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Arginine deficiency induced by acute liver failure compromises the blood-brain barrier by inhibiting the mTORC1-S6K1/4EBP1 pathway and inducing autophagy - A review of the evidence

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1. ABSTRACT

The analyzed study evaluated whether acute liver failure can damage the blood-brain barrier through the loss of arginine, an amino acid necessary for various cellular processes. The authors used human and rat brain endothelial cells, as well as rat models with acute liver failure induced by paracetamol or thioacetamide and rats treated with arginase. Arginase is an enzyme that consumes arginine; in the study, its increase in the blood was associated with lower arginine levels, increased barrier permeability, and reduced proliferation of the cells that compose it.The relevant passage regarding "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi concerns S6K1 as an interpreter of nutrient availability and a regulator of the balance between cell growth and autophagy. The study shows, in the experimental models examined, that arginine deficiency reduces mTORC1 signals directed toward S6K1 and 4EBP1, with cell cycle arrest and increased autophagy. Independent research confirms that arginine contributes to the activation of mTORC1 and its effectors; the role of autophagy in the blood-brain barrier, however, varies according to the type and duration of the damage. The confirmation is therefore primarily mechanistic and experimental.

ONLINE ARTICLE

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MISSION

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Through articles, reviews, in-depth analyses and expert contributions, the Journal champions evidence-based medicine and science, fostering a correct interpretation of scientific data and countering misinformation. Every piece of content is developed with the aim of translating the complexity of research into understandable, useful and applicable information, while keeping intact the principles of accuracy, transparency and scientific independence.

Free from political, industrial and commercial influence, the journal stands as an authoritative and independent editorial space, dedicated to promoting scientific culture, professional development and the growth of health awareness among the public.

2. ANALYZED STUDY

The research question was whether acute liver failure damages the blood-brain barrier—the system of cells and junctions that regulates the passage of substances from the blood to the brain—by reducing arginine availability through the release of arginase from the damaged liver.The hypothesis evaluated was structured as a chain: an increase in circulating arginase, a reduction in arginine, lower mTORC1 activity, reduced phosphorylation of S6K1 and 4EBP1, a slowing of cerebral endothelial cell proliferation, and an increase in autophagy. mTORC1 is a protein complex that integrates signals from nutrients and growth factors; S6K1 and 4EBP1 are two of its effectors that help the cell initiate protein synthesis and growth.This research is relevant to section 4.7 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi because it documents an experimental case in which lower availability of a nutrient coincides with lower S6K1 activation and reduced cell proliferation. However, it does not prove that S6K1 alone is the primary nutritional sensor in every tissue, nor that the same sequence causes blood-brain barrier damage in humans with acute liver failure.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The authors combined cellular and animal experiments. As models of the blood-brain barrier in culture, they used human hCMEC/D3 cerebral microvascular endothelial cells and primary rat cerebral microvascular endothelial cells. The cells were exposed to arginine-free medium, arginase, or serum obtained from rats with acute liver failure induced by paracetamol or thioacetamide.Cell viability was measured using CCK-8 assays. Proliferation was assessed via EdU incorporation, a marker that identifies cells duplicating their DNA. The cell cycle was studied using flow cytometry. The authors also measured proteins involved in cell cycle progression, the mTORC1-S6K1/4EBP1 signaling pathway, and autophagy using western blotting, a technique that detects specific proteins.To verify the causal role of certain pathway nodes, S6K1, 4EBP1, TSC2, and Beclin-1 were silenced using small interfering RNAs. TSC2 is a physiological brake on mTORC1; Beclin-1 contributes to the formation of autophagosomes, the vesicles with which the cell initiates the recycling of intracellular components. Insulin, rapamycin, 3-methyladenine, chloroquine, an arginase inhibitor, and arginine supplementation were also used.In the animal models, 8-9-week-old male Sprague-Dawley rats were used. For each of the three main in vivo experiments, eighteen rats were assigned to three groups: control, injury model, and injury model with arginine. Barrier permeability was estimated by measuring the ratio of fluorescein in the brain to that in the plasma. For some cellular results, the samples consisted of four or six replicates; for the main animal groups, there were six rats per group. This information is not reported in the material available for every single supplementary analysis.

