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Review Article

Bidirectional Microbiota-Gut-Brain Axis after Stroke and Its Implications for Treating Ischemic Stroke

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Abstract

The surfaces of the human body, including those of internal organs, are colonized by diverse commensal microbes. As we expand our understanding of these microbes, these micro-organisms increasingly seem to play important roles human disease development. The most abundant and functional among these communities is the gut microbes. They not only influence food digestion in native intestinal habitats but also play an essential role in the neurological system. The communication between the gut microbiota and the brain has been partially revealed by new discoveries in the past few years, which has strengthened our knowledge of how the gut microbiota modulates neurological diseases such as ischemic stroke. Ischemic stroke is characterized by poor outcomes, including high disability and fatality rates. Recent investigations among clinical trials and animal experiments have provided some intriguing hints regarding the role of gut microbiota in ischemic stroke outcomes, and a few experimental therapeutic approaches based on gut microbiota have shown promising results. In this review, we discuss the current findings and theories regarding the interaction between gut microbiota and ischemic stroke, and highlight the potential role of gut microbiota in treating ischemic stroke.

Introduction

Neurovascular diseases are gradually becoming more common due to the increasing burden of vascular risk factors and a prominent aging population, and ischemic stroke (IS) is the most common type among them. IS refers to decreased cerebral blood perfusion as a result of cerebral vascular blockage or severe stenosis caused by cerebral blood circulation disturbance The accompanying ischemia and hypoxia provoke limited brain tissue permanent injury and even necrosis in the area of cerebral vascular blood supply. IS is characterized by high morbidity, recurrence, and mortality rates; currently, it is the second leading cause of death worldwide.[1] The mechanism of cerebral injury is complex, and exploring effective cerebral protection treatment strategies has been a hot topic worldwide. It has mainly focused on the recovery of the ischemic penumbra, the viable brain tissue, which requires the initial application of neuroprotective drugs and vascular reperfusion within a short timeframe. Compared with strict timeframe limitation and contraindications for vascular reperfusion, neuroprotective drugs may reduce ischemic injury by blocking each link of the ischemic cascade, and exhibit a broader prospect.[2] Based on the available data, the development of effective neuroprotective drugs in the clinic remains challenging, and the overall therapeutic effect is not ideal, partially because these drugs that target a single rank in the ischemic cascade reactions are not sufficient to alleviate brain in-

Keywords: Ischemic stroke; Gut microbiota; Immune response; Probiotic; Fecal microbiota transplantation.

Abbreviations: BBB, Blood-brain barrier; BDNF, Brain-derived neurotrophic factor; CRH, Corticotropin releasing hormone; FMT, Fecal microbiota transplantation; HPA, Hypothalamus-pituitary-adrenal axis; IFN-γ, Gamma interferon; IgA, Immunoglobulin-A; IS, Ischemic stroke; MAPK, Mitogen-activated protein kinase; TLR-4, Toll-like receptor-4; TNF, Tumor necrosis factor; Treg, Regulatory T cells; ZO-1, Zonula occludens-1.

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jury, and it is necessary to consider novel supplementary therapy in this circumstance. In addition, a recent study has indicated a mechanistic link between brain ischemia, the immune system, and gut microbes, in the adjustment of cerebral responses to ischemic damage.[3] However, precise descriptions are still lacking in laboratory studies that have shown novel pathways of damage. It is not difficult to notice that a large proportion of post-stroke patients experience gastrointestinal complications, including constipation, dysphagia, even gastrointestinal bleeding.[4] Altered defecation habits are often accompanied by gut microbiota dysbiosis,[5] and a few studies have also indicated that gut microbiota dysbiosis can affect prognoses following IS.[6]

One of the most essential features of the pathological changes in the brain after an IS is inflammation in the damaged areas. Several clinical trials have focused on the immune system for regulating inflammatory response to ischemia.[7] What should not be neglected is that the intestine is the "engine" of our immune system, and gut microbes play a vital role in the development and homeostasis of intestinal lamina propria lymph nodes and mesenteric lymph nodes.[8] The immune system evolved not only to fight off pathogens but also to tolerate beneficial microbes that can symbiose with their hosts. It is well established that microbes directly and indirectly regulate the tolerance process that manipulates the inflammatory response that occurs on the surface of the intestinal epithelium, and a few studies have highlighted long-term effects on extraintestinal organs, such as the brain,[9] changes in microbial abundance and function may lead to the development of inflammation and immune-mediated pathology. IS is a stress disorder that often damages the intestinal barrier. A recent study captured the dynamics of the gut microbial community after stroke.[10] In this review, we present our understanding of the bidirectional gut-brain axis in post-stroke and enhance the view of the effective therapeutic target of gut microbiota in ischemic stroke.

