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Gut-Brain Axis Microbiota in Stroke Pathogenesis

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ABSTRACT

Stroke is the second cause of death globally and the third cause of global disability, causing substantial health and socioeconomic problems. Clinically, Stroke is defined as brain tissue injury caused by a lack of blood supply to a particular area, resulting in permanent nerve damage or death. Stroke is also frequently associated with metabolic causes, including insulin resistance, obesity, hypertension, and type 2 diabetes mellitus (T2DM), significantly influencing the composition and abundance of the gut microbiota. Therefore, identifying potential risk factors influencing stroke prognosis and underlying pathogenic mechanisms is essential for better management and treatment of Stroke. Several studies show the existence of two-way communication between the gut and the brain through the gut microbiota. Intimate two-way interactions between the gut and the brain occur via neural (central, autonomic, and enteric nervous systems), hormonal (endocrine system), and immunological (innate and acquired immune systems) pathways, commonly referred to as the gut-microbiota axis. Brain or gut-brain axis (Gut-Brain Axis/GBA). Gut microbiota and derived metabolites play an important role in brain function by regulating GBA signaling. Recent evidence also suggests that GBA is critical in regulating immune function post-stroke. Intestinal dysbiosis provides a chronic peripheral and central inflammatory response that accelerates stroke pathology. Gut microbial dysbiosis is a significant risk factor positively correlated with poor post-stroke outcomes. This literature review summarizes the pathogenesis of ischemic Stroke, the description of the gut microbiota, and preclinical and clinical evidence indicating the pathogenic influence of gut dysbiosis in cerebral ischemic Stroke.

Keywords: Stroke, Gut-brain axis, dysbiosis

INTRODUCTION

Stroke is the second cause of global death and the third cause of global disability, which causes substantial health and socioeconomic problems.[1] Clinically, stroke is defined as brain tissue injury caused by a lack of blood supply to a particular area, resulting in permanent nerve damage or death.[2] Cerebral ischemic Stroke, which accounts for 85% of all strokes, is caused by vascular occlusion or arterial stenosis. Ischemic Stroke causes a significant health burden, with an annual rate of 24.9 million cases worldwide. In contrast, hemorrhagic strokes, which account for 15% of strokes, are triggered by the rupture of blood vessels, resulting in intraparenchymal and subarachnoid hemorrhages.[1,2]

Intravenous thrombolysis, endovascular thrombectomy, and stroke therapy agents can improve the physical, motor-behavioral, and mental deficits of some patients. However, the overall prognosis for most post-stroke patients remains low.[2] Studies have suggested that 90% of stroke cases are correlated with lifestyle factors, such as a Western-style high-fat diet containing omega-6 fatty acids, smoking, high alcohol consumption, and low physical activity.[3,4]

Stroke is also frequently associated with metabolic causes, including insulin resistance, obesity, hypertension, and type 2 diabetes mellitus (T2DM), significantly affecting the composition and abundance of the gut microbiota. Therefore, identifying potential risk factors influencing stroke prognosis and underlying pathogenic mechanisms is essential for better management and treatment of Stroke. [5,6]

Several studies show the existence of bidirectional communication between the gut and the brain through the gut microbiota.[5,7] Intimate two-way interactions between the gut and the brain occur via neural (central, autonomic, and enteric nervous systems), hormonal (endocrine system), and immunological (innate and acquired immune systems) pathways, commonly referred to as the gut-microbiota axis. Brain or gut-brain axis (Gut-Brain Axis/GBA).[7,8] Gut microbiota and derived metabolites play an important role in brain function by regulating GBA signaling. Recent evidence also suggests that GBA is critical in regulating immune function in post-stroke conditions.[9]

A direct influence of gut dysbiosis on the development of clinical risk factors for Stroke, such as high-fat diet, hypertension, T2DM, obesity, insulin resistance, metabolic syndrome, aging, vascular dysfunction, and inflammation, has been reported.[5,10,11] Since then, the gut microbiome has emerged as a new vital "organ" that significantly influences human health. This microbiome influences the early stages of neurological, immune, and metabolic disorders and pathologies. Previous studies have also linked gut dysbiosis to post-stroke outcomes through multiple factors, including local and systemic inflammation, leaky gut, endotoxemia, bacterial components, metabolites, and the immune and nervous systems. Dysbiosis is also associated with poor treatment outcomes and has been actively explored to produce more effective therapies.[9,11]

Stroke causes intestinal dysmotility and increases intestinal barrier permeability and the translocation of microbes and microbial-derived products, such as lipopolysaccharide (LPS) and trimethyl amine-N-oxide (TMAO), into the bloodstream.[12] These changes accelerate systemic inflammation and worsening of symptoms, contributing to a poor prognosis. Intestinal dysbiosis provides a chronic peripheral and central inflammatory response that accelerates stroke pathology. Gut microbial dysbiosis is a significant risk factor positively correlated with poor post-stroke outcomes.[13,14,15] This literature review summarizes the pathogenesis of ischemic Stroke, the description of the gut microbiota, and preclinical and clinical evidence indicating the pathogenic influence of gut dysbiosis in cerebral ischemic Stroke.

1. Stroke

It is essential to recognize that Stroke and transient ischemic attack (TIA) are two different entities. However, the clinical syndrome and underlying vascular brain injury may share many mechanisms (associated with various risk factors and disease processes). 'Stroke' and 'TIA' are not single or complete diagnoses but are starting points for rational investigation and treatment.[16,17]

TIA is defined as a brief episode of focal neurological dysfunction that is not associated with permanent cerebral infarction and lasts less than 24 hours. A stroke is defined as a focal neurological deficit of sudden onset, with symptoms lasting more than 24 hours (or resulting in death before 24 hours). However, this definition is no longer helpful in clinical practice because stroke treatment is time-sensitive and needs to be initiated as soon as possible after diagnosis; the 24-hour time limit is arbitrary; and 30-50% of patients with clinically defined TIA have evidence of brain ischemia or infarction on diffusion-weighted magnetic resonance imaging (MRI).[4,16] Atypical, usually recurrent, attacks of stereotypic paresthesia and numbness affecting the arms and face are associated with subarachnoid hemorrhage and, therefore, require brain imaging, ideally with

MRI.[16]

There are two main types of Stroke, namely ischemic and hemorrhagic. Ischemic Stroke is caused by a disruption in blood flow to some brain regions. Ischemic strokes account for the majority of all strokes worldwide. Hemorrhagic Stroke is caused by the extravasation of blood into the brain parenchyma or ventricles.[1,17] In this literature review, the gut-brain axis (GBA) will focus on the pathogenesis of ischemic Stroke

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1.1.Ischemic Stroke

1.1.1. Mechanism and pathogenesis of ischemic Stroke

Most cases of ischemic Stroke are thromboembolic in origin, with familiar sources of embolism being extensive arterial atherosclerosis and heart disease, especially atrial fibrillation. Other causes of ischemic Stroke include small vessel disease, which is associated with increased blood pressure, and diabetes mellitus, which is very common in Asia.[18,19] Causes that are less reported overall but are proportionally more common in younger patients are arterial dissection, vasculitis, patent foramen ovale (PFO) with paradoxical embolism (venous thrombus entering the systemic and cerebral circulation), and hematologic disorders. The cause of ischemic Stroke is significant because it can guide therapeutic strategies to prevent recurrent Stroke.[20]

