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REVIEW

The impact of thrombo-inflammation on the cerebral microcirculation

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Abstract

The intertwined processes of thrombosis and inflammation (termed "thrombo-inflammation") are significant drivers of cerebrovascular diseases, and as such, they represent prime targets for drug discovery programs focusing on treatment and management of cerebrovascular diseases. Most cerebrovascular events result from chronic systemic microcirculatory dysfunction due to underlying conditions, for example, hypertension, diabetes mellitus, coronary artery disease, dyslipidemia, and sickle cell disease. Immune cells especially neutrophils play a critical role in the onset and maintenance of neuroinflammatory responses in the microcirculation. Neutrophils have the ability to drive both inflammatory and anti-inflammatory/pro-resolution effects depending on the underlying vascular state (physiological vs. pathological). In this article, we highlight the pathophysiological role of neutrophils in stroke and discuss ongoing pharmacotherapeutic strategies that are focused on identifying potential therapeutic targets for enhancing neuroprotection, mitigating inflammatory pathways, and enabling resolution.

KEYWORDS
inflammation, neutrophils, stroke, thrombosis

1 | INTRODUCTION

Cerebrovascular diseases include a number of conditions that involve thrombosis and inflammation, including stroke, intracranial stenosis, aneurysms, carotid and vertebral stenosis, and vascular malformations. Ischemic stroke is one of the leading causes of morbidity and mortality worldwide, and the leading cause of adult disability, resulting in a significant socioeconomic burden.[1,2] Rapid recanalization with intravenous thrombolysis or endovascular thrombectomy is the mainstay of current and evolving acute ischemic stroke management.[3] However, there is limited indication of rapid recanalization therapies due to a shorter time window and availability of stroke centers, and a large number of patients with ischemic stroke receive no acute therapy resulting in significant residual neurological damage, a long-term recovery period, and further risk of recurrent events and complications.[4] Therefore, there is a rapidly growing clinical need of additional therapies which may not be time-sensitive and can be given to a wider number of stroke patients and in non-specialty centers. In recent years, there has been a considerable advancement in the research and development of neuroprotective and anti-inflammatory agents as potential adjunctive therapies in both pre-clinical clinical models.[5,6] In our own work, we have shown that pharmacologically targeting the Annexin A1/formyl peptide receptor (FPR)-2 pathway can significantly attenuate cerebrovascular thrombo-inflammation by modifying neutrophil-platelet phenotype from pro-inflammatory to pro-resolutory.[7,8]

The cerebral microcirculation (comprising of neurovasculature within the brain <100 μm in diameter) is the most critical part of neurovascular bed, providing all metabolic needs to the cerebral parenchyma.

Therefore, cerebral microvascular dysfunction plays a significant role in the setting of common neurovascular abnormalities, with thrombosis and inflammation working in a concerted fashion. Consequently, understanding the relationship between thrombosis and inflammation, that is, "thrombo-inflammation," in the cerebral microcirculation is critical in devising therapeutic strategies that can aggressively target and resolve any evolving cerebral thrombotic events such as ischemic stroke (Figure 1).[9]

1.1 | Stroke

Stroke is one of the leading causes of death and long-term disability worldwide. In the United States alone, almost 800,000 stroke-related events occur each year.[2] In the majority of cases, stroke is the result of multiple underlying risk factors including hypertension, obstructive sleep apnea, dyslipidemia, atrial fibrillation, and genetic disorders, for example, sickle cell disease (SCD).[10-14] There are two main types of stroke: acute ischemic or hemorrhagic stroke, with the former bringing ten times more prevalent than the latter, but the latter having worse prognosis.[15] Acute ischemic stroke is further classified into etiologic subtypes such as cardioembolic, atherosclerotic, lacunar, cryptogenic, and unusual cases (eg, vasculitis, dissection, drug abuse, SCD, Alzheimer's disease).[16] The type of stroke suffered is dependent upon several factors including different populations, underlying risk factors, and events leading up to the stroke itself. Most of the stroke-related pre-clinical and clinical work is focused on the related pathophysiology, including the involvement of the neurovascular unit and blood-borne cells, for example, neutrophils.[17]

1.2 | Brain microvascular endothelial cells and cerebral blood flow in stroke

Brain microvascular endothelial cells are a central part of the neurovascular unit (formed by neurones, interneurons, astrocytes, basal