4. RESULTS

In rats with acute liver failure induced by paracetamol or thioacetamide, plasma showed higher arginase activity and lower arginine levels compared to controls. In the paracetamol model, the peak of arginase activity was approximately 1,000 units/L, and the minimum arginine level was near 1 µg/mL at 12 hours post-administration. In the thioacetamide model, the maximum imbalance was observed at 36 hours. The authors report statistically significant differences, but the exact values for each comparison are not provided in the available text.When brain endothelial cells were cultured without arginine or treated with arginase, proliferation decreased, and a larger proportion of cells remained in the G1 phase. In simple terms, the cells slowed down before the phase in which they duplicate their genetic material. Cyclin D, CDK2, and CDK4—proteins that support cell cycle progression—decreased. Apoptosis, or programmed cell death, did not increase significantly in the tests described.The key finding for the book section is that arginine deficiency and arginase reduced the phosphorylation of S6K1 and 4EBP1. Phosphorylation is a chemical modification that, in this context, is used as an indicator of pathway activation. Rapamycin, an inhibitor of mTORC1, also reduced cell viability and increased G1 arrest. Furthermore, silencing S6K1 or 4EBP1 reproduced some of the effects of arginine deficiency on hCMEC/D3 cells.Insulin and the silencing of TSC2 attenuated the reduction of p-S6K1 and p-4EBP1 and improved the cellular indices altered

by arginine deficiency. This result is consistent with the involvement of mTORC1, but it does not prove that insulin is a treatment for blood-brain barrier damage in acute liver failure.Arginine deficiency also increased markers consistent with greater autophagic flux: decreased phosphorylation of ULK1, an increased LC3-II/LC3-I ratio, and decreased p62. Inhibition of autophagy with 3-methyladenine or silencing of Beclin-1 only partially reversed the cellular damage and cycle arrest. In rats with acute liver failure or those treated with arginase, fluorescein penetrated the brain more, indicating an increase in barrier permeability. Oral arginine supplementation attenuated many of these brain alterations without improving liver function in the experimental models described.

5. INTERPRETATION

The biological significance of the results is that blood-brain barrier cells appear to depend on the availability of arginine to maintain an active growth and renewal program. When arginine levels decrease, the mTORC1-S6K1/4EBP1 signal is reduced, protein production and cell cycle progression slow down, and autophagy increases.The segment actually documented by the publication subject to the Evidence Review is: arginine deficiency inhibition of mTORC1-S6K1/4EBP1 reduced proliferation of cerebral endothelial cells, with concomitant activation of autophagy. The work therefore directly supports the idea that S6K1 participates in translating nutrient availability into a concrete cellular response.The relationship with barrier integrity is supported in rats because animals with lower arginine levels showed greater fluorescein passage into the brain and because arginine attenuated the alteration. However, in the animal model, several changes occur simultaneously: liver damage, elevated arginase, metabolic modifications, and possible inflammation. For this reason, it is not possible to attribute the entire barrier damage exclusively to S6K1 or autophagy.Furthermore, autophagy is not automatically harmful. It is a generally useful recycling process, but it can contribute to cellular dysfunction if it is excessive, prolonged, or directed against essential components of the barrier. The significance of increased autophagy therefore depends on the experimental context, the duration of the stress, and the type of proteins involved.

6. INDEPENDENT CONFIRMATORY EVIDENCE

Independent confirmation of the upstream pathway comes from Carroll and colleagues, who studied cultures of human cells, fibroblasts, neurons, and embryonic stem cells. Arginine deprivation reduced mTORC1 activation, observed through decreased phosphorylation of S6K1 and 4EBP1, and increased autophagy. Genetic experiments indicated that the mechanism involves TSC2 and Rheb, regulators positioned upstream of mTORC1. This research does not concern the blood-brain barrier, but it provides mechanistic and independent confirmation that arginine can influence the mTORC1-S6K1 signal and the balance between cell growth and autophagy [1].Ban and colleagues studied rat intestinal epithelial cells. The addition of L-arginine increased the activity of p70 S6 kinase, the functional equivalent of S6K1, and the phosphorylation of 4E-BP1; these effects were sensitive to rapamycin. The study therefore confirms the opposite and complementary direction: greater arginine availability can increase the activation of two mTORC1 effectors involved in protein synthesis and growth. The limitation is the different tissue studied and the absence of direct measurements of the blood-brain barrier [2].Kim and colleagues examined the final part of the chain, namely the relationship between autophagy and the integrity of the blood-brain barrier. In murine brain endothelial cells subjected to oxygen and glucose deprivation and in rats with cerebral ischemia, autophagy increased along with the degradation of occludin, a tight junction protein. Inhibition of autophagy with 3-methyladenine reduced the loss of occludin and the increase in permeability. This is a biologically consistent confirmation that, under conditions of severe stress, increased autophagy can contribute to barrier dysfunction; however, it does not confirm that the initial cause is arginine deficiency [3].

