Role of the Gut Microbiota in Stroke Pathogenesis and Potential Therapeutic Implications

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Keywords

Cerebral ischemia · Dysbiosis · Gut microbiota · Inflammation · Stroke

Abstract

Background: Major advances have been made in stroke treatment and prevention in the past decades. However, the burden of stroke remains high. Identification of novel targets and establishment of effective interventions to improve stroke outcomes are, therefore, needed. Recent research highlights the contribution of the gut microbiota to stroke pathogenesis. **Summary:** Compositional and functional alterations of the gut microbiota, termed dysbiosis, are linked to stroke risk factors, such as obesity, metabolic diseases, and atherosclerosis. In acute cerebral ischemia, the gut microbiota plays a key role in bidirectional interactions between the gut and brain, referred to as the microbiota-gut-brain axis. Gut dysbiosis prior to ischemic stroke affects outcomes. Additionally, the brain affects the gut microbiota during acute ischemic brain injury, which in turn impacts outcomes. Interactions between the gut microbiota and stroke pathogenesis are mediated by several factors including bacterial components (e.g., lipopolysaccharide), gut microbiota-related metabolites (e.g., short-chain fatty acids

and trimethylamine N-oxide), and the immune and nervous systems. Clinical studies have reported that patients with acute ischemic stroke exhibit gut dysbiosis, which is associated with host metabolism and inflammation, as well as functional outcomes. Modulation of the gut microbiota or its metabolites improves conditions related to stroke pathogenesis, including inflammation, cardiometabolic disease, atherosclerosis, and thrombosis. **Key Messages:** Accumulating evidence indicates that the gut microbiota plays a possible role in stroke pathogenesis. Modulation of the gut microbiota may provide a novel therapeutic strategy for the treatment and prevention of stroke.

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Introduction

Major advances have been made in the treatment and prevention of stroke in the past few decades including endovascular thrombectomy, thrombolysis with intravenous alteplase, improvement in vascular risk factor management, and development of new antithrombotic drugs such as direct oral anticoagulants [1]. Although stroke mortality rates decreased sharply from 1990 to 2016, the

Fig. 1. Gut microbiota and stroke pathogenesis. Alteration of gut microbiota (dysbiosis) contributes to the development of stroke risk factors such as systemic inflammation, cardiometabolic diseases, and atherosclerosis. In acute cerebral ischemia, dysbiosis induces neuroinflammation, systemic inflammation, and infection, which affect stroke outcomes. These interactions are mediated by several pathways including bacterial components (e.g., LPS), gut microbiota-related metabolites (e.g., SCFAs and TMAO), immune

cells (e.g., T cells), or bacterial translocation. Additionally, the ischemic brain influences the gut microbiota composition via either the neural or hypothalamic-pituitary-adrenal pathways, which in turn also contribute to stroke outcomes. ANS, autonomic nervous system; HPA, hypothalamus-pituitary-adrenal; LPS, lipopolysaccharide; SCFAs, short-chain fatty acids; TMAO, trimethylamine N-oxide.

decrease in incidence has been less steep, and the burden of stroke remains high [2]. Identification of novel targets and establishment of effective interventions to improve stroke outcomes are, therefore, needed.

The human gut harbors diverse microbes that play a fundamental role in host health and physiology [3]. Various factors including diet, xenobiotics, pathogens, and genetics influence the composition of the gut microbiota [4]. Compositional and functional alterations of the gut microbiota, termed dysbiosis, are associated with the pathogenesis of both intestinal and extra-intestinal disorders, and these alterations modulate stroke risk factors such as obesity, metabolic diseases, and atherosclerosis [4]. The microbiota plays a key role in bidirectional gut-brain interactions. This communication is referred to as the microbiota-gut-brain axis [5]. Emerging evidence suggests that the microbiota-gut-brain axis plays a role as a central regulator of the immune system after an acute

ischemic stroke. Dysbiosis possibly exerts systemic harmful effects with development of the systemic inflammatory response after an ischemic stroke [6]. Recent research has explored the effects of the gut microbiota on ischemic brain injury through multiple factors including bacterial components, metabolites, and the immune and neural systems. Here, we focus on the microbial molecules and discuss the current knowledge about the role of the gut microbiota in stroke pathogenesis and the potential for the gut microbiota as a novel therapeutic target for the treatment and prevention of stroke.