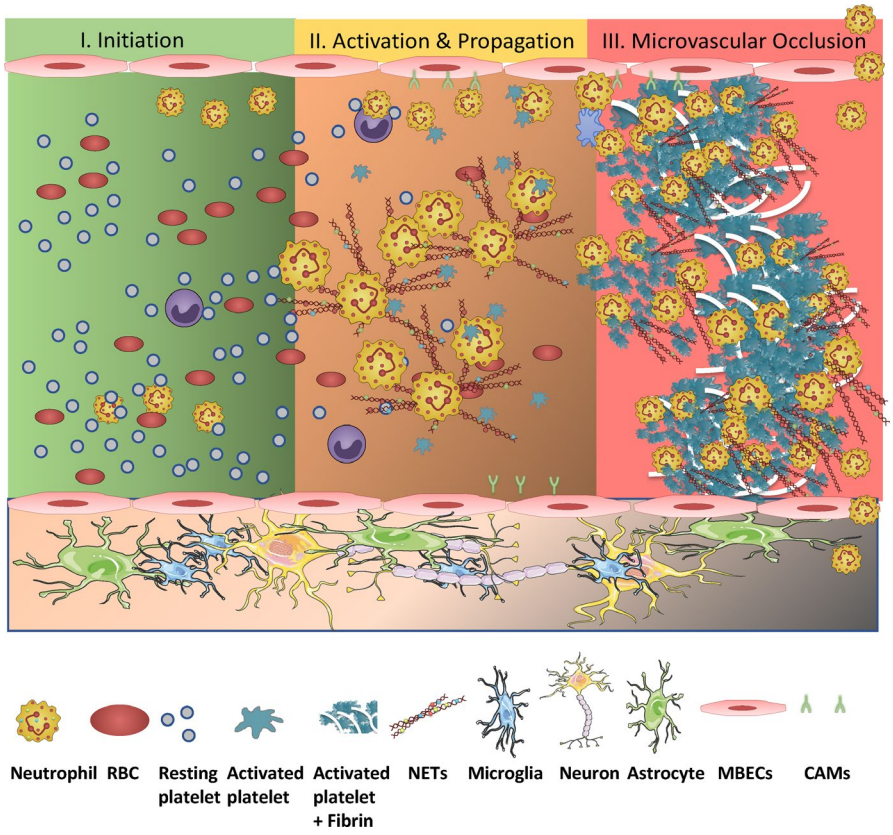

FIGURE 1 Role of thrombo-inflammation in cerebral microcirculatory dysfunction I. Initiation: Under thrombo-inflammatory conditions, there is increased pathophysiological infiltration of circulating leukocytes (including neutrophils) and platelets into the brain through the BBB. II. Activation and propagation: The severe recruitment of neutrophils at the inflammatory site results in increased expression of adhesion molecules such as L-selectin, P-selectin, and intracellular adhesion molecule-1, on microvascular brain endothelial cells (MBECs). Additionally, neutrophils activated at the site of neuro-inflammation express various mediators including neutrophil extracellular traps (NETs) and reactive oxygen species (ROS). NETs are laced with pro-coagulant/pro-thrombotic factors such as citrullinated histone (H3Cit), cathepsin G, and neutrophil elastase (NE) which interact with platelets resulting in platelet activation and aggregation. Activated platelets in itself cause further NET production from neutrophils in a vicious cycle. III. Microvascular Occlusion: This persistent response eventually leads to activation of coagulation cascade culminating in fibrin formation and clot development (thrombus). Within the cerebral microvasculature, neural activity can itself regulate neurovascular flow and activation by releasing neurotransmitters, cytokines, including angiogenesis mediators. In response to cerebral ischemia, microglia produce variety of pro-inflammatory cytokines (eg, interleukin [IL]-1, IL-6, tumor necrosis factor-alpha, ROS, and proteolytic enzymes), which further escalates the pro-thrombo-inflammatory phenotype

lamina covered with smooth muscular cells and pericytes, endothelial cells, and extracellular matrix) and the associated blood-brain barrier (BBB) structures (including tight junctions, basal lamina, pericytes, and parenchymal cells including astrocytes, neurons, and interneurons).[18] This complex neurovasculature regulates cerebral blood flow and nutrient delivery via a wide range of transport mechanisms. In contrast to peripheral endothelial cells, brain microvascular endothelial cells express specific ion transporters and receptors, and contain fewer fenestrations and pinocytotic vesicles, which makes cerebral blood vessels a highly selective molecular transport process, thereby maintaining cerebral homeostasis.[19,20] Much cerebral blood flow regulation is also attained by autoregulation which ensures adequate delivery of oxygen and nutrients through changes in the cerebral vascular tone.[21,22]

The BBB makes the central nervous system (CNS) an immunologically privileged center.[18] Additionally, the absence of traditional lymphatics, lack of major histocompatibility complex class II–expressing antigen-presenting cells, metabolic demands, and other factors contribute to CNS sterilization.[18] Thus, under a normal physiological state there is less communication between the peripheral immune system and the CNS. However, during the setting of acute cerebrovascular events (eg, ischemic stroke), thrombo-inflammatory pathways become activated resulting in pathophysiological infiltration of circulating leukocytes (including neutrophils, monocytes, and lymphocytes) into the brain through the BBB. Neuroinflammatory responses are further orchestrated by the production of cytokines from infiltrated leukocytes within the brain, increased expression of the adhesion molecules on the cerebral endothelium, followed by further migration and recruitment of the leukocytes across the endothelium.[20,23] Neural activity in itself can control the neurovasculature by releasing neurotransmitters (mainly glutamate excitotoxicity from neurons and glial cells)[24] and growth factors (vascular endothelial growth factor) further amplifying cerebrovascular patterning based on the pathophysiological milieu (Figure 1).[25,26]

