Influence of the brain-gut axis on neuroinflammation in cerebral ischemia-reperfusion injury (Review)

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Abstract. Stroke, a debilitating cerebrovascular ailment, poses significant threats to human life and health. The intricate interplay between the gut-brain-microbiota axis (GBMA) and cerebral ischemia-reperfusion has increasingly become a focal point of scientific exploration, emerging as a pivotal research avenue in stroke pathophysiology. In the present review, the authors delved into the nexus between the GBMA and neuroinflammation observed post-stroke. The analysis underscored the pivotal roles of histone deacetylase 3 and neutrophil extracellular traps subsequent to stroke incidents. The influence of gut microbial compositions and their metabolites, notably short-chain fatty acids and trimethylamine N-oxide, on neuroinflammatory processes, was further elucidated. The involvement of immune cells, especially regulatory T-cells, and the intricate signaling cascades including cyclic GMP-AMP synthase/stimulator of interferon genes/Toll-like receptor, further emphasized the complex regulatory mechanisms of GBMA in cerebral ischemia/reperfusion injury (CI/RI). Collectively, the present review offered a comprehensive perspective on the metabolic, immune and inflammatory modulations orchestrated by GBMA, augmenting the understanding of its role in neuroinflammation following CI/RI.

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Abbreviations: GBMA, gut-brain-microbiota axis; CI/RI, cerebral ischemia/reperfusion injury; HDAC3, histone deacetylase 3; NET, neutrophil extracellular traps; SCFA, short-chain fatty acids; TMAO, trimethylamine N-oxide; Treg, regulatory T-cell; GBA, gut-brain axis; NF-kB, nuclear factor kappaB; STAT3, activator of transcription 3; TLR, Toll-like receptor; IFN-gamma, interferon-gamma; TNF-alpha, tumour necrosis factor alpha; IL-6, interleukin-6; ROS, reactive oxygen species; BBB, blood-brain barrier; KLF4, Kruppel-like factor 4; OGD/R, oxygen glucose deprivation reperfusion; PIC, peri-infarct cortex; MMP, matrix metalloproteinase; TJP, tight junction protein; cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes; dsDNA, double-stranded DNA; TBK1, TANK-binding kinase 1; IRF3, interferon-regulating factor 3; CVST, cerebral venous sinus thrombosis; GM, gastrointestinal microbiota; IBD, inflammatory bowel diseases; PD, Parkinson's disease; VB12, Vitamin B12; Hcy, homocysteine; LPS, lipopolysaccharide; PSD, post-stroke depression; 5-HT, 5-Hydroxytryptamine; IEC, intestinal epithelial cell; MPO, myeloperoxidase

Key words: GBMA, CI/RI, cGAS-STING, gut microbial metabolites, neuroinflammation

Contents

1. Introduction

Globally recognized as the second major cause of mortality, stroke also stands as the predominant source of enduring disability in adults (1). The repercussions of a stroke extend beyond the immediate physical manifestations; numerous survivors grapple with significant impediments to their long-term physical functions. A substantial proportion of these survivors, as a result, find themselves reliant on external support, and are predisposed to additional neurological complications, including depression (2,3).

An ischemic stroke (IS) delineates into two principal regions: The core ischemic infarct and the surrounding ischemic penumbra. While neural cells within the infarcted core are often irreversibly damaged, the penumbral tissue, albeit compromised, retains a temporal viability. Swift therapeutic interventions can facilitate cerebral blood flow restoration within the penumbral zone, forestalling further tissue degeneration and subsequent necrosis. Contemporary treatments, notably intravenous thrombolysis deploying recombinant tissue plasminogen activator (rt-PA) and endovascular strategies such as intra-arterial thrombolysis and mechanical thrombectomy, have shown efficacy in preserving

functionality within the ischemic penumbra (1). However, the rapid reperfusion inherent to these treatments, bound by a constrained therapeutic window, risks potential irreversible neurological impairments.

Cerebral ischemia/reperfusion injury (CI/RI) spans both the initial ischemic damage and the ensuing ischemia instigated by reperfusion (4). Intriguingly, the pathophysiological trajectory of CI/RI exhibits associations with gut flora and their metabolic byproducts. The evolution in understanding of the brain-gut dynamics has now shifted focus from the traditionally recognized gut-brain axis (GBA) to the more encompassing gut-brain-microbiota axis (GBMA). This paradigm emphasizes the intricate, bidirectional linkage tethering the gut and brain. GBMA provides an avenue to elucidate the symbiotic interactions at the microbiota-gut-brain interface, facilitated by signaling conduits such as cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) and modulated by intestinal microbial metabolites. Such insights underscore the potential for innovative therapeutic strategies targeting CI/RI via intestinal microbial metabolite modulation.

The methods for selecting articles were as follows: The main things that determined which studies were included in the present review were how they related to the brain-gut axis and how they played a part in neuroinflammation after cerebral ischemia-reperfusion injury. Preference was given to peer-reviewed research articles and reviews published in reputable journals, focusing on those that provided original data, comprehensive reviews, or significant theoretical contributions.

The literature search was extensive and utilized databases such as PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Web of Science (https://www.webofscience.com/wos/). Key search terms included 'gut-brain axis', 'cerebral ischemia/reperfusion injury', 'neuroinflammation', 'ischemic stroke', 'gastrointestinal microbiota', 'the cGAS-STING signaling pathway', 'microglia' and 'astrocytes', 'blood-brain barrier', 'the NF-kappaB signaling pathway', 'the TLR4-NF-kappaB/PI3K-AKT/MAPK signaling pathway', 'the MAPK signaling pathway', 'the PI3K-AKT signaling pathway', 'LPS', 'SCFA', 'VitB12', '5-HT', 'TMAO' and various combinations thereof. Initially, the publication dates were set from 2001 to 2023, with 69.95% of the literature published between 2018 and 2023.

The initial search generated a large number of articles, from which titles and abstracts were screened for relevance. A full-text review was then conducted to assess the depth of the content and the soundness of the methodology. The selection process was iterative, refining search terms and inclusion criteria as needed to ensure comprehensive coverage of the topic.

Each article underwent a rigorous quality assessment focusing on the significance of the study design, methodology, sample size, statistical analyses and findings. Special attention was given to studies that provided novel insights or challenged existing paradigms.

Articles were excluded if they did not address the brain-gut axis associated with CI/RI, lacked scientific rigor or were not peer-reviewed. Duplicate studies and those with outdated or superseded information were also omitted.

The selection process was a collaborative effort that included discussions with co-authors and consultation with other experts in neurology and internal medicine specialists to ensure a multidisciplinary perspective.

As the review progressed, search and selection criteria were continually refined based on emerging themes and findings to ensure comprehensive and up-to-date analyses. The approach to selecting material for review was designed to provide a comprehensive and unbiased overview of current understanding of the effects of the brain-gut axis on neuroinflammation in CI/RI.

2. Crosstalk between CI/RI and neuroinflammation

Microglia in the neuroinflammatory milieu of CI/RI. Microglia, the resident immune cells of the brain, constitute between 6-21% of the brain's total glial cell population (5). Historically, the understanding of microglia's origins underwent a paradigm shift in the early 1990s. Contrary to the previously held belief that microglia solely originated from peripheral macrophages, it was unveiled that they could also arise from bone marrow progenitor cells located in the yolk sac. This novel classification of macrophages was aptly christened 'microglia' by Pío del Río-Hortega, a terminology that has since gained universal acceptance (6,7).

Despite their behavioral and functional parallels with macrophages, which led to their initial identification as such (8), microglia maintain a distinct and irreplaceable role within the central nervous system (CNS). A mere h post-ischemic insult, resident microglia spring into action. These cells undergo notable morphological transformations: Retracting their external protrusions, they adopt an amoeboid form (9). Additionally, based on temporal progression post-injury, microglia can be categorized into acute, subacute and chronic phases, each presenting unique pathological characteristics (10).