Linking microbiota dysbiosis to post-stroke and its potential mechanisms

Gut microbiota dysbiosis due to stroke

Recent studies have found that IS can also affect the gut microbiota composition. IS serves as a stress event, and the gut is one of the main organs of the stress response.[11] Xu *et al*.[10] found that cerebral ischemia quickly results in intestinal ischemia and excessive nitrate production via free radical reactions, which leads to gut dysbiosis with *Enterobacteriaceae* expansion, and that *Enterobacteriaceae* enrichment exacerbates brain infarction by enhancing systemic inflammation and is an independent risk factor for the primary poor outcome of patients with stroke.[10] Karlsson *et al*. compared 12 stroke patients caused by carotid atheromatous plaque sclerosis with age- and sex-matched healthy volunteers, and found that those stroke patients were characterized by an increased abundance of *Collinsella* and a decreased abundance of *Eubacterium* and *Roseburia*.[12] Another case-control study showed that compared with healthy volunteers, patients with IS showed a significant decrease in the relative abundance of *Bacteroides*, *Prevotella*, and *Faecalibacterium*, and the microbiome was related to the severity of stroke.[13] Yamashiro *et al*. showed that an increased relative abundance of *Lactobacillus ruminis* and decreased relative abundance of *Lactobacillus sakei* were detected in IS patients, which can generate the flagella protein and induce the secretion of the proinflammatory cytokine IL-8 when co-cultured with intestinal epithelial cells in vitro.[14] *Lactobacillus sakei* exhibited beneficial functions, such as protecting intestinal mucosa by competitively inhibiting the colonization of pathogenic bacteria.[15] The relationship between stroke severity and gut microbiota dysbiosis has been further explored in animal experiments. Houlden *et al*.[16] tested in mice the hypothesis that brain damage induces changes in the gut microbiota. Experimental stroke altered the composition of cecal microbiota, with specific changes in *Peptococcaceae* and *Prevotellaceae* correlating with the extent of injury, and these effects were found to be mediated by noradrenaline release from the autonomic nervous system with altered cecal mucoprotein production and goblet cell numbers.[16] Singh *et al*.[17] thought that brain injury after stroke could lead to gut microbiota dysbiosis, which in turn promotes inflammation of the nervous system and aggravates brain damage, and that the microbiota diversity decreased and was associated with intestinal epithelial barrier dysfunction as well as weakened intestinal motility. Whether the gut microbiota could affect the stroke outcome was unknown; hence, the authors compared the stroke mice models fed in a conventional environment and germ-free environment. The latter exhibited minor brain damage. Interestingly, germ-free stroke mice accepted fecal microbiota transplantation from the stroke mice fed in a conventional environment exhibited severe brain damage and function defects later, while increased proinflammatory T cell differentiation and lymphocyte metastasis from the gut to the brain were observed in germ-free stroke mice.[17] Benakis *et al*. found that treatment with penicillin before inducing stroke alleviated brain damage.[18] In contrast, the use of broad-spectrum antibiotics reduced the survival rate in stroke mice.[19] Ling *et al*.[20] identified a significant decrease in the richness of *Firmicutes* and their members, including *Clostridium*, *Clostridium*, *Lachnospiraceae*, and *Lachnospiraceae_other*, in age-matched patients with post-stroke cognitive impairment compared with those with non-stroke cognitive impairment. Furthermore, the gut microbiota was strongly linked to Montreal Cognitive Assessment scores and risk factors for IS, including higher baseline National Institutes of Health Stroke Scale scores, higher homocysteine (hcy) levels, higher rates of stroke recurrence, leukodystrophy, and brain atrophy.[20] Xia *et al*. identified bacterial genera that differed significantly between patients and controls, with *Paramecium*, *Oscillospira*, and enriched *Enterobacteriaceae* in patients and *Prevotella*, *Roseobacter*, and enriched *Enterobacter faecium* in healthy controls (Table 1).[21]

Autonomic nervous

Most studies have focused on researching how the gut microbiota affects the outcome of IS, while a few studies have found trends in the gut microbiota after the initial phase of IS. It is well accepted that brain ischemia rapidly induces a reduction of blood flow in intestinal mucosa, and the reperfusion injury could lead to impaired intestinal epithelial barrier,[22] which means the habitat micro-environment of gut microbes has been damaged, thus, the gut microbiota dysbiosis affects the IS outcome. A few studies have investigated possible theories. Houlden *et al*. observed that brain ischemia not only changed the gut microbiota composition, but also affected the intestinal autonomic nervous activity and secretion of intestinal mucin. In order to confirm the abnormalities of intestinal autonomic nerves after brain injury, the concentration of epinephrine, norepinephrine, 5-hydroxytryptamine, neurotransmitter medium P, tyrosine hydroxylase and other key neurotransmitters in the colon tissue were measured,[16] and it was found that epinephrine andnorepinephrine significantly increased on the third day after stroke while increased tyrosine hydroxylase in the colon was positively correlated to neurological damage. Moreover, nor-