1.3 | Cerebral microcirculation and thrombo-inflammation

The microcirculation is the terminal vascular network of the systemic circulation. Recent studies have given a clearer understanding of cerebral microcirculatory circuits and flow mainly involving leukocytes, platelets, and erythrocytes and their interaction with brain microvascular endothelial cells.[9,18] Leukocytes (in particular neutrophils) are the most abundant and the first line of defensive cells to be recruited (within minutes), transported and transmigrated through the endothelium.[9] Neutrophils have been classified as the main armor of the innate immune system with diverse functions in both acute and chronic inflammatory responses.[27] Neutrophils enable thrombo-inflammation, by their ability to express various pro-thrombotic/pro-inflammatory mediators such as neutrophil extracellular traps (NETs), reactive oxygen species (ROS), and neutrophil serine proteases which can all independently, or collectively, upregulate thrombo-inflammatory processes.[7,8,28-31] Neutrophils are rarely found in the brain parenchyma under a physiological setting, due to the presence of the BBB, and are only sparsely seen in cerebral spinal fluid, meninges, and pia mater where they provide immune surveillance.[32] However, during neurovascular insults, such as ischemic stroke or traumatic brain injury, there is a huge influx of neutrophils which can significantly influence and impede normal microcirculatory rheology along the affected neurovascular sites.[32,33] These interactions have been shown to lead to an increase in selectins (eg, P- and E-selectin) and adhesion molecule (eg, intracellular adhesion molecule-1) expression on murine brain endothelial cells hindering vascular flow and predisposing the brain to the vaso-occlusive phenomena.[34] The initial neutrophil response also leads to interactions with platelets via a variety of different mechanisms such as Mac-1 (CD11b/CD18)/glycoprotein Ib (CD42) and P-Selectin/P-selectin glycoprotein ligand-1, formation of fibrin cross-links (via Mac-1/fibrin interaction), and induction of extrinsic Tissue factor/factor IIa pathway, generating thrombin.[30] These neutrophil-platelet aggregates have been associated with stroke progression, but it is still unclear as to whether the direct interaction between platelets and neutrophils is critical for the pathogenesis of ischemic stroke, although evidence suggests they are heavily involved.

1.4 | Neutrophil cross-talk in stroke

Neutrophils are one of the earliest players of stroke pathogenesis, interacting with platelets, microvascular brain endothelial cells, microglial cells, and the cerebral microvessels.[35] Blood-borne neutrophils can migrate within few minutes of ictus (stroke onset) with the zenith achieved between 48 and 72 h after the ischemic stroke event.[36] The interaction of activated neutrophils with surrounding vascular milieu actively promotes BBB disruption, collagen degradation, and hemorrhagic transformation through the release of proteases such as matrix metalloproteases, neutrophil serine proteinases (eg, neutrophil elastase, cathepsin G, and proteinase 3), and ROS. Neutrophil-dependent ROS can further disrupt neurovascular architecture in stroke through modification of microvascular brain endothelial cells, pericytes, astrocytes, and neurons.[36] This rich thrombo-inflammatory milieu results in further platelet aggregation and coagulation, aided by the production of NETs and contributing to secondary thrombotic events.[28,37] Presence of citrullinated histone H3 which is characteristic of NETs, in thrombi, is known to be associated with adverse stroke prognosis and usually secondary to cardioembolic etiology (Figure 1).[38]

1.5 | Neutrophil-platelet interactions in cerebral thrombo-inflammation

Compelling evidence from in vivo animal models and in vitro human studies has demonstrated neutrophils work intimately with platelets via three distinct mechanisms to promote thrombo-inflammation:

(1) The first mechanism involves the production of NETs, which are composed of DNA filaments laced with histones, proteases, and granular and cytosolic proteins (all of which are known to be pro-thrombotic in nature).[39] In vitro and in vivo models have been used to provide compelling evidence demonstrating the abundance of pathological NETs in stroke thrombi, including clots obtained from ischemic stroke patients.[40,41] The pro-thrombotic nature of NETs has been attributed to the presence of citrullinated histone proteins, neutrophil elastase, and cathepsin G.[42,43] Furthermore, NETs are decorated with phosphatidylserine which provides functional platforms for platelet microparticles and coagulation factor deposition, aiding thrombogenesis.[41] (2) The second mechanism involves neutrophil serine proteases which can regulate pro-thrombotic and pro-inflammatory responses by interacting with platelets and coagulation factors[28] and binding with, for example, FPRs on neutrophils and monocytes.[44,45] Cathepsin G and neutrophil elastase are the most common neutrophil serine proteases and can induce a pro-thrombotic environment by stimulating platelet aggregation and activating the coagulation cascade.[28] Cathepsin G may also be producing some of these effects by interacting with FPRs[44] or its agonists such as Annexin A1.[45] (3) Finally, the third mechanism involves tissue factor, a cell-surface, integral-membrane protein, and the initiator of the extrinsic pathway. Neutrophils can passively acquire tissue factor from activated monocytes, transferring it to platelets during thrombus formation via tissue factor containing microparticles.[46,47]

