

PREBIOTICS FOR ACUTE ISCHEMIC STROKE

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ABSTRACT

There is two-way communication between the gut and the brain. The condition of the quality and quantity of microbiota in the gut greatly affects the communication process or commonly known as the microbiota-gut-brain axis. Acute ischemic stroke can affect the quality and quantity of microbiota in the gut, which leads to intestinal dysbiosis. Thus, it might produce an inflammatory response that can change immune homeostatic. This can lead to poor clinical outcomes and neurologic function and an increase in mortality. Dysbiosis is a condition where there are qualitative and quantitative changes in the composition, distribution, and metabolic activity of intestinal microbiota which have a detrimental effect on human health, in other words, there is a decrease in the number of probiotic bacteria in the gut, which provide health benefits. The conditions for a good probiotic are that the probiotics have to be kept alive in the digestive tract to obtain health benefits. The approach taken to keep these bacteria alive is the use of prebiotics. Prebiotics are components of food that cannot be digested by the digestive tract enzymatically. Thus, they are fermented by microbiota in the large intestine to produce metabolites, one of which is short-chain fatty acids (SCFA) as a product of fermentation. SCFA (Short Chain Fatty Acid) or short-chain fatty acids play a neuroprotective role, synthesizing neurotransmitters and modulating the immune system. Therefore, this review explains how stroke affects the quantity and quality of microbiota in the gut in the communication process of the microbiota-gut-brain axis and the role of prebiotics in improving dysbiosis. Hence, it can provide better post-stroke clinical outcomes.

Keywords: Dysbiosis, Intestinal microbiota, Prebiotics, SCFA, Acute ischemic stroke

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INTRODUCTION

The incidence of ischemic stroke accounts for about 80% of all strokes [1]. The main causes of ischemic stroke are thrombosis, embolism, and systemic hypoperfusion. All of these cause disruptions of cerebral blood flow. Normal brain tissue receives a blood flow of 50-55 ml/100g/min. When ischemia occurs in the brain, the cerebral blood flow decreased, results in an inadequate supply of oxygen and glucose, which triggers the pathophysiological process of stroke. The pathophysiology of stroke is very complex and involves various processes, such as energy failure, excitotoxicity, oxidative stress, damage to the blood-brain barrier, inflammation, necrosis or apoptosis, and so on [2, 3], referring to as the ischemic cascade.

Based on data from World Health Organization (WHO) in 2016, there were 5.5 million deaths caused by cerebrovascular disease worldwide (2.7 million deaths from ischemic strokes and 2.8 million deaths from hemorrhagic strokes) [4]. Eastern Europe, East Asia, as well as parts of Southeast Asia, Central Asia, and Africa have the highest death rates from stroke [4]. In Indonesia, the prevalence of stroke increased from 7% in 2013 to 10.9% in 2018 and increased with age [5]. Based on the data basis of health research [6], number of patients who have been diagnosed stroke are 2,137,941 patients (12.1%). Stroke patients were mostly found in the age group of 45-54 y, 55-64 y, and 65-74 y [7].

Several previous studies have shown that the gut-brain axis mechanism plays an important role in the pathophysiological process of ischemic stroke [7, 8]. Ischemic conditions cause dysbiosis and disruption of the intestinal barrier [9-11]. Dysbiosis is a condition where there are qualitative and quantitative changes in the composition, distribution, and metabolic activity of microbes that cause detrimental effects on the host [13]. In other words, the number of beneficial microbiota (probiotics) is decreased. Microbiota in the intestine, especially probiotics, will carry out fermentation to produce metabolites, one of which is SCFA (Short Chain Fatty Acid). Several studies have reported that SCFA can affect diseases of the central nervous system [13, 14]. It can also improve brain function directly and indirectly through immunological, endocrine, vagal, and other

humoral pathways [14]. Moreover, SCFA can improve intestinal permeability and the blood-brain barrier [15, 16].