Acute phase. Within mere h post-ischemic injury, microglia are promptly activated by the infiltration of plasma proteins. Once stimulated, they infiltrate both the infarct core and the peri-infarct region. This activation is mediated by a suite of downstream signaling effectors, notably including nuclear factor κB (NF-κB), hypoxia-inducible factor 1 (HIF-1) and activator of transcription 3 (STAT3). Morphologically, peri-infarct microglia exhibit enlarged cell bodies, short branches and amoeboid structures. Adjacent to the ischemic core, particularly large, round microglia display heightened activity, attributed to their low activation thresholds.

Historically, microglia were perceived to polarize into two distinct phenotypes: M1 and M2, in response to activation. The M1 phenotype, characterized by its pro-inflammatory and neurotoxic activities, emerges within the initial 24 h post-injury. This induction is primarily steered by the Toll-like receptor (TLR) and interferon-gamma (IFN-gamma) signaling pathways (11). M1 microglia secrete a plethora of pro-inflammatory factors such as tumour necrosis factor alpha (TNF-alpha), IL-6, IL-12 and IL-1β, alongside reactive oxidative agents such as reactive oxygen species (ROS) and inducible nitric oxide synthase (12,13). These agents, particularly ROS and TNF-alpha, can disrupt the integrity of the blood-brain barrier (BBB), inflicting severe endothelial damage and potentially leading to hemorrhagic transformation (14,15).

Conversely, while the pro-inflammatory M1 phenotype emerges, certain chemokines, including CCL2 and CXCL4, initiate the polarization of microglia towards the

anti-inflammatory M2 phenotype post the 24-h mark (16,17). Distinct from M1, the M2 phenotype secretes anti-inflammatory agents such as IL-4, IL-10 and transforming growth factor beta, aiming to curb inflammation (18). These cells also release growth factors and neurotrophic agents including insulin-like growth factor 1, vascular endothelial growth factor and brain-derived neurotrophic factors to foster vascular repair and prevent neuronal cell apoptosis in the ischemic zone (19). Furthermore, the M2 phenotype is nuanced, with subcategories M2a, M2b and M2c (20). Each subtype is characterized by its induction mechanism and function (21).

However, the once clear-cut M1/M2 distinction is now acknowledged as an oversimplification. Contemporary understanding recognizes the vast functional overlaps and asserts that the complete array of microglial activation states *in vivo* extends beyond just the conventional M1 or M2 categories (22). For instance, post-ischemia, the presence of M1-like microglia amplifies from day one and peaks around the second week, whereas M2-like microglia peak around day five and subsequently decline (23). Notably, older mice exhibit significantly fewer M2 microglia compared with their younger counterparts, underscoring the adverse influence of advanced age on post-stroke neuroinflammation (24-26). The intricate nature of microglial responses is epitomized by findings where markers of both inflammatory resistance and promotion co-exist within the same cell. This complexity is a key factor influencing the challenges in treating IS injury with whole microglial cells (27).

Subacute vs. chronic phase. While research efforts into the acute phase of post-stroke neuroinflammation have burgeoned over the years, studies exploring the subacute and chronic phases remain relatively sparse. During these later phases, damage-associated molecular patterns activate microglia and infiltrating macrophages. These activated cells subsequently release inflammatory cytokines such as TNF-alpha and IL-1beta, exacerbating neuronal cell death in the ischemic penumbra (28). Remarkably, even after six months post-injury, blood samples from stroke patients persistently exhibited elevated levels of TNF-alpha (29).

Anti-inflammatory M2-type microglia play a pivotal role during this phase by secreting cytokines, most notably IL-10, which counteract the inflammatory effects incited by IL-1beta and TNF-alpha, providing a protective barrier against further ischemic damage (30). Supporting this, a study by Ooboshi *et al* (31) demonstrated that administering an adenoviral vector encoding the human IL-10 gene into the lateral ventricles of stroke-affected rats substantially reduced cerebral infarct sizes and mitigated hippocampal ischemic injuries. The likely mechanism involves the capacity of IL-10 to dampen the production of inflammatory cytokines and enhance the activity of anti-inflammatory cytokine antagonists (31).

Interestingly, microglia can sustain a state of prolonged activity. While their activity tends to wane after six months within the infarct core, it often radiates to more distant regions, following the brain's corticospinal tracts (32). Modern imaging techniques such as positron emission tomography and magnetic resonance imaging have unveiled that, for certain patients, neuroinflammation migrates from the infarct's initial site to remote brain areas, encompassing the hippocampus, thalamus and basal ganglia, even those situated contralaterally (33). Echoing this, Price *et al* (34) observed a decline in microglial activity in the infarct core three to four weeks post-stroke. Conversely, there was a conspicuous surge of microglia in distant regions of the ipsilateral hemisphere and even in areas within the contralateral cerebral hemisphere (34).

Astrocytes in the neuroinflammatory milieu of cerebral ischemia/reperfusion (I/R). Astrocytes, akin to microglia, contribute significantly to the brain's innate immune response. These versatile cells govern ionic homeostasis, modulate neurotransmitter dynamics and contribute to the structural integrity of the BBB, thus playing pivotal roles in safeguarding both neuronal and vascular functionalities. In the context of CI/RI, astrocytes have been linked to the release of Kruppel-like factor 4 (KLF4), which not only curtails the infarcted area and suppresses oxidative stress but also fortifies the BBB. Intriguingly, KLF4 amplifies the levels of Nrf2 and Trx1 mRNA in the oxygen glucose deprivation/reperfusion (OGD/R) model, resulting in improved BBB repair, diminished malondialdehyde levels and elevated antioxidant SOD levels (35).

Astrocytes are swiftly activated post-brain injury and exhibit a dual-functional paradigm (36). Classically activated A1-like astrocytes, stimulated by IL1 alpha, TNF alpha, and complement component 1q (C1q) from microglia, unleash pro-inflammatory molecules, manifesting neurotoxic implications (37). By contrast, alternatively activated A2-like astrocytes confer neuroprotection via the secretion of anti-inflammatory mediators (38). Notably, post-brain I/R, lipopolysaccharide (LPS)-driven neuroinflammation precipitates the transformation of astrocytes to the A1 type. Research by Li *et al* (39) illuminated that hydrogen sulfide (H2S) curbs this LPS-induced A1-like astrocyte metamorphosis in the murine hippocampus, simultaneously promoting the shift of reactive astrocytes to the A2 type. This transformation was hypothesized to be modulated by the upregulation of the BKCa channel (39). In a complex cascade, A1 astrocytes release LCN2, which subsequently binds to 24p3R on astrocytes, instigating focal death of astrocytes via the NLRP3 mechanism. Yet, mitigating this astrocytic focal death curbs neuronal damage, particularly through attenuating caspase-1 activation, subsequently diminishing neuronal apoptosis (40).

Reactive astrocytes, once activated in the wake of cerebral ischemic injury, orchestrate a neuroglial scar within the peri-infarct cortex (PIC). This scar acts as a protective barrier, isolating the injury site from healthy tissues and impeding the spread of deleterious agents (41). Nevertheless, as cerebral ischemia advances, this very neuroglial scar evolves into an obstacle, hampering post-ischemic brain repair. Yuan *et al* (42) uncovered that p-Hydroxybenzaldehyde, derived from asparagus, curtailed astrocytosis in middle cerebral artery occlusion (MCAO) rat PICs. This led to a reprogramming of astrocytes, transitioning the neuroglial scar back to neuronal progenitor cells, thus promoting neural and vascular rejuvenation within the PIC (42). Another noteworthy study revealed that miR-124, encapsulated within the small extracellular vesicles (EVs) of M2-type microglia, expedited the reprogramming of astrocytes. This reprogramming counteracted the impediments posed by the glial scar in the PIC, catalyzing stroke recuperation (43).