1.6 | The role of resolution biology in thrombo-inflammation

There is a growing evidence of the importance of resolution biology in vascular inflammation. Classic resolution is defined as the "period between peak inflammatory cell influx and the clearance of immune cells from the site of tissue injury and the restoration of functional homeostasis." It involves a tightly regulated engagement of specialized lipid mediators (eg, resolvins, lipoxins, maresins, protectins) and resolver proteins (eg, Annexin 1 and Annexin 1-derived peptides),[7,48-53] which are actively involved in the recovery phase of inflammation in acute and chronic conditions.[49,54-57] Annexin 1 holds a special position in the resolution family of mediators due to the ability of the parent protein and its mimetic peptides to act as a pivotal homeostatic mediator targeting both *endogenous* inflammatory and pro-resolving pathways, and in so doing is associated with a lower degree of secondary effects. Our own studies have shown that Annexin A1 and its mimetic peptide (Ac2-26) afford cerebrovascular protection against ischemic stroke by modifying both neutrophil and platelet phenotypes by engaging endogenous pro-resolving, anti-thrombo-inflammatory circuits.[7,8] A number of other studies have demonstrated an alteration or dysregulation of resolution biology in a mixture of inflammatory conditions including, but not limited to, cerebrovascular diseases, neurodegenerative conditions, metabolic syndromes, and autoimmune diseases.[7,58-62]

More recently, the field has coined a third resolution phase (the first two being inflammation and resolution) in which the affected tissue develops adaptive immunity (whereby innate and adaptive immunity is bridged by the production of local pro-resolution processes, as well as tissue reprogramming).[54] This third phase is termed "post-resolution," and currently evidence suggests that in certain situations, "frustrated resolution" may occur in which adaptive immunity is not fully achieved.[63] Further work is needed in order to continue to develop and expand pharmacological strategies involving resolution biology, for example, in maladaptive immunity, are the roles and mechanisms used by neutrophils and other leukocyte subtypes parallel those in acute inflammation, or is there a shift in immune function?

1.7 | SCD and thrombo-inflammation

Sickle cell disease is a hereditary hemoglobin defect associated with early-onset cerebrovascular events including acute ischemic strokes, silent cerebral infarcts, moyamoya disease, and intracerebral hemorrhages.[64] Sickle hemoglobin polymerizes, causing structural damage and altering the erythrocyte membrane. These effects result in excessive hemolysis which activates the vascular milieu via production of heme microparticles and arginase into the plasma. This persistent, cyclical, and chronic process results in unresolved inflammation,[53] and leukocytes (especially SCD neutrophils) have been associated with the pathogenesis of SCD.[65-67] Neutrophilia in SCD is considered a significant risk factor for evolution of cerebrovascular diseases,[64] and SCD neutrophils are hyperinflammatory particularly during the vaso-occlusive crisis.[68] SCD neutrophils stimulate surrounding platelets and endothelium, therefore activating coagulation cascade. Additionally, heme released from lysed red blood cells activates neutrophils to produce NETs that may bind with sickled red blood cells and platelets, further provoking thrombus formation within the cerebral microvessels.[69] Interestingly, as with other chronic inflammatory conditions, SCD has been known to be associated with impaired resolution process resulting in excessive uncontrolled inflammation.[53] Our work (Ansari et al.) will further highlight the importance of pathological neutrophil function and hampered resolution biology in SCD cerebral phenotype and how exogenous Annexin A1 mimetic peptide Ac2-26 can mitigate neutrophil-dependent thrombo-inflammatory responses and attenuate thrombosis in SCD.[70]

1.8 | History and current treatment options for acute ischemic stroke patients

Prompt reperfusion is the cornerstone of acute ischemic stroke patient management. Recanalization with intravenous thrombolysis with tissue plasminogen activator (tPA) or endovascular treatment in combination with intravenous thrombolysis is the mainstay of acute ischemic stroke management. Despite these approaches, there has been a historic gap between evolution of therapies for

stroke vs. myocardial infarction.[71] Intravenous thrombolysis with tPA has been the most important breakthrough treatment for ischemic stroke since the Food and Drug Administration approval in 1996 based on multiple studies showing significant clinical outcomes.[72] Subsequent studies have emphasized the rapid treatment and favorable outcomes with as much as up to 4.5 h after the onset of ischemic stroke, although with a risk of intracranial hemorrhage in some patients.[73] Recent work has suggested superiority in treatment with a new clot buster "tenecteplase" (TNK). TNK is a variant of alteplase (tPA), but possesses greater fibrin specificity and a longer half-life, making it potentially safer and a more effective drug for stroke. It is highly likely that TNK will be the standard of care in the near future with higher rates of recanalization and reperfusion coupled with ease of intravenous administration.[74] There are also ongoing research and clinical trials on the combination of tPA with other treatment modalities such as hypothermia, eptifibatide, and activated protein C.[75-77]

