·综述·

中性粒细胞在缺血性脑卒中的作用研究进展

江娜，许元，冯林玉，谢敏杰

摘要 缺血性脑卒中是一种急性中枢神经系统疾病，发病率、致死率、致残率居高不下，给患者家庭和社会带来了极大负担。缺血性脑卒中病理机制复杂，治疗手段有限，亟需进一步探索病理机制及新的治疗靶点。既往中性粒细胞在缺血性脑卒中的作用长期被忽视，但近十年的研究提示中性粒细胞活跃参与了缺血性脑卒中后神经损伤与修复过程。同时，中性粒细胞胞外陷阱（neutrophil extracellular traps，NETs）作为中性粒细胞的一种新的调控机制，在缺血性脑卒中后急性炎症、血栓形成、血管损害等病理过程中发挥重要作用，可能是促进缺血性脑卒中后新生血管和功能恢复的关键。本文重点评述了中性粒细胞特别是NETs在缺血性脑卒中神经损伤与修复中的作用以及靶向中性粒细胞和NETs治疗的相关进展。

关键词 缺血性脑卒中；中性粒细胞；中性粒细胞外陷阱

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Role of Neutrophils in Ischemic Stroke *JIANG Na, XU Yuan, FENG Linyu, XIE Minjie. Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*

Abstract Ischemic stroke is an acute central nervous system disease with high morbidity, mortality, and disability rates, imposing a significant burden on patients' families and society. Pathological changes in ischemic stroke are complex, and the available treatment options are limited. Therefore, it is urgent to further explore the pathological mechanisms and identify new therapeutic targets. The role of neutrophils in ischemic stroke has long been neglected. However, research in the past decade has suggested that neutrophils are actively involved in nerve injury and repair after ischemic stroke. In addition, neutrophil extracellular traps (NETs), a new regulatory mechanism of neutrophils, play an important role in acute inflammation, thrombosis, vascular damage, and other pathological processes after ischemic stroke, potentially being a key target in promoting neovascularization and functional recovery post-ischemic stroke. This review focuses on the role of neutrophils, especially NETs, in nerve injury and repair after ischemic stroke, along with advances in treatments targeting neutrophils and NETs.

Keywords ischemic stroke; neutrophils; neutrophil extracellular traps

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1 缺血性脑卒中

脑卒中是一种急性中枢神经系统疾病，其特点是发病率、致残率、死亡率和复发率高。世界卒中组织最近的一项统计显示，卒中已成为世界上第二大死亡原因和第三大死亡和残疾原因，给全球经济带来了巨大负担[1,2]。中国的脑卒中预防和控制也面临着巨大的挑战。据统计，2018年中国脑血管疾病死亡率为149.49/10万，死亡人数为157万人，脑卒中已成为仅次于恶性肿瘤和心脏病的第三大死亡原因[3]。目前临床上急性缺血性脑卒中的治疗主要为静脉溶栓和机械取栓，促进血管再通，拯救缺血半暗带。然而，由于其狭窄的治疗窗口和出血转化（hemorrhagic transformation，HT）的高风险，只有少数患者能得到有效治疗[4]。

脑卒中分为缺血性脑卒中和出血性脑卒中，其中缺血性脑卒中约占全球所有卒中的80%[5]。缺血性脑卒中后，血流中断，大脑代谢所需的氧气和葡萄糖缺乏，能量产生不足，导致一些毒性代谢产物在局部堆积，如兴奋性毒性氨基酸、酸性代谢产物、氧化应激产物和炎症介质等，对组织造成损害并导致广泛神经元死亡，死亡的神经元通过释放损伤相关分子模式（damage-associated molecular patterns，DAMPs），进一步诱发炎症反应[6]。

炎症在缺血性脑卒中病理生理发展中发挥着至关重要的作用，存在于缺血性脑卒中神经损伤与修复的所有阶段，由损伤或死亡细胞释放DAMPs启动[7]。缺血性脑卒中急性期的炎症最终会导致神经元损伤、血脑屏障破坏等一系列脑损伤。因此，针对脑缺血急性期炎症是一种有希望的、潜在的治疗策略[8]。中性粒细胞和中性粒细胞胞外陷阱（neutrophil extracellular traps，NETs）是缺血性脑卒中急性炎症重要组成部分，在急性脑损害中发挥重要作用。靶向中性粒细胞和NETs治疗是缺血性脑卒中潜在的治疗靶点。

2 中性粒细胞

中性粒细胞由骨髓中的粒细胞-单核细胞祖细胞分化而来，是一种终末分化、寿命短的吞噬细胞[9]，在创伤、传染病、代谢性疾病、自身免疫性疾病等不同疾病中发挥着不同的作用。一方面，中性粒细胞在防御感染中发挥积极作用，如抗菌[10]、抗真菌[11]和抗病毒[12]。此外，它们可以吞噬损伤组织，消除凋亡细胞碎片，使损伤局限化，并有利于组织损伤后的组织再生和血管生成[13]。另一方面，中性粒细胞通过多种机制促进病变，比如中性粒细胞被招募到病变部位释放各种颗粒蛋白，例如中性粒细胞弹性酶（neutrophil elastase，NE）、髓过氧化物酶（myeloperoxidase，MPO）和基质金属蛋白酶（matrix metalloproteinase，MMP）、产生大量活性氧（reactive oxygen species，ROS）、分泌细胞因子、形成NETs等，进一步加重组织损伤，甚至发展为慢性炎症[14]。

