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Review

Therapeutic approaches targeting the gut microbiota in ischemic stroke: current advances and future directions

Zhiguo MAO[1, 2a], Jinying ZHANG[1, 2a], Lin GUO[1, 2], Xiaoran WANG[1–3], Zhengwang ZHU[3] and Mingsan MIAO[1, 2*]

[1]*Department of Pharmacology, Henan University of Chinese Medicine, No. 156 Jinshui East Road, Zhengdong New District, Zhengzhou, Henan 450046, China*

[2]*Collaborative Innovation Center of Research and Development on the Whole Industry Chain of Yu-Yao, No. 156 Jinshui East Road, Zhengdong New District, Zhengzhou, Henan Province, China*

[3]*The First Clinical Medical College, Henan University of Chinese Medicine, No. 156 Jinshui East Road, Zhengdong New District, Zhengzhou, Henan 450046, China*

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Ischemic stroke (IS) is the predominant form of stroke pathology, and its clinical management remains constrained by therapeutic time frame. The gut microbiota (GM), comprising a multitude of bacterial and archaeal cells, surpasses the human cell count by approximately tenfold and significantly contributes to the human organism's growth, development, and overall well-being. The microbiota-gut-brain axis (MGBA) in recent years has established a strong association between gut microbes and the brain, demonstrating their intricate involvement in the progression of IS. The regulation of IS by the GM, encompassing changes in composition, abundance, and distribution, is multifaceted, involving neurological, endocrine, immunological, and metabolic mechanisms. This comprehensive understanding offers novel insights into the therapeutic approaches for IS. The objective of this paper is to examine the mechanisms of interaction between the GM and IS in recent years, assess the therapeutic effects of the GM on IS through various interventions, such as dietary modifications, probiotics, fecal microbiota transplantation, and antibiotics, and offer insights into the potential clinical application of the GM in stroke treatment.

Key words: ischemic stroke, gut microbiota, microbiota-gut-brain axis (MGBA), neural pathways, endocrine pathways, immune pathways

INTRODUCTION

Ischemic stroke (IS), a cerebrovascular disorder resulting from inadequate blood flow to the brain caused by occlusion of cerebral blood vessels, notably blood clots, represents the predominant subtype of stroke [1]. With an annual incidence of approximately 30 million cases, IS constitutes a significant contributor to mortality and morbidity [2]. Presently, the existing therapeutic interventions for IS, such as thrombolysis, neuroprotection, anti-inflammation, and antioxidant therapies, fail to adequately address the clinical requirements [3, 4]. Moreover, the limited timeframe for early treatment and the subsequent harm caused by reperfusion can lead to enduring neuronal impairment or even fatality. The quality of life for IS patients is significantly compromised, and society bears a substantial burden. Therefore, the exploration of novel therapeutic strategies assumes paramount significance.

The gut microbiota (GM) is comprised predominantly of a diverse array of eukaryotic, bacterial, and archaeal organisms that colonize the gastrointestinal tract of animals. In recent times, the GM has garnered significant interest owing to its remarkably high microbial density. The advent of high-throughput sequencing technology has facilitated examination of the composition, distribution, and quantitative alterations of the GM, revealing its intricate associations with various organs, such as the gut-brain axis, gut-liver axis, and gut-cardiac axis [5]. Consequently, these interconnections have been linked to the development of numerous diseases, including neurodegenerative, metabolic, and autoimmune disorders [6]. The formation of human GM diversity remains relatively stable from the age of three, but can be influenced by various factors, such as disease, spirituality, age, dietary structure, and smoking. The microbiota-gut-brain axis (MGBA) serves as a pathway connecting the brain to the GM through neurological, endocrine, immune, and metabolic

[a]These authors contributed equally to this work and share first authorship.

*Corresponding author. Mingsan Miao (E-mail: miaomingsan@163.com)

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pathways [7]. In the presence of disease states, the development of IS can lead to changes in the GM, while disorders of the GM can worsen the progression of IS [8]. This paper examines the correlation between the GM and IS, with a particular focus on the potential of enhancing the GM to develop a cure for IS.

ISCHEMIC STROKE

IS is a condition characterized by high fatality and disability rates, predominantly affecting the elderly population. However, there has been a notable rise in the incidence of IS cases and related deaths. Recent trends indicate a shift towards younger age groups, particularly individuals aged 18–49, which has been attributed to risk factors like excessive sodium intake and smoking [9]. This demographic transition not only imposes a burden of disability on younger individuals but also contributes to a decline in national productivity. IS has been shown to result in a reduction in beneficial microorganism levels and an increase in opportunistic pathogenic bacteria [10]. This shift in microbial composition can also impact the ratio of the phylum Firmicutes to the phylum Bacteroides, potentially leading to gastrointestinal disorders [11]. The emerging recognition of the neuroprotective role of gut microbes and their metabolites in IS highlights the potential for modulating the GM as a novel therapeutic approach for treating IS.

GASTROINTESTINAL TRACT AND THE GUT MICROBIOTA

The gastrointestinal tract harbors a significant population of immune cells, constituting approximately 70% of the immune system and representing the most extensive aggregation of immune cells within the body [12]. The gut accommodates a vast number of microorganisms, approximately tenfold the total cell count of the body, which actively contribute to the regulation of the body's homeostasis. Perturbation in the ecological balance of the GM has been associated with the progression of various diseases. The GM is composed of various microorganisms, including bacteria, archaea, protozoa, viruses, and fungi. The phyla Firmicutes (*Ruminococcus*, *Clostridium*) and Bacteroides (*Prevotella* and *Porphyromonas*) are the predominant members, accounting for approximately 90% of the GM [13]. Additionally, the GM contains Actinobacteria (*Bifidobacterium*), *Spirillaceae*, *Fusobacteria*, Cyanophyta, *Verrucomicrobium*, and *Proteus*, among others. The GM is widely regarded as the "second brain" of human beings. It plays a crucial role in various physiological processes, including digestion, absorption of nutrients, and the production of metabolic substances. It also exerts influences on energy metabolism, physical development, and overall body functions. Additionally, the GM regulates tight junction proteins, thereby ensuring the integrity of the gastrointestinal barrier, defending against pathogens, and enhancing cellular immunity [14]. The main regulator of T cells is the GM, as evidenced by various studies [15]. In the presence of pathological conditions, GM disorders lead to a decline in immune function, resulting in local or systemic inflammation, translocation of microorganisms through the circulatory system, and the development of neurological, pulmonary, cardiovascular, and other diseases [16]. The GM serves as a producer of crucial bioactive metabolites, including but not limited to short-chain fatty acids (SCFAs, such as isobutyric, isovaleric, butyric, acetic, propionic, and formic acids) [17], neurotransmitters (such as acetylcholine, 5-hydroxytryptophan, melatonin, norepinephrine, gamma-aminobutyric acid, and dopamine), oxidized trimethylamine, and lipopolysaccharides (LPS). These metabolites not only participate in the synthesis of vitamin B and vitamin K, as well as the absorption and metabolism of essential substances like bile acids and sterols, but also actively contribute to maintenance of the body's immune function and energy metabolism [18]. The neurotransmitters generated by SCFAs activate the immune system and bind to G-protein coupled receptors on endocrine cells, thereby stimulating the secretion of gut hormones, such as cholecystokinin and glucagon-like peptide-1 (GLP-1). LPS have the ability to traverse damaged intestinal barrier cells and enter the bloodstream, resulting in a systemic immune response [19].