The conditions for a good probiotic are that the probiotics have to be kept alive in the digestive tract to obtain the health benefits from it. The approach taken to keep these bacteria alive is the use of prebiotics [18]. Prebiotics are food components that cannot be digested by the human digestive tract enzymatically. Therefore, they might be fermented by the microflora present in the large intestine [19]. Prebiotics in the large intestine will support the growth of probiotic bacteria and suppress pathogenic bacteria. There are various benefits obtained from consuming prebiotics, including preventing constipation, a condition of not being able to defecate regularly, lowering intestinal pH, and being able to restore the microflora in the intestine after changes due to antibiotic use, diarrhea, or stress [20]. In general, foodstuff components that have prebiotic properties include non-starch polysaccharides (such as pectin, cellulose, and xylene), sugars, and oligosaccharides (such as lactose, raffinose, fructooligosaccharides, galactooligosaccharides, and lactulose) [19, 20].

Fruits, vegetables, cereals, and several types of plants are potential sources of carbohydrates as prebiotics, such as tomatoes, artichokes, bananas, asparagus, berries, garlic, onions, chicory, green vegetables, nuts, oats, flaxseeds, barley, and wheat flour [23]. Besides, there are several artificial prebiotics, such as lactulose, galactooligosaccharides, fructooligosaccharides, maltooligosaccharides, cyclodextrins, and lactosaccharides. Fructans, such as inulin and oligofructose, are the most commonly used and effective for producing many probiotic species [22, 23]. Prebiotics can potentially be beneficial for health, especially in relation to maintaining brain function in ischemic conditions. This review discusses various aspects of prebiotics, including the important role of prebiotics in acute ischemic stroke.

Source of information

For writing this comprehensive research review on prebiotic uses for acute ischemic stroke, various databases were searched. For the

collection of relevant information, specific terms such as medical subject headings (MeSH) and key text words, such as “stroke pathophysiology”, “gut dysbiosis”, “microbiota-gut-brain axis”, “prebiotic and acute ischemic stroke”, and “role of SCFA in gut dysbiosis” published till 2020 were used in MEDLINE. Most especially for retrieving all articles pertaining to the prebiotic uses for acute ischemic stroke, electronic bibliographic databases were searched and abstracts of published studies with relevant information on the prebiotics/SCFA mechanism on the gut-brain axis were collected. Furthermore, additional references were included through searching the references cited by the studies done on the present topic. Relevant terms were used individually and in combination to ensure an extensive literature search. For updating the information about a subject and incorporation of recent knowledge, relevant research articles, books, conference proceedings’ and public health organization survey reports were selected and collated based on the broader objective of the review. This was achieved by searching databases, including SCOPUS, Web of Science, EMBASE, Swiss Prot, Pubmed, and Google scholar. From this common methodology, discoveries and findings were identified and summarized in this final review.

The microbiota-gut-brain communication

Several studies on the human body have shown that stroke can change the composition of the gut microbiota. A cohort study in Japan that analyzed the gut microbiota and organic acids present in the feces of subjects with stroke compared to controls gave results that ischemic stroke altered the diversity of several bacterial species. This change was independent of age, type 2 diabetes mellitus, and hypertension. Increased composition of *Lactobacillus ruminis* was positively correlated with inflammatory markers in stroke patients. This change in microbiota was accompanied by a 13% reduction in total organic acid with increased and decreased SCFAs of valeric and acetic acid by 54% and 18% in patients with stroke, respectively. Given that SCFA is a product of bacterial fermentation, it is expected that the change in SCFA is the result of changes in microbiota composition after stroke. However, in that study, it is also possible that the dietary changes in patients with stroke during the stay in hospital may have resulted in differences in the microbiota and metabolites observed [12].

In another study, patients with large-artery atherosclerotic strokes or transient ischemic attacks (TIA) were compared with non-stroke controls with and without carotid atherosclerotic plaque. In control subjects, the gut microbiota was similar regardless of the presence or absence of carotid plaque. The streak gut microbiota

arrangement/TIA showed an increase of several pathogens and decreased the number of beneficial bacteria [11].