中性粒细胞具有不同的亚群和功能特征，它们在病理学中的作用取决于表型而不是数量[7]。Friedlender等[15]将中性粒细胞主要分为促炎型中性粒细胞（N1）和抗炎型中性粒细胞（N2）。最近的证据表明，N1和N2只是以高度异质性和可塑性为特征的极化状态连续体中的极端，机体内还存在广泛的中性粒细胞亚群，比如表现中性粒细胞功能受损和免疫抑制特性的粒细胞样髓源性抑制细胞（granulocytic myeloid-derived suppressor cells，G-MDSCs）[16]、促血管生成中性粒细胞[17]、老年中性粒细胞[18]、反向跨内皮迁移CD54hiCXCR1lo中性粒细胞[19]、具有促进中枢神经系统神经元存活和轴突再生特性的$CD14^{+}Ly6Glo$中性粒细胞[20]。

不同中性粒细胞亚群对缺血性卒中有不同的影响，因此，可以针对特定中性粒细胞亚群靶向治疗，而不需对中性粒细胞数量的全面靶向[21]。

3 缺血性脑卒中后中性粒细胞的招募和变化

生理情况下，脑内并不存在中性粒细胞。缺血性脑卒中后，中性粒细胞数量在短时间内迅速增加，并率先从循环血液中通过破坏的血脑屏障进入病变部位，这一过程由缺血诱导的内皮细胞快速激活驱动[22]。中性粒细胞聚集在缺血性脑卒中再灌注后1～3 d达到峰值，几乎淹没了缺血半球，第7天中性粒细胞显著下降[23,24]。于此同时，血脑屏障部分恢复[25]，中性粒细胞随着时间的推移在浸润的免疫细胞中成为一个次要群体，但仍少量存在，可能与慢性期脑重构有关。

中性粒细胞的跨内皮迁移由粘附分子介导，如细胞间粘附分子-1（intercellular cell adhesion molecule-1，ICAM-1）、血小板内皮细胞粘附分子-1（platelet endothelial cell adhesion molecule-1，PECAM-1）和血管细胞粘附分子1（vascular cell adhesion molecule 1，VCAM-1），它们在缺血性脑卒中后表达增加，并驱动中性粒细胞浸润[26,27]。趋化因子配体1（C-X-C motif chemokine ligand 1，CXCL1）和趋化因子配体2（C-X-C motif chemokine ligand 2，CXCL2）是中性粒细胞趋化的重要因子，内皮VCAM-1和中性粒细胞晚期抗原-4（very late antigen-4，VLA-4）之间的相互作用促进缺血性脑卒中的中性粒细胞浸润[28]。同时，炎症因子如肿瘤坏死因子-α（tumor necrosis factor-α，TNF-α）、白介素-1β（interleukin-1β，IL-1β）、白介素-8（interleukin-8，IL-8）也是中性粒细胞浸润过程中的主要驱动力。

4 中性粒细胞在缺血性脑卒中中的作用

既往研究证明中性粒细胞在急性缺血性脑卒中中主要发挥破坏作用，急性期中性粒细胞高提示卒中预后不良[29,30]。中性粒细胞进入缺血灶后，一方面，中性粒细胞在血管内积聚，堵塞毛细血管血流，加剧缺血后微血管损伤，阻碍血流再灌注。另一方面，中性粒细胞是缺血性脑卒中急性炎症的重要组成成分，中性粒细胞通过DAMPs诱导驻留细胞Toll样受体（toll-like receptor，TLR）激活，释放白三烯、细胞因子、趋化因子、ROS，各种蛋白酶如MPO、NE、MMP-9等直接损伤内皮，导致血脑屏障通透性增加，脑组织水肿，进一步阻塞血管，并释放NETs，进而促进组织和血脑屏障损伤[31]。同时中性粒细胞可参与血栓的形成和扩张，进而损害脑卒中后血循环重建和血管重构，加剧缺血性神经元损伤[32]。

最近研究表明，中性粒细胞在缺血性脑卒中中可能获得不同的表型，如N2抗炎型中性粒细胞，并通过释放抗炎介质减轻炎症，继而减轻脑损害[33]。在缺血性脑卒中慢性修复过程，中性粒细胞可以调节内皮细胞功能，促进脑重塑。中性粒细胞作为血管内皮生长因子（vascular endothelial growth factor，VEGF）的重要来源，可刺激血管生成[34]。此外，中性粒细胞可吞噬凋亡细胞碎片，局限化损伤灶，且对预防全身感染有重要意义。

5 NETs

中性粒细胞被激活后，染色质去致密，释放细胞核和颗粒内容物，形成了以脱氧核糖核酸（deoxyribonucleic acid，DNA）为骨架，附着组蛋白和颗粒蛋白的网状结构，即为NETs[35]。2015年，Perez-de-Puig等[36]在小鼠永久性大脑中动脉阻塞模型中首次发现缺血性脑卒中后损伤灶NETs的形成。NETs是一种强负电荷的网状结构，主要由DNA、组蛋白和其他蛋白质组成，如NE、MPO、高迁移率族蛋白B1（high mobility group box 1 protein，HMGB1）、肽酰基精氨酸脱亚氨酶4（Peptidyl arginine deiminase 4，PAD4）、MMP-9、组织蛋白酶G等[37]。