Microbial Signaling Molecules

The gut microbiota plays a key role in host health and disease through signals that are either structural components of the bacteria or metabolites produced by the gut

microbiota [7]. These signals affect conditions related to stroke pathogenesis such as systemic inflammation, cardiometabolic diseases, and atherosclerosis (Fig. 1). Among bacterial components, lipopolysaccharide (LPS) has been extensively examined for its association with gut dysbiosis and disease pathogenesis. LPS is a major component of the outer membrane of Gram-negative bacteria and is a potent inflammatory stimulus for the innate immune response via toll-like receptor activation [8]. Gut dysbiosis and impaired intestinal barrier functions lead to translocation of LPS into the circulatory system and contribute to the development of obesity and diabetes, a condition known as metabolic endotoxemia [9]. Metabolic endotoxemia elicits chronic systemic inflammation, which may result in high cardiovascular risk and target organ damage [10].

Short-chain fatty acids (SCFAs) are the major metabolites produced by bacterial fermentation of dietary fibers. Acetic acids, propionic acid, and butyric acid are the most prominent in the colon. SCFAs serve as not only an energy source for epithelial cells but also signaling molecules via activation of G-protein-coupled receptors or inhibition of histone deacetylases [11]. SCFAs regulate inflammation [12], glucose metabolism [13, 14], and blood pressure [15], and in the brain, SCFAs affect maintenance of the blood-brain barrier [16] and maturation and activation of microglia [17].

Trimethylamine N-oxide (TMAO) is another important gut microbiota-dependent metabolite generated from dietary phosphatidylcholine and L-carnitine, which are abundant in the Western diet [18–20]. TMAO is a novel predictor of adverse cardiovascular events including stroke [18, 19]. The potential mechanism linking TMAO to ischemic stroke includes atherosclerotic plaque formation [21], thrombosis due to enhanced platelet hyperactivity [22], and atrial fibrillation [23].

Microbiota-Gut-Brain Axis in Acute Cerebral Ischemia

The microbiota is a key component of bidirectional gut-brain interactions in acute cerebral ischemia (Fig. 1). We previously reported that metabolic endotoxemia is associated with LPS-induced neuroinflammation and worse stroke outcomes after focal cerebral ischemia in genetically diabetic (db/db) mice [24]. db/db mice exhibit gut dysbiosis, increased intestinal permeability, and higher plasma LPS levels than nondiabetic mice. The gut microbiota composition shows changes including an in-

creased abundance of *Enterobacteriaceae*, which are a large family of Gram-negative bacteria that includes opportunistic pathogens such as *Escherichia coli*, *Klebsiella*, and *Salmonella*. After experimental stroke, db/db mice have an increased infarct volume and higher expression levels of LPS, toll-like receptor 4, and inflammatory cytokines in the ischemic brain, as well as more severe neurological impairments and reduced survival rates than nondiabetic mice [24].

Chronic systemic inflammation, known as inflammaging, develops with aging and contributes to the pathogenesis of age-related diseases [25]. Age-related changes in the gut microbiota are associated with the inflammatory status and include a decrease in SCFA-producing bacteria [26]. Spychala and colleagues [27] examined the influence of age-related dysbiosis on stroke outcomes. The ratio of Firmicutes to Bacteroides is increased in aged mice compared to young mice. Aged mice have lower fecal levels of SCFAs such as acetate and propionate. Fecal transplantation of microbiota from aged mice to young mice leads to negative outcomes and enhanced systemic inflammation after stroke.