Table 1. Summary of studies on changes of gut microbiota in ischemic stroke

Species	Models	Outcome measures
Human	Patients with acute ischemic stroke	Increased relative abundance of *Collinsella*, and decreased abundance of *Eubacterium* and *Roseburia*[12]
Human	Patients with large-artery atherosclerotic stroke	Decreased relative abundance of *Bacteroides*, *Prevotella* and *Faecalibacterium*[13]
Human	Patients with ischemic stroke	Increased relative abundance of *Lactobacillus ruminis* and decreased relative abundance of *Lactobacillus sakei*[14]
Human	Patients with ischemic stroke	Decreased in the richness of Firmicutes and their members, including *Clostridium*, *Lachnospiraceae*, and *Lachnospiraceae*_other, in age-matched patients with post-stroke cognitive impairment compared with those with non-stroke cognitive impairment[20]
Human	Patients with ischemic stroke	Bacterial genera that differed significantly between patients and controls, with *Paramecium*, *Oscillospira*, and *Enterobacteriaceae* enriched in patients, while *Prevotella*, *Roseobacter*, and *Enterobacter faecium* were enriched in healthy controls[21]
Mice	Middle cerebral artery occlusion	Increased relative abundance of *Peptococcaceae* and decreased relative abundance of *Prevotellaceae*[16]
Mice	Permanent distal middle cerebral artery occlusion	Reduction in microbiota species diversity and intestinal bacterial overgrowth with a preferential expansion of the Bacteroidetes phylum[17]
Mice	Middle cerebral artery occlusion	Cerebral ischemia rapidly induced intestinal ischemia and produced excess nitrates through free radical reactions, leading to gut dysbiosis with *Enterobacteriaceae* expansion[10]

epinephrine was positively correlated with a relative abundance of bacterial *peptococcaceae*, and negatively correlated with *prevotellaceae*.[16] It is well known that autonomic nerves regulate the function of intestinal epithelial goblet cells, and the mucin secreted by goblet cells is involved in maintaining the normal function of the intestinal epithelial biological barrier.[23] Mucus also comprises antimicrobial peptides and immunoglobulin-A (IgA), giving mucus a pivotal role in innate defense. IgA is the most abundant isotype in humans and is secreted in the lumen where it is essential to prevent infections and to assure homeostasis with gut microbiota. IgA plays a critical role in controlling the composition of gut microbiota via binding to the gut microbiota.[24] Researchers have found that the number of goblet cells and secretion of mucin decreased after brain injury.[25] These results suggest that cerebral ischemia drives gut-derived autonomic norepinephrine transmission, which then reduces the number and function of intestinal epithelial cells and mucus secretion, leading to gut microbiota dysbiosis.

Hypothalamus-pituitary-adrenal axis

Other studies have indicated the role of activation of the hypothalamus-pituitary-adrenal axis (HPA) after IS,[26] and a large population control study showed that increased HPA axis-related hormones and adrenal cortisol was detected in stroke patients, both of which were positively correlated with the size of brain obstructed areas, indicating a relationship between stroke outcome and the HPA axis.[26] Another HPA axis hormone, corticotropin releasing hormone (CRH), was found related to the intestinal physical barrier. Intraperitoneal injection of CRH increased the colon epithelial permeability of ions and macromolecules.[27] Cell experimentation further confirmed the CRH up-regulated the expression of tight junction protein Claudin-2 and down-regulated the expression of tight junction protein Occludin and Zo-1 in colon cells,[28] which is crucial for the integrity of intestinal epithelial barrier. Clinical and experimental studies have suggested the importance of intestinal hyperpermeability in the progress of IS. Consistent restoration of intestinal tight junctions to regulate trans-epithelial permeability is important for maintaining intestinal barrier functions and preventing dissemination of bacteria to host blood circulation; thus, a dysfunctional gut barrier has been correlated to post-stroke infection. Infection is one of the most common complications during the acute phase of stroke, and approximately 30% of IS patients are complicated with infection.[29] Furthermore, systemic immunosuppression induced by stroke is related to infectious complications.[30] On the other hand, the translocation of gut bacteria to the extra-intestinal organs causes post-stroke infection. Past studies have considered administering antibiotics to be an effective strategy for preventing post-stroke infection, but more current studies do not support the effectiveness of antibiotic treatment. This is perhaps due to the disrupted effect on the gut microbiota. The source of pulmonary infection after stroke has been verified in the intestine.[29] The germ-free stroke model mice and pathogen-free stroke model mice were exposed to the conventional environment for 24 hours. Surprisingly, the bacteria were detected in the lungs, liver, and spleen of the pathogen-free mice, but no positive results were found in these organs of the germ-free mice.[29] Further evidence came from clinical trials, and it was difficult to detect bacteria in stroke patients with infectious complications. However, the bacteria were successfully detected in the blood, urine, and sputum of eight patients, more than 70% of those bacteria belonging to the gut microbiota.[29]

