高原低氧对药物跨血脑屏障转运的调节作用及机制

刘贵琴[1], 白 雪[1], 杨建鑫[1], 段雅彬[2], 朱俊博[1], 田 露[3], 李向阳[1,4*]

(1. 青海大学高原医学研究中心, 青海 西宁 810001; 2. 青海大学附属医院, 青海 西宁 810001; 3. 青海大学医学院, 青海 西宁 810001; 4. 青海大学三江源生态与高原农牧业国家重点实验室, 青海 西宁 810016)

摘要: 高原低氧环境下机体中枢神经系统功能发生显著改变, 这些变化引发中枢神经系统疾病, 影响药物体内代谢过程。血脑屏障是维持中枢神经系统稳定的必要条件, 在药物代谢调节中发挥关键作用, 屏障结构和功能变化影响药物脑部转运。高原低氧环境下血脑屏障结构和功能及药物跨血脑屏障转运变化, 是受脑微血管内皮细胞、星形胶质细胞和周细胞等细胞调节, 或是受药物转运体和药物代谢酶等药物代谢因素调节。本文通过综述高原低氧环境对血脑屏障结构和功能的影响及血脑屏障变化对药物代谢的影响, 探讨高原低氧环境中血脑屏障与转录因子、炎症因子和核受体等相关通路对药物转运的调节作用及潜在机制。

关键词: 血脑屏障; 高原低氧; 药物转运体; 药物代谢酶; 药物转运

中图分类号: R917 **文献标识码**: A **文章编号**: 0513-4870(2023)03-0659-13

The modulation and mechanisms of high-altitude hypoxia in drug transport across the blood-brain barrier

LIU Gui-qin[1], BAI Xue[1], YANG Jian-xin[1], DUAN Ya-bin[2], ZHU Jun-bo[1], TIAN Lu[3], LI Xiang-yang[1,4*]

(*1. Research Center for High Altitude Medicine, Qinghai University, Xining 810001, China; 2. Affiliated Hospital of Qinghai University, Xining 810001, China; 3. Medical College of Qinghai University, Xining 810001, China; 4. State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining 810016, China*)

Abstract: The function of the central nervous system was significantly altered under high-altitude hypoxia, and these changes lead to central nervous system disease and affected the metabolism of drugs *in vivo*. The blood-brain barrier is essential for maintaining central nervous system stability and plays a key role in the regulation of drug metabolism, and barrier structure and dysfunction affect drug transport to the brain. Changes in the structure and function of the blood-brain barrier and the transport of drugs across the blood-brain barrier under high-altitude hypoxia are regulated by changes in brain microvascular endothelial cells, astrocytes and pericytes, and are regulated by drug metabolism factors such as drug transporters and drug metabolizing enzymes. This article reviews the effects of high-altitude hypoxia on the structure and function of the blood-brain barrier and the effects of changes in the blood-brain barrier on drug metabolism. We investigate the regulatory effects and underlying mechanisms of the blood-brain barrier and related pathways such as transcription factors, inflammatory factors and nuclear receptors on drug transport under high-altitude hypoxia.

Key words: blood-brain barrier; high-altitude hypoxia; drug transporter; drug metabolizing enzyme; drug transport

收稿日期: 2022-10-05; 修回日期: 2022-12-17.

基金项目: 国家自然科学基金资助项目 (82260731); 青海省创新平台建设专项 (2021-ZJ-T03).

*通讯作者 Tel: 86-971-5362082, Fax: 86-971-5362383, E-mail: qhmclxy@163.com

DOI: 10.16438/j.0513-4870.2022-1095

血脑屏障是脑内毛细血管壁与神经胶质细胞形成的选择性运输界面, 严格控制中枢神经系统脑组织和血液间离子、分子和细胞运动, 使脑组织免受细菌、病毒等致病微生物侵害, 维持大脑微环境稳定[1]。高原环境对机体的影响涉及大气物理、生态环境、地球化学等因素, 其中低压、低氧、强辐射和寒冷干燥为影响人类生命活动的主要因素, 低氧为核心因素, 是人类的面临的最大挑战。机体进入高原环境后中枢神经系统受到不同程度损伤, 进而导致血脑屏障结构和功能障碍, 屏障损伤与脑水肿、脑卒中、阿尔茨海默病和外伤性脑损伤等中枢神经系统疾病密切相关, 并参与疾病的发生、发展和转归, 影响药物脑跨血脑屏障转运及治疗和预后[2,3]。本文综述了高原低氧对血脑屏障结构和功能的影响及血脑屏障介导的高原低氧对药物代谢的调节机制, 探究高原低氧环境调节中枢神经系统药物转运的靶点, 为提高中枢神经系统药物发挥疗效和促进高原地区中枢神经系统临床合理用药提供新的理论依据。

1 血脑屏障

血脑屏障由单层无孔的脑微血管内皮细胞、周细胞、星形胶质细胞、小胶质细胞、基底膜和神经元组成[4], 其中脑微血管内皮细胞具有物理、运输和代谢屏障的特性, 这种特性严格控制血液和脑组织间的物质传递[5-7], 如图1。血脑屏障通过被动扩散和主动转运等机制将不同分子量的物质传递至中枢神经系统[8]。被动扩散是小分子物质进入脑组织的主要方式, 主动转运主要包括主动外排和载体介导的转运[9], 主动外排促进中枢神经系统药物外排, 血脑屏障中的外排型药物转运体, 将外源性物质和内源性物质通过内皮细胞腔侧主动泵入细胞血液循环, 使毒性代谢物和异源物质排出, 维持大脑生理功能正常[10]。载体介导的转运促进各种分子的跨细胞运动, 血脑屏障中的溶质转运蛋白是一种独立的能量系统, 帮助药物或内源性物质进入脑组织, 维持大脑营养平衡[11], 如图2。然而, 多发性硬化症、帕金森病、阿尔茨海默病、癫痫、脑卒中、脑肿瘤等疾病和低压、低氧、强辐射等环境因素影响中枢神经系统药物转运[12], 这与血脑屏障结构和功能障碍、药物转运体表达变化及治疗药物受不同药物代谢酶催化密不可分。不同疾病对血脑屏障、药物转运体和药物代谢酶的影响见表1[13-47], 但高原低氧等环境因素对血脑屏障和药物转运的影响仍需进一步探索。

2 高原低氧环境对血脑屏障的影响

高原低氧环境是血脑屏障病理生理变化的初始触发因素, 低氧环境引发水和离子分布改变、炎症因子表达变化、外周免疫细胞向脑组织浸润等反应, 促进脑微血管内皮细胞增殖, 星形胶质细胞和周细胞激活, 影响血脑屏障通透性[48,49]。因此, 本部分将分析高原低氧环境对血脑屏障脑微血管内皮细胞、星形胶质细胞和周细胞的影响和分子机制变化。

2.1 高原低氧对脑微血管内皮细胞的影响

脑微血管内皮细胞紧临血液, 最先感知血氧分压变化。Fischer等[50]模拟低氧环境发现低氧降低闭锁连接蛋白-1 (zonula occludens-1, ZO-1) 的表达水平, 增加血脑屏障通透性。Zhang等[51]研究表明低氧引发内皮细胞和其他损伤细胞内 Ca^{2+} 内流, ATP 含量降低, NO 释放增多, 使氧化磷酸化、糖酵解和三羧酸循环功能紊乱, 导致紧密连接相关蛋白重排, 血脑屏障通透性增加。Berndt等[49]发现低氧环境中血脑屏障通透性增加与脑微血管内皮细胞中闭合蛋白-5 (claudin-5) 和咬合

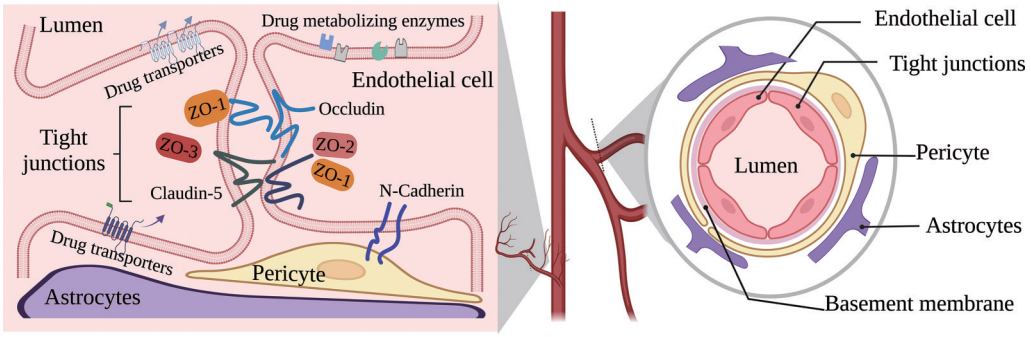

Figure 1 Schematic representation of molecules of the blood-brain barrier. The properties of the blood-brain barrier are determined primarily by endothelial cells, astrocytes and pericytes, both the endothelial cells and the pericytes are enclosed by, the local basement membrane which forms a distinct perivascular extracellular matrix. Endothelial cells are the core of the neurovascular units and their physical, transport and metabolic properties play an important role in maintaining the proper structure and function of the blood-brain barrier. The presence of tight junctions at the edges of endothelial cells, in which proteins such as ZO-1/-2/-3, claudins-5, occludin and cadherin create a virtually impermeable physical barrier, and drug transporters and drug metabolizing enzymes in endothelial cells act as transport and metabolic barriers at the blood-brain barrier, limiting or facilitating the penetration of various substances into the central nervous system. ZO-1/-2/-3: Zonula occludens-1/-2/-3

