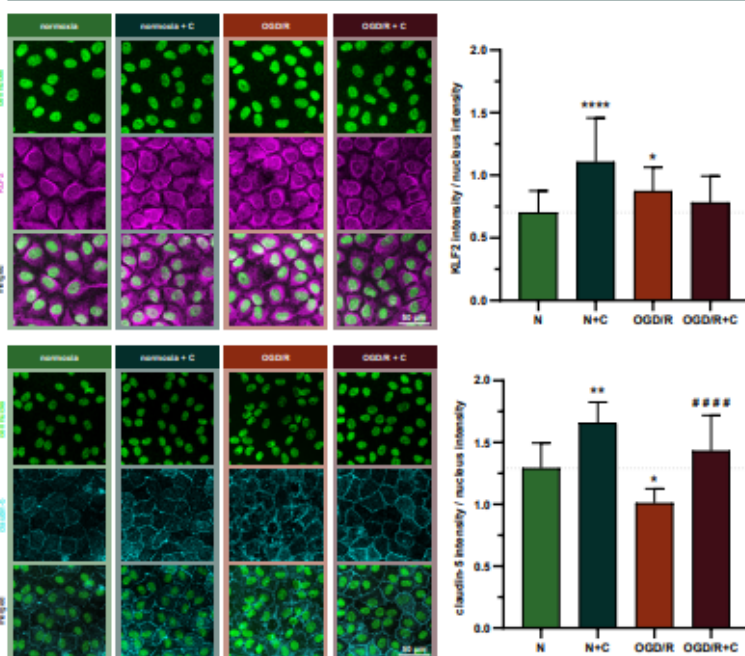
Cellular viability



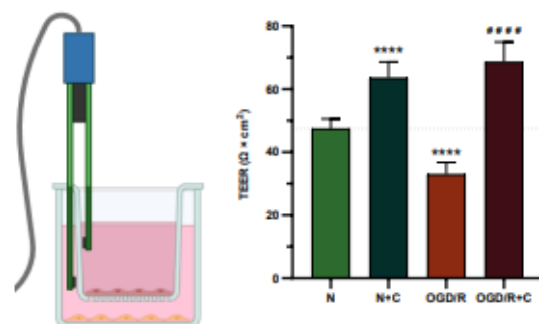
BBB permeability



Immunocytochemistry



Transendothelial electrical resistance (TEER)



Conclusion

OGD/R caused BBB damage including reduced TEER and claudin-5 expression, and increased permeability. Combination-1 was able to protect against these BBB dysfunctions. The protective effect of combination-1 was not related to KLF2, but rather to the increased expression of claudin-5 protein. Our results confirmed that combination-1 has a beneficial effect against ischemic-reoxygenation injury by inducing enhanced barrier properties in endothelial cells and may be a potential therapeutic agent in the treatment of post-ischemic BBB injury.

A.S. was supported by ÚNKP-23-3-SZTE-517 and Gedeon Richter Talentum Foundation. G.P. was supported by the National Academy of Scientist Education Program of the National Biomedical Foundation under the sponsorship of the Hungarian Ministry of Culture and Innovation. S.V. was supported by the National Research, Development and Innovation Office of Hungary (FK 143233).



PROTECTION OF THE BLOOD-BRAIN BARRIER AS A THERAPEUTIC TARGET IN THE CELL CULTURE MODEL OF ISCHEMIC STROKE

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During ischemic stroke, oxygen-glucose deprivation (OGD) and reoxygenation (OGD/R) cause blood brain barrier (BBB) disruption and neuronal death. Our aim was to protect the endothelial cells of the BBB by improving barrier functions via targeting three signalling pathways with a combination of small molecules (Combination1). We cultured human endothelial cells under different conditions: normoxia, OGD, and reperfusion after OGD (OGD/R) and tested the effect of Combination1 against BBB dysfunctions. Following OGD, the viability of cells significantly decreased, but treatment with Combination 1 ameliorated this effect. OGD and OGD/R condition resulted in reduced transendothelial electrical resistance of the cell layers compared to normoxia, suggesting that paracellular barrier is less tight, but this was significantly elevated by Combination 1 treatment. Penetration of BBB marker molecules elevated after OGD/R suggesting increased permeability of the model, but Combination 1 was able to protect this effect. We investigated whether the protective effect of Combination1 against OGD/R is mediated through an increased expression of a transcriptional regulator of blood-flow response, Krüppel-like factor 2 (KLF2). In contrast, immunocytochemical staining showed that OGD/R increased KLF2 levels compared to normoxia, but decreased after Combination1 treatment. Since the cellular mechanism underlying the protective effect was not linked to KLF2, an important BBB tight junction protein, claudin-5 was investigated. Immunocytochemical staining of claudin-5 was reduced after OGD/R but after Combination1 treatment, the staining was similar to normoxia. Our results suggest that tightening the interendothelial junctions via increasing claudin-5 by Combination1 treatment is a promising approach to protect BBB from post-ischemic injury.