Systemic immunosuppression

IS induces a robust inflammatory cascade in the brain and suppresses the peripheral immune system, which is known as stroke-

induced immunosuppression.[31] Systemic immunosuppression protects the brain from further inflammatory damage.[32] The concentration of leukocytes, granulocytes, and lymphocytes in the blood of stroke patients differed from those of healthy people within two weeks. Among them, the number of T lymphocytes decreased significantly within 12 hours after stroke, and the number of CD4+T lymphocytes increased slowly in some stroke patients with infectious complications,[33] especially the significantly increased levels of IL-10, which is produced by monocytes, dendritic cells, and regulatory T cells (Treg), which are mainly involved in the anti-inflammatory immune response.[34] One of the most abundant immune cell populations in the intestine comprise T cells. This is in the intestine, where 70% of immune cells of the whole body are located.[35] Intestinal T cells play an important role in maintaining the barrier function and intestinal homeostasis via interactions with microbes. The balance between T cell-mediated defense and tolerance is fundamental to maintain intestinal homeostasis on general circumstances. Intestinal T cells are constantly contacting with microbes, and stroke-induced immunosuppression might lead to abnormal T-cell-mediated immune responses and inflammatory triggers in the intestine, thus initiating uncontrolled inflammation, and a break in tolerance in turn worsen IS outcome. However, whether gut microbiota dysbiosis was a consequence of immunosuppression is still not clear. It is highly possible that the relationship between dysbiosis and the immune system is bidirectional or an amplifying one. Further studies are required to explain this relationship.

Mechanisms underlying the influence of gut microbiota on IS outcome

Gut microbes and our immune system, likewise, have evolved with us for millions of years and their interactions are important in maintaining normal innate immune responses. Laboratory research and some clinical studies on stroke mice have already shown changes in the gut microbiota composition. However, the impact of such altered microbiota changes in the initial inflammatory state of stroke is yet to be fully understood. Symbiotic microbiota plays a crucial role in maintaining balanced immunity, while imbalanced microbes can also result in imbalanced T-cell subsets, including Th1, Th2, Th17, and Treg.[36] Th1 cells are CD4-positive cells that mainly secrete interleukin 2 (IL-2), gamma interferon (IFN-γ), and tumor necrosis factor (TNF), and participate in the regulation of cellular immunity, assisting in the differentiation of cytotoxic T cells and mediating cellular immune responses.[37] Th2 cells are a subpopulation of T cells capable of secreting Th2-type cytokines (such as interleukins IL-4, IL-5, IL-10, and IL-13). The main effect of Th2 cells is paracellular activation, and the cytokines they secrete promote B cell proliferation, differentiation, and antibody production.[38] Th17 cells are a newly identified subpopulation of T cells capable of secreting interleukin 17 (IL-17), which is important in autoimmune diseases and defense response.[39] Regulatory T cells (Tregs) are essential for maintaining immune tolerance. They suppress the activation and proliferation of potential autoreactive T cells present in normal organisms, thus regulating the immune response.[40] T cells are located mainly at the borders of the infarct and appear several days after the onset of ischemia. The infiltration levels of T lymphocytes and other immune cells vary significantly among stroke models.[7] We have previously explored the possible mechanisms of gut microbiota dysbiosis, in which the intestinal immune system overreacts when faced with stroke-induced microbiota dysbiosis, which can, in turn, influence stroke progression. Benakis *et al*.[18] showed that treatment of mice with amoxicillin and clavulanic acid before inducing laboratory stroke reduced approximately 60% of the damaged area in brain tissue. Similar results were observed in conventional mice that accepted fecal microbiota transplantation from mice treated with antibiotics, and increased regulatory T cells and decreased IL-17+ T cells in blood were detected.[18] Another study showed that germ-free mice accepted fecal microbiota transplantation from stroke model mice and conventional mice, and experimental stroke surgery was performed in these germ-free mice. The former showed a larger brain damage area, more T cell differentiation in the intestine, and increased migration of effector T cells from the gut to the brain.[17] Houlden *et al*. studied the outcome of focal cerebral ischemia in mice after 8 weeks of decontamination with a broad-spectrum antibiotic cocktail in five combinations, and microbiota-deficient mice with middle cerebral artery occlusion were found to have significantly reduced survival rates.[16] These results suggest that the gut microbiota can modulate the immune response and thus influence brain lesions induced by stroke (Fig. 1).