INTERACTIONS BETWEEN ISCHEMIC STROKE AND THE GUT MICROBIOTA

In recent years, the treatment of patients with IS has been notably impacted by alterations in the GM. Specifically, cerebral ischemic injury is associated with a significant decrease in GM diversity, primarily characterized by changes in the abundance of the phyla Firmicutes and Bacteroidetes [20]. Notably, immune cells in the gastrointestinal tract can be activated through various stimuli, such as injury-associated molecular patterns, exosomes, and antigens. This activation leads to the release of substantial quantities of cytokines, which, in conjunction with chemokines, traverse the blood-brain barrier, thereby intensifying the neuroinflammatory response in IS disease [21]. Additionally, this injury leads to compromised intestinal function, characterized by increased permeability of the intestinal wall and weakened intestinal motility. In contrast, a stable GM has the potential to ischemically safeguard injured brain tissue by modulating intestinal immune cells, specifically Treg cells. Consequently, it can be inferred that the brain, gut, and microbiota engage in intricate interactions following cerebral ischemia, which contribute to the progression of the disease. Therefore, comprehending the interplay among these three components assumes a crucial role in the effective management of IS.

The MGBA serves as a communication system facilitating the interaction between the gastrointestinal tract and the nervous system [22]. This interaction is primarily dependent on neural, endocrine, immune, and metabolic pathways. Through the MGBA, the brain's nervous system can regulate the gastrointestinal tract's functions, such as intestinal motility and secretion, to maintain homeostasis. Simultaneously, the gastrointestinal tract can also influence the neurological processes of the brain's nervous system by regulating neuroimmune and endocrine pathways via the MGBA. Therefore, the MGBA plays a crucial role in maintaining overall bodily homeostasis [23].

Neural pathways

The MGBA is bi-directionally regulated through the central nervous system (CNS), autonomic nervous system (ANS), spinal vagus nerve, and enteric nervous system (ENS). The vagus nerve is responsible for innervating around 70% of the parasympathetic nerves. This nerve pathway serves as the primary means by which gut microbes influence the brain. Research findings indicate that germ-free mice, which lack certain microorganisms, exhibited

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significantly heightened functional brain networks and stronger connections compared with mice with standard microbiota. These enhanced networks were approximately twice as robust as those observed in mice with normal microbiota. However, the functional networks of germ-free mice displayed a greater density, less organization, and reduced efficiency in compensating for cerebral ischemic stress. The potential factors contributing to changes in dendritic spine density and immature microglia resulting from GM deletion have been identified [24]. The GM plays a significant regulatory role in modulating the functional connectivity and neuroinflammation in the hippocampal neurons, which are responsible for memory, learning, and cognition, the cingulate cortex, which is closely associated with working memory and executive attention [25], and the thalamus, a crucial brain region for regulating consciousness, alertness, and sleep, in mice with IS [26]. The study found a positive correlation between decreased strength of hippocampal neuronal functional connectivity and the presence of *Corynebacterium diphtheriae*, *Pasteurella*, and *Proteus*. Conversely, a negative correlation was observed between decreased strength of hippocampal neuronal functional connectivity and the relative abundance of *Bartonella* and *Enterococcus faecalis* [27].

Endocrine pathways

IS deteriorates intestinal barrier permeability and the GM composition through the levels of hormones, such as norepinephrine, epinephrine, and dopamine, released through endocrine pathways such as the HPA axis [28]. Conversely, it has also been noted that the release of endocrine hormones, such as norepinephrine, can be regulated by the GM, with *Escherichia coli* being able to stimulate the release of norepinephrine, leading to neuroprotective effects on the brain [29]. During cerebral ischemia, GLP-1 is secreted by intestinal secretory cells. This peptide activates the GLP-1 receptors located in the ischemic regions of the brain, thereby reducing the inflammation mediated by interleukin-6 (IL-6) and ultimately improving the condition of cerebral ischemic injury. Cerebral ischemia triggers a substantial release of IL-6 into the bloodstream, leading to stimulation of the hypothalamus, pituitary, and adrenal glands, resulting in secretion of the hormones [30]. Furthermore, estrogen plays a crucial role in regulating the initiation of immune responses through its promotion of the reestablishment of GM equilibrium, characterized by an increase in beneficial bacteria and a decrease in harmful bacteria. Estrogen also reduces the release of LPS, a metabolite associated with immune activation, while simultaneously enhancing the production of SCFAs [31]. Consequently, the incidence of stroke is significantly lower in women compared with men, and the administration of estrogen-treated fecal microbiota transplantation (FMT) yields noteworthy neurological enhancements in elderly female patients diagnosed with IS [32].