缺血性脑卒中后，中性粒细胞渗入缺血脑组织，并被进一步激活形成NETs。缺血性脑卒中后NETs的形成主要受HMGB1-TLR4、JAK-STAT和cGAS-STING通路的调节[38,39]。NETs的生物标志物主要有瓜氨酸组蛋白3（citrullinated histone H3，CitH3）、MPO-DNA[40,41]。NETs通过多种机制参与缺血性脑卒中的病理过程。

NETs介导缺血性脑卒中的炎症反应[42]。NETs的主要成分组蛋白和颗粒蛋白如MMPs、MPO、NE等具有细胞毒性，同时也是强有力的促炎因子，能够诱发急性炎症[43]。同时，NETs可介导炎症小体的激活，炎症小体促进IL-18和IL-1β的合成和释放来诱导诱导NETs的形成，加重炎症反应[44]。

NETs促进血栓形成并介导缺血性脑卒中后的溶栓抵抗。Fuchs等[45]研究表明NETs可为血小板结合和聚集提供支架和刺

激，并对血栓稳定性起重要作用，DNA酶或抗凝剂肝素可破坏NETs支架并阻止血栓形成。Laridan等[38]对接受血管内治疗的68例缺血性脑卒中患者的血栓进行了表征，在所有血栓中均广泛检测到中性粒细胞和NETs的标志物H3Cit的存在。阿替普酶可诱导中性粒细胞脱颗粒形成NETs，导致出血转化和溶栓抵抗，当使用阿替普酶联合DNase1治疗时，效率更高，患者血栓的离体溶解更成功。

NETs介导缺血性脑卒中后的血管损害。活化的内皮细胞产生细胞因子，如IL-1β、IL-8和ROS等促进NETs的形成，而NETs也通过蛋白酶成分，如组蛋白诱导内皮细胞活化[46]。Kang等[32]发现，NETs通过激活STING通路诱导干扰素-β（interferon-β，IFN-β），阻碍卒中后的血管再生和血管重塑。同时，缺血性脑卒中后，HGMB1、MMP-9、MPO和NE等NETs的主要成分可诱导炎症反应，损伤脑血管内皮，抑制血管生成和血运重建[47-50]。此外，血栓和外周血中NETs的存在与数量可预测缺血性脑卒中的严重程度，可能是缺血性脑卒中诊断和预后的潜在生物标志物[40]。

6 靶向中性粒细胞治疗缺血性脑卒中

6.1 减少中性粒细胞生成或耗竭中性粒细胞

粒细胞-集落刺激因子（granulocyte colony-stimulating factor，G-CSF）是一种强大的造血因子，可提高骨髓系细胞的存活率并驱动其分化，从而产生中性粒细胞。阻断G-CSF受体可减少中性粒细胞生成，进而减轻炎症[51]。给予抗Ly6G抗体耗竭中性粒细胞可在小鼠局灶性脑缺血模型中发挥保护作用，减少缺血性脑损伤和神经功能障碍[52]。

6.2 干扰中性粒细胞募集和趋化

阻滞选择素、整合素和黏附分子可减少中性粒细胞在大脑中的浸润，进而在缺血性脑卒中发挥保护作用[53]。在小鼠和灵长类动物局灶性脑缺血中，抗E/P选择素抗体可以减少缺血后梗死灶，改善神经功能[54]。在短暂性大脑中动脉阻塞模型（transient middle cerebral artery occlusion，tMCAO）中，ICAM-1缺失可减少梗死灶[55]。趋化因子对协调中性粒细胞迁移至关重要。阻断CCL5-CXCL4可阻止中性粒细胞募集，进而减轻炎症反应[56]。敲除CCR2后，小鼠梗死体积减少，脑水肿和缺血半球中性粒细胞浸润也会减少[57]。抗VLA-4治疗可抑制中性粒细胞的聚集，进而减轻缺血性脑卒中急性期损害[22]。

6.3 促进中性粒细胞凋亡

细胞周期蛋白依赖性激酶抑制剂能通过诱导半胱天冬酶（Caspases）依赖性的中性粒细胞凋亡，进而减轻小鼠急性和慢性炎症[58]。依克多因是一种天然化合物，存在于多种细菌中。它是一种相容的溶质，通过充当渗透液作为保护物质，从而帮助生物体在极端渗透压力下生存，具有抗炎性，能够阻止炎症微环境中性粒细胞的抗凋亡机制[59]。此外，中性粒细胞的清除是炎症消退的基础[60]。

6.4 减少中性粒细胞分泌的炎性因子

活化的中性粒细胞分泌TNF-α、IL-1β等炎症因子，是炎症因子的主要来源，同时这些炎症因子又会促进中性粒细胞的活化，加重炎症反应[61]。同时，其他细胞如胶质细胞也分泌TNF-α、IL-1β等炎症因子[62]。因此，拮抗炎症因子治疗可以通过减少多种来源的炎症因子进而减轻炎症，继而减少梗死灶，改善预后。IL-1受体拮抗剂治疗可以改善急性缺血性脑卒中患者预后[63]。