Gut microbiota-based treatment options

Probiotics

A number of relevant studies have been published on the effects of probiotic supplementation over the past few decades, with the number of probiotic-related clinical studies increasing each year. There are a few positive experimental results targeting post-stroke outcomes, while most of the claimed effects still lack sufficient evidence. Furthermore, the majority of probiotic applications in humans have been preventive and supportive, rather than curative, in terms of disease management. Akhoundzadeh *et al*. treated laboratory stroke mice with probiotics containing *Bifidobacterium breve*, Lactobacillus casei, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus*, all of which are known to be safe and belong to the anaerobic bacteria of the human gastrointestinal tract. This decreased the infarct size significantly (by 52%) but did not ameliorate neurological function. In addition, pro-inflammatory tumor necrosis factor-alpha levels in ischemic brain tissue were remarkably reduced after probiotic administration.[41] Wanchao *et al*.[42] investigated the protective effects of inactivated *Lactobacillus* on cerebral ischemia-reperfusion injury in rats. Inactivated Lactobacillus decreased cerebral infarction volume and neural cell apoptosis in mice, and inhibited the pro-inflammatory toll-like receptor-4 (TLR-4) and apoptosis of neural cells, along with reducing oxidative stress through inhibition of TLR-4/NF-kappa B signaling.[42] Sun *et al*. identified that *Clostridium butyricum* pretreatment significantly improved neurological deficits, relieved histopathological changes, decreased malondialdehyde content, and increased superoxide dismutase activity in mice with cerebral ischemia-reperfusion injury. *Clostridium butyricum* was shown to exhibit neuroprotective effects in mice with cerebral ischemia-reperfusion injury through antioxidant and anti-apoptotic mechanisms, and reversal of the decrease in brain butyrate content may be part of its neuroprotective effects.[43] Li *et al*. found that pre-administration of *Bacillus licheniformis* resulted in significantly diminished hyperthermia, reduced mortality due to heat stroke, attenuated multi-damages, and reduced serum inflammatory cytokine levels.[44]

These studies have focused on improving the prognosis of stroke, while the influence on gut microbiota and intestinal epithelial barrier was less mentioned, and clinical trial-related results are