A.S. was supported by Gedeon Richter Talentum Foundation. S.V. was supported by the Young Researcher Excellence Program (FK 143233) by National Research, Development and Innovation Office of Hungary. G.P. was supported by the National Academy of Scientist Education (FEIF/646-4/2021-ITM_SZERZ).

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THE HISTONE DEACETYLASE INHIBITOR SUBEROYLANILIDE
HYDROXAMIC ACID PROMOTES BLOOD-BRAIN BARRIER
PROTECTION DURING REPERFUSION IN A CELL CULTURE
MODEL OF ISCHEMIC STROKE

Anikó Szecskó^{1,2}, Gergő Porkoláb¹, Koppány Párdi¹, Janet Adegbite¹, Nárcisz Cser¹, Zuhao Cui^{1,2}, Mária A. Deli¹, Szilvia Veszélka¹

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Ischemic stroke is a leading cause of death worldwide with limited available treatment options. During ischemic stroke, reduced blood flow causes severe neuronal damage and BBB disruption. Thrombolytic therapy with recombinant tissue plasminogen activator (rtPA) which acts by breaking down clots to restore blood flow are the only FDA approved treatment for ischaemic stroke. However, rtPA is effective within a limited time (4.5 h) of symptom onset with a risk of intracranial hemorrhage. Therefore, additional effective therapeutic approaches for treatment of ischaemic stroke are urgently needed. The aim of this study was to investigate the effect of suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, on restoring the function of the BBB to prevent post-stroke consequences.

We tested the effect of SAHA on human co-cultured BBB model under normoxia, oxygen-glucose deprivation (OGD), and during reperfusion after OGD (OGD/R). Our results show that after OGD, SAHA is able to prevent the BBB functions by increasing the resistance, and reducing the permeability of marker molecules across the BBB. SAHA increased the viability of pericyte cells against injury, which were much more damaged than brain endothelial cells. RNA-seq analyses showed that SAHA decreased the expression of genes involved in inflammatory processes and cell proliferation and increased the expression of the genes of glycocalyx enzymes.

Based on our results, SAHA may be a potential therapeutic tool for the treatment of ischaemic stroke, but further research is needed to gain a deeper understanding of its mechanism of action and to develop its potential clinical applicability. A.S. was supported by Gedeon Richter Talentum Foundation. S.V. was supported by the Young Researcher Excellence Program (FK 143233) by National Research, Development and Innovation Office of Hungary. G.P. was supported by the National Academy of Scientist Education (FEIF/646-4/2021-ITM_SZERZ).

22nd January
2025

8th Hungarian Neuroscience Meeting for
Undergraduate Students, Graduate Students
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8th HuN^{Doc} Meeting Debrecen 2025



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University of Debrecen, Learning Center
(Debrecen, Egyetem tér 1., 4032)

The histone deacetylase inhibitor SAHA protects the blood-brain barrier against ischemic injury

Koppány Párdi ¹, Anikó Szecskó ^{1,2}, Zuhao Cui ^{1,2}, Janet Adegbite ¹, Mária A. Deli ¹, Szilvia Veszélka ¹¹ Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary;² University of Szeged, Szeged, Hungary

During an ischemic event, blood flow interruption and the subsequent oxygen-glucose deprivation (OGD) cause blood-brain barrier (BBB) disruption and neuronal death, which may have life-threatening consequences. Protection of the BBB as a therapeutic target is a novel concept in medicinal strategies to treat ischemia-reperfusion injury. Our aim was to investigate the possible protective effect of a clinically used histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), against BBB disruption in the cell culture model of ischemic stroke.

We cultured human brain endothelial cells and brain pericytes under three different conditions: normoxia, OGD, and reoxygenation after OGD (OGD/R) and then tested the effect of SAHA on BBB dysfunctions. SAHA promoted BBB protection against OGD/R by enhancing several barrier functions: it increased transendothelial electrical resistance (TEER), decreased the permeability of trans- and paracellular tracers, increased the protein level of the important tight junction protein claudin-5 measured by immunofluorescent intensity, and also increased the intensity of the glycocalyx constituent sialic acid. We also observed that SAHA had barrier-strengthening effects only in the presence of brain pericytes, suggesting that pericytes have a pivotal role in the protective mechanism of SAHA.