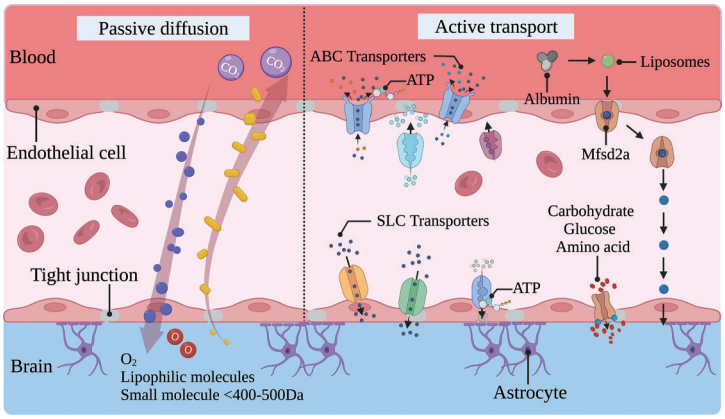

Figure 2 Transport mechanism across the blood-brain barrier. The transport mechanism of the blood-brain barrier is divided into passive diffusion and active transport. Passive diffusion is the main way in which O_2, CO_2 and specific lipid-soluble, small molecular weight substances (< 400−500 Da) across the blood-brain barrier. Active transport includes the active efflux of ABC transporters promotes the excretion of toxic metabolites and xenobiotics. SLC transporters facilitate the uptake of specific substrates, such as carbohydrate, glucose and amino acid transport, and the binding of Mfsd2a to liposomes to facilitate the transport of drugs across the blood-brain barrier. ABC: ATP-binding cassette; SLC: Solute carrier; Mfsd2a: Major facilitator superfamily domain-containing protein 2a

蛋白 (occludin) 表达下调有关。Park 等[52]研究显示低氧通过调节紧密连接相关蛋白及外排转运体、营养素等受体磷酸化和蛋白质再分配, 改变血脑屏障通透性。以上研究结果主要通过模拟低氧环境发现紧密连接相关蛋白重排或表达水平变化影响脑微血管内皮细胞, 增加血脑屏障通透性, 而高原实地环境对脑微血管内皮细胞的影响及其调控机制还需要进一步实验验证。

2.2 高原低氧对星形胶质细胞的影响

星形胶质细胞与脑微血管内皮细胞共同作用调节血脑屏障。研究显示高原低氧环境中星形胶质细胞分泌白介素-1β (interleukin-1β, IL-1β)、肿瘤坏死因子-α (tumor necrosis factor-α, TNF-α)、血管内皮生长因子 (vascular endothelial growth factor, VEGF) 和单核细胞趋化蛋白-1 (monocyte chemotactic protein-1, MCP-1) 等炎症因子促进白细胞在血脑屏障中渗透, 使紧密连接相关蛋白连接紊乱, 屏障通透性增加[53]。Lu 等[54]研究表明低氧时星形胶质细胞中基质金属蛋白酶-2, 9, 13 (matrix metalloproteinase, MMP-2, 9, 13) 活性增加, 而 MMPs 会引起紧密连接相关蛋白重组并减弱屏障功能。Mojsilovic-Petrovic 等[55,56]发现低氧环境中星形胶质细胞以氧依赖性方式产生 MCP-1, MCP-1 由 Rho 信号介导的紧密连接重排增加脑微血管内皮细胞通透性。此外, 低氧环境下星形胶质细胞也可维持血脑屏障的完整性, 细胞感受氧供减少后启动代偿性低氧保护机制, 通过增加无氧糖酵解、降低自身氧耗和释放促进血管新生因子等途径, 保护血脑屏障结构和功能稳定[57]。

2.3 高原低氧对周细胞的影响

周细胞是维持和促进血脑屏障通透性修复的关键。研究表明, 适度低氧使周细胞分泌 VEGF、血管生成素 (angiopoietin, Ang)、转化生长因子-β (transforming growth factor-β, TGF-β)、促红细胞生成素 (erythropoietin, EPO)、胶质细胞源性神经营养因子 (glial cell derived neurotrophic factor, GDNF) 和神经营养因子 (neurotrophin, NT) 等细胞因子维持血脑屏障通透性, 其中分泌最多的是 VEGF 和 Ang[58,59]。Nomura 等[60]发现脑缺氧期间周细胞表达血管内皮细胞生长因子受体-1 (vascular endothelial growth factor receptor 1, VEGFR-1), 其在空间上限制 VEGF 的信号传导, 进而维持血脑屏障结构和功能正常。Ribatti 等[61]表明周细胞分泌的 Ang-1 通过磷脂酰肌醇 3 激酶−丝氨酸−苏氨酸激酶信号途径促进血管平滑肌细胞成活, 维持血管完整性、减少血管外渗。高原低氧环境中 Ang-1 分泌量增加[62], 但血脑屏障中 Ang-1 如何变化还未见报道, 提示这可能是高原低氧环境下周细胞保护屏障通透性的另一种机制。

如前所述, 高原低氧环境使血脑屏障结构和功能发生一定变化, 如图 3, 但它们对低氧所致屏障变化对药物代谢的影响, 尤其是屏障功能障碍与药物转运间的联系常常被忽视。作者将结合现有文献分析探讨高原低氧环境中血脑屏障变化对药物代谢的影响及药物跨血脑屏障转运的调节作用和分子机制。

3 高原低氧环境中血脑屏障变化对药物代谢的影响和药物转运的调控机制

3.1 高原低氧环境中血脑屏障变化对药物代谢的影响

3.1.1 高原低氧环境中血脑屏障变化对药物转运体的影响 药物转运体参与药物吸收和跨屏障分布过程, 介导不同组织对药物及代谢产物的排泄[63]。主要协同转

Table 1 Effect of common central nervous system diseases on blood-brain barrier and drug transporters and associated drug metabolizing enzymes. ZO-1: Zonula occludens-1; P-gp: P-glycoprotein; BCRP: Breast cancer resistance protein; MRP: Multidrug resistance-associated protein; Mfsd2a: Major facilitator superfamily domain-containing protein 2a; OATP: Organic anion-transporting polypeptide; CYP: Cytochrome P. Protein expression, ↑: Increase; ↓: Decrease

Disease	Blood-brain barrier	Drug transporter	Drug metabolizing enzyme	Ref.
Multiple sclerosis	Tight junctions related protein expression changed	P-gp, ↓ BCRP, ↓ MRP1, ↓	CYP2B6 CYP2C9 CYP27B1 CYP2R1	[13−16]
Traumatic brain injury	Claudin-5, ↓ Caveolin-1, ↑	P-gp, ↓ Mfsd2a (possible), ↓	CYP1B1 CYP1A21 CYP2B1 CYP2D1 CYP3A2	[17−21]
Parkinson's disease	ZO-1, ↓ Occludin, ↓	P-gp, ↓ MRP2, ↑	CYP2D6 CYP2C19 CYP2B6 CYP1A2	[22−27]
Alzheimer's disease	Tight junctions abnormal	P-gp, ↓ MRP1, ↓ BCRP, ↑ MRP4, ↑ OATP2B1, ↓ Mfsd2a (possible), ↓	CYP3A CYP2D6	[17, 28−33]
Epilepsy	Tight junctions related protein expression changed	P-gp, ↑ BCRP, ↑ MRP1, ↑ MRP2, ↑	CYP3A4 CYP3A5 CYP2B6 CYP2D6	[16, 17, 34−37]
Stroke	Claudin-5, ↓ ZO-1, ↓ Caveolin-1, ↑	P-gp, ↑ MRP4, ↑ BCRP, ↑ MRP1, ↓ OATP1A2, ↑ Mfsd2a (possible), ↓	CYP2C19	[34, 38−43]
Brain tumor	ZO-1, ↓ Claudin-5, ↓	P-gp, ↑ BCRP, ↑ Mfsd2a (possible), ↓	CYP46A1 CYP1B1 CYP3A4	[17, 44−47]

运蛋白超家族是最大和最多样化的膜转运蛋白, 主要促进调解超家族蛋白2A (major facilitator superfamily domain-containing protein 2a, Mfsd2a) 是其成员之一, 主要功能为维持血脑屏障完整性、抑制中枢神经系统内皮细胞转运和减轻血脑屏障损伤[64]。Han等[65]提出Mfsd2a通过控制血脑屏障内皮细胞的脂质成分抑制小窝介导的胞吞作用调节血脑屏障通透性, 从而转运大分子药物。Shang等[66]发现低氧诱导因子-1α (hypoxia inducible factor-1α, HIF-1α) 和血红素加氧酶-1 (heme oxygenase 1, HO-1) 表达增加使Mfsd2a表达降低, 血脑屏障通透性增加, 但高原低氧环境下血脑屏障结构与功能变化对Mfsd2a及药物跨血脑屏障转运的影响还尚未可知。