Benakis and colleagues [28] showed that commensal bacteria affect stroke outcomes by regulating some immune cells in mice. Antibiotic-induced dysbiosis increases the number of regulatory T cells in the lamina propria of the small intestine and suppresses the development of interleukin-17-positive $\gamma\delta$ T cells. This results in suppression of trafficking of interleukin-17-positive $\gamma\delta$ T cells from the intestine to the leptomeninges after stroke, thereby reducing inflammation-related ischemic injury.

In addition to the effects of prestroke gut dysbiosis on stroke outcomes, gut dysbiosis induced by large stroke lesions is detrimental to stroke outcomes. Poststroke dysbiosis induces pro-inflammatory Th1- and Th17-cell polarization in the intestine [29]. Migration of intestinal lymphocytes to the ischemic brain may be associated with an increased infarct volume [29]. Another experimental study showed that stroke induces gut dysbiosis that involves a decrease in the levels of *Peptococcaceae* and an increase in the level of *Prevotellaceae* via altered autonomic activity and mucoprotein production [30]. A study that examined the mucosal microbiota composition showed that the main characteristics of stroke-induced changes are an increased abundance of *Akkermansia muciniphila* and an excessive abundance of clostridial species [31]. Stroke-induced gut dysbiosis changes the predicted functional potential of the gut microbiota, with significant increases in pathways associated with infec-

tious diseases, membrane transport, xenobiotic degradation, lipid metabolism, and signaling [31].

Infections that present subsequent to stroke complicate up to a third of cases of stroke and may worsen outcomes [32]. Recently, translocation and dissemination of bacteria from the host-gut microbiota has been revealed as a mechanism leading to poststroke infection. Stanley and colleagues [33] showed that the majority of the microorganisms detected in infected stroke patients are commensal bacteria that normally reside in the intestinal tracts. Moreover, using animal models of ischemic stroke, they showed that the source of the bacteria forming the microbial community in the lung of poststroke mice is the host small intestine. Stroke-induced gut barrier permeability and dysfunction precede bacterial translocation [33]. Wen and colleagues [34] showed that aging exacerbates intestinal barrier dysfunction after experimental stroke, which promotes bacterial translocation and contributes to increased lung infection in mice. Another study showed that stroke induces gut permeability and bacterial translocation in both young and aged mice, but only young mice are able to resolve these changes [35]. Aged mice have prolonged sepsis with immune dysfunction and worse outcomes after stroke [35]. Moreover, immobilization stress before cerebral ischemia induces colonic inflammation and bacterial translocation, leading to worse stroke outcomes in rats [36].

Although the gut microbiota may have deleterious effects on the host through inflammation and bacterial translocation, the gut microbiota also plays a protective role during acute stroke. Extensive depletion of the gut microbiota by broad-spectrum antibiotic treatment prior to cerebral ischemia causes severe acute colitis and decreases survival after stroke in mice [37]. These events are prevented by continuous antibiotic treatment or recolonization with microbiota [37]. In addition, systemic immunodepression may contribute to poor stroke outcomes in the microbiota-depleted state. The gut microbiota per se is cerebroprotective via lymphocyte-driven protective neuroinflammation in the ischemic brain [38]. Bacterial colonization in germ-free mice improves stroke outcomes that are mediated by microbiome-induced T-cell priming [38].

Gut Dysbiosis in Patients with Acute Ischemic Stroke

Characteristics of the gut microbiota in patients with acute ischemic stroke have been examined in clinical studies (Table 1). We evaluated the fecal gut microbiota

composition and fecal organic acid concentration in a Japanese cohort of ischemic stroke patients and age- and sex-matched controls [39]. Ischemic stroke is associated with increased bacterial counts of *Atopobium* cluster and *Lactobacillus ruminis* and decreased numbers of the *Lactobacillus sakei* subgroup. Changes in the fecal number of *Lactobacillus ruminis* are positively correlated with serum interleukin-6 levels. Further, ischemic stroke is associated with decreased acetic acid concentrations and with increased valeric acid concentrations. Changes in acetic acid concentrations are negatively correlated with the levels of glycated hemoglobin and low-density lipoprotein cholesterol, whereas changes in valeric acid concentrations are positively correlated with the level of high-sensitivity C-reactive protein and white blood cell counts. These findings suggest that gut dysbiosis in patients with ischemic stroke is associated with altered host metabolism and systemic inflammation.