BBB. The BBB is a complex and multifaceted structure, comprising astrocytes, pericytes, the extracellular matrix and the endothelial cells that line the brain's microvasculature. Its pivotal role is to safeguard the CNS. Not only does the BBB facilitate the entry of vital metabolic substrates such as oxygen and glucose into the brain, but it also acts as a vigilant sentinel, preventing the infiltration of pathogens, non-CNS cells, and other potentially harmful agents. In doing so, the BBB plays an indispensable role in preserving the delicate homeostasis of the brain (44).

Astrocyte-derived Matrix metalloproteinase-2/9 (MMP-2/9) have been implicated in disrupting the integrity of BBB. Specifically, these enzymes catalyze the degradation of tight junction protein (TJP) ZO-1, thereby compromising BBB permeability (45). Corroborating this, research has revealed that MMPs degrade the BBB's TJPs. However, agents such as BB-1101 have been shown to counteract this degradation, thereby bolstering BBB integrity (46).

In the context of I/R, the cyclic RNA of FoxO3 (circ-FoxO3) has been identified as a crucial player. It interacts synergistically with mTOR and E2F1, enhancing autophagic activity through the inhibition of mTORC1. This cascade of molecular events ultimately attenuates BBB-associated neuro-pathologies stemming from damage (47).

The cGAS-STING signaling pathway. The cGAS-STING pathway is a central molecular signaling cascade underpinned by two primary proteins: Cyclic GMP-AMP synthase (cGAS) and the stimulator of interferon genes (STING). Acting as an intrinsic immune sensor, cGAS detects an array of cytoplasmic double-stranded DNA (dsDNA) sources, encompassing viral, bacterial, mitochondrial, micronuclear and reverse transcription-derived DNA (48). Once bound to dsDNA, cGAS undergoes an enzymatic transformation, leading to the synthesis of the secondary messenger, cGAMP, from ATP and GTP. This messenger is subsequently identified by STING, a homodimeric protein anchored in the endoplasmic reticulum (ER) membrane. Upon cGAMP binding, STING undergoes a conformational shift, facilitating its oligomerization. This modified STING then translocates to the Golgi apparatus through the ER-Golgi intermediate compartment (ERGIC). Within the Golgi, a palmitoylation event occurs at STING's cysteine residues (Cys88 and Cys91), enabling its C-terminus to liaise with TANK-binding kinase 1 (TBK1). This interaction culminates in the phosphorylation of the STING protein at Ser366, leading to the recruitment and subsequent phosphorylation of interferon-regulating factor 3 (IRF3). As a result, IRF3 dimerizes, undergoes nuclear translocation, and induces target gene expression (49). Parallelly, STING also stimulates the nuclear factor kappaB (NF-kB) pathway, further influencing type 1 IFN expression (50). Experimental data derived from IS models, wherein cGAS was deleted or STING silenced, revealed reduced cerebral infarct injury and enhanced neuronal survival (51). By contrast, overactivation of this cGAS-STING axis has been linked to heightened neuroinflammation (52) (Fig. 1).

Modulating the cGAS-STING pathway has demonstrated therapeutic potential in mitigating neuroinflammation. Specifically, suppressing this pathway diminishes the inflammatory response elicited by LPS/IFN-gamma in BV-2 microglia (53). Such modulation has been noted to ameliorate learning and memory deficits in Alzheimer's disease, making cognitive impairments less pronounced. In addition, downregulation of the cGAS-STING pathway not only inhibited the attenuation of microglia M1 polarization but also reduced the expression of A1-type astrocytes to achieve an anti-inflammatory effect (54,55). The inhibitor RU.521, targeting cGAS, has shown promise in several contexts. It curbs the expression of NF-kB-associated inflammatory cytokines, thereby safeguarding neurons from potential damage. Furthermore, RU.521 effectively counteracts microglial pyroptosis, which is typically incited by the activation of cytosolic gasdermin-D and NLRP3 post cerebral venous sinus thrombosis (CVST) (52). Notably, RU.521 can also counteract the mtDNA-driven activation of the cGAS-STING pathway and modulate autophagy, further restraining the M1 polarization of microglia (56). Consequently, RU.521's modulation of the STING pathway offers a therapeutic avenue for mitigating IS. The evolving understanding and manipulation of the cGAS-STING signaling in microglia underscore its potential as a therapeutic target for managing neuroinflammation following cerebral I/R.

In the context of cerebral I/R, the downstream STING-TBK1-IRF3 pathway of cGAS is adeptly regulated by histone deacetylase 3 (HDAC3). The BCl-2-associated X-gene (BAX) is known to complex with IRF3, promoting immune cell apoptosis-a process that, if unchecked, can amplify post-stroke neuroinflammation (57,58). Both IRF3 and NF-kB, being downstream effectors of STING, can intensify neuroinflammation, especially evident in the OGD/R model (51). However, pharmacological intervention using HDAC3 inhibitors, such as Entinostat and Trichostatin A, has revealed efficacy in curtailing I/R injuries and the consequent neuroinflammation (59). Post-I/R, HDACs undergo oxidative activation. Different HDAC subclasses can either bolster or hinder cell viability and neurogenesis. For instance, inhibiting HDAC1, HDAC2, HDAC3 and HDAC6 enhances cell survival and dampens neuroinflammation (60,61). Conversely, augmenting the activity of HDAC4 and HDAC5 promotes both cell viability and neurogenesis (62).

Neutrophil extracellular traps (NETs) have emerged as central players in the vascular dynamics of stroke. They stimulate platelets, fostering a conducive environment for thrombosis, impede the repair of damaged vessels, breach the sanctity of the BBB, and underlie degenerative shifts observed in the protracted aftermath of stroke (63). Notably, tissue plasminogen activator (tPA), the primary therapeutic agent for thrombolysis and recanalization in IS, instigates neutrophils to yield NETs. Coincidentally, tPA treatment amplifies the cGAS expression, synchronously boosting STING functionality. This chain of events precipitates enhanced phosphorylation of TANK-binding kinase 1 (pTBK1) and ignites TBK1-facilitated activation of interferon regulatory factor 3 (IRF3), culminating in a surge of type I interferon (IFN-I) and interleukin-6 (IL-6) in ischemic zones. A study led by Rosell *et al* (64) underscored the instrumental role of NETs activation in the cGAS-STING pathway, especially post-tPA administration (64).

In a notable experiment, neutrophils treated with A23187 spawned EVs laden with DNA via NETs. These EV-DNAs, upon internalization by macrophages, activate an alternate