Studies in animal models of stroke have provided further evidence of stroke-induced gut dysbiosis and helped identify the mechanisms by which stroke affects the gut and microbiota. In the experimental animal studied, MCAO (Middle Cerebral Artery Occlusion) was carried out in the proximal part to indicate the condition of ischemic stroke. Stroke causes intestinal dysbiosis, intestinal paralysis, increased intestinal dysbiosis, loss of cholinergic innervation in the ileum, and increased sympathetic activity [23, 31, 32]. Loss of cholinergic signaling that supports this adrenergic signaling is known to increase inflammation in the gut. Increased intestinal adrenergic stimulation following MCAO processing was also associated with decreased goblet cell counts in the cecum and impaired production of the major glycoprotein mucin in gastrointestinal mucus [28]. The mucus layer, which acts as a protective barrier between the epithelium and the lumen of the intestine, is a home for bacteria that helps communication between the host and the luminal microbiota. After the proximal MCAO process, intestinal permeability and bacterial translocation were increased in young and old mice [29]. Therefore, 24 h after proximal MCAO was performed, >60% of the microbiota in the lungs was deemed to originate from the small intestine [32, 35]. However, an increase in bacterial translocation after stroke has not been observed in many studies [25]. Overall, intestinal dysbiosis due to stroke is the precursor to post-stroke infection, which is a major cause of the increased length of stay and mortality after stroke.

The extent of the interaction mechanism between the gut microbiota and non-gastrointestinal organs is still being studied in various studies. However, evidence suggests that there is two-way communication between the gut, microbiota, and brain. This two-way communication is known as the microbiota-gut-brain axis. Signaling between the brain and gut occurs via neuronal and non-neuronal mechanisms (fig. 1 and 2). For signaling from the brain to the intestine, the intestinal wall receives direct communication through parasympathetic and sympathetic nerve fibers or indirectly follows stimulation of the enteric nervous system, namely the system of nervous connections located in the submucosa and the myenteric plexus of the intestine (fig. 2). This neuronal mechanism affects intestinal motility, intestinal permeability, microbiota composition, and activation of immune cells. Not only direct neuronal mechanisms, but the hypothalamic-pituitary-adrenal axis is also very important as a communication pathway in response to stress (fig. 1).

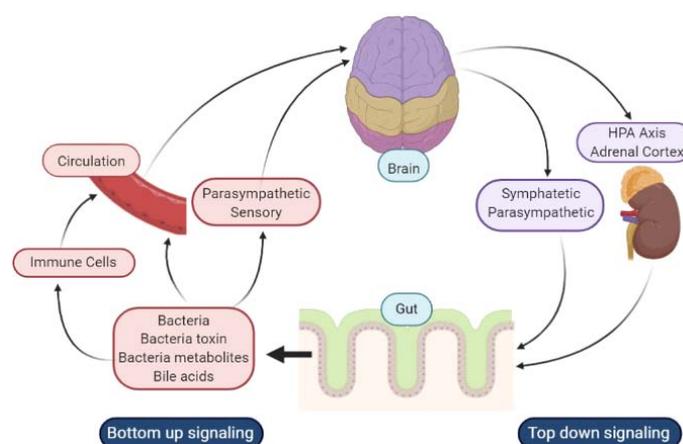


Fig. 1: 2-way communication between gut and brain [9]

Signaling from the gut to the brain is expected to occur through several different mechanisms. The first one is through the vagus nerve, which consists of 80% afferent fiber and 20% efferent fiber, which has a dual role in conveying signals between the intestine and the brain (fig. 2). This afferent fiber can be stimulated by microbial compounds,

metabolites, and hormones (for example, serotonin, cholecystokinin, glucagon-like peptide-1, and peptide YY) released by enteroendocrine cells from the epithelial layer of the intestine to initiate signaling from the intestine to the brain. Stimulation of signals from these afferent nerve fibers goes throughout the brain, including the nerve cells in the

hypothalamus that regulate the secretion of the pituitary gland and the nucleus of the solar nerve tract. Second, immunogenic endotoxin from microbiota, such as lipopolysaccharide (LPS), can induce neuronal inflammation either directly or through activation of peripheral immune cells, which can then migrate to the brain (fig. 1) [23, 24].