ATP结合 (ATP-binding cassette, ABC) 转运蛋白和可溶性载体 (solute carrier, SLC) 转运蛋白共同参与药物跨血脑屏障转运[67]。血脑屏障中的ABC转运体为P-糖蛋白 (P-glycoprotein, P-gp)、乳腺癌耐药蛋白 (breast cancer resistance protein, BCRP) 和多药耐药相关蛋白 (multidrug resistance-associated protein, MRP) 等, 它们构成血脑屏障的主要转运系统, 一方面限制大多数药物通过血脑屏障, 导致中枢神经系统疾病治疗效果不佳; 另一方面介导药物在脑中的清除和分布, 维持中枢神经系统内稳态[68]。Ozgür等[69]探讨低氧对血脑屏障和药物转运体的影响, 发现低氧引起血脑屏障通透性增加和P-gp表达上调, 且P-gp底物地高辛双向运输研究表明地高辛在血脑屏障中的流出率较高, 提示低氧环境中P-gp表达上调减少药物转运, 影响中枢神经系统药物疗效。Zolotoff等[70]构建体外血脑屏障

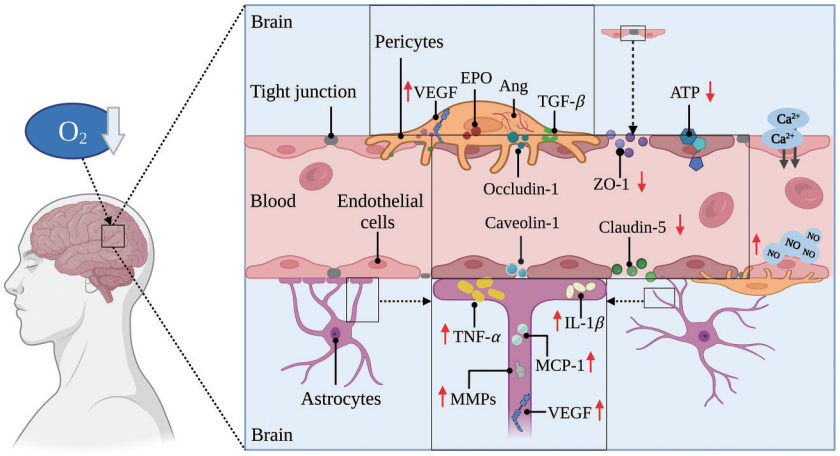

Figure 3 Changes of the structural and functional of blood-brain barrier under hypoxia. Under the condition of high-altitude hypoxia, Ca^{2+} influx, ATP content decreased, NO release increased, the expression of the tight junction proteins ZO-1 and claudin-5 was decreased in endothelial cells. The expression of IL-1β, TNF-α, MCP-1, VEGF increased, and the activity of MMP increased in astrocytes. Secretion cytokines of VEGF, Ang, TGF-β, EPO in pericytes. IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor-α; MCP-1: Monocyte chemotactic protein-1; VEGF: Vascular endothelial growth factor; MMP: Matrix metalloproteinase; Ang: Angiopoietin; TGF-β: Transforming growth factor-β; EPO: Erythropoietin

模型发现慢性间歇低氧 6 h 引发血脑屏障结构和功能障碍, P-gp 表达上调, BCRP 表达下调, 进而影响药物转运。Chatard 等[71]研究发现, 低氧应激增加血脑屏障通透性, 但通过上调 P-gp 和 MRP-1 表达建立防御机制, 减少血脑屏障损伤和药物转运障碍。SLC 转运蛋白主要包括有机阴离子转运多肽 (organic anion-transporting polypeptide, OATP)、有机阴离子转运蛋白 (organic anion transporters, OAT)、有机阳离子转运蛋白 (organic cation transporter, OCT) 和葡萄糖转运蛋白 1 (glucose transporter 1, GLUT1) 等, 它们介导多种药物转运[72]。Thompson 等[73]研究低氧/复氧条件下血脑屏障他汀类药物 OATP1A4 的调节和功能表达, 发现低氧/复氧 (6% O_2, 60 min, 21% O_2, 10 min) 后脑微血管内皮细胞 OATP1A4 表达上调, 且 OATP 抑制剂降低了他汀类药物的脑摄取, 表明 OATP1A4 参与了大脑药物转运。研究表明, 脑缺氧导致大脑能量代谢和葡萄糖利用发生变化, 血脑屏障中 GLUT1 表达增加, 促进 GLUT1 介导葡萄糖跨血脑屏障进入神经胶质细胞和神经元, 推测高原低氧环境中葡萄糖包裹的纳米药物载体具有将药物转运到中枢神经系统的潜力[74]。另外, 本课题组研究发现, 高原低氧环境下药物转运体功能发生变化, P-gp、MRP2、OATP2B1、BCRP 底物药物的吸收受到影响, 罗丹明 123、长春新碱、格列本脲的吸收均加快, 哌唑嗪的吸收减慢[75]。

以上研究表明, 高原低氧环境导致的血脑屏障障碍使不同药物转运体的表达发生变化, 影响药物分布和中枢神经系统疾病治疗, 见表 2[49-51,66,69-71,73,74]。不同药物转运体根据其功能和位置, 可保护中枢神经系统免受外源性毒素的干扰, 促进药物跨血脑屏障转运, 或限制药物进入血脑屏障成为中枢神经系统治疗的障碍。药物转运体的特殊功能提示血脑屏障透过率低的中枢神经系统药物, 可通过调控药物转运体的功能和修饰药物结构等技术, 增加药物与转运体的亲和力促进药物透过屏障。药物转运体在中枢神经系统药物转运中起关键作用, 探明高原低氧环境下血脑屏障变化对药物转运体的影响, 对促进药物跨血脑屏障转运具有重要意义。

3.1.2 高原低氧环境中血脑屏障变化对药物代谢酶的影响 药物代谢酶负责催化药物等外源性物质的代谢, 主要分为 I 相和 II 相药物代谢酶[76], 临床上近 90% 的药物相互作用是由 I 相药物代谢酶细胞色素 P450 (cytochrome P450, CYP450) 变化引起的。近年来, 脑组织中发现了多种 CYP450, 包括 CYP1、CYP2、CYP3 家族和 CYP46A1 等近 24 个 CYP450, 其中 CYP1A1、CYP1A2、CYP1B1、CYP2B6、CYP2D6 和 CYP3A4 表达较为丰富, 这些 CYP450 的表达、功能和调节在血脑屏障药物代谢中的作用越来越受到重视[77,78]。高原低氧环境中血脑屏障结构和功能变化后 CYP450 的表达也发生一定变化, Lu 等[79]研究显示, 新生小鼠大脑缺氧后血脑屏障通透性增加, 导致负责形成 24*S* 羟基胆固醇的 CYP46A1 表达上调, 从而促进 24*S* 羟基胆固醇排出中枢神经系统。Liu 等[80]研究发现, 低氧 3 h 后大鼠星形胶质细胞出现凋亡现象, 且 CYP2C11 表达显著增加。Jacob 等[81]在低氧环境干扰人脑微血管内皮细胞

芳香烃受体通路研究中发现低氧增加内皮细胞通透性，下调芳香烃受体靶基因CYP1A1和CYP1B1表达。血脑屏障的Ⅱ相药物代谢酶主要有尿苷5′-二磷酸葡萄糖醛酸转移酶 (UDP-glucuronosyl transferase, UGT) 和谷胱甘肽S-转移酶 (glutathione S-transferase, GST)[82,83]。研究报道，UGT1A催化多种药物、神经递质和神经甾体在大鼠不同脑区域的葡萄糖醛酸化，影响药物在血脑屏障的药理作用[84]。Xiong等[85]发现低温低氧环境导致大鼠血脑屏障中GST和谷胱甘肽过氧化物酶表达显著升高，影响中枢神经系统药物等内源性物质转运。以上结果显示，高原低氧环境中血脑屏障通透性增加影响药物代谢酶，使药物代谢和中枢神经系统药物转运发生变化，见表2[79-81,84,85]。

本课题组通过实地研究表明，大鼠急进氧分压为15.1 kPa的2 800 m低氧环境3天后CYP1A2的蛋白和mRNA表达均显著降低60.4%和51.1%，慢性饲养30天后显著降低62.0%和32.9%，急进氧分压为12.4 kPa的4 300 m低氧环境3天后CYP1A2的蛋白和mRNA表达均显著降低65.8%和37.2%，慢性饲养30天后显著降低64.8%和30.7%。在4 300 m低氧环境慢性饲养30天后CYP3A1的蛋白和mRNA表达分别降低27.5%和33.7%，CYP2E1分别降低33.9%和52.0%，CYP2D1均显著升高23.5%和34.7%，但急进4 300 m低氧环对CYP3A1、CYP2E1和CYP2D1的蛋白和mRNA表达无显著影响[86,87]。以上研究表明，高原低氧环境对药物代谢酶的活性和表达的影响是双向的，提示高原低氧环境可促进或降低咪达唑仑、对乙酰氨基酚和右美沙芬等中枢神经系统药物的体内代谢。此外，本课题组前期研究表明高原低氧环境下对乙酰氨基酚的药物代谢动力学参数发生显著变化，主要表现为急性和慢性低氧组半衰期 ($t_{1/2}$) 均显著延长21.7%和40.9%，血药浓度曲线下面积 (AUC) 均显著增加91.9%和133.9%，清除率 (CL) 均显著降低48.1%和56.9%[88]。截至目前，尚有研究表明苯巴比妥[89]、苯妥英钠[90]、哌替啶[91]、地西泮[92]、丁螺环酮[93]等中枢神经系统药物在高原低氧环境中的体内代谢减慢，但血脑屏障变化对药物代谢和药物转运的影响仍需进一步实验验证。