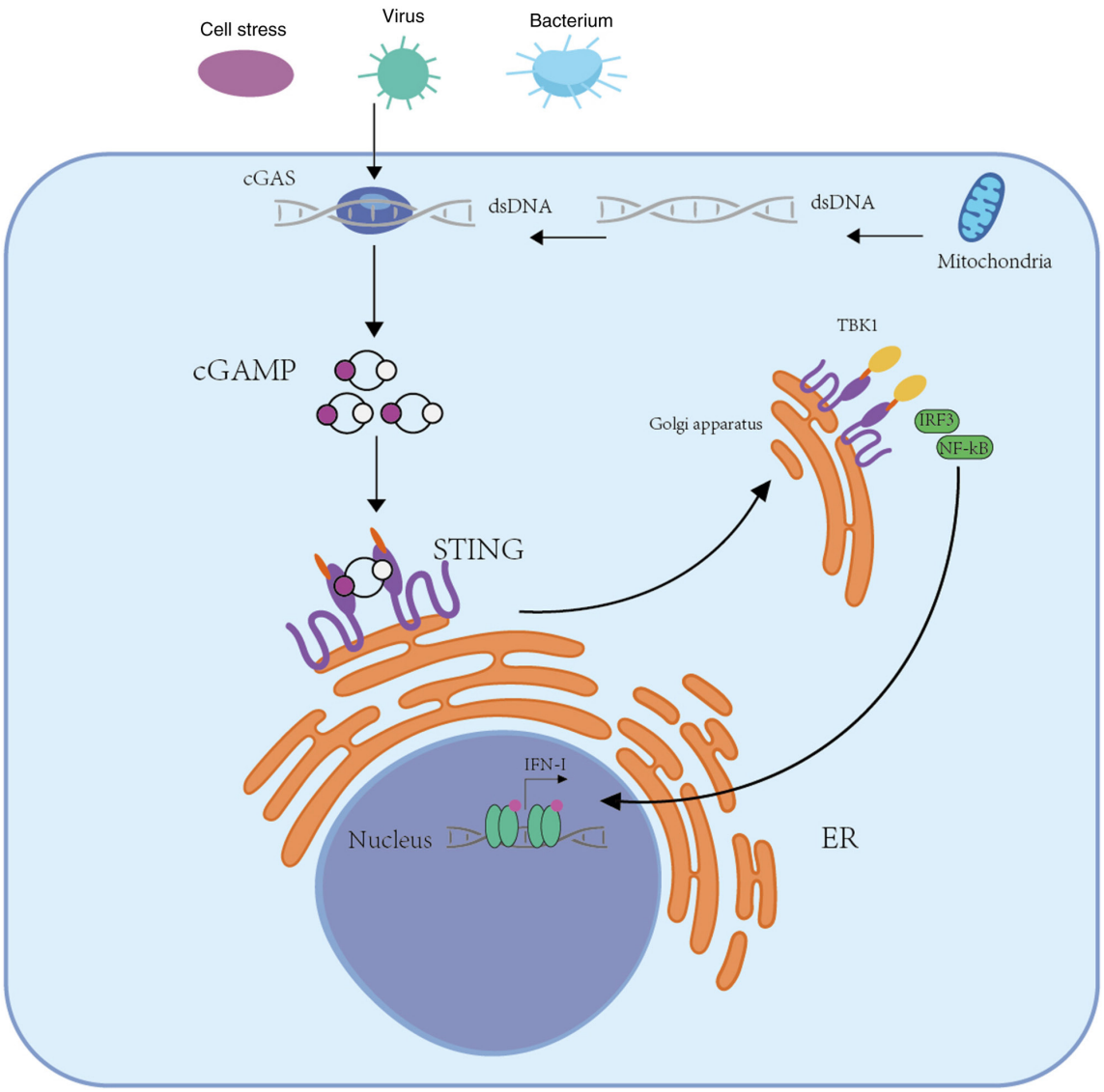

Figure 1. Overview of canonical cGAS-STING signalling. cGAS is a protein that is found in the cytoplasm and has an N-terminal structural domain that is longer than the two-lobed catalytic structural domain. It encounters its agonist DNA substrate (shown in gray), which consists predominantly of long DNA (standard single- or double-stranded molecules >40 bp). Agonist DNA can be derived from a variety of sources, including exogenous sources (DNA viruses, cell death, intracellular bacteria) and endogenous sources [stressed mitochondria, release of mitochondrial DNA (mtDNA), and chromatin fragments in the cytoplasm and cytosol]. Upon binding to dsDNA, cGAS is activated. The cGAS dimer assembles into a dimer on dsDNA, leading to enzymatic activation of cGAS and synthesis of 2'3' cyclic GMP-AMP (cGAMP) (cGAMP is shown as a ring structure connected by purple and white dots). cGAMP binds to STING dimer located in the ER membrane. Binding of the dimer results in a profound conformational change that triggers oligomerization of STING, liberating it from anchoring factors such as STIM1 (not shown in the figure). After crossing the endoplasmic reticulum-Golgi intermediate compartment and the Golgi apparatus, STING recruits TBK1, which promotes TBK1 autophosphorylation, phosphorylation of STING Ser366 and recruitment of IRF3. TBK1 phosphorylates IRF3, which dimerizes and translocates IRF3 to the nucleus, inducing transcription of the type I interferon gene. cGAS-STING signaling also leads to the expression of pro-inflammatory cytokine genes via NF-κB. cGAS, cyclic GMP-AMP synthase; STING, stimulators of the interferon gene; dsDNA, double-stranded DNA; ER, endoplasmic reticulum; TBK1, TANK-binding kinase 1; IRF3, interferon regulatory factor 3; NF-κB, nuclear factor kappaB.

cGAS-STING trajectory that propels NF-kB to synthesize IL-6-a process distinctly independent of the conventional cGAS-STING-TBK1-IRF3 signaling mechanism (65). These A23187-induced EVs provide invaluable insights into neutrophil-driven inflammatory cascades under ischemic conditions, offering potential therapeutic avenues to address neuroinflammation, infections, and autoimmunity in stroke patients.

Currently, tPA thrombolytic therapy is the only available treatment for reperfusion in areas affected by cerebral infarction. However, this treatment still carries the risk of cerebral hemorrhage (66,67). NETs or cGAS as a treatment could reduce the risk of hemorrhage post-tPA thrombolysis in IS, offering a new strategy to enhance the safety of tPA thrombolysis (68). By targeting NETs, it is also possible to influence cGAS-STING activation, further reducing M1-type microglia activation induced by STING/IFN-gamma (53). This reduction can alleviate neurotoxic effects, protect the BBB, and prevent further death of neurons in the ischemic penumbra (14,37). Additionally, this approach appears capable of addressing the prolonged elevated levels of TNF-alpha in the serum of CI/RI patients, which can last up to six months (29).

Additional insights suggest that LPS serves as a catalyst for NET production (69), with inflammatory markers, such as TNF-alpha, IL-6 and IFN, correlating with inflammatory

intensity of IS (14,70,71). Targeted interventions, such as the JAK 1/2 inhibitor Ruxolitinib (Rux), have shown promise in curbing this inflammation via the JAK2/STAT3 pathway, ultimately reducing cerebral edema and lessening infarct volume post-stroke (72). Caspase1, as part of the NLRP3 inflammasome, contributes to increased neurodegeneration and neurological impairment post-cerebrovascular sinus thrombosis (CVST) (52). Repeated cranial magnetic stimulation, operating through TLR4-NF-kB-NLRP3 pathways, enhances TNF-alpha and IFN-gamma while reducing stroke-adjacent areas. Caspase1 alleviates neuronal stress and limb movement disorders following the reperfusion of cerebral ischemia (71). Furthermore, NLRP3 mediates astrocyte death; targeting STING can suppress NLRP3 production, and inhibiting astrocyte death can reduce neuronal damage (40,73). The efficacy of the cGAS inhibitor RU.521 in reducing nerve damage through NLRP3 inhibition further confirms this (52). Targeting the downregulation of the cGAS-STING/NLRP3, TLR4-NF-κB/NLRP3, or NF-κB/NLRP3 axes to inhibit NLRP3 can alleviate astrocyte pyroptosis. This approach represents a promising future method for treating neuroinflammation following CI/RI (74,75).

3. CI/RI, GBMA and neuroinflammation

Gut microbiome. The gastrointestinal (GI) tract stands as a cornerstone of the body's immunological defense, harboring an extensive congregation of immune cells. Remarkably, over 70% of the human immune system finds its home within the GI immune system. Resident within this GI tract is a diverse community of microorganisms collectively termed the GI microbiota (GM). Advances in microbiome research have led to the evolution of the classical GBA concept, ushering in the more comprehensive GBMA. This holistic perspective encompasses the GI neuroendocrine system, the GI immune system, the enteric nervous system, and, pivotally, the GM.

Within the GBMA, GM emerges as a pivotal player, intricately influencing metabolic regulation, nutritional physiology, anticancer responses, immunomodulation, and crucially, neurological functions (76,77). Dominating the GI flora are *Firmicutes* (~48%) and *Bacteroidetes* (roughly 51%), with the residual composition being a mix of *Actinobacteria*, *Proteobacteria*, and other microorganisms (78). Notably, a marked reduction in the abundance of *Proteobacteria* and certain *Firmicutes* (such as *Clostridia* and *Lachnospiraceae*) has been observed in post-stroke cognitive impairment patients, setting them apart from non-stroke counterparts (79).