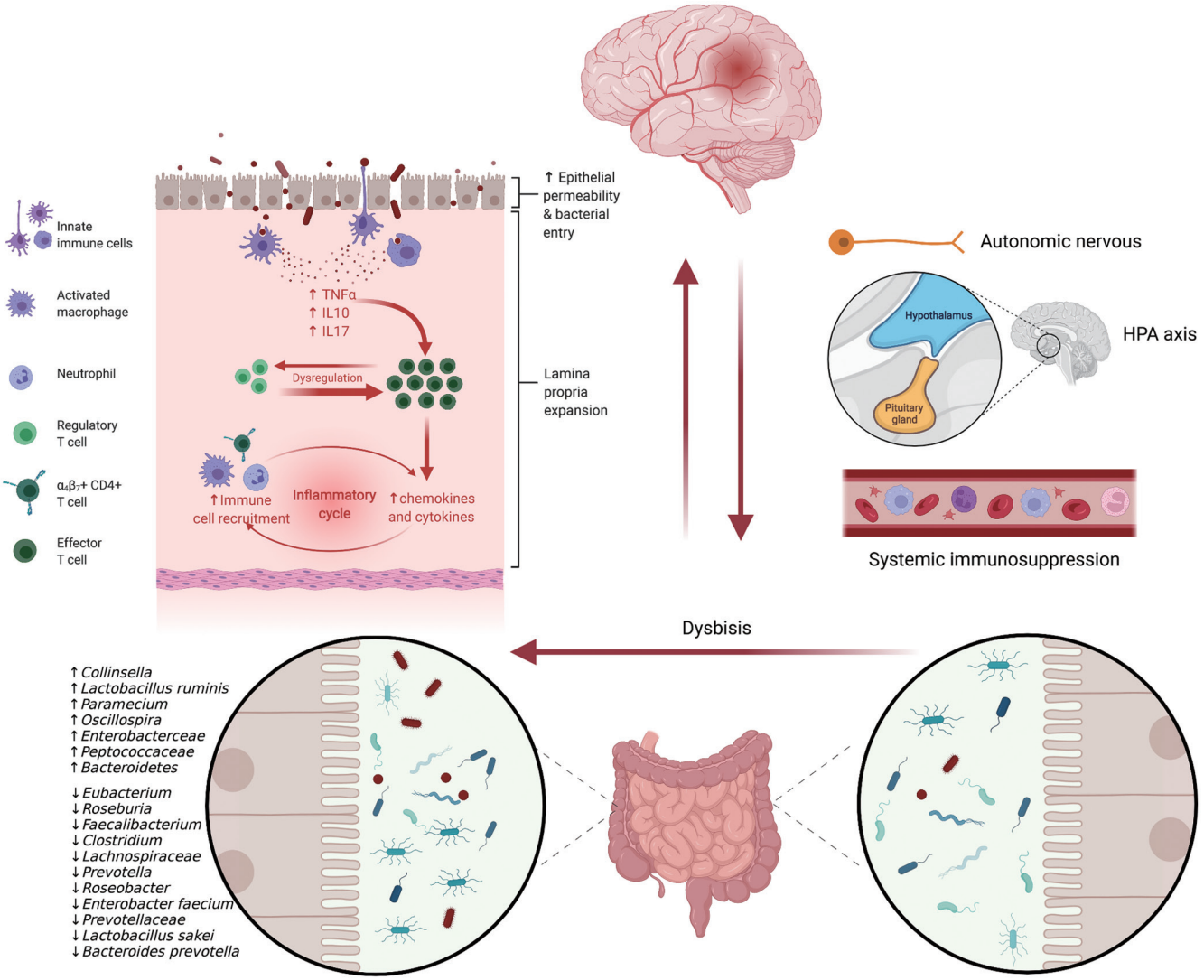

Fig. 1. The interaction between gut microbiota and ischemic stroke. Stroke causes gut microbiota dysbiosis through autonomic nervous, HPA axis, and inducing systemic immunosuppression, which in turn affects stroke outcome by activating the immune system to produce a variety of pro-inflammatory cytokines. HPA, hypothalamus-pituitary-adrenal axis.

still not available. The use of probiotic supplements to restore the balance of the gut microbiota may become an innovative strategy for the management of IS in the future. Furthermore, prebiotics can be considered as a substitute for probiotics or as supplementary support for probiotics. In particular, they can be considered as other beneficial properties of prebiotics in terms of their resistance to acids, proteases, and bile salts in the digestive tract.

Fecal microbiota transplantation

Besides supplementation with probiotics or prebiotics, fecal microbiota transplantation (FMT) exhibits the advantages of safety and stability in modulating gut microbiota composition. FMT refers to the infusion of complete microbiota extracted from the feces of a healthy person into the recipient's intestine through a nasal feeding tube, and is now the most efficient gut microbiota intervention measure. It is widely accepted that FMT has an effect on recurrent *Clostridium difficile* infection.[45] However, to our knowledge, studies on IS with FMT are limited to animal studies. Singh *et al*. found that FMT treatment beginning on the day of stroke induction and administered once daily during the survival period markedly decreased post-stroke lesions in stroke model mice, which was related to an increase in the number of Foxp3+ Treg cells in peripheral immune organs and ischemic brain after stroke.[17] Spychala *et al*. altered the microbiota in older mice after experimental stroke to resemble that of young mice which increased survival rates, and improved recovery rates, as well as increased protective cytokines such as IL-4 and G-CSF in brain tissues.[46] Benakis *et al*. found that mice receiving FMT had a 54 ± 8% reduction in infarct volume (72 h after MCAO).[18] Xia *et al*. found that laboratory ischemic stroke mice receiving fecal transplants from patients with a high stroke dysbiosis index developed severe brain damage and elevated intestinal IL-17+ γδ T cells compared to mice receiving transplants from patients with a low stroke dysbiosis index.[21] This may be due to changes in the gut

Table 2. The effects of gut microbiota-based therapy on post-stroke

Interventions	Models	Dose	Duration	Outcomes
Probiotics	Middle cerebral artery occlusion mice model	107 CFU/mL	2 weeks	Probiotics containing *Bifid bacterium breve*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus* markedly diminished the infarct size by 52% but failed to improve the neurological functions. In this regard, malondialdehyde levels and tumor necrosis factor-α levels in ischemic brain tissue were significantly reduced after probiotic administration[41]
Probiotics	Middle cerebral artery occlusion rat model	108 CFU/mL	One time	Inactivated lactobacillus decreased cerebral infarction volume and neural cell apoptosis of rats. Lactobacillus also inhibited the pro-inflammatory toll-like receptor-4 [TLR-4] and the apoptosis of neural cells, together with reducing oxidative stress through inhibition of TLR-4/NF-kappa B signaling[42]
Probiotics	Middle cerebral artery occlusion mice model	None mentioned	2 weeks	*Clostridium butyricum* pretreatment was able to significantly improve neurological deficits, relieve histopathological changes, and decrease malondialdehyde content and increase superoxide dismutase activity in mice with cerebral ischemia-reperfusion injury[43]
Probiotics	Heat stroke rats model	108 CFU/mL	1 week	Pre-administration of Bacillus licheniformis resulted in significant diminished hyperthermia, reduced mortality due to HS, attenuated multi-organ damage, and reduced serum inflammatory cytokine levels[44]
FMT	Permanent distal middle cerebral artery occlusion mice model	None mentioned	None mentioned	Improved stroke outcome was related to an increase in the number of Foxp3+ Treg cells in peripheral immune organs and ischemic brain after stroke[17]
FMT	Right middle cerebral artery occlusion mice model	50μl	5 days	FMT altered the microbiota of aged mice to resemble that of young mice which increased survival and improved the recovery after experimental stroke, as well as increased protective cytokines such as IL-4, G-CSF in brain tissues[46]
FMT	Middle cerebral artery occlusion mice model	200μl	2 weeks	The mice receiving FMT had a 54±8% reduction in infarct volume (72 hours after MCAO)[18]
FMT	Middle cerebral artery occlusion mice model	200μl	2 weeks	The laboratory ischemia stroke mice receiving fecal transplants from high stroke dysbiosis index patients developed severe brain injury with elevated IL-17+ γδ T cells in gut compared to mice receiving transplants from low stroke dysbiosis index patients[21]