3.2 高原低氧环境中血脑屏障变化对药物转运的调控机制

前期研究认为，高原低氧环境中外周循环中性粒细胞以快速、大流量方式渗透中枢神经系统，主要堆积在脑微血管内皮细胞内，局部释放蛋白水解酶、活性氧类等，使血脑屏障中紧密连接蛋白ZO-1、claudin-5、occludin等重新分布，引起血脑屏障结构和功能改变，但高原低氧环境导致血脑屏障生理病理变化和药物代谢变化的内在调节机制有待进一步研究。综合文献报道，作者主要从转录因子、炎症因子、核受体、微小RNA (microRNA, miRNA) 和肠道菌群与药物转运体和药物代谢酶间的联系探讨相关机制。

3.2.1 转录因子 转录因子参与高原低氧环境所致的血脑屏障损伤，其中HIF-1α对血脑屏障及药物转运体和药物代谢酶具有重要调控作用。Engelhardt等[94]发现HIF-1α稳定表达刺激大鼠脑微血管内皮细胞中总蛋白激酶C，使总蛋白激酶C亚型从细胞质向紧密连接转移，引起ZO-1、claudin-5、occludin重排及磷酸化，增加血脑屏障通透性。研究显示，HIF-1α的靶基因VEGF促进ZO-1和occludin的酪氨酸磷酸化，导致紧密连接失调和血脑屏障通透性增加[95]。以上研究表明，HIF-1α通路激活诱导紧密连接相关蛋白和VEGF磷酸化，引起血脑屏障功能障碍。同时，HIF-1α可与低氧反应元件结合，调控血脑屏障P-gp、BCRP、MPR1和CYP2C11、CYP4A的表达，影响药物代谢[80,96,97]。由此，推测HIF-1α等转录因子的表达及分子机制变化可能成为调节药物跨血脑屏障转运影响药物代谢的潜在靶点。

3.2.2 炎症因子 血脑屏障是炎症因子的调节界面，可选择性转运炎症因子，低氧诱导血脑屏障表达TNF-α、IL-1β、白介素-6 (interleukin-6, IL-6)、TGF-β和成纤维细胞生长因子 (basic fibroblast growth factor, bFGF) 等炎症因子[98,99]。Witt等[100]报道低氧环境中血脑屏障

Table 2 Changes in the blood-brain barrier and drug transporters and drug metabolizing enzymes under hypoxia. OAT: Organic anion transporters; OCT: Organic cation transporter; GLUT1: Glucose transporter 1; UGT: UDP-glucuronosyl transferase; GST: Glutathione *S*-transferase. Protein expression, ↑: Increase; ↓: Decrease

Blood-brain barrier	Drug transporter	Drug metabolizing enzyme	Ref.
ZO-1, ↓	Mfsd2a (possible), ↓	CYP1A1, ↓	[49−51]
Claudin-5, ↓	P-gp, ↑	CYP1B1, ↓	[66, 69−71, 73, 74]
Occludin, ↓	BCRP, ↓	CYP2C11, ↑	[79−81, 84, 85]
Caveolin-1, −	MRP-1, ↑	CYP46A1, ↑	
Increased permeability	OATP1A4, ↑	UGT1A1, −	
	OAT, −	GST, ↑	
	OCT, −		
	GLUT1, ↑		

TNF-α、IL-1β和IL-6表达变化使白细胞外渗至脑实质, 导致紧密连接失调, 血脑屏障通透性增加。此外, 低氧环境中TNF-α、IL-6和IL-1β参与调节肝脏CYP1A2、CYP2C19、CYP2D6和CYP2E1表达[68], 而血脑屏障中也存在大量药物代谢酶, 表明炎症因子也可能参与调节血脑屏障药物代谢酶的表达。Han等[65]发现Mfsd2a基因敲除小鼠具有较高的血脑屏障转运率, 且TGF-β和bFGF表达量发生变化, 表示血脑屏障中Mfsd2a表达受TGF-β和bFGF信号通路的协同调控。也有研究表明, 低氧使TGF-β和bFGF表达升高[99], 但是否会通过TGF-β和bFGF表达升高导致Mfsd2a水平失调, 增加胞吞作用, 改变血脑屏障通透性, 影响药物转运还需进一步研究和验证。

3.2.3 核受体 核受体是调控药物代谢酶和药物转运体的重要调节因子, 包括孕烷X受体 (pregnane X receptor, PXR) 和组成型雄烷受体 (constitutive androstane receptor, CAR) 等。血脑屏障中PXR和CAR被许多内源性和外源性物质激活, 调节下游药物代谢酶的表达与活性, 如CYP1A1、CYP2B6、CYP2D6、CYP3A4和CYP2E1等[101]。低氧显著降低PXR和CAR的表达, 研究发现低氧增加血脑屏障通透性引发的信号级联反应通过磷酸化和核因子κB (nuclear factor-kappa B, NF-κB) 激活调节血脑屏障PXR和CAR的表达, 而NF-κB直接或间接调节药物代谢酶表达[102]。同样, 血脑屏障中PXR和CAR也参与P-gp和BCRP等药物转运体的表达调控, 影响脑中药物代谢[103]。以上研究表明, 低氧环境可能通过增加血脑屏障通透性和激活NF-κB信号通路抑制或促进核受体的表达, 并与药物代谢酶和药物转运体相互作用, 调节药物跨血脑屏障转运。

3.2.4 miRNA miRNA参与脑内药物的生物转化, 脑微血管内皮细胞中有166种miRNA, 其中miR-34a、miR-99b、miR-20b和miR-337-3p在内皮细胞中表达量较高, 且在血脑屏障的功能调节中发挥重要调控作用[104]。Bukeirat等[105]发现miR-34a表达触发血脑屏障功能障碍, 减少线粒体氧化磷酸化, 降低细胞色素C水平, 导致内源性物质跨膜转运变化, 表明miR-34a通过调控血脑屏障通透性和线粒体功能, 影响中枢神经系统药物转运。Wang等[106]研究表明脑缺血缺氧使血脑屏障miR-337-3p水平升高, 大鼠尾静脉给予化学修饰的miR-337抑制剂后, 其可跨血脑屏障运输, 引发miR-337-3p下调并减少缺血缺氧引起的脑损伤和神经功能障碍。本课题组研究显示Caco-2细胞中miRNA-873-5p介导低氧环境对MDR1和PXR表达, 发挥调节药物代谢作用, 表明低氧环境中miRNA调控药物转运体和核受体表达[107]。根据以上研究, 高原低氧环境中血脑屏障miRNA是否参与药物转运体和核受体表达调控, 影响药物跨血脑屏障转运还需进一步研究验证, 且低氧环境中血脑屏障miRNA表达变化及其与药物代谢间的联系有望成为诊断中枢神经系统疾病的新靶点。

3.2.5 肠道菌群 肠道菌群是影响药物体内生物转化的重要因素, 研究显示肠道菌群参与合成和释放γ-氨基丁酸、褪黑激素、儿茶酚胺、组胺、乙酰胆碱、血清素、犬尿氨酸等, 这些神经活性分子和炎症因子通过血液进入血脑屏障, 参与中枢神经系统药物代谢过程[108]。Li等[109]将酪酸梭菌定植于无菌小鼠后紧密连接相关蛋白上调, 血脑屏障通透性降低。Welcome[110]发现肠道菌群紊乱使变形杆菌等致病菌促进毒性物质和炎症因子产生, 造成肠屏障损伤, 激活肠神经系统, 引起脑肠轴功能紊乱和血脑屏障功能障碍, 引发中枢神经系统炎症反应。Yang等[111]研究表明低氧通过诱导信号分子, 向脑肠轴提供反馈, 使肠道菌紊乱和中枢神经系统功能障碍, 表明脑肠轴或可成为影响血脑屏障转运内源性物质的重要通道。课题组研究发现高原低氧环境中厚壁菌、阿克曼菌和乳杆菌等菌群紊乱调节药物代谢酶和药物转运体转录, 影响药物代谢[112,113]。以上研究显示, 肠道菌群作为机体重要的调控参与者是中枢神经系统疾病的易感因素, 低氧环境中特征性菌群紊乱可通过激活脑肠轴途径间接引起血脑屏障结构和功能变化, 参与药物代谢酶和药物转运体调节, 影响中枢神经系统药物转运。