The human gut serves as a sanctuary for an array of microorganisms pivotal in either bolstering health or predisposing to disease, additionally playing a central role in immune homeostasis (47). Deviations in the GM's composition and function, termed dysbiosis, carry potent associations with a plethora of health concerns. Internally, within the GI domain, GM dysregulation correlates with inflammatory bowel diseases (IBD) such as ulcerative colitis, Crohn's disease, colorectal cancers and irritable bowel syndrome (80). Externally, beyond the confines of the gut, an imbalanced GM has links with neurodegenerative ailments including AD, IS and Parkinson's disease (PD) (81-83). Intriguingly, IBD patients exhibit a heightened prevalence of PD compared with those without IBD (84). Beyond merely being bystanders, GM can actively sway the onset of stroke by influencing risk factors including hypertension, diabetes, hyperlipidemia and atherosclerosis (85). Furthermore, the role of GM extends to modulating stroke outcomes through its interactions with neural pathways, endocrine-mediated neurotransmitter pathways, metabolite production and intricate immune signaling cascades.

GBMA and neuroinflammatory crosstalk in cerebral I/R

Changes in microbial metabolites in the GI tract. The intricate interplay of microorganisms significantly influences neuroinflammatory responses in the host. These effects emanate from their ability to metabolize substrates that interact with receptors both within and beyond the GI environment, orchestrating changes in affiliated signaling pathways.

Short-chain fatty acids (SCFAs) epitomize the pivotal products resulting from the microbial fermentation of dietary fibers within the GI realm, exerting considerable protective influences on IS. The triumvirate of principal SCFA constituents encompasses acetate, propionate and butyrate (86). The synthesis of butyrate in SCFAs is orchestrated from diverse precursors such as carbohydrates, organic acids, glutamic acid and lysine, facilitated predominantly by microbial communities within the gut. Renowned producers of butyrate include bacterial families *Lachnospiraceae* (including *Eubacterium rectale, Roseburia inulinivorans* and others) and *Ruminococcus* (spanning species such as *Faecalibacterium prausnitzii* and *Coprococcus comes*) (87). Vancomycin treatment is known to suppress these butyrate-producing bacteria, namely *Lachnospiraceae* and *Ruminococcus* (88). Comparative analyses revealed that SCFA levels, particularly butyrate, were appreciably diminished in Sprague-Dawley (SD) rats afflicted by MCAO in contrast to their healthy counterparts. This decline underscores the potential of butyrate to ameliorate GM during IS by mitigating harmful bacteria and bolstering beneficial lactic acid bacterial populations (89). Intriguingly, an inverse correlation emerges between SCFA levels and the prognosis of reperfused stroke, particularly in elderly patients contending with pronounced stroke events. Empirical studies demonstrated that aged mice subjected to MCAO exhibited attenuated neuroinflammation and reduced neurological deficits when administered fecal transplants from young, SCFA-rich donors (90). Given the presence of SCFA receptors on microglial cells, SCFAs are poised to modulate microglial functionality (91). Sodium butyrate, identified as a histone deacetylase inhibitor, is known to confer neuroprotective benefits against neonatal hypoxic-ischemic brain afflictions, primarily via the BDNF-TrkB signaling cascade (92). Furthermore, the capability of SCFAs to traverse the BBB underscores their influence on both central and peripheral immune cells, which, in turn, modulates post-ischemic cerebral recovery dynamics (93).

Trimethylamine N-oxide (TMAO) emerges from the transformation of trimethylamine, a derivative of GM, facilitated by hepatic flavin monooxygenases. This metabolite has garnered attention due to its association with adverse outcomes in IS patients (94,95). The biosynthesis of TMAO primarily roots from dietary sources, encompassing marine fish, shellfish (96), mushrooms, specific meat sources such as liver and kidneys, various fruits, cruciferous vegetables and

chicken (97). The GI flora, including bacterial phyla such as *Firmicutes*, *Proteobacteria* and *Actinobacteria*, play a pivotal role by metabolizing ingested choline, L-carnitine, and betaine into trimethylamine, which subsequently gets converted to TMAO in the liver (98). Central to the hepatic conversion process is the enzyme flavin monooxygenase 3. Interestingly, certain nutritional supplements, such as L-alpha glycerol-phosphoryl-choline (alpha-GPC), can metabolize TMAO (99).

Empirical evidence underscores that serum TMAO concentrations exhibit a positive correlation with the risk factors of stroke and the ensuing neurological deficits post-ischemic brain injuries. Notably, average serum TMAO levels were observed to be ~49% elevated in stroke-afflicted patients compared with their healthy counterparts (100). Beyond being a standalone predictor for IS, TMAO has been identified to correlate positively with atherosclerotic tendencies and the incidence of atrial fibrillation (101-103). Reinforcing its clinical significance, TMAO not only enhances traditional cardiovascular disease risk predictions but also stands out as a more sensitive and specific independent prognostic marker (104).

Commonly perceived as beneficial, foods including seafood, mushrooms and animal liver, as well as nutritional supplements such as alpha-GPC, can be metabolized in the liver into TMAO (96,97,99). This indicates the importance of diet in stroke patients, as the conversion of the aforementioned foods to TMAO can impact the recovery from CI/RI through the GBMA. However, the specific quantities for consumption still require further experimental validation. Cytidine diphosphate-choline (CDP-choline), commonly used in clinical settings, is typically regarded as a neuroprotective and neural restorative agent (105). Yet, recent studies have questioned its efficacy (106,107). There is also evidence that CDP-choline can be converted to TMAO (108). However, there is no consensus on the rate of this conversion. Further investigation into the clinical efficacy of CDP-choline is needed in the future.

Vitamin B12 is pivotal in safeguarding the intestinal epithelium, especially in inflammatory bowel conditions such as colitis. Its contributions encompass nurturing beneficial intestinal microbes, curbing pathogenic strains and thereby ensuring the stability of the GM (109). Methyl-cobalamin, often in synergy with adenosyl-cobalamin or paired with hydroxocobalamin and cyanocobalamin, serves as a supplement to vitamin B12 (VB12) (110). Deficiencies in both VB12 and folic acid have been linked to heightened stroke risk (111). As VB12 is essential for fatty acid synthesis in the citric acid-pyruvic acid cycle, its absence can impede the recuperation of myelinated nerve fibers post-ischemic events (112). Homocysteine (Hcy) is converted into methionine through the enzyme methionine synthase. VB12 and folate, key cofactors for this enzyme, when deficient, escalate Hcy levels. This in turn, as found by Wang *et al* (113), during I/R injury, impedes the PI3K-AKT- and ERK-dependent mTOR pathways, detrimentally amplifying autophagy and compromising the viability of neural stem cells in specific brain regions (113). Additionally, VB12 fosters the growth of gut bacteria including *Akkermansia and E. faecalis*, known for promoting SCFA production (114).

LPS, a key component of the outer membrane of Gram-negative bacteria, is a potent modulator of innate immunity. Structurally, it is comprised of lipid A, core oligosaccharides, and extended polysaccharides (O antigens) (115). Recognized by the intricate TLR4-MD-2 complex, LPS can instigate an inflammatory response within the GM and associated lymphoid tissues (116). Following an IS, LPS can enter the brain via the bloodstream, through compromised gut and BBB. This promotes the transformation of astrocytes into the A1 subtype, while inhibiting their transformation into the A2 subtype (117). Notably, folic acid can mitigate LPS synthesis (118), providing relief from LPS-induced cognitive impairments and modulating post-injury inflammatory responses (119).

