

# Gut Microbiota and Stroke

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## Abstract

Ischemic stroke remains a significant health problem, which is expected to increase owing to an aging population. A considerable proportion of stroke patients suffer from gastrointestinal complications, including dysphagia, gastrointestinal hemorrhage, and constipation. Often, these complications adversely affect stroke outcomes. Recent research postulates the role of “brain-gut axis” in causing gut microbiota dysbiosis and various complications and outcomes. In this review, we present our current understanding about the interaction between commensal gut microbiome and brain in determining the course of stroke.

**Keywords:** Gut dysbiosis, gut microbiota, stroke

## INTRODUCTION

In recent years, there has been increasing interest with regard to the role of gut microbiota in the pathogenesis of disease, and the therapeutic potential of altering the gut microbiota. Microbiota refers to the microbes that colonize each individual, including our gut and play an imperative role in maintaining homeostasis within the host's health. The gut microbiota is complex and provides an array of genomic material, containing at least 100 times as many genes as the human genome.<sup>[1]</sup>

Dysbiosis is a commonly used term that describes the disturbances in the microbiome structure.<sup>[2]</sup> In this review, we present the current understanding about the interaction between gut microbiota and cerebrovascular disease, more specifically ischemic stroke.

## WHAT IS GUT-BRAIN AXIS AND MICROBIOME-GUT-BRAIN AXIS?

The gut-brain axis (GBA) comprises a two-way communication network between brain and the gastrointestinal tract, which links central cognitive and emotive centers of the brain with peripheral intestinal processes. GBA helps to regulate homeostasis, inflammation, and immune responses and is modulated by signaling pathways, which involve the central, autonomic, and enteric nervous systems and the hypothalamic–pituitary–adrenal axis. Recent research has explored the importance of gut microbiota in influencing the GBA and led toward coining the term “microbiome–GBA,” which explains how gut flora initiates the biochemical signaling events between the gastrointestinal tract and the brain.<sup>[3]</sup> This interaction is bidirectional, through to-and-fro signaling between gut-microbiota and brain by means of neural, endocrine, immune, and humoral links.<sup>[4]</sup>

## HOW DOES “GUT MICROBIOTA” ENHANCE “ATHEROSCLEROSIS” AND “STROKE RISK?”

Trimethylamine N-oxide (TMAO), a metabolite of gut microbiota, is one of the most well-studied links between gut

microbiota and stroke risk. Initial animal studies suggested a role for the gut microbiota in the pathogenesis of atherosclerosis in patients with a diet rich in phosphatidylcholine (contributory major dietary sources include eggs, pork, and beef).<sup>[5,6]</sup> Intestinal microbiota metabolizes dietary phosphatidylcholine, leading to the intermediate formation of trimethylamine, which finally gets converted into TMAO by hepatic flavin-containing monooxygenases.<sup>[7]</sup> More recently, a large clinical study evaluated the relationship between fasting plasma TMAO levels and major adverse cardiovascular events (death, myocardial infarction, or stroke) during 3 years of follow-up in patients undergoing elective coronary angiography.<sup>[8]</sup> This study found that the production of TMAO from dietary phosphatidylcholine is dependent on metabolism by the intestinal microbiota, and increased TMAO levels are associated with an increased risk of major adverse cardiovascular events.<sup>[6]</sup> This strong association has been demonstrated in various other studies. A recent nested case–control study of Chinese patients with hypertension revealed that higher TMAO levels were associated with an increased risk of index stroke.<sup>[9]</sup> Another multicenter study of patients with severe carotid artery stenosis who underwent carotid artery stenting (CAS) reported that plasma TMAO levels before CAS were significantly higher in patients with new diffusion-weighted image lesions poststenting, than in patients without lesions.<sup>[10]</sup>

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**Submitted:** 18-Sep-2019 **Accepted:** 18-Sep-2019 **Published:** 25-Feb-2020

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**DOI:** 10.4103/aian.AIAN\_483\_19

While the association between TMAO, atherosclerosis, and stroke risk appears well established, the mechanism by which TMAO mediates atherosclerosis remains poorly understood. One suggested mechanism for elevated cardiovascular risk and atherosclerosis is the TMAO-mediated increase of pro-inflammatory monocytes and vascular inflammation, as seen in a cohort of patients with prior ischemic stroke, where elevated TMAO levels demonstrated a dose-dependent association with the risk of recurrent stroke. Furthermore, a significant correlation was elucidated between TMAO levels and percentage of pro-inflammatory intermediate CD14<sup>+</sup>CD16<sup>+</sup> monocytes.<sup>[11]</sup> Other proposed mechanisms of TMAO have been on its effects on platelet hyperreactivity,<sup>[12]</sup> cholesterol metabolism,<sup>[13]</sup> and mediation of foam cell formation.<sup>[6]</sup> In addition, plasma TMAO levels have been shown to be associated with other risk factors of ischemic stroke including atrial fibrillation<sup>[14]</sup> and diabetes mellitus,<sup>[15]</sup> which on their own, can result in elevated stroke risks.