One of the most common adverse outcomes of recanalization therapy is reperfusion injury which in some patients results in life-threatening complications such as hemorrhagic transformation (for which there is a tenfold increased risk after tPA administration).[36] Ischemia-reperfusion injury is a well-known phenomenon observed with microcirculatory dysfunction in almost all cerebrovascular abnormalities and mainly consists of two fundamental processes: the initial ischemic insult and the subsequent revascularization with enhanced activation of both innate and adaptive immune systems and cell death programs.[78] Initial tissue hypoxia and production of ROS activate innate and adaptive immune systems, and both leukocytes (mainly neutrophils) and platelets have been observed and quantified in post-ischemic cerebral microvessels. Interestingly, increased neutrophil counts at the time of reperfusion with tPA are known to be associated with increased risk of hemorrhagic transformation (incidence ranging from 8.5% to 40%)[79] and high neutrophil-to-lymphocyte ratio is predictive of hemorrhagic transformation in acute ischemic stroke patients.[79] Thus, targeting neutrophils at ischemic stroke onset may mitigate reperfusion-related complications.[80,81]

Endovascular treatment approaches such as mechanical thrombectomy have rapidly changed the overall management strategy and prognosis of AIS, with substantial improvement in functional neurological outcomes. Additionally, the mechanical thrombectomy has longer window period with up to 24 h if evidence of sizable ischemic penumbra.[82] There is also evidence of benefit of collateral flow augmentation and hemodynamic regulation during proximal vessel acute ischemic stroke.[83]

Secondary stroke prevention is the most important risk-reducing strategy for management of strokes. The major modifiable risk factors include hypertension, diabetes mellitus, smoking, dyslipidemia, and physical inactivity. Two additional mechanisms that are amenable to secondary prevention include atrial fibrillation and carotid artery stenosis. The risk of stroke is particularly increased if there is presence of two or more risk factors.[3,84] The current guidelines for secondary stroke prevention include blood pressure control, use of anti-thrombotics (such as aspirin and clopidogrel), statin therapy (eg, atorvastatin), and lifestyle modification including smoking cessation. Some patients with symptomatic carotid artery stenosis may benefit from revascularization, and atrial fibrillation with long-term anticoagulation[85] or left atrial appendage closure devices.[86]

1.9 | Evolving therapeutics and diagnostics for targeting the vascular inflammation and microcirculatory factors for the management and prevention of stroke

The management of cerebrovascular diseases has undergone a significant progress in last two decades due to research and drug discovery programs with the production of various systemic and local thrombolytic (both medical and mechanical) and neuroprotective strategies.[87] Furthermore, there has been considerable advancement of high-resolution imaging techniques (including computed tomography, magnetic resonance imaging, and functional magnetic resonance imaging) and their tracers, resulting in accurate assessment of core-penumbra volumes and more rapid and appropriate therapy with less complications.[88] In our recent work, we created a viable fluorescent probe that has the ability to target and detect activated neutrophils by binding with Fpr2/ALX surface receptors and track inflammation.[89] Development of such novel small molecule imaging probes will further help in detecting neurovascular inflammation and guide appropriate therapeutics. Clinical trials and designs have targeted neuro-inflammation including decreased leukocyte recruitment, inhibiting the production of neutrophil-derived thrombo-inflammatory mediators and enhancement of anti-inflammatory/pro-resolving pathways in stroke (Table 1). Additional focus has been given on targeting platelets and endothelial cells for mitigation of ongoing inflammation. Here, we will focus more on the neutrophil-dependent targets and pathways including manipulation of resolution biology to attenuate thrombo-inflammation (Figure 2).

1.10 | Targeting neutrophil-dependent thrombo-inflammatory mediators

Neutrophils play a major role in cerebral thrombo-inflammatory processes by promoting thrombosis and atherosclerosis. Thrombosis in particular is enhanced by the release of pro-thrombotic mediators and direct interaction with platelets and clotting factors. Targeting thrombo-inflammatory mediators have an important role in preventing and treating various stroke-related complications by mitigating inflammation and enhancing neuroprotection. As discussed above, all major players of thrombo-inflammation including NETs, neutrophil serine proteases, and ROS, when targeted have shown to significantly reduce stroke complications and preserve neurological functions. Furthermore, neutrophil-derived anti-inflammatory/pro-resolving mediators, which are known to be altered in chronic

TABLE 1 Selected clinical trials targeting thrombo-inflammatory mediators in stroke