6.5 促进中性粒细胞向N2型转变

在特定刺激下，中性粒细胞可从N1型向N2型转变，进而保护神经细胞，该策略也被认为是治疗缺血性脑卒中的有效措施。在小鼠tMCAO模型中，维甲酸受体激动剂贝沙罗汀可增加N2型中性粒细胞，显著减少血脑屏障损伤和神经功能缺损，且不影响脑血流量，可有效用于缺血性卒中的急性治疗[64]。在其他研究中，TLR4敲除[65]和IL-27[66]治疗可促进中性粒细胞向N2型转变，并对大脑起到保护作用，提示N2型中性粒细胞具有神经保护作用。

7 靶向NETs治疗缺血性脑卒中

NETs作为中性粒细胞最重要最强大的杀伤机制，在急性缺血性脑卒中增强急性炎症反应，参与血栓形成，促进血管损伤，并介导溶栓抵抗，大大加重了急性脑损伤。抑制NETs的形成或促进NETs的降解在缺血性脑卒中中起到保护作用，成为治疗缺血性脑卒中新的靶向。

7.1 抑制NETs的形成

PAD4抑制剂通过抑制NETs的形成可增加缺血性脑卒中后新生血管和血管修复，减少血脑屏障损害，改善功能恢复，很有潜力被开发为缺血性脑卒中治疗剂[67]。PAD4抑制剂如GSK484[68]、GSK199[40]、新生儿NET抑制因子（neonatal NET-inhibitory factor，nNIF）[40]、氯胺[69]均被证明可减少NETs的形成，减轻炎症。HMGB1不仅参与NETs的形成过程，而且是NETs的重要组成部分。血小板是HMGB1的主要来源之一，耗竭血小板或特异性敲除血小板HMGB1可显著降低缺血性脑卒中后血浆HMGB1和NETs水平，并大大改善缺血性脑卒中预后[40]。NETs的形成与ROS的产生密切相关。氧自由基清除剂依达拉奉在MCAO小鼠中减少NETs，进而改善神经功能[70]。因此，ROS抑制剂可通过抑制NETs的形成对急性缺血性脑卒中起保护作用。其他药物如环孢菌素减少IL-8介导的NETs形成，并在缺血性卒中的治疗中发挥神经保护作用[71]。辛伐他汀具有抑制中性粒细胞介导的炎症反应和NETs形成的能力，减轻卒中后的脑损伤，改善阿替普酶诱导的出血转化[72]。

7.2 促进NETs的降解

除了抑制NETs的形成外，破坏DNA骨架、拮抗组蛋白、MPO和NE以促进NETs的降解为缺血性脑卒中治疗提供了另一种潜在的方法。DNA降解剂DNase治疗通过降解NETs来减少缺血性脑卒中小鼠的梗死体积并改善卒中预后。此外，DNase加速脑卒中血栓的溶解，DNase和阿替普酶联合治疗可以减少阿替普酶的剂量和副作用，并扩大治疗时间窗口，同时降解血栓中的纤维蛋白和NETs[32,38]。组蛋白是NETs重要组成成

分，降解组蛋白可以促进NETs的降解。临床研究表明，组蛋白降解剂3K3A-APC可增加小鼠缺血性脑卒中后神经发生，改善神经功能[73]。NE和MPO都是NETs的组成部分，也是中性粒细胞的重要酶，在炎症相关疾病中发挥着关键作用。研究证实NE抑制剂和MPO抑制剂对脑缺血具有保护作用[74,75]。

8 小结

中性粒细胞作为缺血性脑卒中急性炎症重要组成部分，在缺血性脑卒中后，中性粒细胞第一个从血液循环系统进入缺血灶，分泌各种炎性因子和蛋白酶，并释放NETs，加重内皮损伤和血脑屏障损害，与缺血性脑卒中不良预后成正相关。同时，中性粒细胞和NETs参与血栓形成，阻碍血管重构，加剧缺血性神经元损伤。已有研究证实调控进入缺血半球的中性粒细胞数目与功能可以减轻缺血性脑卒中急性炎症，改善脑水肿，减少梗死灶体积，改善神经功能。

既往中性粒细胞在缺血性脑卒中中的作用长期被忽视，但近十年的研究提示中性粒细胞和NETs在缺血性脑卒中后急性炎症、血栓形成、血管损害等病理过程中发挥重要作用，可能是促进缺血性脑卒中后新生血管和功能恢复的关键。因此，靶向中性粒细胞及NETs是急性缺血性脑卒中治疗的潜在靶点，有希望促进未来缺血性脑卒中治疗的发展。然而，如何避免中性粒细胞靶向治疗的不良反应如感染等问题值得进一步研究。同时，由于流式技术和质谱技术的发展，目前已经发现了多种具有不同作用的中性粒细胞亚群，如何精准靶向中性粒细胞某一亚群而不影响其他中性粒细胞亚群仍需继续探索。

参考文献

[1] GBD 2019 Stroke Collaborators. Global, regional, and national burden of stroke and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019[J]. Lancet Neurol, 2021, 20: 795-820. DOI: 10.1016/S1474-4422(21)00252-0.

[2] Feigin VL, Brainin M, Norrving B, et al. World Stroke Organization (WSO): Global Stroke Fact Sheet 2022[J]. Int J Stroke, 2022, 17: 18-29. DOI: 10.1177/17474930211065917.