高原低氧环境中, 血脑屏障结构和功能变化是否通过转录因子、炎症因子、核受体、miRNA和肠道菌群等相互作用影响药物代谢和转运亟需探究与验证。综上, 作者推测了以下五种分子机制: ① 低氧环境激活转录因子和炎症因子, 使紧密连接相关蛋白重排并磷酸化, 引发血脑屏障结构和功能障碍, 药物转运体和药物代谢酶功能和表达变化。② 高原低氧导致Mfsd2a水平失调, 增加胞吞作用和血脑屏障通透性, 改变药物转运体和药物代谢酶的功能和表达, 影响药物转运。③ 高原低氧增加血脑屏障通透性和诱导NF-κB信号通路, 调节核受体表达, 转录调控药物转运体和药物代谢酶, 影响药物转运。④ 高原低氧上调或下调血脑屏障中关键性miRNA表达, 直接或间接参与药物转运体或核受体调控, 使药物跨屏障转运发生变化。⑤ 高原低氧环境中特征性菌群紊乱使肠上皮细胞通过上行途径向中枢神经系统传递信号, 导致脑肠轴功能障碍及血脑屏障结构和功能变化, 间接参与药物代谢酶和药物转运体调节, 影响中枢神经系统药物转运, 如图4。

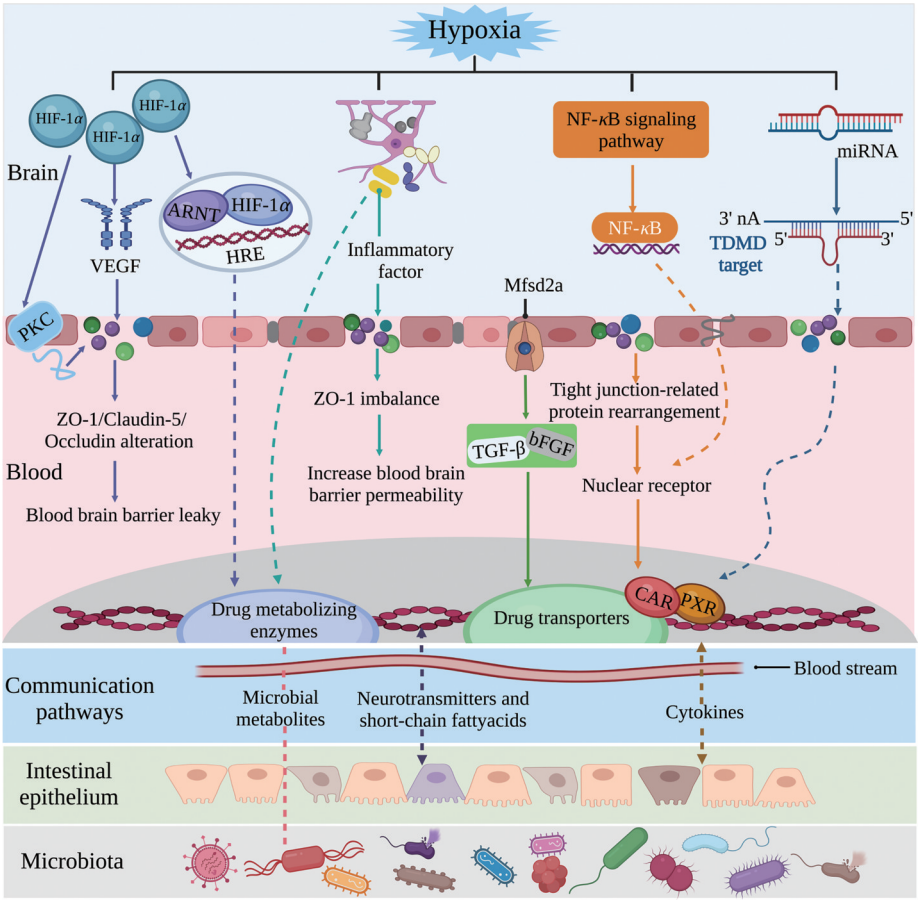

Figure 4 A hypothetical network view on the regulation of blood-brain barrier and drug transport in the central nervous system under hypoxia. The network contains of the five regulatory mechanisms of drug transport across the blood-brain barrier under high-altitude hypoxia. ① The high-altitude hypoxic environment activates transcription factors and inflammatory factors, rearrangement and phosphorylation of tight junction-associated proteins, inducing structural and functional disorders of the blood-brain barrier, and changes in the function and expression of drug transporters and drug metabolizing enzymes. ② Hypoxia causes imbalance of Mfsd2a levels, increases endocytosis and blood-brain barrier permeability, alters the function and expression of drug transporters and drug metabolizing enzymes, and affects drug metabolism. ③ High-altitude hypoxic increases blood-brain barrier permeability and induces NF-κB signaling pathway, regulates nuclear receptor expression, transcriptional regulation of drug transporters and drug metabolizing enzymes, and affects drug transport. ④ High-altitude hypoxic up or down-regulates critical miRNA expression in the blood-brain barrier, and involved in drug transporter or nuclear receptor regulation, resulting in changes of drug transport across the barrier. ⑤ Characteristic microbiota disorders cause intestinal epithelial cells to transmit signals to the central nervous system *via* the enteric nervous system under high-altitude hypoxia, it leads to brain-gut axis and barrier dysfunction, indirectly involved in the regulation of drug metabolizing enzymes and drug transporters, affecting central nervous system drug transport. HIF-1α: Hypoxia inducible factor-1α; ARNT: Aryl hydrocarbon receptor nuclear translocator; HRE: Hypoxia responsive element; PKC: Protein kinase C; bFGF: Basic fibroblast growth factor; NF-κB: Nuclear factor-kappa B; PXR: Pregnane X receptor; CAR: Constitutive androstane receptor; miRNA: microRNA

4 小结与展望

高原低氧环境对机体物质代谢具有显著影响, 阻碍机体中枢神经系统正常功能, 导致药物在体内的药物代谢动力学特征、药物转运体和药物代谢酶的活性与表达发生变化, 进一步影响药物疗效。研究高原低氧环境中药物跨血脑屏障转运的调节作用及机制, 可以促进中枢神经系统药物转运, 提升药物疗效, 避免不良反应的发生。此外, 探究高原低氧环境调节中枢神经系统药物转运的靶点, 可以为新药研发和高原地区中枢神经系统临床合理用药提供新的理论依据。

近年来, 人类高原活动日渐频繁, 高原旅游、商贸、体育训练等人群日益增多, 急进高原引起的头晕、头痛、共济失调、颅内压升高和脑水肿等中枢神经系统疾病严重威胁人类健康。充分了解低氧环境中枢神经系统解剖、生理病理变化, 对临床认识和治疗中枢神经系统疾病有很大帮助。大多数研究者把目光投向中枢神

经系统的特异性细胞—神经元, 但低氧环境在引起神经元损伤前, 血脑屏障通透性增加或功能异常早已发生, 且血脑屏障变化与药物转运密不可分。因此, 研究高原低氧环境中血脑屏障结构和功能及其分子机制变化不乏成为高原地区中枢神经系统临床合理用药和疾病治疗的关键, 这同样也是中枢神经系统药物开发亟待解决的难题。

低氧环境诱导的血脑屏障结构和功能异常可通过屏障通透性变化指导中枢神经系统用药, 但单纯通过低氧促进或抑制药物进入血脑屏障减少或增加用药量, 虽具有一定用药指导意义, 但改变后的用药量将对脑组织或机体其他组织产生毒副作用。目前, 大量动物实验表明低氧增加血脑屏障通透性, 影响药物正常转运过程, 药物转运涉及屏障物理类、运输类和代谢类等转运系统。据此推断, 高原低氧环境中血脑屏障经历各种生理病理变化, 并通过多种调节机制影响药物转运系统, 这种调节包括屏障结构和功能、药代动力学特征、药物转运体和药物代谢酶等变化, 其中药物转运体和药物代谢酶作为调节药物代谢重要因素, 对中枢神经系统药物转运起关键和核心作用。

血脑屏障中药物转运体限制药物进入中枢神经系统, 成为中枢神经系统药物转运的巨大障碍, 许多研究都集中于开发抑制剂或发现调节信号通路, 如开发P-gp的抑制剂或将药物修饰成类似于P-gp底物的结构, 被血脑屏障P-gp识别后, 促进药物向中枢神经系统转运[8]。这些策略不仅可抑制药物转运体的功能和表达升高, 还有效降低了游离药物浓度, 减轻脱靶效应, 提高中枢神经系统药物疗效。因此, 了解药物转运体在血脑屏障的调节机制有助于促进大脑对中枢神经系统药物的摄取, 提升药物有效性。血脑屏障中药物代谢酶影响中枢神经系统药物代谢和处置, 在减少大脑药物蓄积中发挥重要作用, 明确血脑屏障药物代谢酶的调控途径可为屏障通透性调节和药物转运提供参考依据。然而, 作者对高原低氧环境中血脑屏障药物转运体和药物代谢酶的表达和调节机制及它们与中枢神经系统疾病间关系的了解尚未全面, 且血脑屏障药物转运体作为多种中枢神经系统疾病治疗靶点的体内和体外研究数据十分有限。

本课题组前期研究表明高原低氧环境对大鼠血液、肝脏、肠道中的药物代谢动力学、药物代谢酶和药物转运体具有一定影响, 但低氧环境下血脑屏障与药物代谢的研究还鲜有报道, 且血脑屏障介导高原低氧对药物代谢的影响是通过药物转运体和药物代谢酶、转录因子、炎症因子及核受体等多因素调控的机制, 其表达变化是抑制还是促进药物跨血脑屏障转运尚未有定论, 仍需大量实验进行验证。明确血脑屏障介导高原低氧对中枢神经系统药物转运的影响虽具有挑战性, 但对高原地区血脑屏障结构和功能变化的认识, 有助于解释脑部疾病的诊断和预后以及制定合理的药理和非药理干预策略, 促进中枢神经系统药物向血脑屏障转运。另外, 研究高原低氧环境下血脑屏障变化与药物代谢的联系, 对高原人群中枢神经系统临床用药重新评估给药剂量, 制定符合高原地区的中枢神经系统用药标准具有重要意义。

综上, 高原低氧环境中血脑屏障变化与药物代谢间的关系对药物转运具有十分重要的作用和意义, 深入研究血脑屏障在高原低氧环境中与药物代谢之间的关系可为高原地区的人群提供更有效和更安全的药物治疗。

作者贡献: 李向阳、刘贵琴撰写稿件并总结图表; 白雪、杨建鑫、段雅彬负责论文内容核对; 朱俊博、田露参与论文相关内容补充。

利益冲突: 无任何利益冲突。

References

[1] Daneman R, Prat A. The blood-brain barrier [J]. Cold Spring Harb Perspect Biol, 2015, 7: a020412.