Clinical trial	Phase	Intervention	Primary outcome	Protocol no.
Combining Fingolimod with Alteplase Bridging with Mechanical Thrombectomy in Acute Ischemic Stroke (FAMTAIS)	2	Fingolimod	Salvaged ischemic tissue index (%)	NCT02956200
Efficacy and safety of FTY720 for acute stroke	2	Fingolimod	Clinical improvement	NCT02002390
Safety and efficacy of intravenous natalizumab in acute ischemic stroke (ACTION2)	2	Natalizumab	Percentage of participants with composite global measure of functional disability excellent outcome at day 90	NCT02730455
Vinpocetine inhibits NF-κB-dependent inflammation in acute ischemic stroke	2,3	Vinpocetine	Changes in lesion volume changes in lesion volume from baseline (DWI) to day 7 (Flair), brain inflammatory level, brain inflammatory level (MRS) at day 7, extent of clinical improvement	NCT02878772
SCIL-STROKE (subcutaneous interleukin−1 receptor antagonist in ischemic stroke)	2	IL−1Ra	Difference in concentration of log (interleukin−6) as area under the curve to day 3	NCT74236229
Minocycline to Improve Neurologic Outcome in Stroke (MINOS)	2	Minocycline	Maximally tolerated dose of IV minocycline, response to therapy at 90 days on the Modified Rankin Scale	NCT00630396
Use of anti-ICAM−1 therapy in ischemic stroke	3	Enlimomab	Response to therapy at 90 days on the Modified Rankin Scale	Enlimomab acute stroke trial
Hu23F2G phase III stroke trial (HALT)	3	Hu23F2G	Response to therapy at 90 days on the Modified Rankin Scale	Monoclonal antibody (humanized) against the neutrophil CD11/CD18 cell adhesion molecule (Hu23F2G, LeukArrest®)
Colchicine for Prevention of Vascular Inflammation in Non-cardio Embolic Stroke (CONVINCE)	3	Colchicine	Recurrence of non-fatal ischemic stroke, any recurrence of non-fatal ischemic stroke, non-fatal hospitalization for unstable angina, myocardial infarction, cardiac arrest, vascular death	NCT02898610
Atorvastatin in acute stroke treatment	4	Atorvastatin	NIHSS at 72 h, differences in msRankin score	NCT02225834
Secondary prevention with HMG-CoA reductase inhibitor against stroke	3	Atorvastatin	Incidence Rate of Stroke and TIA	NCT00221104
Neuroprotection of pioglitazone in acute ischemic stroke	2	Pioglitazone	Modified Rankin Scale (mRS)	NCT02195791
Intravenous autologous bone marrow-derived stem cells therapy for patients with acute ischemic stroke	2	Bone Marrow–derived Stem Cells	Modified Barthel Index score at six-month post-randomization	NCT01501773
Pilot investigation OF STEM CELLS IN STROKE	1	CTX0E03 neural stem cells	Incidence of adverse events	NCT01151124

inflammatory/metabolic disease, have been studied to counter regulate stroke pathogenesis by enabling resolution, clearance, and homeostasis.

A number of studies (some of which are included in Table 2, that focuses on emerging therapeutics targeting ischemic stroke pathogenesis) have shown neutrophil-derived serine proteases and nucleosomes contribute to cerebrovascular thrombosis by enhancing coagulation pathways. Specifically, pre-clinical models have shown targeting cathepsin G and neutrophil elastase can potentially inhibit platelet thrombus formation. Pharmacologic inhibition of cathepsin G improved CBF and attenuated brain injury in an animal stroke model.[90] In a similar fashion, neutrophil elastase has shown