Immune pathways

The gastrointestinal system harbors a significant proportion of the body's immune cells, including regulatory T-cells, B-cells, dendritic cells, macrophages, and T-cells. IS-induced inflammatory responses can lead to alterations in the number of immune cells in the gut. Monocytes, lymphocytes, and neutrophils in the gut can also migrate into brain tissue, leading to secondary neurodegenerative diseases [33]. The GM, which plays a significant role in the immune system, serves as a crucial regulator of T-cell homeostasis [34]. Furthermore, it serves as a vital determinant in the progression of inflammation associated with IS. The involvement of the GM in the development of cerebral ischemic disease can be attributed to its impact on Treg cells, which release the anti-inflammatory factor IL-10 for neuroprotection, as well as γδ T cells, known for their innate immune response and secretion of IL-17, which exacerbates neuroinflammation [35]. These findings suggest that the GM can potentially mitigate brain damage in cases of cerebral ischemic disease. Following the initiation of IS, intestinal T cells are instructed to migrate to the brain and release IL-17 in order to amplify the inflammatory response in the brain. Conversely, Treg cells were found to be elevated solely in the gut and facilitated the secretion of IL-10 and transforming growth factor-β (TGF-β) into the brain via the MGBA, thereby mitigating inflammation. Even after a span of 12 months post-IS in crab-eating monkeys, heightened levels of the pro-inflammatory factors interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and IL-6 were still observed in plasma. However, the persistence of systemic inflammation was diminished through regulation of the GM. Transplantation of GM into sterile animals experiencing cerebral ischemia resulted in the polarization of Th1 and Th17 cells, leading to increased levels of IFN-γ and IL-17. Additionally, more than 25% of the T-cell infiltrate in the ischemically injured brain tissue originated from the intestinal immune population. Conversely, FMT in a healthy state effectively regulates the peripheral immune system by reducing the number of pro-inflammatory cells, such as Th1 and Th17 cells, while increasing the number of Treg cells. This intervention also contributes to the reduction in cerebral infarction volume in ischemic mice. Moreover, the SCFA and LPS metabolites derived from the GM play a crucial role in regulating the inflammatory response. Specifically, the elevation of SCFAs influences G protein-coupled receptors and histone deacetylases, thereby facilitating the upregulation of anti-inflammatory factors (IL-10 and TGF-β) through immune cells. Additionally, SCFAs enhance the activation of Treg cells by augmenting the expression of forkhead box protein P3 (Foxp3), CD4, and CD25 proteins, ultimately mitigating cerebral ischemia/reperfusion injury in mice [36]. In contrast, LPS has the ability to traverse the intestinal barrier, activate B cells, and induce the release of pro-inflammatory factors such as IL-6, TNF-α, and chemokines from inflammatory cells via the Toll-like receptor 4 (TLR4)-myeloid differentiation factor 88 (MyD88) pathway. This activation can compromise the integrity of the blood-brain barrier, leading to cerebral edema and neurological damage [37]. Furthermore, following cerebral infarction, disruption of the GM can result in a sustained reduction in SCFAs and an enduring elevation in plasma LPS levels, thereby compromising the intestinal barrier and exacerbating the systemic immune response. The suppression of pathogenic bacterial abundance, such as *Enterobacteriaceae*, in the gastrointestinal tract following IS has been shown to effectively reduce inflammation and cerebral ischemic injury.

IMPROVEMENT OF THE GUT MICROBIOTA IN THE TREATMENT OF ISCHEMIC STROKE

Dietary interventions

Low-protein diets increase the abundance and diversity of GM (mainly in the phyla Firmicutes and Bacteroidetes), reverse pro-inflammatory T-cell polarisation, and exert anti-inflammatory

effects [38]. Furthermore, calorie-restricted diets that involve a reduction of 30–40% in caloric intake may have a protective effect against IS through the regulation of GM, specifically the enrichment of *Bifidobacterium* [39]. Likewise, an elevated protein intake raises the likelihood of stroke, while a moderate diet with a reduced protein content (7% or 8% protein diet) effectively improves neurological harm, the blood-brain barrier, swelling, and inflammation in the brains of IS mice. However, it is crucial to avoid excessive reduction of dietary protein, as it can lead to extensive protein-energy malnutrition, worsen motor dysfunction in rats with cerebral ischemic injury, and intensify the inflammatory response in ischemic brain tissue. Additionally, the protective effect on ischemic brain tissue is not significantly influenced by the type of protein, as both soy protein and casein exhibit similar effects.

Probiotics

Probiotics, including species, such as *Bifidobacterium* spp., *Bacillus* spp., *Streptococcus* spp., *Enterococcus* spp., *Lactobacillus*, *Schizosaccharomyces papyrifera*, and *Saccharomyces cerevisiae*, have been found to positively impact body homeostasis when present in sufficient quantities. Multiple studies have demonstrated that the prolonged utilization of probiotics, specifically *Lactobacillus* and *Clostridium butyricum*, can impact the concentrations of brain-derived neurotrophic factor, serotonin, and neurotransmitters like γ-aminobutyric acid. Additionally, they can impede apoptosis, particularly through inhibition of the PI3K/Akt pathway, within ischemic brain regions. Furthermore, probiotics exhibit antioxidant enzyme activities that effectively mitigate neuronal damage and cognitive impairments, thereby ameliorating brain injuries. These findings have been supported by various sources (Table 1). The administration of probiotics to modulate the GM has demonstrated considerable therapeutic potential for cerebral ischemic diseases. Nevertheless, given the intricate nature of the intestinal microecological environment, the selection and formulation of probiotics pose significant considerations.

Fecal microbiota transplantation

Following ischemic brain injury, GM disorders can elicit microglia-associated inflammatory responses and cognitive dysfunction, which may serve as a potential risk factor for cerebral ischemic diseases [40]. Both FMT and SCFAs demonstrated a reduction in nuclear factor-κB (NF-κB) and extracellular regulated protein kinase (ERK) protein activity in ischemic brain tissues by enhancing mitochondrial function, specifically the electron transport chain and oxidative phosphorylation. Additionally, FMT and SCFAs inhibited microglia polarization to M2 type and upregulated the level of tight junction proteins in the blood-brain barrier. Consequently, this pathway attenuated cerebral ischemic injury by suppressing NF-κB and ERK protein activities in ischemic brain tissues and preventing the upregulation of downstream pro-inflammatory factors [41]. These findings have been supported by various sources (Table 2).