1.1.1.1.Mechanism of Stroke Due to Arterial Atherosclerosis

One of the common causes of ischemic Stroke is an embolus in the cerebral blood vessels originating from ulcerated and usually stenotic atherosclerotic plaque in the aortic arch, neck, or intracranial blood vessels. In patients with atherosclerosis, thrombi can form when the lipid core of atherosclerotic plaques is exposed to the bloodstream, which can be caused by inflammation and ulceration of the plaque's fibrous cap. These thrombi can occlude atherosclerotic blood vessels or, more commonly in large blood vessels relevant to Stroke, can cause distal embolism.[18] In Western populations, the location of atherosclerotic plaque that most frequently causes ischemic Stroke is the internal carotid artery, just after its branch from the common carotid artery.[4] This condition is hypothesized to be related to reduced shear stress in the arterial wall at that location. Low shear stress is associated with intimal thickening and reduced nitric oxide release, which is thought to mediate this susceptibility to cholesterol plaque development. Although intracranial atherosclerosis has been observed in patients in Western countries, especially in heavy smokers and individuals with diabetes mellitus, this condition is more common in Asia.[21]

Intracranial atherosclerosis is reported to cause 30-50% of ischemic strokes in Asian patients compared to 5-10% of strokes in white.[22] This disorder presents a challenge for standard thrombectomy, as it is associated with a higher rate of reocclusion after thrombectomy and has an increased need for stent placement, the latter carrying a greater risk of complications, especially bleeding associated with the use of antiplatelet drugs to maintain stent patency.[21]

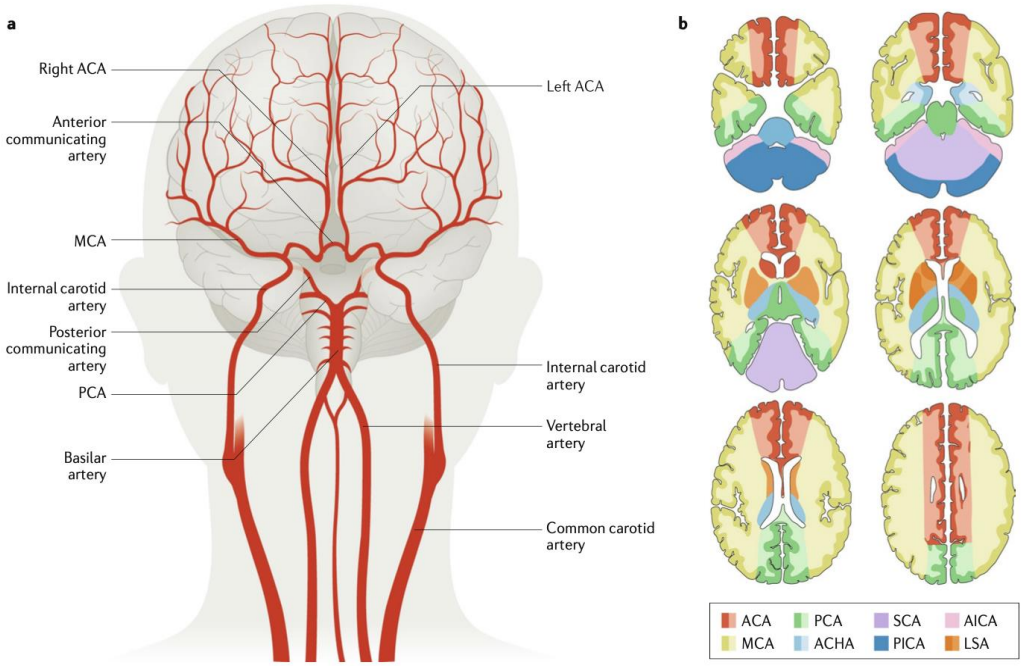

Figure 1 ***Cerebral Vasculature***.[4]

The brain's main arteries (part a) and their vascular territories (part b). Although simplified here for illustrative purposes, an ischemic stroke in any of these blood vessels can cause tissue damage in the highlighted areas. ACA, anterior cerebral artery; ACHA, anterior choroidal artery; AICA, anterior inferior cerebellar artery; LSA, lenticulostriate artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; PICA, posterior inferior cerebellar artery; SCA, superior cerebellar artery.[4]

Small Vessel Diseases

Small vessel disease affects the small arteries and arterioles of the brain. Small vessel disease can manifest in several ways, including lacunar Stroke, leukoaraiosis (white matter changes observed as T2 hyperintensity on MRI or hypodensity on Computed Tomography [CT]), cerebral microhemorrhage and intracerebral hemorrhage. Deep subcortical and brainstem structures are supplied by small-caliber perforating arteries that arise from the larger arteries of the circle of Willis, exposing small blood vessels to high pressures that can predispose to lipo hyalinosis (narrowing of the small cerebral blood vessels).[23]

Lipohyalinosis is not the only cause of small subcortical infarcts, and the traditional clinical pattern defined as lacunar syndrome has limited specificity for Stroke associated with small vessel disease. Atherosclerosis of the parent artery with occlusion of the origin of the perforating vessel is another important mechanism of the clinical lacunar syndrome. In rare cases, monogenic disorders can cause small vessel disease, such as Cerebral arteriopathy, autosomal dominant, with subcortical infarcts and leukoencephalopathy (CADASIL), which usually presents with migraine followed by lacunar infarcts and then dementia.[4,24]

Arterial Dissection

Dissection or tear of the intimal layer of the artery with intramural thrombus is an essential cause of Stroke, especially in younger patients. Most dissections that cause ischemic Stroke are in the extracranial carotid arteries and vertebral arteries and can occlude the arteries at the surgical site or cause thrombus formation and distal embolism. Dissection often occurs spontaneously. Some collagen and connective tissue diseases may predispose to arterial dissection. However, apart from

fibromuscular dysplasia and Ehlers-Danlos syndrome, these disorders can rarely be identified with currently available tests, and genetic contribution is limited to the risk of dissection. As a result, testing for underlying connective tissue disorders is not routinely performed.[25]

Cerebral Vasculitis

Vasculitis is a disease characterized by inflammation of blood vessels and leukocytes in the walls of blood vessels. Compromise of blood vessel lumina and distal tissue ischemia increases the risk of ischemic Stroke. Meanwhile, loss of blood vessel integrity increases the risk of hemorrhagic Stroke due to aneurysm bleeding.[26] Cerebral artery vasculitis is rare but may occur as primary central nervous system (CNS) angiitis or as a manifestation of systemic vasculitis. In this condition, inflammation of the blood vessel walls can cause luminal narrowing and thromboembolism, leading to ischemic Stroke and sometimes intracerebral hemorrhage.[4]

Reversible Cerebral Vasoconstriction Syndrome

Reversible cerebral vasoconstriction syndrome presents with recurrent thunderclap headaches and can lead to ischemic Stroke, intracerebral hemorrhage, or focal subarachnoid hemorrhage via vasospasm and vascular dysregulation. The exact etiology is unknown, and vasospasm may be absent during initial arterial imaging. This condition is a separate entity from vasospasm that occurs after aneurysmal subarachnoid hemorrhage, which can also cause ischemic Stroke.[4]

2. Gut Microbiota

The human gastrointestinal (GI) tract is home to trillions of microorganisms, such as viruses, bacteria, fungi, and protozoa. These microbes are usually called the gut microbiota and are called the gut microbiome when combined with their functional characteristics and genetic material.[27.28]

Gut microbiota is known to have the function of maintaining body homeostasis by regulating digestive, metabolic, immune, and neurological functions through a complex, interconnected physiological pathway called GBA.[5,7] Gut microbes maintain the integrity of the intestinal epithelial barrier and stimulate intestinal cell regeneration and production of mucin and other metabolites, including bile acids, ethanol, acetaldehyde, acetate, and other short-chain fatty acids (SCFA). The gastrointestinal tract is considered a major immune organ, containing the largest pool of immune cells (>70% of the entire immune system).[29] The majority of gut bacteria belonged to the phylum Bacteroidetes or Firmicutes (approximately 51% or 48%, respectively), whereas Actinobacteria (including the genera Bifidobacteria), Spirochaetes, Proteobacteria, Cyanobacteria, Leptosphaeria, Fusobacteria, and the phylum Verrucomicrobia were found in relatively lower abundance.[30]