Yin and colleagues [40] reported that patients with large-artery atherosclerotic stroke have more opportunistic pathogens, such as *Enterobacter*, *Megasphaera*, and *Desulfovibrio* and fewer commensal or beneficial genera including *Bacteroides*, *Prevotella*, and *Faecalibacterium*. In particular, *Faecalibacterium* is a critical butyrate acid-producing beneficial bacterium. Another study from China reported that SCFA-producing bacteria including *Odoribacter*, *Akkermansia*, *Ruminococcaceae*_UCG_005, and *Vectivallis* are increased in patients with ischemic stroke compared to controls [41]. In addition, the levels of genus *Christensenellaceae*_R-7_group, norank_f_Ruminococcaceae, and *Enterobacter* are correlated with stroke severity [41].

Xia and colleagues [42] developed an index to measure gut dysbiosis in acute stroke patients that is correlated with early stroke outcomes. The Stroke Dysbiosis Index is positively correlated with stroke severity and poor functional outcomes. A higher Stroke Dysbiosis Index includes an increased abundance of pathogenic bacteria such as *Enterobacteriaceae* and decreased abundance of *Faecalibacterium* that have anti-inflammatory properties through butyrate production. Furthermore, mice that received fecal transplants from patients with a high Stroke Dysbiosis Index had worse stroke outcomes and larger infarct volumes than those in mice given transplants from patients with a low Stroke Dysbiosis Index [42]. Tan and colleagues [43] reported a lack of SCFA-producing bacteria (*Roseburia*, *Bacteroides*, *Lachnospiraceae*, *Faecalibacterium*, *Blautia*, and *Anaerostipes*) and an overgrowth of opportunistic pathogens (*Enterobacteriaceae* and *Porphyromonadaceae*) as well as *Lactobacillaceae* and *Akkermansia* in patients with acute ischemic stroke. Addition-

Table 1. Clinical studies examining the gut microbiota in patients with acute ischemic stroke

Authors	Study population	Dysbiosis of microbiota	Main findings
Yamashiro et al. [39]	Forty-one patients with AIS and 40-matched control subjects	Ischemic stroke was associated with increased bacterial counts of <i>Atopobium</i> cluster and <i>Lactobacillus ruminis</i> and decreased numbers of the <i>Lactobacillus sakei</i> subgroup	Patients with AIS had altered microbial composition and reduced fecal acetic acid levels. Gut dysbiosis was associated with changes in serum metabolic and inflammatory marker levels
Yin et al. [40]	One-hundred forty-one AIS and TIA patients with large-artery atherosclerosis and 94 asymptomatic persons	Increase in opportunistic pathogens (<i>Enterobacter</i> , <i>Megasphaera</i> , and <i>Desulfovibrio</i>) and decrease in commensal or beneficial genera including <i>Bacteroides</i> , <i>Prevotella</i> , and <i>Faecalibacterium</i> (butyrate acid producer) in patients	Patients with AIS and TIA had more opportunistic pathogens and fewer commensal or beneficial genera
Li et al. [41]	Thirty patients with AIS and 30 healthy controls	Increase in SCFA-producing bacteria including <i>Odoribacter</i> , <i>Akkermansia</i> , <i>Ruminococcaceae</i> _UCG_005, and <i>Victivallis</i> in patients	The gut microbiota of patients with AIS had more SCFA producers. Gut dysbiosis was correlated with stroke outcomes
Xia et al. [42]	One-hundred four patients with AIS and 90 healthy individuals	A higher Stroke Dysbiosis Index includes an increased abundance of pathogenic bacteria such as <i>Enterobacteriaceae</i> and decreased abundance of butyrate-producing bacteria such as <i>Faecalibacterium</i>	The Stroke Dysbiosis Index was an independent predictor of severe stroke and early unfavorable outcomes
Tan et al. [43]	One-hundred forty patients with AIS and 92 healthy controls	A lack of SCFA-producing bacteria (<i>Roseburia</i> , <i>Bacteroides</i> , <i>Lachnospiraceae</i> , <i>Faecalibacterium</i> , <i>Blautia</i> , and <i>Anaerostipes</i>) and an increase in opportunistic pathogens (<i>Enterobacteriaceae</i> and <i>Porphyromonadaceae</i>) and <i>Lactobacillaceae</i> and <i>Akkermansia</i> in patients	Reduced fecal acetate level was associated with an increased risk of 90-day poor functional outcomes
Karlsson et al. [44]	Twelve patients with symptomatic carotid plaques (including minor ischemic stroke and TIA) and 13-matched controls	The genus <i>Collinsella</i> was enriched in patients, whereas <i>Eubacterium</i> and <i>Roseburia</i> were enriched in controls. Samples from patients were enriched in genes encoding peptidoglycan biosynthesis, and samples from controls had enriched levels of phytoene dehydrogenase in metagenomes	Patients with symptomatic carotid plaques had altered gut metagenomes associated with host inflammatory pathways
AIS, acute ischemic stroke; SCFAs, short-chain fatty acids; TIA, transient ischemic attack.			