[2] Bonkowsky JL, Son JH. Hypoxia and connectivity in the developing vertebrate nervous system [J]. Dis Model Mech, 2018, 11: dmm037127.

[3] Langen UH, Ayloo S, Gu C. Development and cell biology of the blood-brain barrier [J]. Annu Rev Cell Dev Biol, 2019, 35: 591-613.

[4] Kadry H, Noorani B, Cucullo L. A blood-brain barrier overview on structure, function, impairment, and biomarkers of integrity [J]. Fluids Barriers CNS, 2020, 17: 69.

[5] Abdullahi W, Tripathi D, Ronaldson PT. Blood-brain barrier dysfunction in ischemic stroke: targeting tight junctions and transporters for vascular protection [J]. Am J Physiol Cell Physiol, 2018, 315: C343-C356.

[6] Miller DS. Regulation of ABC transporters at the blood-brain barrier [J]. Clin Pharmacol Ther, 2015, 97: 395-403.

[7] Kuban W, Daniel WA. Cytochrome P450 expression and regulation in the brain [J]. Drug Metab Rev, 2021, 53: 1-29.

[8] Harilal S, Jose J, Parambi DGT, et al. Revisiting the blood-brain barrier: a hard nut to crack in the transportation of drug molecules [J]. Brain Res Bull, 2020, 160: 121-140.

[9] Pandit R, Chen L, Götz J. The blood-brain barrier: physiology and strategies for drug delivery [J]. Adv Drug Deliv Rev, 2020, 165-166: 1-14.

[10] Wang Q, Zuo Z. Impact of transporters and enzymes from blood-cerebrospinal fluid barrier and brain parenchyma on CNS drug

uptake [J]. Expert Opin Drug Metab Toxicol, 2018, 14: 961-972.

[11] Neumaier F, Zlatopolskiy BD, Neumaier B. Drug penetration into the central nervous system: pharmacokinetic concepts and *in vitro* model systems [J]. Pharmaceutics, 2021, 13: 1542.

[12] Liebner S, Dijkhuizen RM, Reiss Y, et al. Functional morphology of the blood-brain barrier in health and disease [J]. Acta Neuropathol, 2018, 135: 311-336.

[13] Kooij G, Mizee MR, van Horssen J, et al. Adenosine triphosphate-binding cassette transporters mediate chemokine (C-C motif) ligand 2 secretion from reactive astrocytes: relevance to multiple sclerosis pathogenesis [J]. Brain, 2011, 134: 555-570.

[14] Nicolas JM, Chanteux H, Nicolai J, et al. Role of P-glycoprotein in the brain disposition of seletalisib: evaluation of the potential for drug-drug interactions [J]. Eur J Pharm Sci, 2020, 142: 105122.

[15] de Castro FA, Simões BP, Coelho EB, et al. Enantioselectivity in the metabolism of cyclophosphamide in patients with multiple or systemic sclerosis [J]. J Clin Pharmacol, 2017, 57: 784-795.

[16] Martinez-Hernandez A, Perez-Guerrero EE, Macias-Islas MA, et al. Polymorphisms CYP2R1 rs10766197 and CYP27B1 rs10877012 in multiple sclerosis: a case-control study [J]. J Immunol Res, 2021, 2021: 7523997.

[17] Rodriguez-Grande B, Ichkova A, Lemarchant S, et al. Early to long-term alterations of CNS barriers after traumatic brain injury: considerations for drug development [J]. AAPS J, 2017, 19: 1615-1625.

[18] Badaut J, Ajao DO, Sorensen DW, et al. Caveolin expression changes in the neurovascular unit after juvenile traumatic brain injury: signs of blood-brain barrier healing? [J]. Neuroscience, 2015, 285: 215-226.

[19] Han L, Jiang C. Evolution of blood-brain barrier in brain diseases and related systemic nanoscale brain-targeting drug delivery strategies [J]. Acta Pharm Sin B, 2021, 11: 2306-2325.

[20] Lidin E, Sköld MK, Angéria M, et al. Hippocampal expression of cytochrome P450 1B1 in penetrating traumatic brain injury [J]. Int J Mol Sci, 2022, 23: 722.

[21] Ma J, Wang J, Cheng J, et al. Impacts of blast-induced traumatic brain injury on expressions of hepatic cytochrome P450 1A2, 2B1, 2D1, and 3A2 in rats [J]. Cell Mol Neurobiol, 2017, 37: 111-120.

[22] Sweeney MD, Zhao Z, Montagne A, et al. Blood-brain barrier: from physiology to disease and back [J]. Physiol Rev, 2019, 99: 21-78.

[23] Chen XS, Lan X, Roche I, et al. Caffeine protects against MPTP-induced blood-brain barrier dysfunction in mouse striatum [J]. J Neurochem, 2008, 107: 1147-1157.

[24] Kortekaas R, Leenders KL, van Oostrom JC, et al. Blood-brain barrier dysfunction in parkinsonian midbrain *in vivo* [J]. Ann Neurol, 2005, 57: 176-179.

[25] Chen T, Li Y, Li C, et al. Pluronic P85/F68 micelles of baicalein could interfere with mitochondria to overcome MRP2-mediated efflux and offer improved anti-parkinsonian activity [J]. Mol Pharm, 2017, 14: 3331-3342.

[26] Ur Rasheed MS, Mishra AK, Singh MP. Cytochrome P450 2D6 and Parkinson's disease: polymorphism, metabolic role, risk and protection [J]. Neurochem Res, 2017, 42: 3353-3361.

[27] Agúndez JA, García-Martin E, Alonso-Navarro H, et al. Anti-Parkinson's disease drugs and pharmacogenetic considerations [J]. Expert Opin Drug Metab Toxicol, 2013, 9: 859-874.

[28] Bruckmann S, Brenn A, Grube M, et al. Lack of P-glycoprotein results in impairment of removal of beta-amyloid and increased intraparenchymal cerebral amyloid angiopathy after active immunization in a transgenic mouse model of Alzheimer's disease [J]. Curr Alzheimer Res, 2017, 14: 656-667.

[29] Krohn M, Lange C, Hofrichter J, et al. Cerebral amyloid-β proteostasis is regulated by the membrane transport protein ABCC1 in mice [J]. J Clin Investig, 2011, 121: 3924-3931.

[30] Xiong H, Callaghan D, Jones A, et al. ABCG2 is upregulated in Alzheimer's brain with cerebral amyloid angiopathy and may act as a gatekeeper at the blood-brain barrier for Aβ_{1-40} peptides [J]. J Neurosci, 2009, 29: 5463-5475.

[31] Wijesuriya HC, Bullock JY, Faull RL, et al. ABC efflux transporters in brain vasculature of Alzheimer's subjects [J]. Brain Res, 2010, 1358: 228-238.

[32] Storelli F, Billington S, Kumar AR, et al. Abundance of P-glycoprotein and other drug transporters at the human blood-brain barrier in Alzheimer's disease: a quantitative targeted proteomic study [J]. Clin Pharmacol Ther, 2021, 109: 667-675.

[33] Kagawa Y, Yamamoto Y, Ueno A, et al. Impact of CYP2D6, CYP3A5, and ABCB1 polymorphisms on plasma concentrations of donepezil and its metabolite in patients with Alzheimer disease [J]. Ther Drug Monit, 2021, 43: 429-435.

[34] Liu JS, He YJ, Zhang J, et al. Functionalized nanocarrier combined seizure-specific vector with P-glycoprotein modulation property for antiepileptic drug delivery [J]. Biomaterials, 2015, 74: 64-76.

[35] Dazert P, Suofu Y, Grube M, et al. Differential regulation of transport proteins in the periinfarct region following reversible middle cerebral artery occlusion in rats [J]. Neuroscience, 2006, 142: 1071-1079.