Our results suggest that SAHA has beneficial effects on the integrity of the BBB after OGD and may prove useful as a therapeutic drug in the treatment of ischemia-reperfusion injury.

This work was funded by the project no.143233, which has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the FK_22 funding scheme. A.S. was supported by the Gedeon Richter Talentum Foundation.



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The histone deacetylase inhibitor SAHA protects blood-brain barrier during reperfusion in a cell culture model of ischemic stroke

Koppány Párdi¹, Anikó Szecskó^{1,2}, Janet Adegbite¹, Mária A. Deli¹, Szilvia Veszélka¹¹ Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary;² University of Szeged, Szeged, Hungary

Introduction

Oxygen-glucose deprivation (OGD) causes blood-brain barrier (BBB) disruption which contributes to neuronal injury. Our aim was to investigate the barrier-protecting effects and mechanisms of the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) against ischemia-reperfusion injury on the cell culture model of the BBB. We cultured endothelial cells and pericytes under three different conditions: normoxia, OGD, and reperfusion after OGD (OGD/R) and then tested the effect of SAHA on BBB dysfunctions.

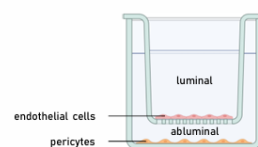
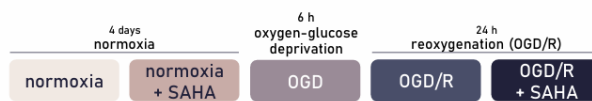
Methods

- transendothelial electrical resistance (TEER): evaluates the integrity of interendothelial tight junctions (TJs)
- permeability: measures the penetration of transcellular (Evans blue albumin [EBA]) and paracellular (Na-fluorescein [SF]) markers through the endothelial cell layer by spectrofluorimeter
- immunocytochemistry: staining of glycocalyx constituent sialic acid and tight junction protein claudin-5

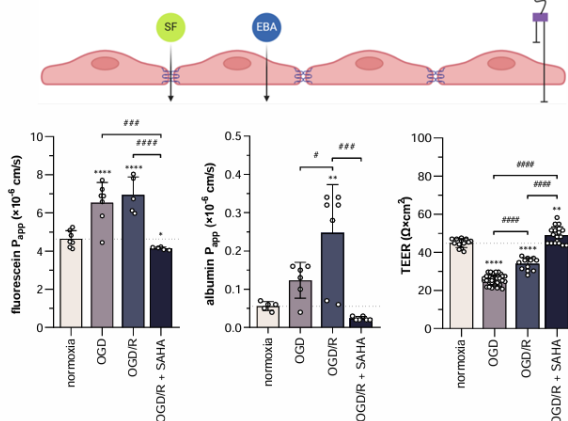
Results & Conclusion

OGD disrupts the BBB by decreasing TEER and increasing fluorescein permeability, but treatment with SAHA has the opposite, protective effect in OGD/R. Increased sialic acid claudin-5 intensity and elevated claudin-5 protein levels further contribute to strengthened BBB integrity.

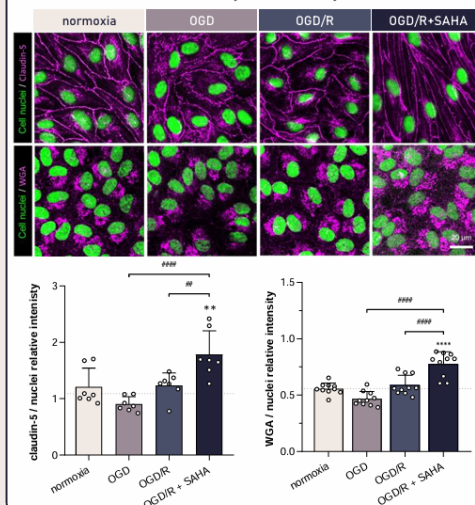
Experimental setup



Blood-brain barrier functions



Immunocytochemistry





Annual Meeting of the Hungarian Neuroscience Society



HUNGARIAN
NEUROSCIENCE
SOCIETY

ABSTRACT BOOK

23-24 January 2025, Debrecen, Hungary

P3.22 Expressional changes of claudin-5 and PDGFRbeta, two key blood brain barrier proteins, in a culture model of ischemic stroke

Csilla Kovács¹, Anikó Szecskó¹, Koppány Párdi¹, Gergő Porkoláb¹, Szilvia Veszélka¹, Zsófia Hoyk¹,
Mária A. Deli¹