In addition, gut microbiota is a crucial regulator of T-cell homeostasis and plays a vital role in the maturation of the immune system. Pathological changes in the composition and abundance of gut microbes, which lead to gut immune and neuroimmune status changes, are called gut dysbiosis or gut microbial dysbiosis.[31] Gut dysbiosis, often associated with increased gut barrier dysfunction and local inflammation, typically contributes to disrupted GBA signaling, resulting in pathophysiological consequences. Increased translocation of bacteria and their toxic products into the systemic circulation predisposes to various GI, metabolic, endotoxemic, cardiovascular, and neurological disorders.[10,11]

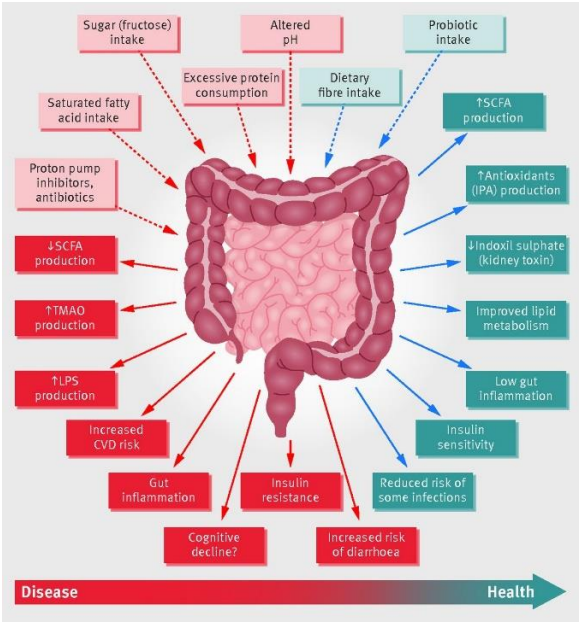

Figure 2. Schematic representation of the role of gut microbiota in health and disease.[32] CVD=cardiovascular disease; IPA=indolepropionic acid; LPS = lipopolysaccharide; SCFA = short-chain fatty acids; TMAO=trimethylamine N-oxide.[32]

2.1.Molecules or Metabolites of the Gut Microbiota

Gut microbiota produces bioactive metabolites important in maintaining homeostasis, immune maturation, mucosal integrity, and host energy metabolism. Short-chain fatty acids (SCFA), such as butyrate, acetate, and propionate, are significant metabolites in the colon produced by anaerobic fermentation of dietary fiber and resistant starch. SCFA is an important energy source and has immunomodulatory and neuroactive properties.[33] SCFAs regulate the physiological functions of host cells through various mechanisms, including epigenetic changes related to histone acetylation, cell proliferation, and activation of G-protein-coupled receptors. In addition, SCFAs maintain glucose metabolism, inflammation, and blood pressure and regulate blood-brain-barrier (BBB) maintenance and microglial physiology.[34]

Reduced abundance of SCFA-producing bacteria was observed in metabolic diseases and models of cardiovascular and neuropsychiatric diseases, including Stroke, hypertension, insulin resistance, obesity, and T2DM. These gut microbes are involved in the production of vitamins B and K and the absorption and metabolism of essential substances, such as bile acids, sterols, and drugs. Gut bacteria are also known to synthesize and stimulate several neurotransmitters, such as acetylcholine; γ-aminobutyric acid (GABA); serotonin (5-HT); melatonin and its precursor, N-acetyl serotonin; glutamate; dopamine, and noradrenaline, and modulates immune system activation.[27,33]

SCFAs also interact with G-protein-coupled receptors on enteroendocrine cells (specialized intestinal cells) and trigger the secretion of intestinal hormones, such as glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK) and peptide YY (PYY), both indirectly (via the systemic circulation) or directly (via the vagus nerve) via the GBA. Gut bacterial metabolites, such as SCFA, nitrite, TMAO, indole, and hydrogen sulfide, affect the cardiovascular and cerebrovascular systems profoundly. SCFA and hydrogen sulfide have vasorelaxant properties, and the abundance of bacterial species, such as Gram-positive Firmicutes, producers of these beneficial compounds, was found to be decreased in animal models of hypertension and human studies compared with healthy subjects.[9]

In contrast, higher levels of TMAO, a metabolite that increases reactive oxygen

species (ROS) production and impairs endothelial vasodilation, was found to be positively correlated with adverse cardiovascular events, including ischemic Stroke in patients with atherosclerosis, due to increased platelet hyperactivity and atrial fibrillation.[32] Thus, TMAO is considered a new predictor of Stroke. LPS, a component of the outer membrane of Gram-negative bacteria, often called an endotoxin, can enter the blood circulation when the integrity of the intestinal barrier is compromised by bacterial translocation and intestinal dysbiosis, leading to systemic inflammation.[9]

Table 1. Gut-derived bioactive metabolites and associated microorganisms.[9]

Gut-Derived Metabolites	Microorganisms
Acetate and propionate	Bacteroidetes (Gram-negative microorganisms), mainly *Bacteroides thetaiotaomicron* and *Bifidobacterium* species (Phylum: Actinobacteria).
Butyrate	Firmicutes (Gram-positive microorganisms), particularly *Faecalibacterium prausnitzii* (Phylum: Firmicutes), *Clostridium leptum* (Family: Ruminococcaceae), and *Eubacteriumrectale* and *Roseburia* species (Family: *Lachnospiraceae*).Other potential butyrate producers include *Eubacteriumhallii* and *Anaerostipes* spp. and members of the phyla Actinobacteria, Bacteroidetes, Fusobacteria, Proteobacteria, Spirochaetes, and Thermotogae.
Lipopolysaccharide	Gram-negative members of *Enterobacteriaceae*, such as *Escherichia coli*, *Klebsiella*, and *Salmonella*.
Neurotransmitters (acetylcholine, GABA, 5-HT, glutamate, dopamine, and noradrenaline)	*Lactobacillus* species secrete acetylcholine and GABA; *Bifidobacterium* species produce GABA; *Escherichia* produce norepinephrine, 5-HT, and dopamine; *Streptococcus* and *Enterococcus* produce 5-HT; and *Bacillus* species produce norepinephrine and dopamine.
Gut hormones (cholecystokinin, glucagon-like peptide-1, peptide YY, glucose-dependent insulinotropic polypeptide, or gastric inhibitory polypeptide and 5-HT (acts as a local hormone in the gut and as neurotransmitter in the brain)	Indigenous spore-forming microbes from *Clostridial* species, *Corynebacterium* spp., *Streptococcus* spp., and *Escherichia coli* synthesize 5-HT; *Odoribacter*, *Akkermansia*, *Ruminococcaceae*_UCG_005, and *Victivallis* are well-known producers of SCFAs that regulate the released gut hormones in response to nutrients by enteroendocrine cells.
Trimethylamine-N-oxide (TMAO)	Gut microbes *Anaerococcushydrogenalis*, *Clostridium asparagiforme*, *Clostridium hathewayi*, *Clostridium sporogenes*, *Edwardsiellatarda*, *Escherichia fergusonii*, *Proteus penneri*, and *Providencia rettgeri* metabolize dietary choline, L-carnitine, and betaine to form trimethylamine and TMAO.