ally, fecal SCFA levels are lower in acute ischemic stroke patients than in healthy controls. Reduced acetate levels are associated with an increased risk of 90-day poor functional outcomes [43].

Karlsson and colleagues [44] performed shotgun sequencing of the gut metagenome and found that the genus *Collinsella* is enriched in patients with symptomatic atherosclerotic plaques (who had undergone carotid endarterectomy for minor ischemic stroke, transient ischemic attack, or amaurosis fugax), whereas *Eubacterium* and *Roseburia* are enriched in controls. Further, patient metagenomes are enriched in genes encoding peptidoglycan biosynthesis, suggesting a contribution of bacterial molecules to symptomatic atherosclerosis by priming the innate immune system. Controls have enriched levels of phytoene dehydrogenase in metagenomes and elevated levels of associated metabolites, including antioxidants such as β -carotene in the serum [44]. Gut metagenomes may contribute to the development of symptomatic atherosclerosis that regulates host inflammatory pathways.

Although gut dysbiosis has been demonstrated in patients with acute ischemic stroke, some discrepancies were observed regarding specific bacterial changes among studies (Table 1). Furthermore, studies including ours showed reduced SCFA-producing bacteria or fecal SCFA levels in patients with acute ischemic stroke [39, 40, 42, 43], whereas another study showed increased SCFA producers [41]. These discrepancies may be due to differences in the background of the study participants such as age, the severity of stroke, and dietary habits. Few studies have investigated the gut microbiota in patients with acute ischemic stroke. Additional studies are needed to explore how the gut microbiota affects the pathology of acute ischemic stroke.

Gut Microbiota Modulation as a Therapeutic Strategy for Prevention and Treatment of Stroke

High plasma LPS levels are associated with incident carotid atherosclerosis and cardiovascular disease [45]. Moreover, plasma LPS activity rises during ischemic stroke, and high LPS activity is associated with unfavorable outcomes in patients with acute ischemic stroke [46]. Recent evidence indicates that the gut microbiota is an origin of circulating LPS in type 2 diabetes [9, 10]. In our study, gut dysbiosis in diabetic mice is associated with increased intestinal permeability and circulating LPS levels, which are further enhanced after cerebral ischemia [24].

We further showed that modulation of the gut microbiota with oral administration of a nonabsorbable antibiotic improves metabolic endotoxemia and stroke outcomes; these effects are associated with a reduction in LPS levels and neuroinflammation in the ischemic brain [24]. These data indicate that gut microbiota modulation may be a potential therapeutic strategy for stroke treatment and prevention via reduction of circulating LPS levels. In this regard, supplementation with probiotics such as *Bifidobacterium* [47] or prebiotics such as oligofructose [48] may be useful for reducing plasma LPS levels. Additionally, probiotics and prebiotics improve lipid and glucose metabolism in overweight patients and those with diabetes mellitus [49]. Probiotics may also have beneficial effects for stroke prevention by reducing blood pressure [50].