[3] Wang YJ, Li ZX, Gu HQ, et al. China Stroke Statistics 2019: A Report From the National Center for Healthcare Quality Management in Neurological Diseases, China National Clinical Research Center for Neurological Diseases, the Chinese Stroke Association, National Center for Chronic and Non-communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention and Institute for Global Neuroscience and Stroke Collaborations[J]. Stroke Vasc Neurol, 2020, 5: 211-239. DOI: 10.1136/svn-2020-000457.

[4] Phipps MS, Cronin CA. Management of acute ischemic stroke[J]. BMJ, 2020, 368: l6983. DOI: 10.1136/bmj.l6983.

[5] Toyoda K, Yoshimura S, Nakai M, et al. Twenty-Year Change in Severity and Outcome of Ischemic and Hemorrhagic Strokes[J]. JAMA Neurol, 2022, 79: 61-69. DOI: 10.1001/jamaneurol.2021.4346.

[6] Walter K. What Is Acute Ischemic Stroke? [J]. JAMA, 2022, 327: 885. DOI: 10.1001/jama.2022.1420.

[7] Endres M, Moro MA, Nolte CH, et al. Immune Pathways in Etiology, Acute Phase, and Chronic Sequelae of Ischemic Stroke[J]. Circ Res, 2022, 130: 1167-1186. DOI: 10.1161/CIRCRESAHA.121.319994.

[8] Maida CD, Norrito RL, Daidone M, et al. Neuroinflammatory Mechanisms in Ischemic Stroke: Focus on Cardioembolic Stroke, Background, and Therapeutic Approaches[J]. Int J Mol Sci, 2020, 21: 6454. DOI: 10.3390/ijms21186454.

[9] Silvestre-Roig C, Braster Q, Ortega-Gomez A, et al. Neutrophils as regulators of cardiovascular inflammation[J]. Nat Rev Cardiol, 2020, 17: 327-340. DOI: 10.1038/s41569-019-0326-7.

[10] Thanabalasuriar A, Scott BNV, Peiseler M, et al. Neutrophil Extracellular Traps Confine Pseudomonas aeruginosa Ocular Biofilms and Restrict Brain Invasion[J]. Cell Host Microbe, 2019, 25: 526-536.e4. DOI: 10.1016/j.chom.2019.02.007.

[11] Song Z, Huang G, Chiquetto Paracatu L, et al. NADPH oxidase controls pulmonary neutrophil infiltration in the response to fungal cell walls by limiting LTB4[J]. Blood, 2020, 135: 891-903. DOI: 10.1182/blood.2019003525.

[12] Iversen MB, Reinert LS, Thomsen MK, et al. An innate antiviral pathway acting before interferons at epithelial surfaces[J]. Nat Immunol, 2016, 17: 150-158. DOI: 10.1038/ni.3319.

[13] Castanheira FVS, Kubes P. Neutrophils and NETs in modulating acute and chronic inflammation[J]. Blood, 2019, 133: 2178-2185. DOI: 10.1182/blood-2018-11-844530.

[14] El-Benna J, Hurtado-Nedelec M, Marzaioli V, et al. Priming of the neutrophil respiratory burst: role in host defense and inflammation[J]. Immunol Rev, 2016, 273: 180-193. DOI: 10.1111/imr.12447.

[15] Fridlender ZG, Sun J, Kim S, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN[J]. Cancer Cell, 2009, 16: 183-194. DOI: 10.1016/j.ccr.2009.06.017.

[16] Sagiv JY, MichaeliI J, Assi S, et al. Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer[J]. Cell Rep, 2015, 10: 562-573. DOI: 10.1016/j.celrep.2014.12.039.

[17] Ballesteros I, Rubio-Ponce A, Genua M, et al. Co-option of Neutrophil Fates by Tissue Environments[J]. Cell, 2020, 183: 1282-1297. DOI: 10.1016/j.cell.2020.10.003.

[18] Gullotta GS, De Feo D, Friebel E, et al. Age-induced alterations of granulopoiesis generate atypical neutrophils that aggravate stroke pathology[J]. Nat Immunol, 2023, 24: 925-940. DOI: 10.1038/s41590-023-01505-1.

[19] Weisenburger-Lile D, Dong Y, Yger M, et al. Harmful neutrophil subsets in patients with ischemic stroke: Association with disease severity [J]. Neurol Neuroimmunol Neuroinflamm, 2019, 6: e571. DOI: 10.1212/NXI.0000000000000571.

[20] Sas AR, Carbajal KS, Jerome AD, et al. A new neutrophil subset promotes CNS neuron survival and axon regeneration[J]. Nat Immunol, 2020, 21: 1496-1505. DOI: 10.1038/s41590-020-00813-0.

[21] Chen C, Huang T, Zhai X, et al. Targeting neutrophils as a novel therapeutic strategy after stroke[J]. J Cereb Blood Flow Metab, 2021, 41: 2150-2161. DOI: 10.1177/0271678X211000137.

[22] Neumann J, Riek-Burchardt M, Herz J, et al. Very-late-antigen-4 (VLA-4)-mediated brain invasion by neutrophils leads to interactions with microglia, increased ischemic injury and impaired behavior in experimental stroke[J]. Acta Neuropathol, 2015, 129: 259-277. DOI: 10.1007/s00401-014-1355-2.

[23] Grønberg NV, Johansen FF, Kristiansen U, et al. Leukocyte infiltration in experimental stroke[J]. J Neuroinflam, 2013, 10: 115. DOI: 10.1186/1742-2094-10-115.