Beyond TMAO, other cross-sectional studies have demonstrated a different profile of gut microbiota in patients stratified according to stroke risk. Specifically, in the high-risk group, opportunistic pathogens and lactate-producing bacteria were enriched, while butyrate-producing bacteria were depleted.<sup>[16]</sup> The significance and implications of these associations remain unclear.

## DOES STROKE LEAD TO GUT MICROBIOTA DYSBIOSIS?

Acute ischemic stroke induces dysbiosis of the microbiome (top-down signaling), and these resultant changes in the gut microbiota, in turn, affect neuroinflammatory processes and influence stroke outcomes (bottom-up signaling).

Several animal-based studies have demonstrated differences in microbiota composition between poststroke models and controls. One such study showed substantial differences in microbiota at all sections of the gastrointestinal tract, even at the phylum level. The main characteristics of the stroke-induced shift in mucosal microbiota composition were an increased abundance of *Akkermansia muciniphila* and an abundance of *Clostridial* species.<sup>[17]</sup> Using mice middle cerebral artery occlusion models, a recent study illustrated substantial changes in gut microbiota composition 3 days after severe stroke through the next-generation sequencing technique.<sup>[18]</sup> These changes included a reduction in the diversity of microbiota species, intestinal bacterial overgrowth with a preferential expansion of the *Bacteroidetes* phylum, and more specific stroke-induced changes on the bacterial genus and at the species level.<sup>[19]</sup>

Limited clinical studies exist in evaluating gut microbiota changes after stroke. A cross-sectional study evaluating the fecal gut microbiota profile and fecal organic acids in a cohort of stroke and control subjects found that bacterial counts of *Lactobacillus ruminis* were significantly higher in stroke patients compared to the controls. Conversely, ischemic stroke was also associated with decreased counts of

other *Lactobacillus* species such as the *Lactobacillus sakei* subgroup.<sup>[19]</sup> In a separate study, shotgun sequencing of the gut metagenome was used to demonstrate that the genus *Collinsella* was enriched in patients with severe symptomatic carotid artery stenosis, whereas *Roseburia* and *Eubacterium* were enriched in healthy controls.<sup>[20]</sup> Most recently, Li *et al.* reported that the gut microbiota of ischemic stroke patients had more short-chain fatty acids producers including *Odoribacter*, *Akkermansia*, *Ruminococcaceae* UCG-005, and *Victivallis* when compared to healthy controls.<sup>[21]</sup>

While differences in gut microbiota between ischemic stroke patients and healthy controls have been established, the precise mechanism underpinning the effect of stroke on gut microbiota dysbiosis remains poorly understood. Some animal studies have attempted to evaluate this difference. One postulated mechanism is the reduced gastrointestinal motility after acute ischemic stroke due to a poststroke stress response, leading to bacterial overgrowth. Reduced species diversity and bacterial overgrowth of bacteroidetes were identified as hallmark of poststroke dysbiosis, which was associated with reduced intestinal motility.<sup>[19]</sup> The autonomic nervous system has also been implicated in mediating the effect of stroke on dysbiosis. Houlden *et al.* concluded that stroke altered the composition of cecal microbiota, with specific changes according to the severity of injury. These effects were mediated by noradrenaline release from the autonomic nervous system, with altered cecal mucoprotein production and goblet cell numbers.<sup>[22]</sup> Furthermore, the poststroke stress response is believed to increase intestinal permeability via the release of corticotropin-releasing and glucocorticoid hormones, which leads to increased bacterial translocation in the gut.<sup>[23,24]</sup> These theories are consistent with clinical data that report impaired gastrointestinal motility as well as increased gastrointestinal complications in patients with severe brain injuries and ischemic stroke.<sup>[25-27]</sup>

Other potential mechanistic links between acute stroke and dysbiosis include direct mediators released from necrotic brain tissue affecting microbiota composition. Specifically, the release of pro-inflammatory alarming such as adenosine triphosphate, HMGB1, and S100 proteins affect intestinal immunity and microbiota composition.<sup>[28,29]</sup>

## HOW DOES GUT MICROBIOTA INFLUENCE STROKE OUTCOMES?

Recent studies have explored the mechanisms by which changes in the gut microbiota affect poststroke neuroinflammation and impact stroke outcomes. In a proof of concept study with sham controls, microbiota obtained from mice that suffered from stroke was transplanted into germ-free recipient mice. Subsequently, cortical strokes were induced in these recipient mice. The study found that mice that had received transplanted microbiota from the stroke mice had significantly larger infarct volumes compared to mice