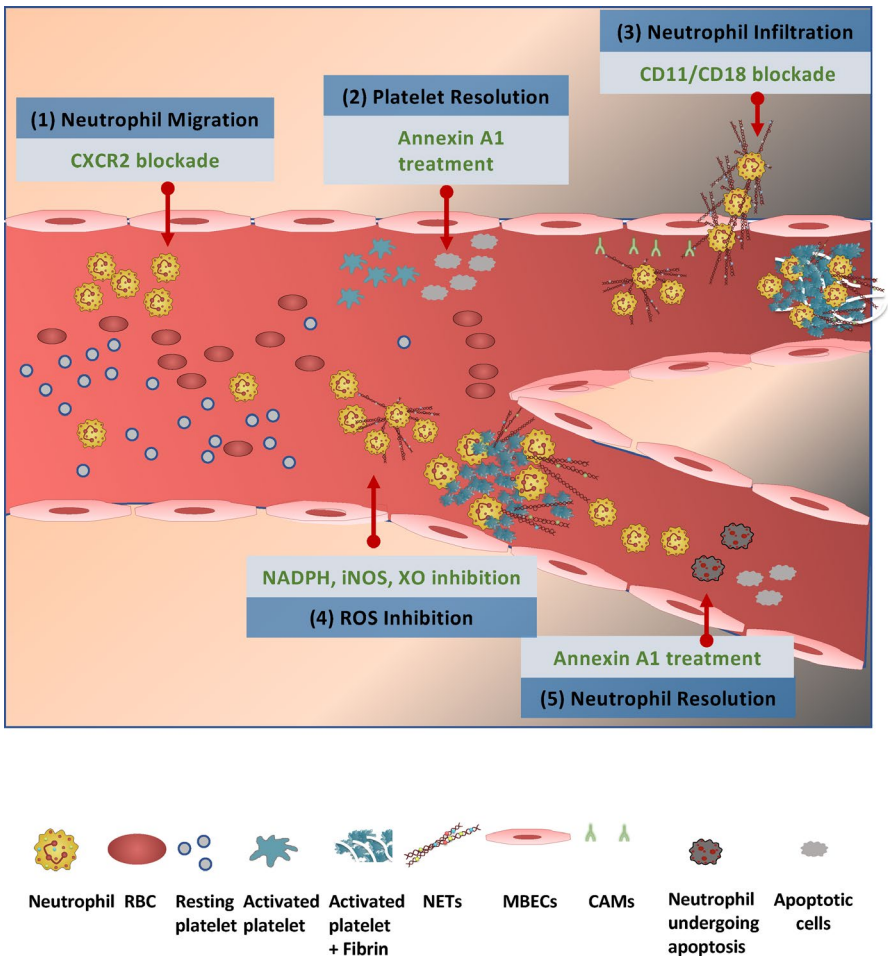

FIGURE 2 Schematic depiction of potential thrombo-inflammatory targets in neurovascular inflammation. Depiction of various aspects of neurovascular inflammation that can be therapeutically targeted. (1) Blockade of CXCR2 can reduce neutrophil activation and migration to the neurovascular insult site and mitigate inflammation. (2) Targeting platelet-dependent resolution with FPR2/ALX agonists such as Annexin A1 has shown to significantly impede cerebral thrombo-inflammatory processes and maybe neuroprotective in ischemic events. (3) CD11/CD18 antagonists have shown diminished neutrophil infiltration and transmigration. (4) Targeting reactive oxygen species attenuates neurovascular inflammation by reducing oxidative stress, inflammation, maintaining blood-brain barrier, reducing edema formation, and finally enabling apoptosis and autophagy. (5) Enabling neutrophil resolution by Annexin A1 treatment can reduce neutrophil activation and release of pro-thrombotic mediators subsequently resulting in neutrophil apoptosis and clearance

to mount a pro-thrombotic environment with the ability of neutrophil elastase to colocalize and facilitate degradation of tissue factor pathway inhibitor.[28] At present, no clinical trials are in place testing the effects of inhibiting serine proteases in the management of cerebrovascular diseases.

Neutrophil extracellular traps are chromatin structures laced with pro-thrombotic, pro-coagulant factors and are known to significantly contribute to venous and arterial thrombosis. A large body of pre-clinical work has been focused on targeting NETs to alleviate thrombo-inflammation. One of the earlier evidences was based on using PAD4 knockout mice and PAD4 inhibitors which demonstrated reduced thrombosis in murine model.[91,92] In our own work, we have demonstrated the important role that neutrophils play in SCD, revealing that making sickle cell mice neutropenic significantly reduce the thrombotic potential in the cerebral microvessels, our more recent findings have further reported on the mechanistic insights and pharmacological approaches of targeting neutrophil-dependent thrombo-inflammation to promote resolution.[69]

1.11 | Targeting neutrophil transmigration

Under chronic inflammatory states and especially during heightened crisis such as uncontrolled glucose, severe hypertension, or uncontrolled hyperlipidemia, there is increased activation and recruitment of neutrophils in the cerebrovascular milieu.[93] In addition, during acute stroke events, there is an enhanced neutrophil recruitment into the ipsilateral side resulting in localized inflammation, which is often coupled to a secondary ischemic insult of the penumbra. As such, preventing neutrophil recruitment, adhesion, and transmigration has been considered a viable strategy by many pre-clinical and clinical models.

TABLE 2 Emerging therapeutics ischemic stroke pathogenesis

Pathophysiology	Intervention	Therapeutic mechanism	Clinical situation	Ref
Neutrophil migration	CXCR2 blockade	Decreased neutrophil recruitment, increased neutrophil apoptosis, reduced CXCR2 levels, inducible NOS and NOX2 expression of BM neutrophils	Ischemic stroke	1
Neutrophil infiltration	CD11/CD18 blockade (LFA–1)	Reduced adhesivity for both quiescent and cytokine-activated endothelial monolayers	Ischemic stroke	2
Neutrophil resolution	AnxA1 treatment	Reduces infarct size and edema Reduces neutrophil migration Reduces leukocyte-platelet interaction, Restoration of BBB Inhibits lipopolysaccharide and SCD-induced cerebral inflammation Inhibits neutrophil-dependent cerebral thrombosis Reduces $H3Cit^{+}$ rich NET production	Ischemic stroke	3-5
Platelet resolution	AnxA1 treatment	Reduced platelet activation and aggregate formation, delayed thrombosis, enhanced phagocytosis by neutrophils	Ischemic stroke	4
ROS inhibition	ROS scavengers, NADPH inhibitors, iNOS inhibitors, XO inhibitors	Inhibition of initial and later phase ROS-induced inflammatory damage	Ischemic stroke	6

Abbreviations: AnxA1, Annexin A1; BBB, blood-brain barrier; CXCR2, CXC chemokine receptor 2; iNOS, inducible isoform nitric oxide synthase; NADPH, nicotinamide adenine dinucleotide phosphate; NETs, neutrophil extracellular traps; NOX2, NADPH oxidase 2; ROS, reactive oxygen species; XO, xanthine oxidase.