3. The Role of Microbiota Dysbiosis in Ischemic Stroke

3.1. Changes in Gut Microbiota in Stroke Patient

Brain ischemia dysregulates GBA signaling through neural pathways or the hypothalamic–pituitary–adrenal (HPA) axis in stroke-induced gut dysbiosis. Metabolic endotoxemia occurs with elevated plasma LPS, contributing to increased intestinal permeability. Metabolic endotoxemia activates innate immune cells, contributing

to chronic systemic inflammation with increased migration of immune cells to the brain, resulting in BBB damage and neuroinflammation.[13,35]

Experimental stroke models in rodents show reduced gut microbiome diversity after Stroke. Clinical trials in stroke patients have demonstrated gut dysbiosis with altered Firmicutes-to-Bacteroidetes ratios, increased abundance of opportunistic pathogens (Megasphaera, Enterobacter, and Desulfovibrio), and decreased abundance of beneficial SCFA-producing bacteria (Blautia, Roseburia, Anaerostipes, Bacteroides, Lachnospiraceae, and Faecalibacterium).[36]

A clinical study investigating the microbiome of patients after cerebral Stroke demonstrated a reduced abundance of Roseburia, Bacteroides, and Faecalibacterium prausnitzii and an increased abundance of Enterobacteriaceae, Bifidobacteriaceae, and Clostridium difficile in gut samples when compared with healthy subjects, intensive care patients, and active ulcerative colitis patients. Patients with transient ischemic symptoms or mild ischemic Stroke show an altered gut microbiome compared with patients with asymptomatic atherosclerosis and without atherosclerotic changes.[36,37]

3.2.Microbiota Dysbiosis and Prognosis of Stroke Patients

Approximately 50% of stroke patients report GI symptoms, supporting the presence of gut dysbiosis. Ischemic Stroke directs aberrant signals to the intestine via the GBA, resulting in impaired intestinal barrier integrity, reduced mucus secretion, and translocation of intestinal bacteria into the circulation and extraintestinal organs. Gut dysbiosis affects local immune cells in the gut and brain. In the early phase of Stroke, microglial activation is accompanied by infiltration of peripheral immune cells, mainly monocytes, as well as T and B lymphocytes. In mouse stroke models, intestinal dysbiosis intensifies the influx of Th17- and IL17-secreting γδ T-cells (γδ T-cells) into the CNS from the intestine, causing chronic systemic and nervous inflammation. Higher numbers of pro-inflammatory lymphocyte populations are negatively correlated with stroke outcomes, reflected as more significant infarct size, brain edema, and neurological deficits.[35]

3.3.Gut Microbiota in the Pathogenesis of Ischemic Stroke

Reports from rodent and human clinical studies suggest that gut dysbiosis affects several pathways involved in developing risk factors associated with cerebral Stroke, such as hypertension, and metabolic diseases, such as obesity, T2DM, atherosclerosis, and vascular dysfunction.[9] Other metabolic disorders are accompanied by increased plasma levels of the endotoxin LPS, indicating increased gut leakage. Changes in the ratio of Bacteroidetes to Firmicutes and increased capacity to obtain energy from food were found in genetically obese mice and humans.[37]

Germ-free (GF) mice that received Faecal Microbiota Transplantation (FMT) from obese mice showed a marked increase in total body fat compared with GF mice that received FMT from lean mice, indicating an essential role for gut microbiota in regulating metabolic state. Overall, this explains the pathogenic role of gut dysbiosis in the onset, progression, and outcome of Stroke.[39] Therefore, potential therapeutic approaches targeting gut dysbiosis may be considered in treating and managing Stroke.

(a) Macrofag and monocyte;
(b) Lymphocytes T: T helper (Th) CD4+ (Th1-, Th17-, dan γδ T which secretes IL17), T cell CD8+, Treg cell, and T cell natural killer (NK);
(c) Lymphocytes B
(d) Microglial
(e) Astrocytes
(f) Dendritic cell
(g) Neutrophils
(h) Mast cell

3.3.1. Innate Immune Signaling

The pathophysiology of stroke-induced intestinal dysbiosis includes a series of

inflammatory responses induced by the activation of intestinal immune cells, increased intestinal permeability with increased levels of endotoxins, and their trafficking pathways to the brain. After Stroke, an innate immune response is produced by innate immune cells, such as neutrophils, macrophages, microglia, mast cells, lymphocytes (γδ T-cells), and NK cells, followed by activation of the adaptive immune response mediated by T and B lymphocytes. Pathological cascade after Stroke begins with releasing damage-associated molecular patterns (DAMP), cytokines from the infarct area, and activated microglia combined with increased levels of pro-inflammatory cytokines and chemokines from intestinal immune cells.[9,38]

In the presence of pro-inflammatory microbiota (an increase in pathogenic microbes, such as Bacteroidetes, Proteobacteria, and Clostridia), these DAMPs, pathogen-associated molecular patterns (PAMPs), such as interferon-1 (IFN-1) and cytokines trigger innate and adaptive immune responses in ischemic areas of the brain and the periphery via special pattern recognition receptors (Toll-like receptors (TLRs)). This process, in turn, triggers endothelial cells to express adhesion molecules with subsequent translocation of a large number of inflammatory cells and peripheral immune cells, such as macrophages or monocytes, neutrophils, dendritic cells, Th17 cells, and regulatory T cells (Treg) from Patch Peyer's in the small intestine to the site of stroke injury.[40]

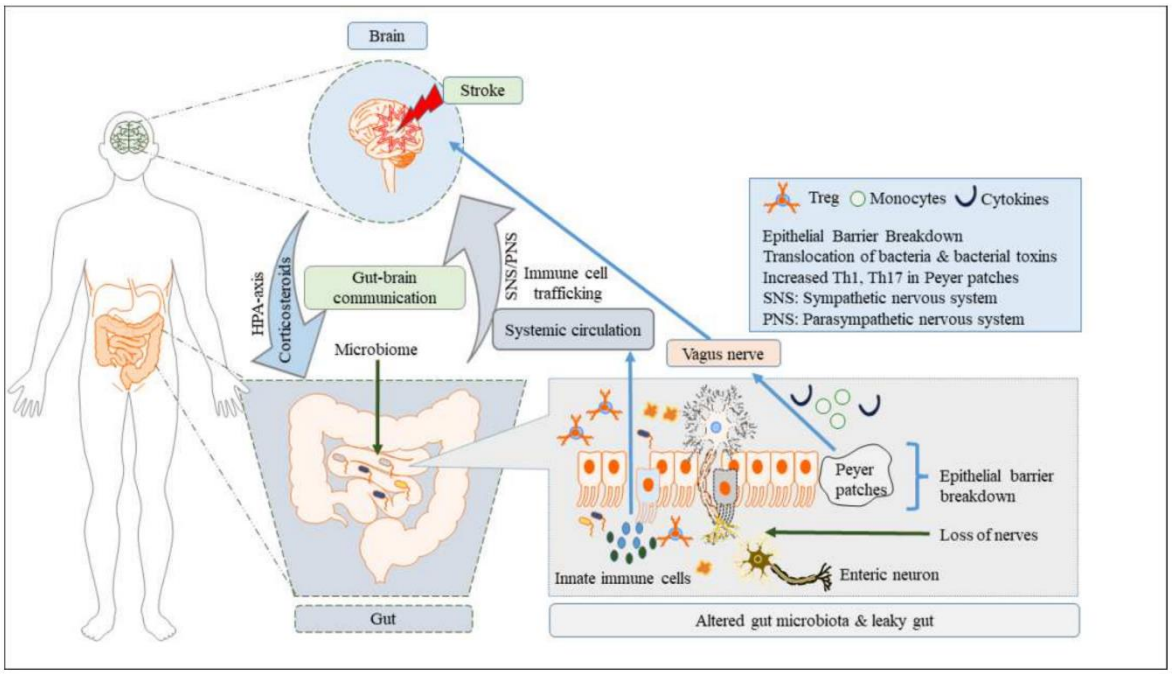

Figure 3. Effects of Stroke on the Gut-Brain Axis.[9]