5-Hydroxytryptamine (5-HT), a pivotal monoamine neurotransmitter, facilitates communication between nerve cells in both central and peripheral nervous systems. Selective serotonin reuptake inhibitors (SSRIs) have demonstrated efficacy in treating post-stroke depression (PSD) (120). The onset of PSD might be attributed to diminished neurotransmission owing to damage to the mood-regulating frontal/temporal-basal ganglia-ventral brainstem loop and interruptions in 5-HTergic neurons and pathways. This damage, in turn, compromises neurotransmitter synthesis (121). Notably, 5-HT's precursor is the essential amino acid tryptophan, and a staggering 90% of the body's 5-HT is localized within the GI tract cells (122). Intriguingly, there is a positive feedback mechanism between GI-produced 5-HT and NLRP3, with excessive 5-HT amplifying the activation of NLRP3 inflammatory vesicles (123). NLRP3 is implicated in triggering inflammatory signaling cascades and exacerbating neuronal damage (124).

Scutellarin has demonstrated its potential in reversing neuronal deterioration and cognitive impairments by acting through the cAMP-PKA-CREB-HDAC3 pathway within the GBMA (125). LPS is known to stimulate HDAC3 in the GBMA circulation. By contrast, butyrate attenuates HDAC3 expression, counteracting LPS-induced neurotoxic effects. Additionally, melatonin appears to ameliorate cognitive deficits either by downregulating LPS or enhancing butyrate levels (126). Fiber-rich diets such as high-inulin promote butyrate production to inhibit HDAC3 (127). These findings underscore the pivotal role of HDAC3 in modulating cerebral I/R injury via the GBMA. The aforementioned content is summarized in Table I.

Gut microbiota and the immune system. During ischemia and reperfusion, the gut's immune cells play an instrumental role. The intestinal mucosal lamina propria (LP) is replete with a variety of specialized immune cells, including regulatory T (T-reg) cells, gammadelta T cells, innate lymphocytes and helper T 17 cells. Remarkably, this region boasts the most extensive T cell population in the human body. Within the gut microbiota, gammadelta T cells stand out, as they transmit diverse signals that adjust the host's immune response (128). These immune cells, while influenced by the GM, traverse through peripheral circulation to exert their influence in the brain. Research has noted an uptick in the frequency of gammadelta T cells within the meningeal zones of brains afflicted by ischemic injury (129). The overexpression of IL-17 protein in these cells incites a surge in chemokines in the brain tissue. This results in significant neutrophil infiltration at the injury site, thereby compromising the structural cohesiveness

Table I. Regulation of gut microbial metabolites.

Gut microbial metabolites	Facilitating factors	Inhibitory factors
Short-chain fatty acids	*Lachnospiraceae (Eubacterium rectale, Roseburia inulinivorans, Roseburia intestinalis, Eubacterium hallii, Anaerostipes hadrus), Ruminococcus (Faecalibacterium prausnitzii, Eubacterium rectale, Roseburia, Anaerobic bacteria, Coprococcus comes, Coprococcus catus* and *Coprococcus eutactus*), VB12, high-inulin	Vancomycin
Trimethylamine N-oxide	Marine fish and shellfish, mushrooms, meat products (mainly liver, kidney), fruits, cruciferous vegetables, chicken, intestinal flora (such as Firmicutes, Proteus, Actinobacteria), L-alpha glycerophosphocholine	NA
VB12	Methylcobalamin, adenosylcobalamin, hydroxocobalamin, cyanocobalamin	NA
Homocysteine	NA	VB12, Folic acid
Lipopolysaccharide	NA	Folic acid
5-hydroxytryptamine	Selective serotonin reuptake inhibitors (such as Fluoxetine, Fluvoxamine and Tropisetron)	NA

of BBB (130). Furthermore, IL-17 stimulates the production of ROS and elevates MMP-3 and MMP-9 levels, which further erode BBB integrity by weakening its tight junctions (131,132). Ultimately, IL-17 accentuates neuronal death, through processes including apoptosis and autophagy, exacerbating neuroinflammatory damage (133,134).

Benakis *et al* (135) illuminated the capacity of Tregs to counteract the differentiation of IL-17$^+$ gammadelta T cells via IL-10 (135). Post-stroke, another source of MMP-9 is neutrophils, and Tregs specifically target and inhibit this MMP-9 source, fortifying the integrity of BBB (136). Additionally, sodium butyrate in SCFA amplifies Tregs in regions affected by cerebral I/R, leading to the mitigation of neuroinflammation in the ischemic hemisphere. Notably, Tregs significantly curtail TNF-alpha levels in the contralateral hemisphere, thereby further reducing neuroinflammation (137). In the aftermath of cerebral I/R, Tregs also promote the repair of white matter and augment long-term neurological recovery (138).

Following an IS, neutrophils can be detected in the soft meninges as early as 6 h. Their presence peaks between days 3 to 7 post-stroke and then gradually diminishes, yet they can still be observed up to 14 days after the ischemic incident (63). In the clinical context, neutrophils are predominantly detrimental during IS. These cells are potent catalysts for the abundant secretion of ROS through myeloperoxidase (MPO), intensifying the deleterious impacts of cerebral I/R. Notably, inhibiting MPO has been demonstrated to diminish neuronal death, enhance the prognosis for neuroinflammation, reduce the infarct size, and decrease the levels of the pro-apoptotic protein, p53 (139). Additionally, neutrophils can produce MMP-9, which, when found in elevated concentrations in the core of ischemic regions, can inflict damage on brain tissue and possibly precipitate hemorrhagic transformations (140).

cGAS-STING in the gut. The GM produces an array of dsDNA that activates the STING pathway. The GM has a pivotal role in orchestrating the synthesis of type I IFN through the cGAS-STING-dependent pathway (48). *Lactobacillus rhamnosus* GG, a prevalent probiotic in the GI system, has the potential to enhance the cGAS-STING-TBK1-IRF7-IFN-beta signaling cascade via dendritic cells (DCs), fostering a potent adaptive immune response (141). Conversely, *Helicobacter pylori* infections have been identified to reduce the host's STING expression by 50%, leading to the downregulation of IRF3-dependent type I IFN (142).

Moreover, STING plays a role in precipitating intestinal epithelial cell (IEC) apoptosis. IEC apoptosis is a central factor contributing to the dysfunction of the intestinal barrier. This dysfunction results in heightened intestinal permeability, allowing for the abnormal positioning of intestinal flora (143). Research has highlighted that suppressing STING leads to an augmentation in the TJPs, which bolsters defense against increased intestinal permeability. However, research by Canesso *et al* (144) highlighted the complexity of STING pathway functions. It was found that intact STING signaling is essential for maintaining intestinal homeostasis and regulating CD4$^+$Foxp3$^+$ T regulatory cells in the gut through type I IFN. In STING knockout mice, a reduction in T regulatory cell function and expression was observed, indicating the multifaceted role of the STING pathway in intestinal permeability and homeostasis (144). This cGAS-STING signaling pathway's multifaceted regulatory actions on intestinal permeability, mucosal protection and immune modulation have repercussions on cerebral I/R through the GBMA. Interestingly, butyrate has been identified to inhibit TBK1 and IRF3 phosphorylation in the downstream segments of the cGAS-STING pathway, thereby constraining STING-activated type I IFN expression in DCs (145).