P-selectin and intracellular adhesion molecule-1 are main drivers to neutrophil adhesion to the MBECs and inhibiting P-selectin or intracellular adhesion molecule-1–mediated neutrophil recruitment has been studied as a possible anti-neutrophil adhesive strategy.[87,94,95] Using this hypothesis, CD18 (leukocyte counter-ligand to endothelial intracellular adhesion molecule-1) knockout mice showed diminished neutrophil recruitment and significant neuroprotection in the setting of a cerebral ischemia/reperfusion.[94] However, earlier clinical trials with anti-ICAM antibody enlimomab proved ineffective with no improvement in functional outcome and stroke severity.[96] Additional studies involving anti–E-selectin, anti–L-selectin, and chemokine receptors had none to minimal response in animal models.[36]

1.12 | Targeting oxidative stress

Leukocyte (especially neutrophil) recruitment to the cerebrovascular inflammatory sites results in overwhelming oxidative stress due to production of ROS. Brain resident cells such as microglia and astrocytes are the main sources of ROS within the CNS.[97] Furthermore, during the reperfusion phase of ischemia-reperfusion injury, there is rapid increase in the production of ROS, damaging the vascular and parenchymal structures causing widespread damage.[98] In acute ischemic stroke, ROS has been further implicated in the hemorrhagic transformation and cerebral edema, which are both major complications of revascularization therapy.[98] As with other forms of vascular inflammation, role of oxidative stress and therapeutically targeting ROS has been widely studied in neurovascular inflammatory conditions. As eluded by various studies, targeting ROS production attenuates neurovascular inflammation by reducing oxidative stress, inflammation, maintaining BBB, reducing edema formation, and finally enabling apoptosis and autophagy.[99] Remote ischemic conditioning (which inhibits activation of nicotinamide adenine dinucleotide phosphate oxidase in neutrophils)[100] and hypothermia (which decreases the generation of free radicals, inhibits the induction of oxidative DNA lesions, and suppresses immune system)[101] are non-pharmacologic adjunctive ROS directed therapies that are currently being tested to enhance neurovascular protection in ischemic stroke. Edaravone,[102] uric acid,[103] and citicoline[104] have been utilized to eliminate free radicals and have shown to mitigate ischemic-reperfusion injury and promote neurovascular recovery after stroke.[98]

1.13 | Enhancing resolution of thrombo-inflammation

Endogenous-specialized pro-resolving mediators which enhance the ability of vascular milieu to clear inflammatory phase and enable homeostasis have shown to be of benefit in many pre-clinical models of vascular inflammation.[54,105,106] These mediators act on different stages of the inflammatory cascade, restoring physiological responses by, for example, reducing neutrophil recruitment, inhibiting cytokine release, promoting apoptosis and phagocytosis, decreasing vascular permeability, regulating neutrophil-platelet complexes, and delaying the overall cerebral thrombotic responses.[7,8,57,107,108] More

specifically, in our study models, we have shown that Annexin A1 (and its N-terminal–derived peptide) modifies neutrophil-platelet interaction by activating the Annexin A1/FPR-2 pathway in both neutrophils and platelets, with AnxA1 administration leading to reduced platelet activation and aggregate formation, delayed thrombosis, and enhanced platelet phagocytosis by neutrophils.[7,106] Some of these mechanisms were dependent upon AKT, intracellular Ca^{2+}, and Rap1 and suggested a role for Annexin A1 to affect integrin ($\alpha_{IIb}\beta_3$) activation.[7] In addition, we also showed that aspirin-triggered lipoxin, a specific FPR-2, markedly attenuated inflammatory responses, including neutrophil-platelet aggregates in a murine model of ischemic stroke.[8]

2 | CONCLUDING REMARKS

Neutrophils play a significant role in the pathogenesis of cerebrovascular abnormalities and participate in the onset and propagation of cerebrovascular thrombo-inflammation (especially in the context of ischemic stroke) in the acute and chronic setting. Neutrophils work in a "dichotomous" fashion and have the capacity to exhibit pro- and anti-inflammatory (resolution) phenotype. Although understanding neutrophil resolution in the context of cerebrovascular inflammation has resulted in promising pre-clinical studies, further investigation is necessary to unravel the pathophysiological responses contributing to neurovascular thrombo-inflammation. More specifically, the potential use of endogenous biosynthetic circuits (such as Annexin A1/FPR-2 pathway) may play a significant role in guiding therapies targeting the major players (such as neutrophils and platelets) on the thrombo-inflammatory stage.

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CONFLICT OF INTEREST

None.

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