After the cerebral Stroke, intestinal dysbiosis leads to loss of enteric nerves, increased intestinal barrier permeability, decreased mucus production, loss of goblet cells, thinning of the mucus barrier, and increased sympathetic activity in the intestinal wall, all of which contribute to intestinal inflammation and excessive immune response. These events, in turn, disrupt intestinal and systemic immune homeostasis, resulting in poor stroke treatment prognosis.[9]

3.3.2. Adaptive Immune Signaling

During stroke-induced gut dysbiosis, amplification of chronic inflammation by excessive recruitment or infiltration of peripheral immune cells (Th1 cells, Th17 cells, and monocytes from Peyer's patches to the infarct area), gut-derived toxic

metabolites, and bacterial translocation suppresses anti-Treg cell polarization. -inflammation. Specifically, dendritic cells migrate to mesenteric lymph nodes to promote the differentiation of T cells into Treg cells under the influence of pro-inflammatory microbiota. Reduced Treg cell migration to the lamina propria also promotes γδ T cell differentiation. Pro-inflammatory Th17, Th1, and γδ cells originating from the small intestinal lamina propria migrate to the meninges, accelerate inflammatory damage, and increase infarct size. In addition, DAMPs originating from brain injury and a cytokine storm of activated microglia with increased amounts of IL-6, TNF-α, and IFN-γ stimulate the vagus nerve, leading to intestinal dysmotility, dysbiosis, increased permeability, intestinal injury, and sepsis.[9,12]

These toxic changes in the GI tract can lead to excessive translocation of pathogenic bacteria, bacterial toxins, and toxic metabolites, which leak more intestinal inflammatory cells and immune cells into the circulation and the site of stroke injury. In addition, damage to the intestinal epithelial barrier and BBB, enteric nerve depletion, loss of goblet cells, and thinning of the mucus barrier promote chronic systemic inflammation. Thus, gut dysbiosis creates a vicious pro-inflammatory cycle, which worsens post-stroke treatment outcomes.[35]

3.3.3. Other Key Signaling Pathways in Stroke and Intestinal Dysbiosis

Intestinal dysbiosis primarily influences the pathology or prognosis of Stroke by affecting key immunological signaling pathways. The three most essential pathways have severe implications in gut dysbiosis-related neurological disease.[9]

(a) Inflammatory signaling pathways consist of innate immune signaling complexes that respond to various microbial and endogenous pathogen signals. In colitis models, inflammasome activation by SCFAs leads to the secretion of IL-18, contributing to intestinal homeostasis and preventing intestinal injury. Inflammation-mediated gut dysbiosis impacts neuropsychiatric diseases, such as major depressive disorder. In depressed patients, inflammatory hyperstimulation leads to increased levels of pro-inflammatory cytokines.

(b) Type I interferon (IFN-1) signaling pathway: IFN-1 is a pleiotropic and universal cytokine that plays an essential role in innate and adaptive immunity and contributes to host homeostasis. Host IFN-I can influence gut microbiota composition, suggesting a bidirectional relationship between gut microbiota signaling and IFN-I in Stroke. Thus, an altered IFN-1 pathway causes gut dysbiosis after Stroke. In a murine model, Martin et al. demonstrated that autophagy proteins suppress the microbiota-dependent IFN-I signaling pathway.[5]

Nuclear factor (NF)-κB signaling pathway: The NF-κB pathway is a central hub for inflammatory processes in the host. The NF-κB family consists of several key transcription factors that regulate innate and adaptive immune responses. The NF-κB pathway determines pro-inflammatory and proapoptotic gene expression, thereby maintaining immune homeostasis. In particular, NF-κB signaling is a significant pathway affected by changes in levels of gut-derived microbial products resulting in gut dysbiosis and immune-derived chronic systemic inflammation. Optimal activation of NF-κB activity in intestinal epithelial cells maintains normal intestinal homeostasis by preventing pathogen invasion and intestinal injury.[41]

In humans and rodent models of ischemic Stroke, NF-κB signaling was shown to play a significant role in tissue viability and recovery from ischemic damage. In a mouse model of hypertension, increased matrix metalloproteinase activity indicated hyperactivation of NF-κB, thereby suggesting that inhibiting the NF-κB cascade by anti-inflammatory drugs may be a

promising target for treating ischemia-reperfusion injury.[42] Preclinical studies have shown that potent inhibitors of the NF-κB pathway led to desirable outcomes in rodent models of ischemic Stroke, such as reduction in infarct size and inflammatory biomarkers.[43]

4. Animal Model Research Related to Intestinal Dysbiosis in Ischemic Stroke

Several experimental studies in stroke-related animal models have demonstrated a direct link between dysregulated GBA signaling and intestinal motility, dysbiosis, intestinal permeability, inflammation, and altered immune responses. Various reports also show that dysbiosis increases the abundance of intestinal T cells, which aggravates ischemic lesions. Bacteria originating from the small intestine are the leading cause of post-stroke infections in stroke patients and mouse stroke models. Unlike primary autoimmune diseases of the CNS, TIA triggers a rapid local neuroinflammatory reaction and peripheral immune activation.

Reports of microbial pathogenic changes occurring in animal models of Stroke include decreased species diversity (e.g., specific changes in Peptococcaceae and Prevotellaceae), along with increased abundance of pro-inflammatory microbiota (phylum Bacteroidetes, phylum Proteobacteria, and Clostridium species) and decreased abundance of anti-inflammatory microbiota (phyla Firmicutes and Actinobacteria). Intestinal dysbiosis is usually associated with altered T-cell homeostasis and induction of pro-inflammatory responses by migrating T cells, T helper, and monocytes from Peyer's patches to peri-infarct brain regions, negatively impacting post-stroke outcomes. Additionally, the presence of intestinal dysbiosis tends to induce larger infarct lesions and impaired recovery in ischemic mouse models. Nearly all brain structures are susceptible to gut dysbiosis-mediated ischemic brain injury (Figure 2).

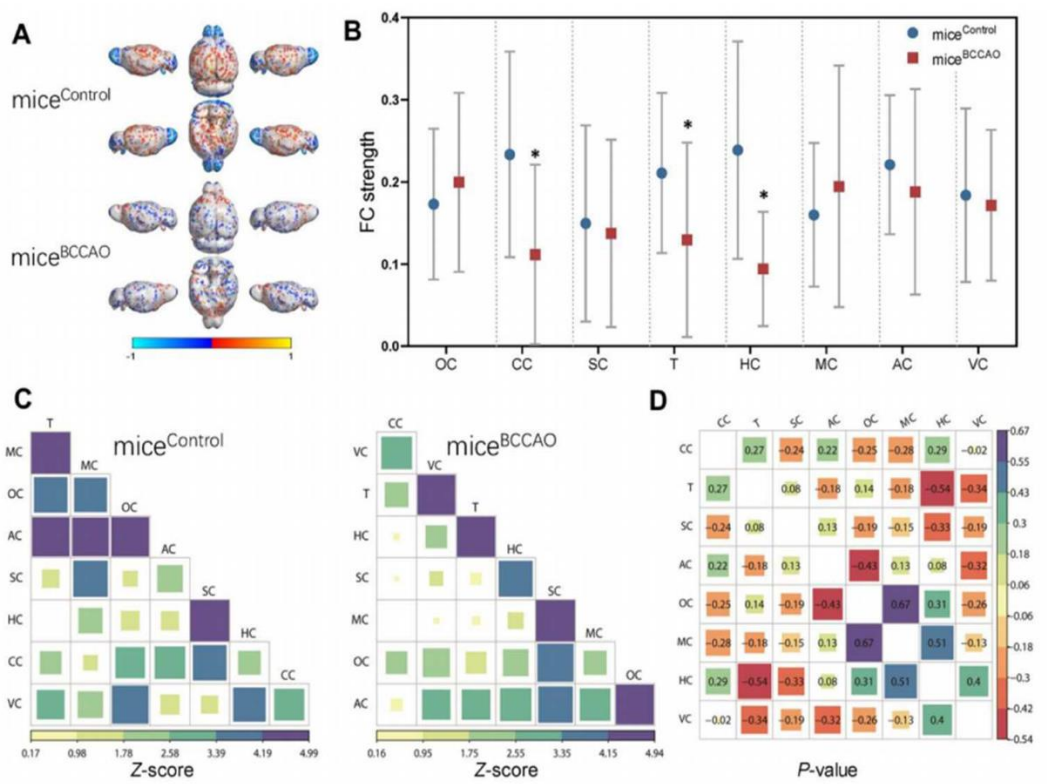

Figure 4. Effects of intestinal dysbiosis after ischemic injury on brain structure and function.[9]