[24] Gelderblom M, Leypoldt F, Steinbach K, et al. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke[J]. Stroke, 2009, 40: 1849-1857. DOI: 10.1161/STROKEAHA.108.534503.

[25] Jiang X, Andjelkovic AV, Zhu L, et al. Blood-brain barrier dysfunction and recovery after ischemic stroke[J]. Prog Neurobiol, 2018, 163-164: 144-171. DOI: 10.1016/j.pneurobio.2017.10.001.

[26] Winneberger J, Schols S, Lessmann K, et al. Platelet endothelial cell adhesion molecule-1 is a gatekeeper of neutrophil transendothelial migration in ischemic stroke[J]. Brain Behav Immun, 2021, 93: 277-287. DOI: 10.1016/j.bbi.2020.12.026.

[27] Kong LL, Wang ZY, Han N, et al. Neutralization of chemokine-like factor 1, a novel C-C chemokine, protects against focal cerebral ischemia by inhibiting neutrophil infiltration via MAPK pathways in rats[J]. J Neuroinflam, 2014, 11: 112. DOI: 10.1186/1742-2094-11-112.

[28] Neumann J, Riek-Burchardt M, Herz J, et al. Very-late-antigen-4

(VLA-4)-mediated brain invasion by neutrophils leads to interactions with microglia, increased ischemic injury and impaired behavior in experimental stroke[J]. Acta Neuropathol, 2015, 129: 259-277. DOI: 10.1007/s00401-014-1355-2.
[29] Hermann DM, Kleinschnitz C, Gunzer M. Implications of polymorphonuclear neutrophils for ischemic stroke and intracerebral hemorrhage: Predictive value, pathophysiological consequences and utility as therapeutic target[J]. J Neuroimmunol, 2018, 321: 138-143. DOI: 10.1016/j.jneuroim.2018.04.015.
[30] 彭伟,汪慧,乔向亮. 中性粒细胞百分比对进展性脑梗死的早期预测价值[J]. 神经损伤与功能重建, 2021, 16: 745-747. DOI: 10.16780/j.cnki.sjssgncj.20200788.
[31] Pena-Martinez C, Duran-Laforet V, Garcia-Culebaras A, et al. Pharmacological Modulation of Neutrophil Extracellular Traps Reverses Thrombotic Stroke tPA (Tissue-Type Plasminogen Activator) Resistance [J]. Stroke, 2019, 50: 3228-3237. DOI:10.1161/STROKEAHA.119.026848.
[32] Kang L, Yu H, Yang X, et al. Neutrophil extracellular traps released by neutrophils impair revascularization and vascular remodeling after stroke[J]. Nat Commun, 2020, 11: 2488. DOI: 10.1038/s41467-020-16191-y.
[33] Cuartero MI, Ballesteros I, Moraga A, et al. N2 neutrophils, novel players in brain inflammation after stroke: modulation by the PPARgamma agonist rosiglitazone[J]. Stroke, 2013, 44: 3498-3508. DOI: 10.1161/STROKEAHA.113.002470.
[34] Gong Y, Koh DR. Neutrophils promote inflammatory angiogenesis via release of preformed VEGF in an in vivo corneal model[J]. Cell Tissue Res, 2010, 339: 437-448. DOI: 10.1007/s00441-009-0908-5.
[35] Pinegin B, Vorobjeva N, Pinegin V. Neutrophil extracellular traps and their role in the development of chronic inflammation and autoimmunity [J]. Autoimmun Rev, 2015, 14: 633-640. DOI: 10.1016/j.autrev.2015.03.002.
[36] Perez-De-Puig I, Miro-Mur F, Ferrer-Ferrer M, et al. Neutrophil recruitment to the brain in mouse and human ischemic stroke[J]. Acta Neuropathol, 2015, 129: 239-257. DOI: 10.1007/s00401-014-1381-0.
[37] Erpenbeck L, Schon MP. Neutrophil extracellular traps: protagonists of cancer progression?[J]. Oncogene, 2017, 36: 2483-2490. DOI: 10.1038/onc.2016.406.
[38] Laridan E, Denorme F, Desender L, et al. Neutrophil extracellular traps in ischemic stroke thrombi[J]. Ann Neurol, 2017, 82: 223-232. DOI: 10.1002/ana.24993.
[39] Zhao Z, Pan Z, Zhang S, et al. Neutrophil extracellular traps: A novel target for the treatment of stroke[J]. Pharmacol Ther, 2023, 241: 108328. DOI: 10.1016/j.pharmthera.2022.108328.
[40] Denorme F, Portier I, Rustad JL, et al. Neutrophil extracellular traps regulate ischemic stroke brain injury[J]. J Clin Invest, 2022, 132: e154225. DOI: http://doi.org/10.1172/JCI154225.
[41] Molek P, Zabczyk M, Malinowski KP, et al. Markers of NET formation and stroke risk in patients with atrial fibrillation: association with a prothrombotic state[J]. Thromb Res, 2022, 213: 1-7. DOI: 10.1016/j.thromres.2022.02.025.
[42] 覃秋燕,曹小丽. NETs及Cdc42在缺血性脑卒中神经损伤中的作用[J]. 神经损伤与功能重建, 2022, 17: 145-147. DOI: 10.16780/j.cnki.sjssgncj.20210203.
[43] Luo H, Guo H, Zhou Y, et al. Neutrophil Extracellular Traps in Cerebral Ischemia/Reperfusion Injury: Friend and Foe[J]. Curr Neuropharmacol, 2023, 21: 2079-2096. DOI: 10.2174/1570159X21666230308090351.
[44] Kahlenberg JM, Carmona-Rivera C, Smith CK, et al. Neutrophil extracellular trap-associated protein activation of the NLRP3 inflammasome is enhanced in lupus macrophages[J]. J Immunol, 2013, 190: 1217-1226. DOI: 10.4049/jimmunol.1202388.