Nutritional habits have been shown to influence the risk of stroke [51]. Furthermore, dietary patterns [52] and the consumption of alcohol [53], tea [54], and nutrients [55] have been found to contribute to gut microbiota composition. Therefore, diet could be one of the factors that cause gut dysbiosis in stroke patients. Accumulating knowledge suggests that the gut microbiota mediates the dietary impact on the host metabolic status [56]. Low fiber intake with high fat and high sugar consumption reduces microbial diversity and SCFA production, leading to metabolic abnormalities and chronic inflammation [57]. Patients with high stroke risk have a low abundance of butyrate-producing bacteria and reduced fecal butyrate concentrations [58]. Conversely, increased dietary fiber intake is associated with better glycemic control in patients with type 2 diabetes [59]. Emerging evidence shows that SCFAs improve type 2 diabetes characteristics such as hyperglycemia, insulin resistance, and inflammation [60]. Additionally, increased dietary fiber intake reduces blood pressure in patients with hypertension [61]. A high-fiber diet alters the gut microbiota composition and increases the abundance of acetic acid-producing bacteria, thus reducing blood pressure in mice [62]. Further, butyrate-producing bacteria lower endotoxemia and reduce systemic inflammation and the extent of atherosclerotic lesions in a murine model [63]. Greater dietary fiber intake is associated with a lower risk of cardiovascular disease [64] and stroke [65]. Such beneficial effects of dietary fiber may be partly related to SCFA production by the gut microbiota. In acute stroke patients, decreased abundance of butyrate-producing bacteria [42] and low fecal acetate levels [43] are associated with poor functional outcomes. Sadler and colleagues [66]

demonstrated that experimental stroke lowers plasma SCFA concentrations, and this is associated with worse outcomes in mice. SCFA supplementation improves recovery of the affected limb motor function. This effect is associated with changes in neuronal plasticity mediated by circulating lymphocytes that affect microglial activation [66]. Additionally, Lee and colleagues [67] reported that stroke outcomes in aged mice are improved by transplantation of a young microbiome that contains high SCFA levels and related bacterial strains. The protective role of a young microbiome is mediated by reduced numbers of interleukin-17⁺ γδ T cells in the brain after stroke [67]. These data indicate the potentially beneficial role of SCFAs for modifying stroke risk factors as well as acute ischemic cerebral injury.

Findings of recent experimental studies have also implicated targeted inhibition of gut microbial metabolites as a potential therapeutic approach to prevent stroke. A structural analog of choline, 3,3-dimethyl-1-butanol, reduces plasma TMAO levels by inhibiting microbial trimethylamine production and attenuates choline diet-enhanced macrophage foam cell formation and atherosclerosis in apolipoprotein E knockout mice [68]. Additionally, an inhibitor of CutC/D, a major microbial trimethylamine-generating enzyme pair, reduces plasma TMAO levels and rescues choline diet-enhanced platelet responsiveness and thrombotic formation without observed toxicity or increased bleeding risk [69].

Conclusion

Accumulating evidence indicates that the gut microbiota plays a possible role in stroke pathogenesis via bacterial molecules and metabolites as well as in the immune and nervous systems. Gut dysbiosis has been demonstrated in patients with acute ischemic stroke. Still, no direct evidence has demonstrated that gut dysbiosis is the cause or result of stroke. Further research is necessary to elucidate how the gut microbiota affects patients with acute ischemic stroke. Modulation of the gut microbiota with probiotics, prebiotics, dietary intervention, or inhibition of production of bacterial metabolites such as TMAO may provide a novel therapeutic strategy for prevention and treatment of stroke.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest with regard to this study.

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Author Contributions

K.Y. wrote the manuscript. N.K., T.U., and N.H. revised the manuscript. All the authors read and approved the final version of the manuscript.

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