Antibiotics

The use of antibiotics causes changes in the GM, which then impact immune cells in the intestines. These changes include modifications in T cells, the movement of intestinal lymphocytes to ischemic brain tissue, and the regulation of Treg and Th17 cells. Additionally, antibiotics can affect the development of cerebral ischemic injury by influencing the peripheral immune system. The regulatory effects of antibiotics on cerebral ischemic diseases have also been investigated, as summarized in Table 3. The majority of these studies suggest that antibiotics may alleviate IS by exerting both anti-inflammatory and neuroprotective effects. However, there are also studies suggesting that antibiotics can

Table 1. Probiotics for the treatment of ischemic stroke (IS)

Year and researcher	Drugs and dosage	Subjects	Results	Reference
Han *et al.*, 2024	*Enterococcus faecalis* (10^7 colony forming unit [CFU]), *Lactobacillus acidophilus* (10^7 CFU), *Bifidobacterium longum* (10^7 CFU); oral	Male C57BL/6L mice	Balances GM, improves brain tissue immune function, and promotes neurological recovery.	[46]
Cruz-Martínez *et al.*, 2024	symbiotic mix of *Enterococcus faecium* (4×10^8 CFU) and agave inulin (860 mg/kg); oral	Male SD rats	Reduces TNF-α levels and increases brain-derived neurotrophic factor expression, promotes neurological recovery.	[47]
Rahman *et al.*, 2023	*Limosilactobacillus reuteri* UBLRu-87, *Lactiplantibacillus plantarum* UBLP-40, *Lacticaseibacillus rhamnosus* UBLR-58, *Ligilactobacillus salivarius* UBLS-22, and *Bifidobacterium breve* UBBr-01 ($6.7–9.8\times10^9$ CFU); oral	Male SD rats	Remodels GM, modulates pro-inflammatory mediators, and improves gut barrier and BBB permeability.	[48]
Daniele *et al.*, 2023	*Bifidobacterium longum* R0175, *Lactobacillus helveticus* R0052 (10^9 CFU); oral	Male C57BL/6L mice	Remodels GM, and promotes neurological recovery.	[49]
Rahmati *et al.*, 2019	*Lacticaseibacillus casei* ZT-Lca.106, *Lactobacillus acidophilus* ZT-Lac.51, *Lacticaseibacillus rhamnosus* ZT-Lrh.54, *Lactobacillus bulgaricus* ZT-Lbu.90, *Bifidobacterium breve* ZT-Bbr.22, *Bifidobacteriumlongum* ZT-Lca.106, and *Streptococcus thermophilus* ZT-Sth.20 (10^9 CFU); oral	Male Swiss albino mice	Reduces apoptosis in brain tissue and improves spatial learning and memory deficits.	[50]
Sun *et al.*, 2016	*Clostridium butyricum* (10^9 CFU); oral	Male ICR mice	Reduces oxidation levels and apoptosis in brain tissue, elevates butyric acid content and protects damaged nerve cells.	[51]

Table 2. Fecal microbiota transplantation (FMT) for the treatment of ischemic stroke (IS)

Year and researcher	Microbiological sources	Subjects	Results	Reference
Wei *et al.*, 2024	Normal male SD rats	Male SD rats	Reduces malondialdehyde levels in the brain, increases GSH and GPX4 expression, and reduces cerebral infarct volume.	[52]
Zeng *et al.*, 2023	Normal young or old male C57BL/6L mice	male C57BL/6L mice	GM from aged mice increases valproic acid levels in the brain, exacerbating inflammatory responses and neurological damage.	[53]
Guo *et al.*, 2023	Male SD rats administered dengzhan shengmai in the model	Male SD rats	Reduces the level of inflammation in the brain and improves neurological damage.	[54]
Li *et al.*, 2022	Male C57BL/6L mice administered Buzhong Yiqi decoction in the model	Male C57BL/6L mice	Reduces levels of apoptosis and neurological damage in brain tissue.	[55]
Wang *et al.*, 2022	Male SD rats administered Zhilong Huoxue Tongyu capsule in the model	Male SD rats	Reduces BBB permeability and neurological damage.	[56]
Wang *et al.*, 2022	Normal male or female C57BL/6L mice	Male or female C57BL/6L mice	GM from female mice reduces systemic inflammatory factors and promotes neurological recovery.	[57]
Li *et al.*, 2022	Male C57BL/6L mice pre-treated with electroacupuncture in the model	Male C57BL/6L mice	Produces indole-3-propionic acid, which exerts antioxidant effects, reduces inflammatory responses, and ameliorates neurological damage.	[42]
Feng *et al.*, 2022	Normal male C57BL/6L mice	Male C57BL/6L mice	Reduces systemic IL-17 expression and improves neurological function.	[58]
Zhang *et al.*, 2021	Male C57BL/6L mice treated with atorvastatin in the model	Male C57BL/6L mice	Regulates immune function, enhances anti-inflammatory effects, and ameliorates nerve damage.	[40]
Lee *et al.*, 2020	Normal young or aged male C57BL/6L mice	Aged male C57BL/6L mice	GM from young mice have higher levels of SCFAs, attenuating inflammatory responses and neurological damage in the brain.	[59]
Zhang *et al.*, 2020	Male SD rats administered Tong-Qiao-Huo-Xue Decoction	Male SD rats	Reduces nerve damage and cerebral infarct volume.	[60]
Chen *et al.*, 2019	Male SD rats administered Puerariae Lobatae Radix with Chuanxiong Rhizoma in the model	Male SD rats	Increases intestinal butyric acid levels, repairs the intestinal barrier, and alleviates cerebral oedema and neurological damage.	[61]
Xia *et al.*, 2019	Individuals of higher Stroke Dysbiosis Index	Male C57BL/6L mice	GM from patients with high Stroke Dysbiosis Index can exacerbate gut inflammation and exacerbate IS.	[62]
Spychala *et al.*, 2018	Normal young or aged male C57BL/6L mice	Male C57BL/6L mice	GM from aged mice exacerbates inflammatory response and nerve damage.	[63]
Singh *et al.*, 2016	Normal male C57BL/6L and Rag1$^{-/-}$ mice	Germ-free (GF) C57BL/6J and GF Rag1$^{-/-}$ female mice	Initiates T_{helper} cells, reduces immune response, and improves neurological function.	[64]
Benakis *et al.*, 2016	Normal male C57BL/6L mice	Male C57BL/6L mice	Increases Treg cells, reduces inflammation levels and provides neuroprotection.	[65]

Table 3. Antibiotics for the treatment of ischemic stroke (IS) via the gut microbiota (GM)