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P3.7 Effect of the histone deacetylase inhibitor SAHA on the gene expression of brain endothelial cells after ischemic injury

Zuhao Cui^{1,2}, Anikó Szecskó^{1,2}, Koppány Párdi¹, László Dér³, Krisztina Nagy³, Mária A. Deli¹, Szilvia Veszélka¹

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P3.28 The histone deacetylase inhibitor SAHA protects the blood-brain barrier against ischemic injury

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The histone deacetylase inhibitor SAHA protects blood-brain barrier during reperfusion in a cell culture model of ischemic stroke

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¹ Biological Barriers Research Group, Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary; ² University of Szeged, Szeged, Hungary

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Oxygen-glucose deprivation (OGD) causes blood-brain barrier (BBB) disruption which contributes to neuronal injury. Our aim was to investigate the barrier-protecting effects and mechanisms of the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) against ischemia-reperfusion injury on the cell culture model of the BBB. We cultured endothelial cells and pericytes under three different conditions: normoxia, OGD, and reperfusion after OGD (OGD/R) and then tested the effect of SAHA on BBB dysfunctions.

Methods

- transendothelial electrical resistance (TEER): evaluates the integrity of interendothelial tight junctions (TJs)
- permeability: measures the penetration of transcellular (Evans blue albumin [EBA]) and paracellular (Na-fluorescein [SF]) markers across the endothelial layer by spectrofluorimeter
- immunocytochemistry: staining of glycocalyx constituent sialic acid and tight junction protein claudin-5

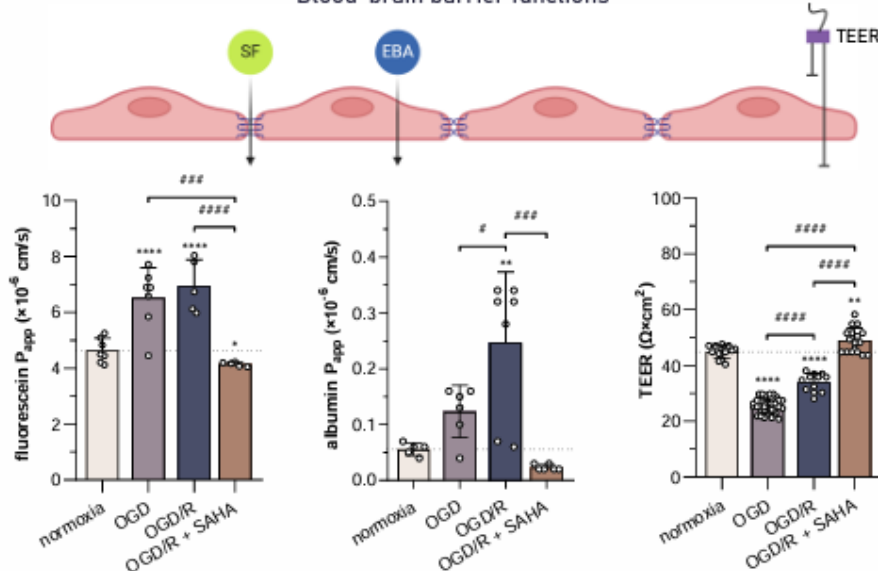
Results & Conclusion

SAHA protected the BBB against OGD/R injury by increasing TEER and decreasing the permeability of SF and EBA markers. SAHA increased the level of the tight junction protein claudin-5 and the glycocalyx constituent sialic acid. SAHA had barrier-strengthening effects only in presence of brain pericytes, suggesting that pericytes have a pivotal role in the protective mechanism of SAHA.