Eight particular regions of the brain, such as orbitofrontal cortex (OC), somatosensory cortex (SC), cingulate cortex (CC), hippocampus (H), motor cortex (MC), thalamus (T), auditory cortex (AC), and visual cortex (VC), in rats subjected to cerebral ischemia/reperfusion injury caused by bilateral typical carotid artery occlusion (BCCAO group) or sham surgery (control group). (A) Functional connectivity between specific regions of interest from the control and BCCAO groups is displayed in a virtual graph. (B) Average functional connectivity strength within brain networks was measured using two-way repeated measures ANOVA with Tukey's multiple comparisons as a post hoc test. (C) The average functional connectivity matrix shows the strength of functional connectivity between pairs of brain regions in the standard and stroke groups. (D) Correlation analysis in the region (8) of interest in the animal brain; the color scale indicates the strength of functional connectivity.[9]

Using animals in contact with human flora (mice developed using FMT replicate the human microbial ecosystem) and GF mice devoid of complex microbiota, developed by growing under GF conditions or treatment with antibiotics, are considered powerful models for studying the ecosystem and the influence of gut flora man. Animal and human clinical studies have confirmed that FMT from healthy donors improves cognitive and neurobehavioral function in recipients.[44]

Stroke induction in GF mice was associated with a larger infarct area than specific pathogen-free or recolonized mice, indicating that intestinal bacterial composition is vital in modulating post-stroke outcome. For example, aberrant gut microbiota transferred by FMT from human hypertensive donors causes hypertension in GF mice.[44] Additionally, induction of large hemispheric lesions by proximal middle cerebral artery occlusion in rats (as a model of ischemic Stroke) causes intestinal dysbiosis, intestinal paralysis, increased intestinal permeability, loss of cholinergic innervation in the ileum, decreased goblet cell number and mucin production, and increased sympathetic activity. Young and old mice undergoing stroke procedures showed increased intestinal permeability, with increased translocation of intestinal bacteria and bacterial toxins. In addition, antibiotic-induced intestinal dysbiosis significantly reduces the survival rate of mice after stroke induction.[37,45]

A study examining the role of aging-related dysbiosis on post-stroke outcomes showed that old mice (≥20 months) had a 9-fold higher Firmicutes/Bacteroidetes ratio compared with young mice (≥3 months). Furthermore, FMT from young to old mice reduced mortality, improved locomotor function and anxiety, and increased motor strength during recovery from proximal MCAO. Intestinal dysbiosis in mice with genetic diabetes (db/db) shows increased intestinal permeability, higher plasma LPS levels, and increased abundance of the phylum Bacteroidetes and members of the Enterobacteriaceae family (mainly Escherichia coli, Klebsiella, and Salmonella).[46] Firmicutes, Actinobacteria, and Tenericutes were lower in diabetic mice than non-diabetic mice. Induction of focal cerebral ischemia in diabetic mice leads to larger infarct volumes and subsequent increased expression levels of LPS, TLR4, and inflammatory cytokines in the ischemic brain, in addition to inducing severe neurological deficits and higher mortality rates compared with non-diabetic mice.[47]

Benakis et al. demonstrated that mice with an anti-inflammatory gut microbiome (induced by antibiotics to reduce species abundance and diversity) showed reduced cerebral infarct volume (60%) and better-preserved sensory-motor function for at least 1 week after proximal middle cerebral artery occlusion (MCAO). Furthermore, FMT from healthy donors with mild inflammation to recipient naïve mice decreased infarct volume by 54% under MCAO-stroke conditions.[35]

In a study of male C57BL/6J GF mice receiving FMT from male mice with cerebral

ischemia/reperfusion injury caused by bilateral typical carotid artery occlusion (BCCAO group) or control mice) the gut microbial composition changed significantly in the BCCAO group compared to the control group on day 29 after FMT. In addition, the results of microbial analysis showed that the relative abundance of microbial colonization of 20 genera was significantly different between the BCCAO and control groups. These genera belong to the phyla Bacteroidetes (7/20), Firmicutes (9/20), Cyanobacteria (1/20), and Proteobacteria (3/20). These results indicated that microbial colonization from the BCCAO group hurt the gut microbiota of GF mice. Resting-state MRI imaging performed to assess (FC) between specific brain regions showed a significant reduction in FC in the cingulate cortex and thalamus of BCCAO mice compared with control mice.[48]

Acute ischemic Stroke in mice was shown to increase the expression of autophagy marker proteins, such as light-chain 3-II Beclin-1 and autophagy-related gene (Atg) 12, with increased levels of ROS, NADPH oxidase 2/4 (NOX2/4), malondialdehyde lipid peroxide, homocysteine, and free fatty acids (triglycerides and cholesterol), reflecting ischemia-induced nerve damage.[49,50] However, the levels of total antioxidant capacity and activities of superoxide dismutase (SOD) and reduced glutathione (GSH) were found to be decreased in brain tissue of stroke rats.[15] Overall, these preclinical studies reveal that gut microbiota can be manipulated to improve or worsen post-stroke outcomes. Generally, a healthy gut microbiota (eubiosis) stabilizes the gut wall and regulates low-grade inflammation, protecting against gut barrier dysfunction and infection. However, conditions that produce inflammation and dysbiosis in the gut are known to accelerate brain injury and negatively impact prognosis in stroke models via GBA.

5. Clinical Studies Related to Intestinal Dysbiosis in Ischemic Stroke

Changes in the gut microbiota ecology are associated with many diseases, including cerebrovascular disease. Several clinical investigations have reported on stroke-induced intestinal dysbiosis and demonstrated the presence of gastrointestinal symptoms after the onset of Stroke. In addition, clinical studies have also described an essential relationship between the gut microbiome and Stroke, confirming significant changes in the diversity and abundance of fecal microbial samples of stroke patients.[7,13]

Patients with large artery atherosclerotic stroke or TIA have a different gut microbial composition, along with increased abundance of opportunistic pathogens (e.g., Megasphaera, Enterobacter, Desulfovibrio, and Oscillibacter) and decreased abundance of commensal or beneficial genera. (Prevotella, Bacteroides, and Faecalibacterium) Compared with asymptomatic controls with or without carotid atherosclerotic plaque. In addition, blood TMAO levels were reported to be lower in patients with large artery atherosclerotic stroke or acute cerebral ischemia.[50]

A Japanese cohort study revealed that ischemic Stroke was associated with increased abundance of the Lactobacillus ruminis Atopobium cluster and decreased abundance of Lactobacillus sakei subgroups, hypertension, independent of age, and T2DM when compared with control subjects. Ischemic Stroke was associated with decreased levels of acetic acid (negatively correlated with levels of low-density lipoprotein cholesterol and glycated hemoglobin) and with increased levels of valeric acid (correlated positively with levels of white blood cell count and high-sensitivity C-reactive protein), suggesting that ischemic Stroke induces changes in host metabolism and systemic inflammation. Patients with coronary artery disease showed a pattern of dysbiosis with enrichment of Enterococcus and Escherichia-Shigella and depletion of Faecalibacterium, Roseburia, Subdoligranulum, and Eubacterium rectale.