Mitochondria, often termed the 'powerhouses' of cells, are chiefly tasked with energy synthesis. Paradoxically, instead of aiding in the restoration of ischemic areas after reperfusion brought about by the reintroduction of oxygen and nutrients, further cellular damage is catalyzed. This damage is propelled by an accumulation of mitochondrial Ca^{2+}, a surge in open mitochondrial permeability transition pores and a pronounced release of ROS (146). Mitophagy stands as a sentinel, governing both the number and functionality of mitochondria. It achieves this by systematically eliminating

dysfunctional or overabundant mitochondria, leading to enhanced neuronal viability and improved neurobehavioral outcomes. A deficiency in XBP1 hampers the efficiency of mitochondrial autophagy. When mitochondria sustain extensive damage, they release ROS and initiate mitochondrial (mt)DNA clustering. The ensuing recognition of mtDNA by macrophage cGAS instigates STING activation, intensifying cGAS-dependent cellular pyroptosis (147). Notably, the collaboration of the Parkin (an E3 ubiquitin ligase) and PINK1 (a ubiquitin kinase) pathways fosters mitochondrial autophagy. This synergy curtails mtDNA release, suppresses cGAS activation and dampens STING-driven neuroinflammation (148). Innovative formulations such as Fucoidan-proanthocyanidin nanoparticles have showcased potential in kindling mitochondrial autophagy while simultaneously inhibiting the cGAS/STING expression (149). Harnessing and modulating the intricacies of mitophagy post-cerebral I/R has revealed promising avenues for mitigating neuroinflammation and catalyzing neurological rejuvenation.

4. Cerebral I/R under the influence of the GBMA through signalling pathways

Emerging research underscores the profound interplay between cerebral I/R and various signaling pathways, influenced by the GBMA. Several pathways have been identified that amplify the severity of cerebral infarction and intensify neuroinflammation following cerebral I/R. These pathways include: The cGAS-STING signaling pathway (51), the NF-kB signaling cascade (71), the MAPK signaling conduit (150), and the integrated TLR4-NF-kB/PI3K-AKT/MAPK signaling mechanism (151).

Conversely, the PI3K-AKT signaling pathway has been spotlighted for its neuroprotective role, demonstrating potential in mitigating neurological injury post-cerebral I/R (152,153). Intriguingly, pathways downstream of cGAS-STING exhibit the capacity to activate either NF-kB or IRF3, signifying a multifaceted modulation of the neuroinflammatory response in cerebral I/R dynamics.

The cGAS-STING signaling pathway. Butyrate effectively inhibits the phosphorylation of both TBK1 and IRF3, a process that is activated in the presence of STING. This action in turn impedes the production of IFN-I (145). Upon stimulation by LPS, Kupffer cells release mtDNA through the voltage-dependent anion channel 1, thereby initiating the cGAS-STING cascade. Notably, within this cascade, IRF3 is revealed to elevate the expression of NLRP3, instigating inflammatory reactions and apoptosis. It is noteworthy that by suppressing STING, the inflammatory and apoptotic effects of LPS are significantly diminished (154).

However, there remains an ambiguity regarding the role of IRF3 in the LPS-driven TLR4-NF-kB-NLRP3 signaling pathway. Further comprehensive studies are essential to elucidate the interrelation between the STING-IRF3 pathway and the TLR4 signaling mechanisms.

Fluvoxamine, recognized as an SSRI, exhibits a dual inhibitory action on both cGAS and STING (155). The modulation of the cGAS-STING signaling pathway by these specific gut microbial metabolites within the GBMA framework opens promising avenues for potential targeted pharmaceutical interventions or nutritional strategies to address cerebral I/R.

The NF-kB signaling pathway. Pathology NF-kB mediates neuroinflammation by modulating the expression of multiple inflammatory factors such as IL-6, TNF-α and COX2 (156,157). L-alpha-GPC, a dietary supplement, undergoes metabolic transformation *in vivo*, leading to the formation of TMAO, which in turn activates the NF-kB and MAPK signaling pathways (99). Notably, the effects of cholinergic and anti-cholinergic agents on IS might be attributed to their modulation of TMAO. Furthermore, LPS and homocysteine (Hcy) has been identified as activators of the NF-kB signaling cascade (158,159).

Interestingly, certain SSRIs such as Fluoxetine (160) and Tropisetron (161) have been demonstrated to inhibit the NF-kB signaling pathway in cerebral I/R scenarios. Their anti-inflammatory properties serve to confer neuroprotective benefits.

The TLR4-NF-kB/PI3K-AKT/MAPK signaling pathway. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB, also known as AKT) signaling pathway has been identified as a key regulator of apoptosis-associated protein expression and cell survival in CI/RI, thus playing a significant neuroprotective role (162,163). Conversely, the mitogen-activated protein kinase (MAPK) signaling pathway is implicated in inflammatory processes in CI/RI (164). Activation of the MAPK pathway triggers a substantial release of inflammatory factors (165). Inhibiting this pathway can reduce astrocyte apoptosis, attenuate astrocyte-mediated inflammatory responses, and protect brain neurons from I/R injury (166). Additionally, blocking the MAPK pathway may alleviate oxidative stress-induced damage and reduce microglial activation and the overexpression of inflammatory factors (167).

Aloe-emodin acts as a potent modulator, attenuating the LPS-induced TLR4-NF-kB pathway while simultaneously enhancing the TLR4-PI3K-AKT signaling. This dual activity offers promising therapeutic potential for mitigating neurological damage and inflammation associated with IS (168). Several other agents have displayed similar inhibitory effects on the LPS-triggered TLR4-NF-kB pathway. Notably, the depletion of Polymerase δ-interacting protein 2 (169), reduction in Cofilin (170), neuroprotective agents (171) and Escin (172) have all shown substantial inhibitory effects on this pathway.

Furthermore, several compounds, including Esculentoside A (173), Imperatorin (174), Progesterone (175), Probucol (176), Hydroxysafflor Yellow A (177) and steppogenin (178), have been identified to impede the LPS-mediated TLR4-NF-kB/MAPK pathway, specifically in BV-2 microglia. This suggests their potential utility in protecting the brain and supporting neuronal health, either as prophylactic agents before IS or as therapeutic agents during cerebral I/R, by targeting the LPS-driven TLR4 pathway within the GBMA framework.

The implications of the MAPK signaling pathway in IS. The MAPK signaling pathway, particularly its p38 branch, plays a crucial role in the cellular responses post-IS. A notable influencer of this pathway is Hyperhomocysteinemia (hHcy).

Table II. Signaling pathways affected by gut microbiota metabolites in GBMA.

Signal path	Facilitating factors	Inhibitory factors
cGAS-STING signaling pathway	Lipopolysaccharide, Histone deacetylase 3	Butyrate, 5-Hydroxytryptamine
NF-kappaB signaling pathway	Trimethylamine N-oxide, Homocysteine, lipopolysaccharide	5-Hydroxytryptamine
MAPK signaling pathway	Trimethylamine N-oxide, High homocysteine	NA
TLR4-NF-kappaB signaling pathway	NA	Aloe-emodin, Poldip2 consumption, Cofilin reduction, Neuroprotective, Escin, Esculentoside A, Imperatorin, Progesterone, Probucol, Hydroxysafflor Yellow A, steppogenin
TLR4-PI3K-AKT signaling pathway	Aloe-emodin	NA
TLR4-MAPK signaling pathway	NA	Esculentoside A, Imperatorin, Progesterone, Probucol, Hydroxysafflor Yellow A, Steppogenin
PI3K-AKT signaling pathway	Short-chain fatty acids (sodium butyrate)	Homocysteine

cGAS, cyclic GMP-AMP synthase; STING, stimulators of the interferon gene.