Year and researcher	Drugs and dosage	Subjects	Results	Reference
Liu *et al.*, 2022	Ampicillin (1 g/L), vancomycin (500 mg/L), ciprofloxacin (200 mg/L), meropenem (250 mg/L) and metronidazole (1 g/L); delivered in the drinking water	Male Wistar rats	The simultaneous down-regulation of inflammatory in the cortex, effectively inhibits the initiation of inflammatory responses and facilitates the recovery process.	[66]
Benakis *et al.*, 2020	Ampicillin (1 g/L), vancomycin (0.5 g/L); delivered in the drinking water	Male C57BL/6L mice	Decrease the number of pro-inflammatory γδT cells and IL-17, to improves both the volume of cerebral infarction and neurological recovery following a stroke.	[67]
Chen *et al.*, 2019	Neomycin (450 mg/L), polymyxin B (150 mg/L); delivered in the drinking water	Male SD rats	Reduce nerve damage and cerebral infarct volume.	[61]
Benakis *et al.*, 2016	Vancomycin (0.5 g/L); delivered in the drinking water	Male C57BL/6L mice	By augmenting regulatory T cells, reducing γδ T cells, and regulating immune response occurrence, the improvement of cerebral ischemic injury can be achieved.	[65]
Winek *et al.*, 2016	Ampicillin (1 g/L), vancomycin (500 mg/L), ciprofloxacin (200 mg/L), imipenem (250 mg/L), and metronidazole (1 g/L); delivered in the drinking water	Female C57BL/6L mice	By disrupting the protective effect of GM on the gut, thereby exacerbating the detrimental effects of ischemic injury.	[68]

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worsen neurologic injury and the cerebral infarct volume by promoting the expansion of proinflammatory cells and reducing anti-inflammatory cells, thereby affecting Treg and Th17 cells. Hence, there is a need for further investigation into the use of antibiotics in the treatment of cerebral ischemic diseases. It is important to note that the effects of specific GM on cerebral ischemic diseases may vary. Additionally, GM are integral to a symbiotic relationship with the organism. Therefore, caution must be exercised when employing broad-spectrum antibiotics.

Other interventions

Changes in the GM can significantly influence the prognosis of IS. In addition to the aforementioned approaches, electroacupuncture preconditioning and treatment for cerebral ischemia/reperfusion injury have been found to restore microbial diversity and elevate the levels of the gut microbial metabolite isopropanol, which is known for its potent hydroxyl radical scavenging properties [42, 43], thereby exerting neuroprotective effects. Furthermore, exercise preconditioning has been shown to enhance the integrity of the intestinal barrier by augmenting the abundance of lactic acid bacteria and *Alistipes*, while reducing the presence of *Ruminococcus* [44]. This, in turn, leads to the inhibition of inflammatory factors such as NLR family pyrin domain-containing protein 3 (NLRP3), ultimately resulting in improved cognitive function within the brain [45].

SUMMARY AND PROSPECTS

This paper summarises the relationship between transgenics and IS, as well as the treatment of IS by modulating transgenics. As the largest immune organ in the human body, the gut is susceptible to systemic immune responses induced by changes in the GM. Therefore, modulating the GM provides new ideas for treating cerebral ischemic diseases. The following are worth noting: 1. A unified GM platform (containing detailed information on each genus of microorganisms and their roles, mechanisms, and interactions with other microbiota) needs to be established and improved. 2. Detailed molecular mechanisms of GM alteration of neurotransmitters and immune cells affecting ischemic brain tissues need to be further investigated. 3. Detailed studies are needed to investigate how drugs modulate the GM and their metabolites. 4. GM differences between rodents and humans should not be ignored in experimental studies. 5. Large differences in the GM exist between individuals, especially those residing in different regions, and personalised treatment of IS based on the GM is also a noteworthy topic. In conclusion, GM regulation plays an important role in the treatment of cerebral ischemic diseases, and elucidating its detailed mechanism of action will provide new ideas and options for the treatment of IS.

AUTHOR CONTRIBUTIONS

Zhiguo Mao and Jinying Zhang equally contributed to the conception and design of the research; Mingsan Miao contributed to the design of the research; Mingsan Miao, Lin Guo, Xiaoran Wang, and Zhengwang Zhu critically revised the manuscript; and Zhiguo Mao drafted the manuscript. All authors critically revised the manuscript, agreed to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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REFERENCES

1. Liu J, Gu Y, Guo M, Ji X. 2021. Neuroprotective effects and mechanisms of ischemic/hypoxic preconditioning on neurological diseases. CNS Neurosci Ther 27: 869–882. [Medline] [CrossRef]
2. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Cheng S, Delling FN, *et al.* American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee 2021. Heart disease and stroke statistics-2021 update: a report from the american heart association. Circulation 143: e254–e743. [Medline] [CrossRef]
3. Zheng W, Lei H, Ambler G, Werring DJ, Lin H, Lin X, Tang Y, Wu J, Lin Z, Liu N, *et al.* 2023. A comparison of low- versus standard-dose bridging alteplase in acute ischemic stroke mechanical thrombectomy using indirect methods. Ther Adv Neurol Disord 16: 17562864221144806. [Medline] [CrossRef]
4. Mao Z, Tian L, Liu J, Wu Q, Wang N, Wang G, Wang Y, Seto S. 2022. Ligustilide ameliorates hippocampal neuronal injury after cerebral ischemia reperfusion through activating PINK1/Parkin-dependent mitophagy. Phytomedicine 101: 154111. [Medline] [CrossRef]
5. Koszewicz M, Jaroch J, Brzecka A, Ejma M, Budrewicz S, Mikhaleva LM, Muresanu C, Schield P, Somasundaram SG, Kirkland CE, *et al.* 2021. Dysbiosis is one of the risk factor for stroke and cognitive impairment and potential target for treatment. Pharmacol Res 164: 105277. [Medline] [CrossRef]
6. El-Sayed A, Aleya L, Kamel M. 2021. Microbiota and epigenetics: promising therapeutic approaches? Environ Sci Pollut Res Int 28: 49343–49361. [Medline] [CrossRef]
7. Bi C, Guo S, Hu S, Chen J, Ye M, Liu Z. 2022. The microbiota-gut-brain axis and its modulation in the therapy of depression: comparison of efficacy of conventional drugs and traditional Chinese medicine approaches. Pharmacol Res 183: 106372. [Medline] [CrossRef]
8. Lee J, Peesh P, Quaicoe V, Tan C, Banerjee A, Mooz P, Ganesh BP, Petrosino J, Bryan RM Jr, McCullough LD, *et al.* 2023. Estradiol mediates colonic epithelial protection in aged mice after stroke and is associated with shifts in the gut microbiome. Gut Microbes 15: 2271629. [Medline] [CrossRef]
9. Fan J, Li X, Yu X, Liu Z, Jiang Y, Fang Y, Zong M, Suo C, Man Q, Xiong L. 2023. Global burden, risk factor analysis, and prediction study of ischemic stroke, 1990–2030. Neurology 101: e137–e150. [Medline] [CrossRef]
10. Zhang H, Hui X, Wang Y, Wang Y, Lu X. 2022. Angong Niuhuang Pill ameliorates cerebral ischemia/reperfusion injury in mice partly by restoring gut microbiota dysbiosis. Front Pharmacol 13: 1001422. [Medline] [CrossRef]
11. Tan C, Wu Q, Wang H, Gao X, Xu R, Cui Z, Zhu J, Zeng X, Zhou H, He Y, *et al.* 2021. Dysbiosis of gut microbiota and short-chain fatty acids in acute ischemic stroke and the subsequent risk for poor functional outcomes. JPEN J Parenter Enteral Nutr 45: 518–529. [Medline] [CrossRef]
12. Zheng D, Liwinski T, Elinav E. 2020. Interaction between microbiota and immunity in health and disease. Cell Res 30: 492–506. [Medline] [CrossRef]
13. Manoharan L, Roth B, Bang C, Stenlund H, Ohlsson B. 2023. An okinawan-based nordic diet leads to profound effects on gut microbiota and plasma metabolites linked to glucose and lipid metabolism. Nutrients 15: 15. [Medline] [CrossRef]
14. Mallesh S, Ten Hove AS, Schneider R, Schneiker B, Efferz P, Kalff JC, de Jonge WJ, Wehner S. 2022. Sympathetic innervation modulates mucosal immune homeostasis and epithelial host defense. Cells 11: 11. [Medline] [CrossRef]
15. Akagbosu B, Tayyebi Z, Shibu G, Paucar Iza YA, Deep D, Parisotto YF, Fisher L, Pasolli HA, Thevin V, Elmentaite R, *et al.* 2022. Novel antigen-presenting cell imparts T_{reg}-dependent tolerance to gut microbiota. Nature 610: 752–760. [Medline] [CrossRef]
16. Chidambaram SB, Essa MM, Rathipriya AG, Bishir M, Ray B, Mahalakshmi AM, Tousif AH, Sakharkar MK, Kashyap RS, Friedland RP, *et al.* 2022. Gut dysbiosis,