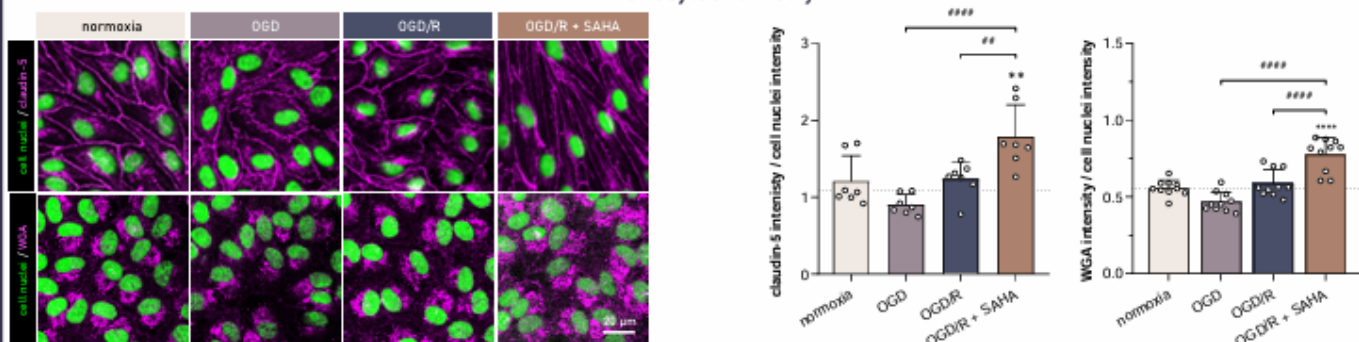
Experimental setup



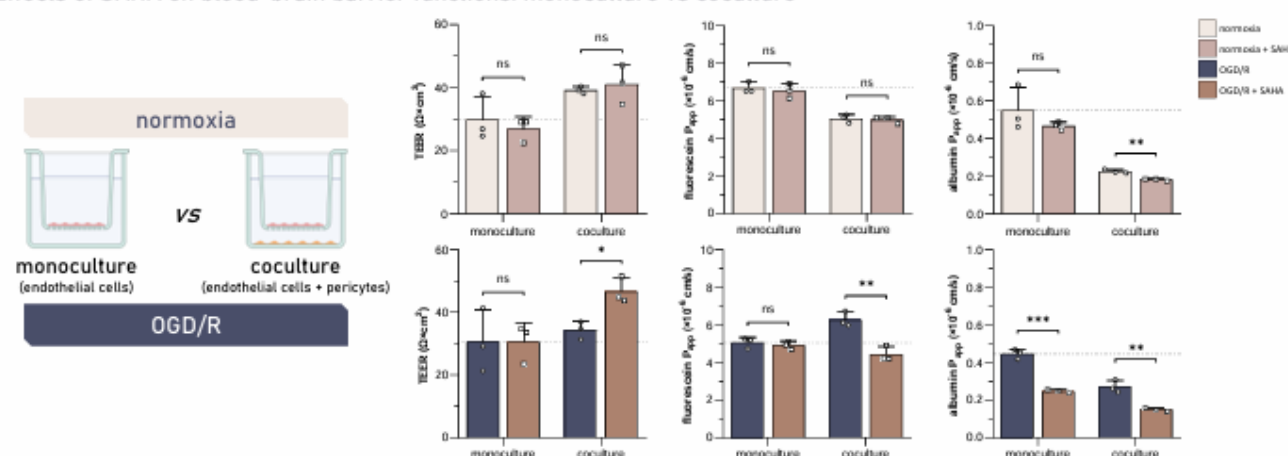
Blood-brain barrier functions



Immunocytochemistry



Effects of SAHA on blood-brain barrier functions: monoculture vs coculture



IGAZOLÁS

Párdi Koppány, a Szegedi Tudományegyetem végzős BSc hallgatója a Szegedi Biológiai Kutatóközpont Biológiai Barrierék Kutatócsoport tagjaként rendszeres résztvevője a kutatóintézet által szervezett tudománynépszerűsítő eseményeknek.

Koppány elsősorban a látogatók kísérésben és a teaházban nyújtott segítséget a minden év szeptemberében megtartott *Kutatók Éjszakája* országos rendezvényen, valamint a minden év márciusában megrendezett *Agykutatás Hete* nemzetközi rendezvénysorozaton.

Párdi Koppány a tudomány iránt elkötelezett hallgató, aki lelkesen kiveszi részét kutatócsoportjának tudománynépszerűsítő tevékenységében.

Szeged, 2024. június 25.

Dr. Hoyk Zsófia
főszervező

Helyszín: I. emelet, 148.

+ Cser Nárcisz, Párdi Koppány, Szecskó Anikó

+ **Mi is az a vér-agy gát, ami megnehezíti az idegrendszeri betegségek gyógyítását?**

A program során az érdeklődők betekintést nyerhetnek a vér-agy gát működésébe, melynek fő feladata az agy táplálása és káros anyagoktól való védelme, ugyanakkor védő mechanizmusaival megnehezíti a gyógyszerek bejutását is az agyba. A látogatók fáziskontraszt mikroszkóp segítségével élő agyi sejteket: agyi endotélsejteket, pericita sejteket és asztroglia sejteket láthatnak, valamint megismerkedhetnek a sejtenyésztés módszereivel és a sejtenyésztő laboratórium különböző műszereivel

Elérhető turnusok:

- 18:00 – 18:30, max 8 fő
- 18:30 –19:00, max 8 fő