[36] van Vliet EA, Redeker S, Aronica E, et al. Expression of multidrug transporters MRP1, MRP2, and BCRP shortly after status epilepticus, during the latent period, and in chronic epileptic rats [J]. Epilepsia, 2005, 46: 1569-1580.

[37] Makuch-Kocka A, Andres-Mach M, Zagaja M, et al. Effect of chronic administration of 5-(3-chlorophenyl)-4-hexyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (TP-315)-a new anticonvulsant drug candidate-on living organisms [J]. Int J Mol Sci, 2021, 22: 3358.

[38] Huang L, Chen Y, Liu R, et al. P-glycoprotein aggravates blood

brain barrier dysfunction in experimental ischemic stroke by inhibiting endothelial autophagy [J]. Aging Dis, 2022, 13: 1546-1561.

[39] Al-Ahmady ZS, Jasim D, Ahmad SS, et al. Selective liposomal transport through blood brain barrier disruption in ischemic stroke reveals two distinct therapeutic opportunities [J]. ACS Nano, 2019, 13: 12470-12486.

[40] Abdullahi W, Davis TP, Ronaldson PT. Functional expression of P-glycoprotein and organic anion transporting polypeptides at the blood-brain barrier: understanding transport mechanisms for improved CNS drug delivery? [J]. AAPS J, 2017, 19: 931-939.

[41] Albekairi TH, Vaidya B, Patel R, et al. Brain delivery of a potent opioid receptor agonist, biphalin during ischemic stroke: role of organic anion transporting polypeptide (OATP) [J]. Pharmaceutics, 2019, 11: 467.

[42] Knowland D, Arac A, Sekiguchi KJ, et al. Stepwise recruitment of transcellular and paracellular pathways underlies blood-brain barrier breakdown in stroke [J]. Neuron, 2014, 82: 603-617.

[43] Wang Y, Meng X, Wang A, et al. Ticagrelor versus clopidogrel in CYP2C19 loss-of-function carriers with stroke or TIA [J]. N Engl J Med, 2021, 385: 2520-2530.

[44] Robey RW, Pluchino KM, Hall MD, et al. Revisiting the role of efflux pumps in multidrug-resistant cancer [J]. Nat Rev Cancer, 2018, 18: 452-464.

[45] Tiwary S, Morales JE, Kwiatkowski SC, et al. Metastatic brain tumors disrupt the blood-brain barrier and alter lipid metabolism by inhibiting expression of the endothelial cell fatty acid transporter mfsd2a [J]. Sci Rep, 2018, 8: 8267.

[46] Han M, Wang S, Yang N, et al. Therapeutic implications of altered cholesterol homeostasis mediated by loss of CYP46A1 in human glioblastoma [J]. EMBO Mol Med, 2020, 12: e10924.

[47] Ziegler N, Awwad K, Fisslthaler B, et al. β-Catenin is required for endothelial CYP1b1 regulation influencing metabolic barrier function [J]. J Neurosci, 2016, 36: 8921-8935.

[48] Tsao CC, Baumann J, Huang SF, et al. Pericyte hypoxia-inducible factor-1 (HIF-1) drives blood-brain barrier disruption and impacts acute ischemic stroke outcome [J]. Angiogenesis, 2021, 24: 823-842.

[49] Berndt P, Winkler L, Cording J, et al. Tight junction proteins at the blood-brain barrier: far more than claudin-5 [J]. Cell Mol Life Sci, 2019, 76: 1987-2002.

[50] Fischer S, Renz D, Kleinstück J, et al. *In vitro* effects of anaesthetic agents on the blood-brain barrier [J]. Anaesthesist, 2004, 53: 1177-1184.

[51] Zhang Y, Li X, Qiao S, et al. Occludin degradation makes brain microvascular endothelial cells more vulnerable to reperfusion injury *in vitro* [J]. J Neurochem, 2021, 156: 352-366.

[52] Park TE, Mustafaoglu N, Herland A, et al. Hypoxia-enhanced blood-brain barrier chip recapitulates human barrier function and shuttling of drugs and antibodies [J]. Nat Commun, 2019, 10: 2621.

[53] Amin N, Chen S, Ren Q, et al. Hypoxia inducible factor-1α attenuates ischemic brain damage by modulating inflammatory response and glial activity [J]. Cells, 2021, 10: 1359.

[54] Lu DY, Yu WH, Yeh WL, et al. Hypoxia-induced matrix metalloproteinase-13 expression in astrocytes enhances permeability of brain endothelial cells [J]. J Cell Physiol, 2009, 220: 163-173.

[55] Mojsilovic-Petrovic J, Callaghan D, Cui H, et al. Hypoxia-inducible factor-1 (HIF-1) is involved in the regulation of hypoxia-stimulated expression of monocyte chemoattractant protein-1 (MCP-1/CCL2) and MCP-5 (Ccl12) in astrocytes [J]. J Neuroinflammation, 2007, 4: 12.

[56] Stamatovic SM, Keep RF, Kunkel SL, et al. Potential role of MCP-1 in endothelial cell tight junction 'opening': signaling *via* Rho and Rho kinase [J]. J Cell Sci, 2003, 116: 4615-4628.

[57] Barialai L, Strecker MI, Luger AL, et al. AMPK activation protects astrocytes from hypoxia induced cell death [J]. Int J Mol Med, 2020, 45: 1385-1396.

[58] Tjakra M, Wang Y, Vania V, et al. Overview of crosstalk between multiple factor of transcytosis in blood brain barrier [J]. Front Neurosci, 2020, 13: 1436.

[59] Al Ahmad A, Gassmann M, Ogunshola OO. Maintaining blood-brain barrier integrity: pericytes perform better than astrocytes during prolonged oxygen deprivation [J]. J Cell Physiol, 2009, 218: 612-622.

[60] Nomura M, Yamagishi S, Harada S, et al. Possible participation of autocrine and paracrine vascular endothelial growth factors in hypoxia-induced proliferation of endothelial cells and pericytes [J]. J Biol Chem, 1995, 270: 28316-28324.

[61] Ribatti D, Nico B, Crivellato E. The role of pericytes in angiogenesis [J]. Int J Dev Biol, 2011, 55: 261-268.

[62] Tanaka T, Nangaku M. Angiogenesis and hypoxia in the kidney [J]. Nat Rev Nephrol, 2013, 9: 211-222.

[63] Jala A, Ponneganti S, Vishnubhatla DS, et al. Transporter-mediated drug-drug interactions: advancement in models, analytical tools, and regulatory perspective [J]. Drug Metab Rev, 2021, 53: 285-320.

[64] Wood CAP, Zhang J, Aydin D, et al. Structure and mechanism of blood-brain-barrier lipid transporter MFSD2A [J]. Nature, 2021, 596: 444-448.

[65] Han L, Qu Q, Aydin D, et al. Structure and mechanism of the SGLT family of glucose transporters [J]. Nature, 2022, 601: 274-279.

[66] Shang J, Yamashita T, Tian F, et al. Chronic cerebral hypoperfusion alters amyloid-β transport related proteins in the cortical blood vessels of Alzheimer's disease model mouse [J]. Brain Res, 2019, 1723: 146379.

[67] He YZ, Wang H, Fang JH, et al. Research progress on the traditional Chinese medicine-pharmaceutical drug interaction mediated by the ABC transporter family [J]. Acta Pharm Sin (药学学报),

2021, 56: 1778-1788.

[68] Taccola C, Cartot-Cotton S, Valente D, et al. High brain distribution of a new central nervous system drug candidate despite its P-glycoprotein-mediated efflux at the mouse blood-brain barrier [J]. Eur J Pharm Sci, 2018, 17: 68-79.

[69] Ozgür B, Helms HCC, Tornabene E, et al. Hypoxia increases expression of selected blood-brain barrier transporters GLUT-1, P-gp, SLC7A5 and TFRC, while maintaining barrier integrity, in brain capillary endothelial monolayers [J]. Fluids Barriers CNS, 2022, 19: 1-17.

[70] Zolotoff C, Voirin AC, Puech C, et al. Intermittent hypoxia and its impact on Nrf2/HIF-1α expression and ABC transporters: an *in vitro* human blood-brain barrier model study [J]. Cell Physiol Biochem, 2020, 54: 1231-1248.

[71] Chatard M, Puech C, Roche F, et al. Hypoxic stress induced by hydralazine leads to a loss of blood-brain barrier integrity and an increase in efflux transporter activity [J]. PLoS One, 2016, 11: e0158010.

[72] Schäfer AM, Meyer Zu Schwabedissen HE, Grube M. Expression and function of organic anion transporting polypeptides in the human brain: physiological and pharmacological implications [J]. Pharmaceutics, 2021, 13: 834.

[73] Thompson BJ, Sanchez-Covarrubias L, Slosky LM, et al. Hypoxia/reoxygenation stress signals an increase in organic anion transporting polypeptide 1a4 (Oatp1a4) at the blood-brain barrier: relevance to CNS drug delivery [J]. J Cereb Blood Flow Metab, 2014, 34: 699-707.

[74] Veys K, Fan Z, Ghobrial M, et al. Role of the GLUT1 glucose transporter in postnatal CNS angiogenesis and blood-brain barrier integrity [J]. Circ Res, 2020, 127: 466-482.