defective autophagy and altered immune responses in neurodegenerative diseases: tales of a vicious cycle. Pharmacol Ther 231: 107988. [Medline] [CrossRef]
17. de Vos WM, Tilg H, Van Hul M, Cani PD. 2022. Gut microbiome and health: mechanistic insights. Gut 71: 1020–1032. [Medline] [CrossRef]
18. Ishida K, Nagatake T, Saika A, Kawai S, Node E, Hosomi K, Kunisawa J. 2023. Induction of unique macrophage subset by simultaneous stimulation with LPS and IL-4. Front Immunol 14: 1111729. [Medline] [CrossRef]
19. Jiang H, Shi GF, Fang YX, Liu YQ, Wang Q, Zheng X, Zhang DJ, Zhang J, Yin ZQ. 2022. Aloin A prevents ulcerative colitis in mice by enhancing the intestinal barrier function via suppressing the Notch signaling pathway. Phytomedicine 106: 154403. [Medline] [CrossRef]
20. Zhang Q, Deng P, Chen S, Xu H, Zhang Y, Chen H, Zhang J, Sun H. 2023. Electroacupuncture and human iPSC-derived small extracellular vesicles regulate the gut microbiota in ischemic stroke *via* the brain-gut axis. Front Immunol 14: 1107559. [Medline] [CrossRef]
21. Huang A, Ji L, Li Y, Li Y, Yu Q. 2023. Gut microbiome plays a vital role in post-stroke injury repair by mediating neuroinflammation. Int Immunopharmacol 118: 110126. [Medline] [CrossRef]
22. Muhammad M, Muchimapura S, Wattanathorn J. 2023. Microbiota-gut-brain axis impairment in the pathogenesis of stroke: implication as a potent therapeutic target. Biosci Microbiota Food Health 42: 143–151. [Medline] [CrossRef]
23. Tait C, Sayuk GS. 2021. The Brain-Gut-Microbiotal Axis: a framework for understanding functional GI illness and their therapeutic interventions. Eur J Intern Med 84: 1–9. [Medline] [CrossRef]
24. Aswendt M, Green C, Sadler R, Llovera G, Dzikowski L, Heindl S, Gomez de Agüero M, Diedenhofen M, Vogel S, Wieters F, *et al.* 2021. The gut microbiota modulates brain network connectivity under physiological conditions and after acute brain ischemia. iScience 24: 103095. [Medline] [CrossRef]
25. Higarza SG, Arboleya S, Arias JL, Gueimonde M, Arias N. 2022. The gut-microbiota-brain changes across the liver disease spectrum. Front Cell Neurosci 16: 994404. [Medline] [CrossRef]
26. Bairamian D, Sha S, Rolhion N, Sokol H, Dorothée G, Lemere CA, Krantic S. 2022. Microbiota in neuroinflammation and synaptic dysfunction: a focus on Alzheimer's disease. Mol Neurodegener 17: 19. [Medline] [CrossRef]
27. Wang H, Ren S, Lv H, Cao L. 2021. Gut microbiota from mice with cerebral ischemia-reperfusion injury affects the brain in healthy mice. Aging (Albany NY) 13: 10058–10074. [Medline] [CrossRef]
28. Zhou Z, Yang W, Yu T, Yu Y, Zhao X, Yu Y, Gu C, Bilotta AJ, Yao S, Zhao Q, *et al.* 2023. GPR120 promotes neutrophil control of intestinal bacterial infection. Gut Microbes 15: 2190311. [Medline] [CrossRef]
29. Li C, Wang Y, Xing Y, Han J, Zhang Y, Zhang A, Hu J, Hua Y, Bai Y. 2022. Regulation of microglia phagocytosis and potential involvement of exercise. Front Cell Neurosci 16: 953534. [Medline] [CrossRef]
30. Zhai Z, Su PW, Ma LY, Yang H, Wang T, Fei ZG, Zhang YN, Wang Y, Ma K, Han BB, *et al.* 2023. Progress on traditional Chinese medicine in treatment of ischemic stroke via the gut-brain axis. Biomed Pharmacother 157: 114056. [Medline] [CrossRef]
31. Group EBCTC. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Electronic address: bc.overview@ctsu.ox.ac.uk Early Breast Cancer Trialists' Collaborative Group (EBCTCG). 2023. Anthracycline-containing and taxane-containing chemotherapy for early-stage operable breast cancer: a patient-level meta-analysis of 100 000 women from 86 randomised trials. Lancet 401: 1277–1292. [Medline] [CrossRef]
32. Park MJ, Pilla R, Panta A, Pandey S, Sarawichitr B, Suchodolski J, Sohrabji F. 2023. Correction to: Reproductive senescence and ischemic stroke remodel the gut microbiome and modulate the effects of estrogen treatment in female rats. Transl Stroke Res 14: 278–279. [Medline] [CrossRef]
33. Iadecola C, Buckwalter MS, Anrather J. 2020. Immune responses to stroke: mechanisms, modulation, and therapeutic potential. J Clin Invest 130: 2777–2788. [Medline] [CrossRef]
34. Hanna BS, Wang G, Galván-Peña S, Mann AO, Ramirez RN, Muñoz-Rojas AR, Smith K, Wan M, Benoist C, Mathis D. 2023. The gut microbiota promotes distal tissue regeneration via RORγ^{+} regulatory T cell emissaries. Immunity 56: 829–846. e8. [Medline] [CrossRef]
35. Díaz-Marugan L, Gallizioli M, Márquez-Kisinousky L, Arboleya S, Mastrangelo A, Ruiz-Jaén F, Pedragosa J, Casals C, Morales FJ, Ramos-Romero S, *et al.* 2023. Poststroke lung infection by opportunistic commensal bacteria is not mediated by their expansion in the gut microbiota. Stroke 54: 1875–1887. [Medline] [CrossRef]
36. Sadler R, Cramer JV, Heindl S, Kostidis S, Betz D, Zuurbier KR, Northoff BH, Heijink M, Goldberg MP, Plautz EJ, *et al.* 2020. Short-chain fatty acids improve poststroke recovery via immunological mechanisms. J Neurosci 40: 1162–1173. [Medline] [CrossRef]
37. Brioschi S, Wang WL, Peng V, Wang M, Shchukina I, Greenberg ZJ, Bando JK, Jaeger N, Czepielewski RS, Swain A, *et al.* 2021. Heterogeneity of meningeal B cells reveals a lymphopoietic niche at the CNS borders. Science 373: 373. [Medline] [CrossRef]
38. Silva de Carvalho T, Singh V, Mohamud Yusuf A, Wang J, Schultz Moreira AR, Sanchez-Mendoza EH, Sardari M, Nascentes Melo LM, Doeppner TR, Kehrmann J, *et al.* 2022. Post-ischemic protein restriction induces sustained neuroprotection, neurological recovery, brain remodeling, and gut microbiota rebalancing. Brain Behav Immun 100: 134–144. [Medline] [CrossRef]