[45] Fuchs TA, Brill A, Wagner DD. Neutrophil extracellular trap (NET) impact on deep vein thrombosis[J]. Arterioscler Thromb Vasc Biol, 2012, 32: 1777-1783. DOI: 10.1161/ATVBAHA.111.242859.
[46] Saffarzadeh M, Juenemann C, Queisser MA, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones[J]. PloS One, 2012, 7: e32366. DOI: 10.1371/journal.pone.0032366.
[47] Kim HJ, Wei Y, Wojtkiewicz GR, et al. Reducing myeloperoxidase activity decreases inflammation and increases cellular protection in ischemic stroke[J]. J Cereb Blood Flow Metab, 2019, 39: 1864-1877. DOI: 10.1177/0271678X18771978.
[48] Kim SW, Lee JK. Role of HMGB1 in the Interplay between NETosis and Thrombosis in Ischemic Stroke: A Review[J]. Cells, 2020, 9: 1794. DOI: http://doi.org/10.3390/cells9081794.
[49] Kim SW, Lee H, Lee HK, et al. Neutrophil extracellular trap induced by HMGB1 exacerbates damages in the ischemic brain[J]. Acta Neuropathol Commun, 2019, 7: 94. DOI: 10.1186/s40478-019-0747-x.
[50] Christoffersson G, Vågesjö E, Vandooren J, et al. VEGF-A recruits a proangiogenic MMP-9-delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue[J]. Blood, 2012, 120: 4653-4662. DOI: 10.1182/blood-2012-04-421040.
[51] Lee MC, Mccubbin JA, Christensen AD, et al. G-CSF Receptor Blockade Ameliorates Arthritic Pain and Disease[J]. J Immunol, 2017, 198: 3565-3575. DOI: 10.4049/jimmunol.1602127.
[52] Yan H, Kawano T, Kanki H, et al. Role of Polymorphonuclear Myeloid-Derived Suppressor Cells and Neutrophils in Ischemic Stroke[J]. J Am Heart Assoc, 2023, 12: e028125. DOI: 10.1161/JAHA.122.028125.
[53] Mitroulis I, Alexaki VI, Kourtzelis I, et al. Leukocyte integrins: role in leukocyte recruitment and as therapeutic targets in inflammatory disease [J]. Pharmacol Ther, 2015, 147: 123-135. DOI: 10.1016/j.pharmthera.2014.11.008.
[54] Mocco J, Choudhri T, Huang J, et al. HuEP5C7 as a humanized monoclonal anti-E/P-selectin neurovascular protective strategy in a blinded placebo-controlled trial of nonhuman primate stroke[J]. Circ Res, 2002, 91: 907-914. DOI: 10.1161/01.res.0000042063.15901.20.
[55] Jickling GC, Liu D, Ander BP, et al. Targeting neutrophils in ischemic stroke: translational insights from experimental studies[J]. J Cereb Blood Flow Metab, 2015, 35: 888-901. DOI: 10.1038/jcbfm.2015.45.
[56] Vajen T, Koenen RR, Werner I, et al. Blocking CCL5-CXCL4 heteromerization preserves heart function after myocardial infarction by attenuating leukocyte recruitment and NETosis[J]. Sci Rep, 2018, 8: 10647. DOI: 10.1038/s41598-018-29026-0.
[57] Dimitrijevic OB, Stamatovic SM, Keep RF, et al. Absence of the chemokine receptor CCR2 protects against cerebral ischemia/reperfusion injury in mice[J]. Stroke, 2007, 38: 1345-1353. DOI: 10.1161/01.STR.0000259709.16654.8f.
[58] Rossi AG, Sawatzky DA, Walker A, et al. Cyclin-dependent kinase inhibitors enhance the resolution of inflammation by promoting inflammatory cell apoptosis[J]. Nat Med, 2006, 12: 1056-1064. DOI: 10.1038/nm1468.
[59] Sydlik U, Peusche H, Paunel-Görgülü A, et al. Recovery of neutrophil apoptosis by ectoine: a new strategy against lung inflammation[J]. Eur Respir J, 2013, 41: 433-442. DOI: 10.1183/09031936.00132211.
[60] Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators[J]. J Clin Invest, 2018, 128: 2657-2669. DOI: 10.1172/JCI97943.
[61] Lambertsen KL, Biber K, Finsen B. Inflammatory cytokines in experimental and human stroke[J]. J Cereb Blood Flow Metab, 2012, 32: 1677-1698. DOI: 10.1038/jcbfm.2012.88.
[62] SHICHITA T, OOBOSHI H, YOSHIMURA A. Neuroimmune mechanisms and therapies mediating post-ischaemic brain injury and repair [J]. Nat Rev Neurosci, 2023, 24(5): 299-312.http://doi.org/10.1038/s41583-023-00690-0.
[63] Emsley HCA, Smith CJ, Georgiou RF, et al. A randomised phase II study of interleukin-1 receptor antagonist in acute stroke patients[J]. J Neurol Neurosurg Psychiatry, 2005, 76: 1366-1372. DOI: 10.1136/jnnp.2004.054882.
[64] Certo M, Endo Y, Ohta K, et al. Activation of RXR/PPARγ underlies neuroprotection by bexarotene in ischemic stroke[J]. Pharmacol Res, 2015, 102: 298-307. DOI: 10.1016/j.phrs.2015.10.009.
[65] Garcia-Culebras A, Duran-Laforet V, Pena-Martinez C, et al. Role of TLR4 (Toll-Like Receptor 4) in N1/N2 Neutrophil Programming After Stroke[J]. Stroke, 2019, 50: 2922-2932. DOI: 10.1161/STROKEAHA.119.