microbiota following FMT, which rebalances the intestinal environment, thereby limiting the inflammatory response and controlling disease progression. Further animal studies and clinical trials are needed to determine whether FMT can be used to treat IS and whether it is safe. Further animal experiments and clinical trials are needed to determine whether FMT can be used for the treatment of IS and its safety (Table 2, Fig. 2).[41-44]

Short-chain fatty acids

The gut microbiota produces a large number of bioactive metabolites that may influence brain function by modulating transmission through the immune system or neural pathways. In particular, the metabolic substrates of short-chain fatty acids (SCFAs) acetate, butyrate, and propionate have been shown to easily cross the blood-brain barrier and affect brain function. Recently, the role of SCFAs in post-stroke recovery in the chronic phase after cerebral ischemia, and their potential therapeutic activity, have been investigated. Sadler *et al*.[47] found that supplementation of short-chain fatty acids in drinking water in mice significantly improved recovery of motor function in the affected limb. Using in vivo wide-field calcium imaging, they observed that SCFAs induced alterations in contralateral cortical connections. This was associated with SCFAs-dependent changes in spine and synaptic density. Forebrain cortical RNA sequencing suggested that microglia may be involved in structural and functional remodeling. Further analysis confirmed that SCFAs have a strong effect on microglia activation, depending on T cell recruitment in the infarcted brain. The findings suggest that SCFAs affect the maturation of peripheral lymphocytes or the drainage from their primary lymphoid tissue, and that lymphocytes then indirectly mediate the effects of SCFAs on the brain microenvironment through the overall reduction in the response of SCFAs to brain invasion or the polarity of secreted cytokine signaling.[47] Another study showed that aged stroke mice receiving young fecal transplants exhibited less behavioral deficits