39. Huang JT, Mao YQ, Han B, Zhang ZY, Chen HL, Li ZM, Kong CY, Xu JQ, Cai PR, Zeng YP, *et al.* 2021. Calorie restriction conferred improvement effect on long-term rehabilitation of ischemic stroke via gut microbiota. Pharmacol Res 170: 105726. [Medline] [CrossRef]
40. Zhang P, Zhang X, Huang Y, Chen J, Shang W, Shi G, Zhang L, Zhang C, Chen R. 2021. Atorvastatin alleviates microglia-mediated neuroinflammation via modulating the microbial composition and the intestinal barrier function in ischemic stroke mice. Free Radic Biol Med 162: 104–117. [Medline] [CrossRef]
41. Li HJ, Li DQ, Zhang YL, Ding XF, Gao HT, Zhu Y, Liu J, Zhang LX, Chen J, Chen G, *et al.* 2023. Modulation of gut microbiota alleviates cerebral ischemia/reperfusion injury in rats by inhibiting M1 polarization of microglia. Front Pharmacol 14: 1123387. [Medline] [CrossRef]
42. Li S, Zhao X, Lin F, Ni X, Liu X, Kong C, Yao X, Mo Y, Dai Q, Wang J. 2022. Gut flora mediates the rapid tolerance of electroacupuncture on ischemic stroke by activating melatonin receptor through regulating indole-3-propionic acid. Am J Chin Med 50: 979–1006. [Medline] [CrossRef]
43. Deng P, Wang L, Zhang Q, Chen S, Zhang Y, Xu H, Chen H, Xu Y, He W, Zhang J, *et al.* 2022. Therapeutic potential of a combination of electroacupuncture and human ipsc-derived small extracellular vesicles for ischemic stroke. Cells 11: 11. [Medline] [CrossRef]
44. Reinholdsson M, Abzhandadze T, Palstam A, Sunnerhagen KS. 2022. A register-based study on associations between pre-stroke physical activity and cognition early after stroke (part of PAPSIGOT). Sci Rep 12: 5779. [Medline] [CrossRef]
45. Lv H, Wang S, Tian M, Wang L, Gao J, Zhao Q, Li Z, Jia X, Yu Y. 2022. Exercise preconditioning ameliorates cognitive impairment in mice with ischemic stroke by alleviating inflammation and modulating gut microbiota. Mediators Inflamm 2022: 2124230. [Medline] [CrossRef]
46. Han Y, Xu H, Tao S, Zhu Y, Wei ZZ, Zhao Y, Zhang Y. 2024. Bifico ameliorates neurological deficits after ischemic stroke in mice: transcriptome profiling. In Vivo 38: 699–709. [Medline] [CrossRef]
47. Cruz-Martínez Y, Aguilar-Ponce L, Romo-Araiza A, Chávez-Guerra A, Martiñón S, Ibarra-García AP, Arias-Santiago S, Gálvez-Susano V, Ibarra A. 2024. Supplementation with a symbiotic induced neuroprotection and improved memory in rats with ischemic stroke. Biomedicines 12: 12. [Medline] [CrossRef]
48. Rahman Z, Bhale NA, Dikundwar AG, Dandekar MP. 2023. Multistrain probiotics with fructooligosaccharides improve middle cerebral artery occlusion-driven neurological deficits by revamping microbiota-gut-brain axis. Probiotics Antimicrob Proteins. [Medline] [CrossRef]
49. Daniele E, Nazer Y, Kortebi I, Casasbuenas DL, Fan Y, Trinh M, Tompkins TA, Faiz M. 2023. Oral probiotic therapy improves motor function in a rodent model of sensorimotor stroke. Exp Brain Res 241: 1931–1943. [Medline] [CrossRef]
50. Rahmati H, Momenabadi S, Vafaei AA, Bandegi AR, Mazaheri Z, Vakili A. 2019. Probiotic supplementation attenuates hippocampus injury and spatial learning and memory impairments in a cerebral hypoperfusion mouse model. Mol Biol Rep 46: 4985–4995. [Medline] [CrossRef]
51. Sun J, Ling Z, Wang F, Chen W, Li H, Jin J, Zhang H, Pang M, Yu J, Liu J. 2016. *Clostridium butyricum* pretreatment attenuates cerebral ischemia/reperfusion injury in mice via anti-oxidation and anti-apoptosis. Neurosci Lett 613: 30–35. [Medline] [CrossRef]
52. Wei J, Wang G, Lai M, Zhang Y, Li F, Wang Y, Tan Y. 2024. Faecal microbiota transplantation alleviates ferroptosis after ischaemic stroke. Neuroscience 541: 91–100. [Medline] [CrossRef]
53. Zeng X, Li J, Shan W, Lai Z, Zuo Z. 2023. Gut microbiota of old mice worsens neurological outcome after brain ischemia via increased valeric acid and IL-17 in the blood. Microbiome 11: 204. [Medline] [CrossRef]
54. Guo HH, Shen HR, Tang MZ, Sheng N, Ding X, Lin Y, Zhang JL, Jiang JD, Gao TL, Wang LL, *et al.* 2023. Microbiota-derived short-chain fatty acids mediate the effects of dengzhan shengmai in ameliorating cerebral ischemia via the gut-brain axis. J Ethnopharmacol 306: 116158. [Medline] [CrossRef]
55. Li Q, Cao M, Wei Z, Mei J, Zhang Y, Li M, Li M, Zhang Y, Wang Z. 2022. The protective effect of Buzhong Yiqi decoction on ischemic stroke mice and the mechanism of gut microbiota. Front Neurosci 16: 956620. [Medline] [CrossRef]
56. Wang R, Liu M, Ren G, Luo G, Wang Z, Ge Z, Pu Q, Ren W, Yang S. 2022. Zhilong Huoxue Tongyu Capsules' effects on ischemic stroke: an assessment using fecal 16S rRNA gene sequencing and untargeted serum metabolomics. Front Pharmacol 13: 1052110. [Medline] [CrossRef]
57. Wang J, Zhong Y, Zhu H, Mahgoub OK, Jian Z, Gu L, Xiong X. 2022. Different gender-derived gut microbiota influence stroke outcomes by mitigating inflammation. J Neuroinflammation 19: 245. [Medline] [CrossRef]
58. Feng Y, Zhang D, Zhao Y, Duan T, Sun H, Ren L, Ren X, Lu G, Liu Y, Zhang Z, *et al.* 2022. Effect of intestinal microbiota transplantation on cerebral ischemia reperfusion injury in aged mice via inhibition of IL-17. Neurogastroenterol Motil 34: e14313. [Medline] [CrossRef]
59. Lee J, d'Aigle J, Atadja L, Quaicoe V, Honarpisheh P, Ganesh BP, Hassan A, Graf J, Petrosino J, Putluri N, *et al.* 2020. Gut microbiota-derived short-chain fatty acids