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for Hypertension Management in Stroke Patients[J]. J Stroke Cerebrovasc Dis, 2018, 27: 460-465. DOI: 10.1016/j.jstrokecerebrovasdis.2017.09.023.
[37] Finger JR, Kurczewski LM, Brophy GM. Clevidipine Versus Nicardipine for Acute Blood Pressure Reduction in a Neuroscience Intensive Care Population[J]. Neurocrit Care, 2017, 26: 167-173. DOI: 10.1007/s12028-016-0349-4.
[38] Donovan AL, Flexman AM, Gelb AW. Blood pressure management in stroke[J]. Curr Opin Anaesthesiol, 2012, 25: 516-22. DOI: 10.1097/ACO.0b013e32835721a5.
[39] 刘晓, 李小静. 右美托咪定对重症脑卒中患者镇静效果及对血清S100β蛋白的影响[J].中国实验诊断学, 2020, 24: 1987-1989.
[40] Campbell TS, Labelle LE, Bacon SL, et al. Impact of Mindfulness-Based Stress Reduction (MBSR) on attention, rumination and resting blood pressure in women with cancer: a waitlist-controlled study [J]. J Behav Med, 2012, 35: 262-271. DOI: 10.1007/s10865-011-9357-1.
[41] Nolan RP, Floras JS, Harvey PJ, et al. Behavioral neurocardiac training in hypertension: a randomized, controlled trial[J]. Hypertension, 2010, 55: 1033-1039. DOI: 10.1161/HYPERTENSIONAHA.109.146233.

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025085.
[66] Zhao X, Ting SM, Liu CH, et al. Neutrophil polarization by IL-27 as a therapeutic target for intracerebral hemorrhage[J]. Nat Commun, 2017, 8: 602. DOI: 10.1038/s41467-017-00770-7.
[67] Franck G, Mawson TL, Folco EJ, et al. Roles of PAD4 and NETosis in Experimental Atherosclerosis and Arterial Injury: Implications for Superficial Erosion[J]. Circ Res, 2018, 123: 33-42. DOI: 10.1161/CIRCRESAHA.117.312494.
[68] Lewis HD, Liddle J, Coote JE, et al. Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation[J]. Nat Chem Biol, 2015, 11: 189-191. DOI: 10.1038/nchembio.1735.
[69] Bonaventura A, Liberale L, Carbone F, et al. The Pathophysiological Role of Neutrophil Extracellular Traps in Inflammatory Diseases[J]. Thromb Haemost, 2018, 118: 6-27. DOI: 10.1160/TH17-09-0630.
[70] Huang Y, Zhang X, Zhang C, et al. Edaravone Dexborneol Downregulates Neutrophil Extracellular Trap Expression and Ameliorates Blood-Brain Barrier Permeability in Acute Ischemic Stroke[J]. Mediators Inflamm, 2022, 2022: 3855698. DOI: 10.1155/2022/3855698.
[71] Gupta AK, GiaglisI S, Hasler P, et al. Efficient neutrophil extracellular trap induction requires mobilization of both intracellular and extracellular calcium pools and is modulated by cyclosporine A[J]. PloS One, 2014, 9: e97088. DOI: 10.1371/journal.pone.0097088.
[72] Yin B, Li DD, Xu SY, et al. Simvastatin pretreatment ameliorates t-PA-induced hemorrhage transformation and MMP-9/TIMP-1 imbalance in thromboembolic cerebral ischemic rats[J]. Neuropsychiatric Disease and Treatment, 2019, 15: 1993-2002. DOI: 10.2147/NDT.S199371.
[73] Wang Y, Zhao Z, Chow N, et al. Activated protein C analog promotes neurogenesis and improves neurological outcome after focal ischemic stroke in mice via protease activated receptor 1[J]. Brain Res, 2013, 1507: 97-104. DOI: 10.1016/j.brainres.2013.02.023.
[74] Pravalika K, Sarmah D, Kaur H, et al. Trigonelline therapy confers neuroprotection by reduced glutathione mediated myeloperoxidase expression in animal model of ischemic stroke[J]. Life Sci, 2019, 216: 49-58. DOI: 10.1016/j.lfs.2018.11.014.
[75] Ikegame Y, Yamashita K, HayashiI SI, et al. Neutrophil elastase inhibitor prevents ischemic brain damage via reduction of vasogenic edema [J]. Hypertens Res, 2010, 33: 703-707. DOI: 10.1038/hr.2010.58.

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