[75] Duan YB, Zhu JB, Yang JX, et al. Effect of hypoxia on drug metabolizing enzymes and transporters and the role of microRNA [J]. Acta Pharm Sin (药学学报), 2021, 56: 50-60.

[76] Guengerich FP. Cytochrome P450 research and the journal of biological chemistry [J]. J Biol Chem, 2019, 294: 1671-1680.

[77] Fanni D, Pinna F, Gerosa C, et al. Anatomical distribution and expression of CYP in humans: neuropharmacological implications [J]. Drug Dev Res, 2021, 82: 628-667.

[78] Borsini A, Nicolaou A, Camacho-Muñoz D, et al. Omega-3 polyunsaturated fatty acids protect against inflammation through production of LOX and CYP450 lipid mediators: relevance for major depression and for human hippocampal neurogenesis [J]. Mol Psychiatry, 2021, 26: 6773-6788.

[79] Lu F, Zhu J, Guo S, et al. Upregulation of cholesterol 24-hydroxylase following hypoxia-ischemia in neonatal mouse brain [J]. Pediatr Res, 2018, 83: 1218-1227.

[80] Liu M, Alkayed NJ. Hypoxic preconditioning and tolerance *via* hypoxia inducible factor (HIF) 1α-linked induction of P450 2C11 epoxygenase in astrocytes [J]. J Cereb Blood Flow Metab, 2005, 25: 939-948.

[81] Jacob A, Potin S, Saubaméa B, et al. Hypoxia interferes with aryl hydrocarbon receptor pathway in hCMEC/D_3 human cerebral microvascular endothelial cells [J]. J Neurochem, 2015, 132: 373-383.

[82] Ouzzine M, Gulberti S, Ramalanjaona N, et al. The UDP-glucuronosyltransferases of the blood-brain barrier: their role in drug metabolism and detoxication [J]. Front Cell Neurosci, 2014, 8: 349.

[83] Koehn LM, Dziegielewska KM, Mollgard K, et al. Developmental differences in the expression of ABC transporters at rat brain barrier interfaces following chronic exposure to diallyl sulfide [J]. Sci Rep, 2019, 9: 5998.

[84] Zhang ZQ, Sheng L, Li Y. Drug glucuronidation and disposition in brain [J]. Acta Pharm Sin (药学学报), 2016, 51: 1674-1680.

[85] Xiong M, Chen LX, Ma SM, et al. Short-term effects of hypothermia on axonal injury, preoligodendrocyte accumulation and oligodendrocyte myelination after hypoxia-ischemia in the hippocampus of immature rat brain [J]. Dev Neurosci, 2013, 35: 17-27.

[86] Li XY, Wang XJ, Li YP, et al. Effect of exposure to acute and chronic high-altitude hypoxia on the activity and expression of CYP1A2, CYP2D6, CYP2C9, CYP2C19 and NAT2 in rats [J]. Pharmacology, 2014, 93: 76-83.

[87] Li XY, Wang XJ, Li YP, et al. The activity, protein, and mRNA expression of CYP2E1 and CYP3A1 in rats after exposure to acute and chronic high altitude hypoxia [J]. High Alt Med Bio, 2014, 15: 491-496.

[88] Nian YQ. Effect of Simulated High Altitude Hypoxia on Pharmacokinetics of Metformin Hydrochloride and Acetaminophen (模拟高原低氧对盐酸二甲双胍和对乙酰氨基酚药代动力学特征的影响) [D]. Xining: Qinghai University, 2019.

[89] du Souich P, Varin F, Courteau H. Effect of hypercapnia and/or hypoxemia and metabolic acidosis on kinetics and concentrations of phenytoin in the cerebrospinal fluid of conscious rabbits [J]. Neuropharmacology, 1986, 25: 857-862.

[90] Anjana GV, Krishna K, Joginder D. Effect of intermittent hypobaric hypoxia on efficacy & clearance of drug [J]. Indian J Med Res, 2012, 135: 211-216.

[91] Ritschel WA, Paulos C, Arancibia A, et al. Pharmacokinetics of meperidine in healthy volunteers after short-and long-term exposure to high altitude [J]. J Clin Pharmacol, 1996, 36: 610-616.

[92] Gong W, Liu S, Xu P, et al. Simultaneous quantification of diazepam and dexamethasone in plasma by high-performance liquid chromatography with tandem mass spectrometry and its application to a pharmacokinetic comparison between normoxic and hypoxic rats [J]. Molecules, 2015, 20: 6901-6912.

[93] Li WB, Wang R, Xie H, et al. Effects on pharmacokinetics of propranolol and other factors in rats after acute exposure to high altitude at 4,010 m [J]. Cell Biochem Biophys, 2015, 72: 27-36.

[94] Engelhardt S, Al-Ahmad AJ, Gassmann M, et al. Hypoxia selec-

tively disrupts brain microvascular endothelial tight junction complexes through a hypoxia-inducible factor-1 (HIF-1) dependent mechanism [J]. J Cell Physiol, 2014, 229: 1096-1105.

[95] Kim Y, Lee S, Zhang H, et al. CLEC14A deficiency exacerbates neuronal loss by increasing blood-brain barrier permeability and inflammation [J]. J Neuroinflammation, 2020, 17: 48.

[96] Ronaldson PT, Davis TP. Regulation of blood-brain barrier integrity by microglia in health and disease: a therapeutic opportunity [J]. J Cereb Blood Flow Metab, 2020, 40: S6-S24.

[97] Zhu J, Wang B, Lee JH, et al. Additive neuroprotection of a 20-HETE inhibitor with delayed therapeutic hypothermia after hypoxia-ischemia in neonatal piglets [J]. Dev Neurosci, 2015, 37: 376-389.

[98] Zhao N, Xu X, Jiang Y, et al. Lipocalin-2 may produce damaging effect after cerebral ischemia by inducing astrocytes classical activation [J]. J Neuroinflammation, 2019, 16: 168.

[99] Chen W, He S, Xiang D. Hypoxia-induced retinal pigment epithelium cell-derived bFGF promotes the migration and angiogenesis of HUVECs through regulating TGF-β1/smad2/3 pathway [J]. Gene, 2021, 790: 145695.

[100] Witt KA, Mark KS, Sandoval KE, et al. Reoxygenation stress on blood-brain barrier paracellular permeability and edema in the rat [J]. Microvasc Res, 2008, 75: 91-96.

[101] Singh AK, Kashyap MP, Jahan S, et al. Expression and inducibility of cytochrome P450s (CYP1A1, 2B6, 2E1, 3A4) in human cord blood $CD34^+$ stem cell-derived differentiating neuronal cells [J]. Toxicol Sci, 2012, 129: 392-410.

[102] Zordoky BN, El-Kadi AO. Role of NF-κB in the regulation of cytochrome P450 enzymes [J]. Curr Drug Metab, 2009, 10: 164-178.

[103] Chan GN, Hoque MT, Cummins CL, et al. Regulation of P-glycoprotein by orphan nuclear receptors in human brain microvessel endothelial cells [J]. J Neurochem, 2011, 118: 163-175.

[104] Ma F, Zhang X, Yin KJ. MicroRNAs in central nervous system diseases: a prospective role in regulating blood-brain barrier integrity [J]. Exp Neurol, 2020, 323: 113094.

[105] Bukeirat M, Sarkar SN, Hu H, et al. MiR-34a regulates blood-brain barrier permeability and mitochondrial function by targeting cytochrome c [J]. J Cereb Blood Flow Metab, 2016, 36: 387-392.

[106] Wang X, Suofu Y, Akpinar B, et al. Systemic antimiR-337-3p delivery inhibits cerebral ischemia-mediated injury [J]. Neurobiol Dis, 2017, 105: 156-163.

[107] Duan YB, Bai X, Yang JX, et al. Exposure to high-altitude environment is associated with drug transporters change: microRNA-873-5p-mediated alteration of function and expression levels of drug transporters under hypoxia [J]. Drug Metab Dispos, 50: 174-186.

[108] Parker A, Fonseca S, Carding SR. Gut microbes and metabolites as modulators of blood-brain barrier integrity and brain health [J]. Gut Microbes, 2020, 11: 135-157.

[109] Li H, Sun J, Du J, et al. Clostridium butyricum exerts a neuroprotective effect in a mouse model of traumatic brain injury *via* the gut-brain axis [J]. Neurogastroenterol Motil, 2018, 30: e13260.

[110] Welcome MO. Gut microbiota disorder, gut epithelial and blood-brain barrier dysfunctions in etiopathogenesis of dementia: molecular mechanisms and signaling pathways [J]. Neuromolecular Med, 2019, 21: 205-226.

[111] Yang X, Yu D, Xue L, et al. Probiotics modulate the microbiota-gut-brain axis and improve memory deficits in aged SAMP8 mice [J]. Acta Pharm Sin B, 2020, 10: 475-487.

[112] Liu GQ, Bai X, Duan YB, et al. Changes in the intestinal flora of rats under high altitude hypoxia [J]. Acta Pharm Sin (药学学报), 2021, 56: 1100-1108.

[113] Bai X, Liu GQ, Yang JX, et al. The effect of high-altitude hypoxia on drug metabolism is mediated by gut microbiota [J]. Acta Pharm Sin (药学学报), 2021, 56: 2787-2796.