promote poststroke recovery in aged mice. Circ Res 127: 453–465. [Medline] [CrossRef]
60. Zhang F, Zhai M, Wu Q, Jia X, Wang Y, Wang N. 2020. Protective effect of tong-qiao-huo-xue decoction on inflammatory injury caused by intestinal microbial disorders in stroke rats. Biol Pharm Bull 43: 788–800. [Medline] [CrossRef]
61. Chen R, Xu Y, Wu P, Zhou H, Lasanajak Y, Fang Y, Tang L, Ye L, Li X, Cai Z, *et al.* 2019. Transplantation of fecal microbiota rich in short chain fatty acids and butyric acid treat cerebral ischemic stroke by regulating gut microbiota. Pharmacol Res 148: 104403. [Medline] [CrossRef]
62. Xia GH, You C, Gao XX, Zeng XL, Zhu JJ, Xu KY, Tan CH, Xu RT, Wu QH, Zhou HW, *et al.* 2019. Stroke dysbiosis index (SDI) in gut microbiome are associated with brain injury and prognosis of stroke. Front Neurol 10: 397. [Medline] [CrossRef]
63. Spychala MS, Venna VR, Jandzinski M, Doran SJ, Durgan DJ, Ganesh BP, Ajami NJ, Putluri N, Graf J, Bryan RM, *et al.* 2018. Age-related changes in the gut microbiota influence systemic inflammation and stroke outcome. Ann Neurol 84: 23–36. [Medline] [CrossRef]
64. Singh V, Roth S, Llovera G, Sadler R, Garzetti D, Stecher B, Dichgans M, Liesz A. 2016. Microbiota dysbiosis controls the neuroinflammatory response after stroke. J Neurosci 36: 7428–7440. [Medline] [CrossRef]
65. Benakis C, Brea D, Caballero S, Faraco G, Moore J, Murphy M, Sita G, Racchumi G, Ling L, Pamer EG, *et al.* 2016. Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. Nat Med 22: 516–523. [Medline] [CrossRef]
66. Liu C, Cheng X, Zhong S, Liu Z, Liu F, Lin X, Zhao Y, Guan M, Xiao T, Jolkkonen J, *et al.* 2022. Long-term modification of gut microbiota by broad-spectrum antibiotics improves stroke outcome in rats. Stroke Vasc Neurol 7: 381–389. [Medline] [CrossRef]
67. Benakis C, Poon C, Lane D, Brea D, Sita G, Moore J, Murphy M, Racchumi G, Iadecola C, Anrather J. 2020. Distinct commensal bacterial signature in the gut is associated with acute and long-term protection from ischemic stroke. Stroke 51: 1844–1854. [Medline] [CrossRef]
68. Winek K, Engel O, Koduah P, Heimesaat MM, Fischer A, Bereswill S, Dames C, Kershaw O, Gruber AD, Curato C, *et al.* 2016. Depletion of cultivatable gut microbiota by broad-spectrum antibiotic pretreatment worsens outcome after murine stroke. Stroke 47: 1354–1363. [Medline] [CrossRef]