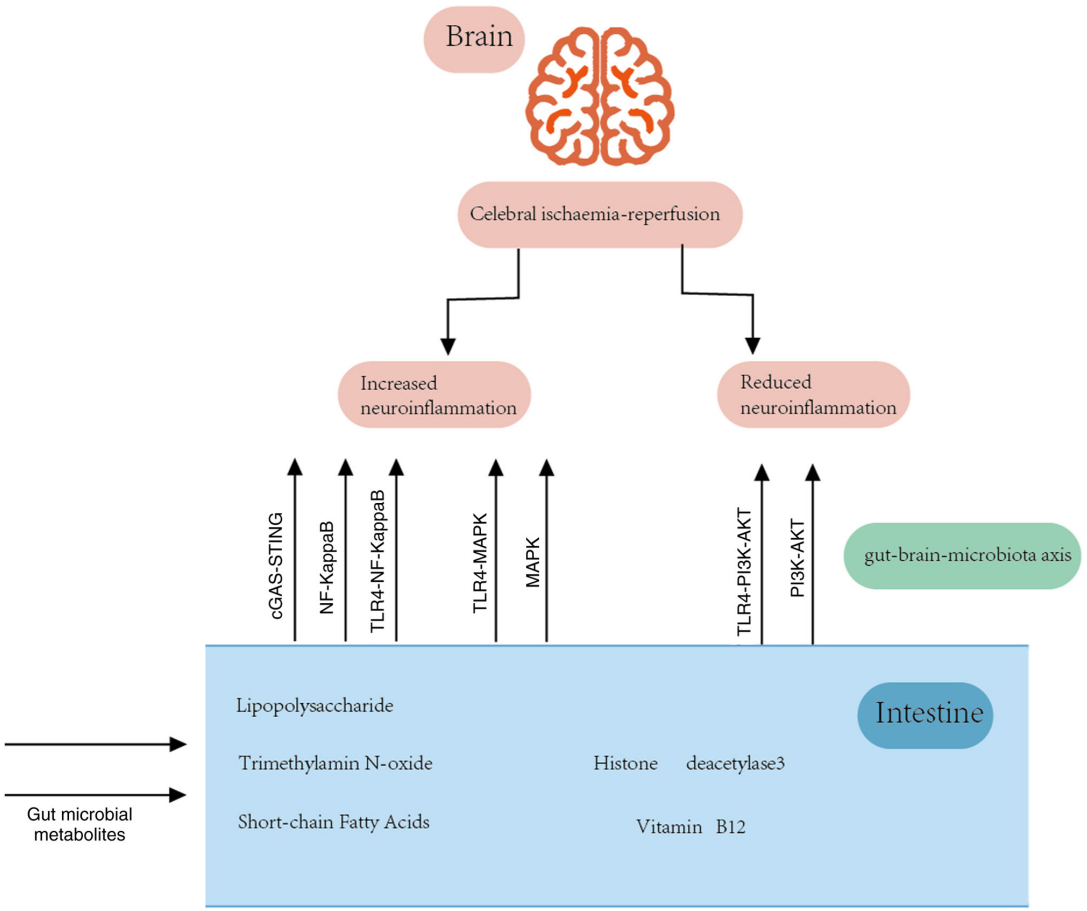

Figure 2. Gut microbial metabolites influence neuroinflammation after cerebral ischemia-reperfusion through signalling pathways. Each individual exhibits unique microbiota characteristics with specific functions such as immunomodulation, defense against pathogens, maintenance of intestinal mucosal barrier integrity and control of nutrient metabolism. The gut possesses the largest commensal flora, and the gut microbiota consists of several species of bacteria, 90% of which are composed of the genera Thick-walled Bacteria and *Mycobacterium*, with the Thick-walled phylum consisting mainly of the genus *Clostridium*. In the gut-brain-microbiota axis, gut microbial metabolites produce Lipopolysaccharide, Trimethylamin N-oxide, short-chain fatty acids, histone deacetylase 3 and Vitamin B12 affecting the cGAS-STING, NF-kB, MAPK, TLR4-NF-kB, TLR4-PI3K-AKT and the PI3K-AKT signaling pathways. These signaling pathways, which achieve signal transduction at the gut-brain-microbiota axis, exacerbate or attenuate neuroinflammation after cerebral ischemia-reperfusion. cGAS, cyclic GMP-AMP synthase; STING, stimulators of the interferon gene; NF-κB, nuclear factor kappaB.

Recognized as a standalone risk factor for stroke, hHcy's interactions with the MAPK pathway heighten its implications. Specifically, hHcy's activation of the p38 MAPK pathway exacerbates neurotoxic effects after an ischemic event (179,180). This correlation underscores the importance of understanding the mechanistic details of hHcy and the MAPK pathway. Exploring this further could unveil potential therapeutic targets that can mitigate the detrimental aftermath of stroke events.

The role of the PI3K-AKT signaling pathway in IS outcomes. The PI3K)-AKT signaling pathway stands as a crucial component in the cellular responses following IS. One noteworthy mediator of this pathway is SCFA. Research has illuminated SCFA's potential in mitigating the adverse effects of IS by activating the PI3K-AKT signaling route, thereby inhibiting neuronal apoptosis, curbing neuroinflammation and enhancing cognitive function (181). Additionally, experiments involving intranasal administration of sodium butyrate have revealed a significant reduction in infarct volume and overall improvement in neurological function, outcomes attributed to the activation of the PI3K-AKT pathway (182). Conversely, hHcy is a noteworthy antagonist in this context. By suppressing the PI3K-AKT pathway, hHcy amplifies neurotoxic effects post-ischemic damage (113), highlighting the pathway's double-edged nature in the realm of IS (Fig. 2). The aforementioned content is summarized in Table II.

5. Conclusion and outlook

Patients with CI/RI exhibit a more disordered gut microbiome compared with healthy individuals. Through the complex bidirectional GBMA communication between the gut and the brain, exploring the connection between gut microbiota and neuroinflammation post CI/RI can illuminate promising pathways for targeted treatment of post-CI/RI neuroinflammation. These gut microbial communities, via signaling pathways such as cGAS-STING, produce cytokines that positively or negatively affect microglia, astrocytes and the BBB in CI/RI patients, thereby influencing the neuroinflammation associated with CI/RI.

Within the GBMA, SCFAs block the cGAS-STING pathway while simultaneously upregulating the PI3K-AKT signaling pathway. This dual action reduces neuroinflammatory damage factors following CI/RI and enhances protective factors (145,182). Future treatments could focus on increasing the presence of gut bacterial families including *Lachnospiraceae* and *Ruminococcus* in IS patients. Additionally, increasing the infusion of VB12 and the intake of high-inulin, fiber-rich diets can elevate SCFA levels (127). TMAO, an independent predictor for IS, can upregulate NF-kB and MAPK signaling pathways, exacerbating neurological damage. In the context of CI/RI's GBMA, on one hand, serotonin (5-HT) can inhibit the cGAS-STING/NF-kB pathway, offering neuroprotection (155,160,161), while on the other hand, excessive 5-HT can enhance NLRP3 inflammasome activation, worsening brain injury (123). More importantly, LPS can activate the TLR4-dependent NF-kB/MAPK signaling pathways, exacerbating neuroinflammatory damage post-CI/RI (151,167), but can also activate TLR4-dependent PI3K/AKT signaling pathways, participating in the protection of the brain from CI/RI-induced damage (162,183).

In summary, these gut microbial metabolites, produced by various gut microbial communities, suggest that broad anti-inflammatory strategies are not suitable for treating neuroinflammatory damage in CI/RI. The development and application of targeted antibiotics are crucial. A new balance between different gut microbial communities and their metabolites and the signaling pathways of cGAS-STING/NF-kB/MAPK/PI3K-AKT is sought. This balance aims to improve post-CI/RI neuroinflammation. Through the GBMA, especially via various neural pathways, understanding of how to reduce detrimental microbial communities and factors (including LPS, TMAO, hcy, NET and HDAC3) or enhance beneficial ones (such as SCFA, VB12 and 5-HT) can improve. This approach could target treatment and even prevent neuroinflammation in patients, promote early recovery in the ischemic penumbra, and reduce the disease burden. Future research should validate these findings from animal models to human diseases, identify the minimum levels needed for therapeutic efficacy, and ultimately introduce new and promising strategies for treating neuroinflammation following CI/RI.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

YZ and HY reviewed the literature, drafted and revised the manuscript. SH and YX discussed and revised the manuscript. YQW provided critical comments. YZ and HY drew figures and translated the manuscript. YZ and YQW revised and finalized the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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