Metagenomic analysis of fecal samples from patients with chronic heart failure revealed a lower abundance of Faecalibacterium prausnitzii and an increased abundance of Ruminococcus gnavus.[51]

A clinical study in China showed that microbial α diversity and composition were similar between cerebral ischemic stroke patients and healthy controls. However, the gut microbiota of stroke patients had an increased number of SCFA-producing taxonomies, such as Akkermansia, Odoribacter, Ruminococcaceae_UCG_005, and Victivallis. Another clinical study investigated the gut microbiome of 141 participants (aged 60 years or older) by classifying them into low, moderate, and high-risk groups based on known risk factors, including diabetes mellitus, hypertension, dyslipidemia, atrial fibrillation, and hypertension. Fibrillation, smoking, overweight, sedentary lifestyle, and family history of Stroke. The main results showed that individuals at high risk of Stroke exhibited increased levels of intestinal opportunistic pathogens (e.g., Betaproteobacteria, Desulfovibrionaceae, Enterobacteriaceae, Actinomycetaceae, Veillonellaceae, Veillonella, Megasphaera, Acidaminococcus, and Sutterella) and lactate-producing bacteria (e.g., Lactobacillus and Bifidobacterium) in abundance. Lower butyrate-producing bacteria (Lachnospiraceae and Ruminococcaceae) compared with individuals at low risk of Stroke. The relative abundance of Proteobacteria, Lactobacillales, Bacilli, Streptococcaceae, and Fusobacterium was also higher in the high-risk group than in the low-risk group. Increased abundance of Lactobacillus and Bifidobacterium was reported in high-risk patients. Likewise, fecal butyrate levels were also lower in the high-risk group than in the low-risk group.[53]

A clinical study revealed a lack of SCFA-producing bacteria, including Roseburia, Blautia, Bacteroides, Faecalibacterium, Lachnospiraceae, and Anaerostipes, and an increased abundance of opportunistic pathogens, such as Akkermansia, Lactobacillaceae, Enterobacteriaceae, and Porphyromonadaceae, confirming intestinal dysbiosis in patients with acute ischemic Stroke, especially in those with increased severity, when compared with healthy controls. Patients with higher stroke severity had reduced fecal SCFA levels. Compared with healthy controls, an increased Bacteroidetes/Firmicutes ratio was observed in acute ischemic stroke patients.[52, 53,54]

Karlsson et al. showed that patients with atherosclerotic plaque symptoms showed an abundance of the genera Bacteroides, Ruminococcus, Eubacterium, and Faecalibacterium and a low abundance of the butyrate-producing bacteria SSC/2. Shotgun sequencing of gut metagenomes showed that the genus Collinsella was enriched in stroke patients, whereas Eubacterium and Roseburia were enriched in healthy controls. In addition, intestinal metagenomes showed increased genes related to peptidoglycan biosynthesis. In contrast, the gene encoding phytoene dehydrogenase K10027 was decreased in patients, along with reduced levels of antioxidants, such as β-carotene, compared to control subjects. These results suggest that gut microbiota may contribute to atherosclerosis symptoms by favoring associated inflammation and innate immune system pathways.[36]

Overall, these clinical studies demonstrate that toxic changes in gut microbial composition and abundance correlate directly with the magnitude of stroke severity. However, the timing of microbial changes and their impact on ischemic Stroke in patients remain poorly understood

6. New Therapeutic Strategy in Modulating Gut Microbiota for Prevention and Treatment of Stroke

6.1.Probiotic / Prebiotic / Sinbiotic

Probiotics and prebiotics have been shown to help establish eubiosis in stroke patients. However, the exact mechanisms for its beneficial effects remain largely unknown and are a topic of intensive research. Probiotics refer to live microorganisms that

can provide health benefits when ingested by humans or animals. Prebiotics refer to indigestible fibers or antioxidant compounds that are selectively metabolized in the small intestine and promote the growth of symbiotic species. Synbiotics are synergistic mixtures of probiotics and prebiotics that benefit the host by selectively promoting the growth of beneficial microbes. Supplementation with probiotics or prebiotics may improve post-stroke outcomes by reducing gut leakage and plasma LPS levels, as these supplements improve lipid and glucose metabolism in overweight people and those with diabetes.[55,56]

Another study showed that probiotic supplementation reduced blood pressure, one of the main risk factors for Stroke.[57] Supplementation with probiotics alters the composition and abundance of the gut microbiome by modulating cytokine release and neuroinflammatory responses. Therefore, probiotics represent another adjunct therapeutic approach for the management of cardiometabolic disorders, including arterial hypertension and acute Stroke. Known mechanisms of action of probiotics include suppression of TNF-α and free radicals via TLRs in the intestinal epithelium, reduction of TMAO levels, increased production of brain-derived neurotrophic factor (BDNF), inhibition of apoptosis, and increased abundance of several symbionts.[48]

Pretreatment with Clostridium butyricum attenuated hippocampal apoptosis and improved neurological deficit scores in mice after BCCAO. Intragastric pretreatment with 1 × 10 CFU C. butyricum (200 µL once daily for 2 consecutive weeks) before bilateral typical carotid artery occlusion (for 20 min) showed increased butyrate levels. It decreased oxidative stress in the brains of male ICR mice, suggesting neuroprotective effects (Wang et al., 2012). In a mouse model of Stroke, oral pretreatment with a mixture of probiotic bacteria (Bifidobacterium breve, Lactobacillus casei, Lactobacillus acidophilus, and Lactobacillus bulgaricus of 107 CFU/mL via daily oral gavage for 14 days) reduced cerebral ischemia by 52% and significantly improved neurological outcomes.[51]

Prebiotics help restore gut microbiota and are negatively correlated with the risk of cardiometabolic diseases. A diet rich in fiber and plant polyphenols shifts the gut microbial ecology to eubiosis. Fiber and polyphenols are converted into biologically active compounds by intestinal microbes and maintain colonic metabolic homeostasis while they function as antioxidants to neutralize ROS and reactive nitrogen species (RNS).[58] A high-fiber diet increases the number of acetic acid-producing bacteria, which causes a decrease in blood pressure in hypertensive mice. High-fiber diets also increase the abundance of butyrate-producing bacteria, associated with reduced gut leakage, endotoxemia, systemic inflammation, and susceptibility to atherosclerotic lesions in murine models.[59] Overall, these results demonstrate that greater dietary fiber intake is inversely correlated with cardiovascular disease and stroke development and treatment outcomes. Nonetheless, it must be acknowledged that there is significant methodological heterogeneity across studies describing the impact of probiotics, prebiotics, and synbiotics on Stroke.