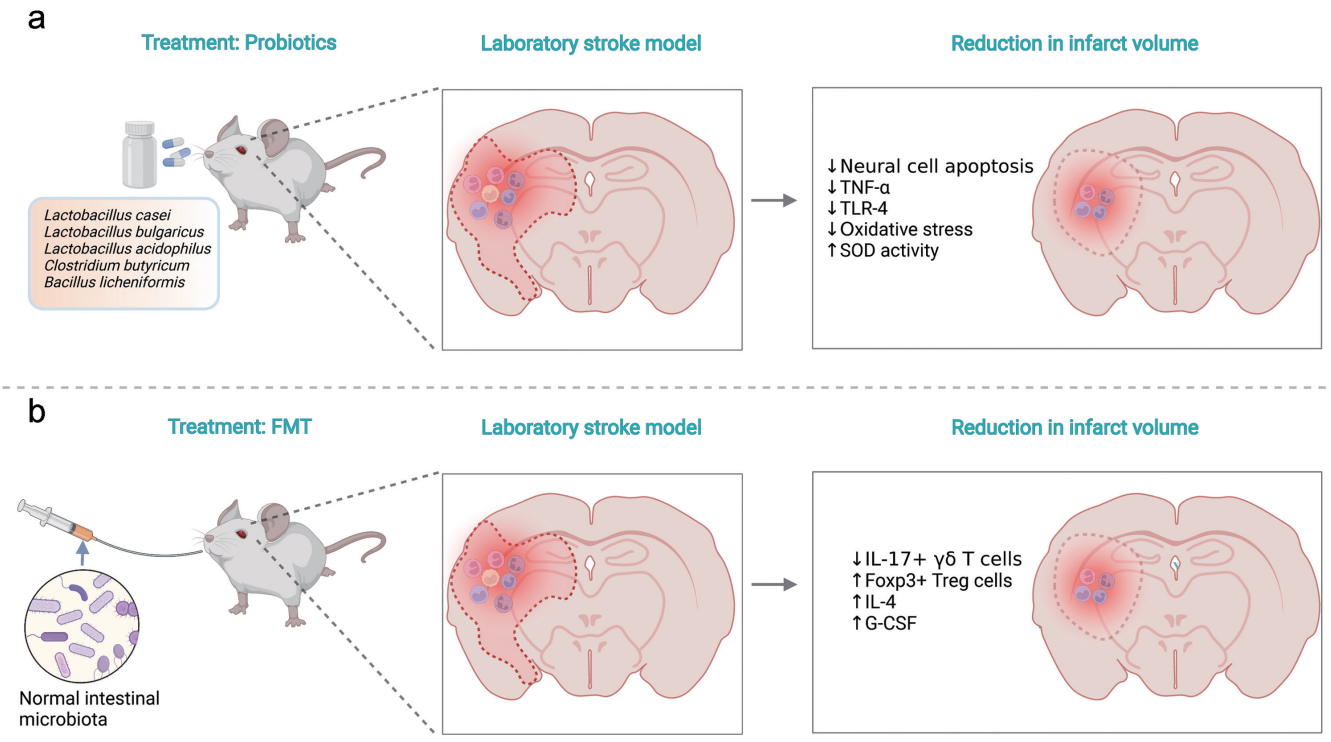

Fig. 2. Gut microbiota-based treatment options on ischemic stroke. (a) and (b) exhibits the beneficial effects of probiotics and FMT on post-stroke outcome respectively. FMT: Fecal microbiota transplantation, SOD, superoxide dismutase, G-CSF, granulocyte colony-stimulating factor.

and brain or intestinal inflammation. Data based on microbial sequencing and metabolomic analysis showed that young fecal grafts contained higher levels of short-chain fatty acids and associated strains, and four short-chain fatty acid producers (Bifidobacterium longum, Clostridium symbioticum, Aspergillus commonus and Lactobacillus fermentum) were selected for transplantation. These short-chain fatty acid producers attenuate neurological deficits and inflammatory responses after stroke.[48] A clinical study including 53 patients with acute ischemic stroke showed that plasma SCFA concentrations were not associated with the severity of stroke at presentation. However, elevated levels of SCFAs were associated with increased inflammatory markers, shorter recovery time from admission to discharge, and increased symptom burden at discharge.[49] The effect of oral SCFAs on the outcome of neurological injury is currently under investigation and has not been fully and adequately tested in human individuals with neurological injury. However, SCFAs appear to have a relatively safe profile and can be readily ingested orally.[50] Therefore, although more studies are needed to assess their effectiveness in humans, oral SCFAs may eventually become a safe and readily available adjunct to nerve injury treatment.

Future directions

As mentioned above, gut microbiota is closely linked to the activity of the immune system and the subsequent modulation of neuroinflammation and the consequences of ischemic stroke. Since studies on the role of the gut-brain axis in stroke are still in their beginning stages, to introduce broader insights in the field of stroke, the current rodent models and the areas being explored will yield overall questions for the concerns of researchers in the future.

Conclusions

Studies have described differences in the composition of the gut microbiota between patients with IS and healthy individuals, and most studies have been conducted on animal models with a small number of studies in patients with IS. Understanding the ways in which IS causes dysbiosis of the gut microbiota may allow for the future development of microbiota-based approaches for the personalized diagnosis and treatment of IS. Exploring the relationship between the gut microbiota and stroke has become a novel and important area of science within microbiology and medical research. However, at present, we have not yet conducted any clinical trials to explore how the gut microbiota is involved in stroke prognosis. A few animal experiments have highlighted that microbiota-related immune activation affects the inflammatory response post-stroke, while the repair process of brain injury is a process that requires the involvement of more mediators. Other possible mechanisms by which gut microbiota affects IS outcome need to be explored. A few interventions have been described to restore a more balanced microbiota composition, such as prebiotics, probiotics, and FMT in experimental stroke mice, and these treatments need to be further examined in a systematic approach to assess their potential in improving IS outcomes. A large-scale clinical trial is required to establish a more definitive connection between IS and microbiota dysbiosis.

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Conflict of interest

Dr. Zhi-hua Liu has been an editorial board member of *Exploratory Research and Hypothesis in Medicine* since December 2018. The authors have no other conflicts of interest to note.

Author contributions

Conceptualization (LZ and LC), Funding acquisition (LZ), Supervision, (LC, LC and LL), Original draft preparation (LC and CE), Review & Editing (TL and LL). All authors have made a significant contribution to this study and have approved the final manuscript.

Data sharing statement

The datasets during the current study are available from the corresponding author on request.

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