7. CONFLICTING EVIDENCE

A relevant result, but with a different outcome, was observed by Yang and colleagues under conditions of short-term fasting and hypoxia. In brain endothelial cells, a zebrafish experimental model, and patient samples with stroke, autophagy promoted the

elimination of caveolin-1 and cytoplasmic claudin-5 aggregates, helping to limit damage to the intestinal barrier. Pharmacological or genetic blockage of autophagy worsened the impairment of the barrier [4].This evidence does not directly contradict the effect of arginine deficiency on the mTORC1-S6K1 signal. However, it reduces the possibility of considering autophagy as always harmful to the blood-brain barrier. The direction of the effect may depend on the type of stress, the duration, the intensity of autophagy, and the proteins being degraded. In the analyzed work, the inhibition of autophagy produced only a partial recovery, consistent with an important but not exclusive contribution of this process.

8. QUADRO COMPARATIVO E SINTESI DELLE EVIDENZE

The table distinguishes the publication subject to the Evidence Review from the independent research used to verify the individual steps of the narrative.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Cellular and animal	Human and rat brain endothelial cells; rats with acute liver failure or arginase	Arginine deficiency reduced p-S6K1 and p-4EBP1, increased autophagy, slowed proliferation, and increased barrier permeability in rats	direct confirmation	moderate
[1]	Cellular and genetic	Human cells, fibroblasts, neurons, and embryonic stem cells	Arginine deprivation reduced mTORC1-S6K1/4EBP1 signaling and increased autophagy via the TSC2-Rheb axis	direct confirmation	moderate
[2]	Cellular	Rat intestinal epithelial cells	Arginine supplementation increased S6K1 activity and 4E-BP1 phosphorylation in a rapamycin-sensitive manner	indirect confirmation	low
[3]	Cellular and animal	Murine brain endothelial cells and rats with cerebral ischemia	Increased autophagy contributed to occludin degradation and increased barrier permeability	biologically consistent evidence	moderate
[4]	Cellular, animal, and observational tissue study	Brain endothelial cells, zebrafish, and samples from stroke patients	Autophagy limited barrier damage by promoting the control of claudin-5 redistribution	discordant evidence	moderate

9. SINTESI EDITORIALE E CONCLUSIONI

CONCLUSIONS

The study analyzed convincingly supports, in its cellular and animal models, that arginine reduction can decrease mTORC1-S6K1/4EBP1 signaling in blood-brain barrier cells. This reduction is accompanied by slowed proliferation, cell cycle arrest, and increased autophagy.Independent evidence confirms that arginine is a regulator of the mTORC1 pathway and its effectors S6K1 and 4EBP1. It is also experimentally supported that, under certain conditions of brain injury, increased autophagy may contribute to the loss of barrier integrity. However, autophagy can also have protective functions, and its significance cannot be generalized.The book's narrative is therefore well-supported at the molecular and cellular level. Human validation remains necessary to establish the extent to which this chain contributes to hepatic encephalopathy and to clarify whether arginine correction can produce safe and effective clinical benefits.

Aggiornato al 24/11/2026. Questo contenuto riflette una sintesi divulgativa delle evidenze scientifiche al momento della pubblicazione e non sostituisce il parere medico.

10. TRANSPARENCY AND SCIENTIFIC RESPONSIBILITY

Artificial Intelligence was used solely as a tool for editorial and documentary support. All content, interpretations, bibliographic citations and conclusions have been verified, reviewed and approved by the author, who assumes full scientific responsibility for them.

The manuscript was also subjected to independent peer review prior to publication.

To ensure the greatest transparency, the complete history of the article, including the dates of submission, revision, acceptance and publication, as well as information on the peer review process, is available on the dedicated Article History page.

11. RIFERIMENTI BIBLIOGRAFICI

[1] **Control of TSC2-Rheb signaling axis by arginine regulates mTORC1 activity**
Carroll et al., 2016
eLife
DOI: https://doi.org/10.7554/eLife.11058

[2] **Arginine and Leucine regulate p70 S6 kinase and 4E-BP1 in intestinal epithelial cells**
Ban et al., 2004
International Journal of Molecular Medicine
DOI: https://doi.org/10.3892/ijmm.13.4.537

[3] **Autophagy-mediated occludin degradation contributes to blood–brain barrier disruption during ischemia in bEnd.3 brain endothelial cells and rat ischemic stroke models**
Kim et al., 2020
Fluids and Barriers of the CNS
DOI: https://doi.org/10.1186/s12987-020-00182-8

[4] **Autophagy alleviates hypoxia-induced blood-brain barrier injury via regulation of CLDN5 (claudin 5)**
Yang et al., 2021
Autophagy
DOI: https://doi.org/10.1080/15548627.2020.1851897