that received sham transplants. In addition, mice that had received transplants from the poststroke mice had higher expression of the inflammatory T-cells Th1 and Th17. Further analyses using fluorescent-labeled immune tissue revealed that T-cells from the mice intestines had migrated to the brain 2–3 days after the stroke, where they exacerbated neurological injury.<sup>[19]</sup> In another animal study, altering intestinal microbial diversity using broad-spectrum antibiotics (amoxicillin/clavulanic acid) before stroke conveyed an anti-inflammatory neuroprotective effect and resulted in a reduction of infarct volume by 60% and preservation of sensorimotor function. Antibiotic use resulted in intestinal dysbiosis and modulation of the neuroinflammatory response after stroke by T-cell mediated mechanisms. Specifically, the authors attribute the neuroprotective effect to an increase in regulatory T-cells, a reduction in interleukin (IL)-17<sup>+</sup>  $\gamma\delta$  T-cells through altered dendritic cell activity, and suppressed trafficking of effector T-cells from the gut to the leptomeninges after stroke.<sup>[30]</sup> Further experimental mice studies found that the microbiota composition in young and aged mice are significantly different (ratio of *Firmicutes* to *Bacteroidetes* increased ~9-fold in aged mice compared to young). Altering the microbiota in young by fecal gavage to resemble that of aged mice increased cytokine levels and increased mortality following middle cerebral artery occlusion. Conversely, altering the microbiota in aged to resemble that of young mice increased survival and improved recovery following middle cerebral artery occlusion.<sup>[31]</sup>

There has been a relative paucity of clinical studies correlating gut dysbiosis with clinical outcomes after acute ischemic stroke. The study by Yamashiro *et al.* reported that changes in the prevalence of *L. ruminis* were positively correlated with serum IL-6 levels.<sup>[19]</sup> In addition, ischemic stroke was associated with increased concentrations of valeric acid (fecal organic acid), which were positively correlated with inflammatory markers like high sensitivity C-reactive protein levels and white blood cell counts.<sup>[19]</sup> Authors concluded that gut dysbiosis in patients with ischemic stroke affects host metabolism and inflammation, but no associations were observed between patients' bacterial counts and National Institutes of Health Stroke Scale (NIHSS).<sup>[20]</sup> A novel study established a Stroke Dysbiosis Index (SDI)<sup>[32]</sup> to be an independent predictor of severe stroke (NIHSS  $\geq 8$ ) and early unfavorable outcome (Modified Rankin Scale  $> 2$  at discharge).<sup>[33]</sup> The SDI was developed by analyzing gut microbial taxonomic features of acute ischemic stroke patients and healthy controls using sequencing of 16S ribosomal RNA genes, and subsequent identification of 18 bacterial genera that demonstrated a profound difference between the acute stroke and control groups.<sup>[34]</sup> The same study had an animal experiment phase which also showed that mice receiving fecal transplants from high-SDI patients developed severe brain injury with elevated IL-17<sup>+</sup>  $\gamma\delta$  T-cells in gut, compared to mice receiving transplants from low-SDI patients after inducing stroke.<sup>[34]</sup>

## COULD MICROBIOTA SERVE AS A THERAPEUTIC TARGET?

Recent research on gut microbiota and its relation to stroke pathogenesis and outcomes might offer a new potential therapeutic target. Efforts to “drug the microbiome” are still in their infancy but carry a great hope for stroke prevention and treatment. Earlier studies revealed that plasma levels of TMAO were markedly suppressed after the administration of broad-spectrum antibiotics and then increased after withdrawal of the antibiotics, which limited its utility since prolonged antibiotic use would carry other harmful effects like *Clostridium difficile* colitis.<sup>[9]</sup> More recently, preliminary studies have described the development of potent inhibitors of gut microbiota-dependent TMAO generation that might be capable of reducing thrombosis and stroke risk.<sup>[34]</sup> In animal models, a single oral dose of a CutC/D inhibitor significantly reduced plasma TMAO levels for up to 3 days and rescued diet-induced enhanced platelet responsiveness and thrombus formation, without observable toxicity or increased bleeding risk.<sup>[35]</sup>

Fecal microbiota transplantation has been widely utilized as a safe and effective treatment modality for patients with recurrent *Clostridium difficile* infection.<sup>[35]</sup> In animal experiments, fecal transplantation and colonization with normal stool microbiota improved stroke outcome.<sup>[19,32]</sup> Probiotics also have the ability to alter the gut microbiome, modulate cytokine release, influence neuroinflammatory response, and hence may represent another therapeutic strategy for acute stroke.<sup>[36,37]</sup> An animal study demonstrated that probiotic treatment with *Clostridium butyricum* attenuated cerebral ischemia/reperfusion injury in mice.<sup>[38]</sup> Nonetheless, the safety and efficacy of both fecal microbiota transplantation and probiotics in human stroke patients remain unknown, and this needs to be evaluated in the future research.

## CONCLUSIONS

Interaction between gut microbiome and central nervous system in health and disease is a relatively new and evolving focus for research. Since gastrointestinal complications are quite common in patients after stroke, the role played by alterations in gut microbiota and expression or suppression of various biomarkers are being evaluated extensively. Most of our current understanding of the GBA in stroke is derived from animal models, and various therapeutic targets in gut microbiome have been identified. Current research findings appear exciting and increasing number of research studies are being extended to human ischemic stroke patients and new treatment approaches are expected to evolve in next few years that may improve outcomes.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.



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