6.2.Feces microbiota therapy (FMT)

FMT therapy refers to administering feces from healthy donors via enema, mesenteric, nasogastric, or endoscopic routes (upper endoscopy, sigmoidoscopy, colonoscopy) or oral capsules that establish a new gut microbiota community. Studies have reported that the effects of FMT may vary, depending on study design, use of different donors (i.e., quality control), route of delivery, and use of different antibiotics. However, FMT can be used as an adjunct therapy or combined with other treatment methods to modulate the gut microbiome toward a healthy state of eubiosis. The combination treatment method was found to promote healthy gut microbiota and repair

intestinal damage, including intestinal permeability. Additionally, the FMT method is cost-effective. FMT has evolved as a potential new strategy for reversing gut microbial dysbiosis, which is involved in the complex pathology of several clinical conditions, including metabolic syndrome, alcoholic liver disease, autoimmune disorders, and cardiovascular and neurological diseases. Recent studies in animal models also demonstrate the benefits of FMT in improving post-stroke outcomes.[60]

In the MCAO rat model, intragastric nonabsorbable antibiotics (vancomycin (100 mg/kg), neomycin sulfate (200 mg/kg), metronidazole (200 mg/kg), and ampicillin (200 mg/kg)) were administered daily for 4 days) improves neurological function and reduces cerebral infarction volume. FMT intervention (rich in SCFA-producing bacteria), together with butyric acid supplementation (30 mg/kg) administered intragastrically once daily for 14 days) was influential in the treatment of Stroke by restoring normal gut microbiota and increasing α-diversity, along with significant enrichment of beneficial bacteria, such as Lactobacillus, Butyricicoccus, and Meganonas, along with a reduction in opportunistic pathogens, such as Alistipes, Klebsiella, Bacteroides, Shuttleworth, Fusobacterium, Haemophilus, Faecalibacterium, Proteus, and Papillibacter.[10,13,15]

FMT also significantly reduced infarct volume, neurological deficits, serum total cholesterol, and triglyceride levels; eliminated brain edema; intestinal permeability restored; and increased levels of isobutyric, butyric, and isovaleric acids in feces. In addition, butyric acid supplementation significantly reduced neurological disorders, cerebral infarct volume, serum total cholesterol, triglyceride and fibrinogen levels, whole blood viscosity, and intestinal permeability, meanwhile alleviating cerebral edema. These studies show that FMT, with a high content of SCFA-producing bacteria (especially butyric acid), promotes positive clinical outcomes in cerebral ischemic Stroke by reducing or preventing intestinal dysbiosis, intestinal barrier dysfunction, neurological disorders, cerebral infarct volume, and blood lipids. Level, cerebral edema, neurotoxicity, neuroinflammation, and risk of thrombosis.[9,44,57]

6.3.Natural Bioactive Compounds Used in Stroke Treatment

Several preclinical and clinical studies clearly state that natural bioactive compounds, such as nuts, fruits, vegetables, grapes, olive oil, and seeds consumed as diet foods or as dietary supplements or other plant-derived compounds, have a cardioprotective effect. Many in vitro studies have shown that several molecules with different chemical structures, such as polyphenolic compounds, peptides, oligosaccharides, vitamins, and n-3 fatty acids, were found to be powerful cardioprotective agents. Among them, the most efficient bioactive compounds that show significant cardioprotective effects are long-chain omega-3 polyunsaturated fatty acids, including plant-derived α-linolenic acid, fish oil-derived eicosapentaenoic acid, and docosahexaenoic acid.[61]

The main bioactive molecules found in tea are polyphenols, such as catechins in green tea and aflavins in black tea. Both in vivo and in vitro models of cerebral ischemia/reperfusion (I/R) have demonstrated the beneficial effects of aflavin by eliminating miRNA-128-3p-induced nuclear factor erythroid 2-related factor 2 (Nrf2) suppression and thereby reducing oxidative stress. Another catechin found in green tea, (-)-epigallocatechin-3-gallate, treatment reduces neurological deficits, decreases infarct volume, promotes angiogenesis, enhances the vascular endothelial growth factor receptor two signaling pathway, and increases nuclear levels of Nrf2. Pretreatment with (−)-epicatechin before permanent MCAO reduced infarct volume and improved neurological deficits in Nrf2/2 knockout

(Nrf2−/−) mice compared with wild-type controls. Both in vitro and in vivo models have demonstrated that naringenin and nobiletin in citrus fruits reduce neurological deficits, brain edema, and infarct volume and exert potent antioxidant actions through the involvement of the Nrf2 signaling pathway. Spices have been used medicinally for several centuries, especially garlic, turmeric, chili peppers, and rosemary containing S-allyl cysteine, diallyl trisulfide, dihydrocapsaicin, curcumin, and rosmarinic acid, respectively. In vivo models in mice and rats undergoing I/R injury have proven the neuroprotective effects of this spice. Oleuropein and hydroxytyrosol in olive oil are the most abundant polyphenols, with excellent free radical scavenging properties in experimental models. Anthocyanins, a large subgroup of flavonoids, prevent oxidative injury in the mouse cardiomyocyte cell line H9C2.[62,63]

Among the fruit-based bioactive compounds, Lycium barbarum fruit (Goji berry) contains monoterpenes, flavonols, phenolic acids, and lyceum amide. Lycium amide A provides a protective effect against Stroke. Mangiferin in mango and papaya improved neurological scores and decreased infarct volume and edema in rats with cerebral I/R injury. Procyanidin B is the predominant polyphenol in berries, cereals, legumes, nuts, chocolate, and grapes. Specifically, procyanidin B2 in cocoa, grapes, and apples reduced infarct size, brain edema, and neurological deficits after MCAO by preventing BBB breakdown, attenuating tight junction degradation, and counteracting oxidative stress via the Nrf2 pathway.[64] The antioxidant properties of these natural bioactive polyphenols provide direct or indirect neuroprotection by potentially improving gut dysbiosis associated with stroke model compounds, such as polyphenols and flavonoids, restore commensal microbes and are thus shown to improve post-stroke health.[65]

CONCLUSION

This literature review discusses the relationship of gut dysbiosis to the pathogenesis and treatment outcomes of Stroke. Clinical and preclinical studies over the past decade have demonstrated an association between gut dysbiosis and the development of well-known stroke risk factors, such as dyslipidemia, insulin resistance, obesity, hypertension, T2DM, and cardiovascular, cerebrovascular, and neurological disorders. Findings have confirmed a positive correlation between gut dysbiosis and poor post-stroke outcomes. Pathophysiological mechanisms underlying stroke-induced intestinal dysbiosis include damage to the intestinal epithelial barrier, tight junction proteins and adherens, intestinal dysmotility, altered mucus secretion, loss of goblet cells, altered local immune homeostasis, increased LPS levels, and intestinal inflammation. As a result, immune-driven systemic inflammation or endotoxemia occurs, and these events lead to increased inflammation, BBB breakdown, and neurotoxicity, characterized by increased production of ROS, RNS, TMAO, and NOX2/4 and neuroinflammation.

High levels of LPS, C-reactive protein, TLR4, bacterial toxins, toxic metabolites, abnormally activated immune cells, and pro-inflammatory cytokines in ischemic brain regions cause damaging consequences. These changes result in larger infarct size, neuronal death, synapse loss, and glial dysfunction, leading to poor outcomes. Inflammatory signals from the infarct site further enhance intestinal dysbiosis, producing a pro-inflammatory loop.

In breaking the destructive pro-inflammatory cycle, the gut microbiome has been considered a promising target in treating and preventing Stroke in experimental and clinical studies. Pharmacological and non-pharmacological methods, such as dietary interventions, antibiotics, prebiotics, probiotics, synbiotics, and FMT, can prevent the detrimental effects of intestinal dysbiosis. Several studies have confirmed that gut microbiome dynamics can be regulated toward a healthy state by restoring beneficial microbial populations and increasing SCFA (especially butyric acid) levels. In cerebral

ischemic Stroke, supplements rich in SCFA-producing bacteria have been shown to reduce gut leakage and inflammation significantly, reduce the abundance of pathogenic bacteria, and increase the population of beneficial bacteria, which in turn inhibits neuronal cell apoptosis, oxidative stress, and cerebral infarct volume, leading to the prevention of neurobehavioral disorders. FMT with enriched SCFA-producing bacteria and butyric acid supplementation was found to be an effective treatment for cerebral ischemic Stroke. However, additional large-scale randomized clinical studies are needed in the future to demonstrate the efficacy and safety of FMT in treating patients with Stroke or other cardiovascular complications.

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