Third, the microbiota produces or stimulates the release of several metabolites, such as neurotransmitters, short-chain fatty acids (SCFA), indoles, and bile acids that enter the circulatory system and travel to the brain to modulate the function of neurons, microglia, astrocytes, and the blood-brain barrier (BBB) [25–27].

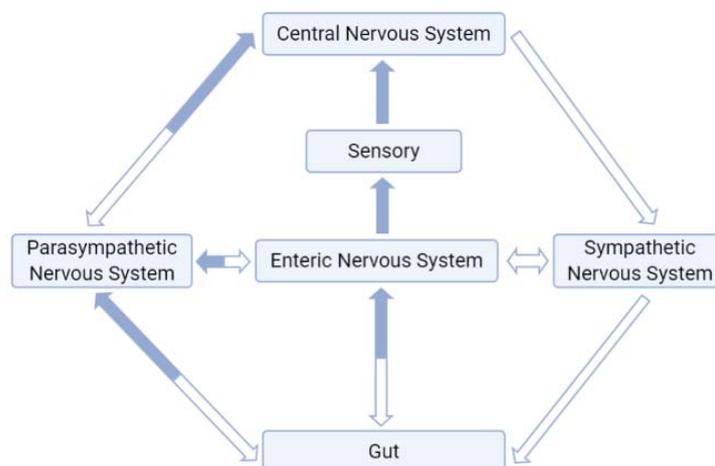


Fig. 2: The neuronal mechanism of communication between the brain and the gut. The blue arrows show that the afferent nerve signaling from the gut to the brain, white arrows show efferent nerve signaling from the brain to the gut [9]

SCFA, such as acetate, butyrate, and propionate, are the main end products of bacterial fermentation of resistant starch and fiber. These SCFAs not only function as a source of energy but also have vasoactive properties. Moreover, SCFAs affect host cells through various mechanisms, including altering histone acetylation and cell proliferation, as well as activation of GPCR (G-protein coupled receptors). An imbalance in the bacterial composition in the gut or a reduction in SCFA-producing bacteria has been described in several models of cardiovascular and metabolic disease, including stroke, hypertension, obesity, and diabetes mellitus [28–30].

As discussed above, the microbiota-gut-brain axis communication is two-way communication. Thus, there is increasing evidence that stroke alters the gut microbial through signaling from the brain to the gut (top-bottom). An imbalance in the composition of the gut microbiota can affect the condition of stroke in gut-to-brain (bottom-top) signaling.

Prebiotics

Prebiotics were first introduced in 1995 by Glenn Gibson and Marcel Roberfroid [38]. Prebiotics are "food substances that cannot be digested and can stimulate the growth and/or activity of one or several bacteria in the large intestine, making them beneficial to one's health." According to this definition, only a few compounds from the carbohydrate group, such as fructooligosaccharides (FOS), inulin, lactulose, and galactooligosaccharides (GOS) can be classified as prebiotics. In 2008, the 6th meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) defined "prebiotics" as "food ingredients that are fermented and produce specific changes in the composition and/or activity of the gastrointestinal microbiota, thus providing benefits to one's health" [38]. The following criteria are used to classify a compound as prebiotic [38]:

- Not digested (or only partially digested)
- Poorly fermented by bacteria in the oral cavity
- Well fermented by probiotic gut bacteria
- Not fermented by potential pathogens in the intestine.

Carbohydrates, like fiber, are prebiotics. Prebiotics and fiber are terms used as alternatives to the undigested components of food in the digestive tract [39,40]. A significant difference between the two terms is that prebiotics is fermented by a certain group of

microorganisms, whereas fiber is used by most of the gut microorganisms [40]. Therefore, taking into account that the interchangeability of the terms is not always correct. Prebiotics are fibers, but the fiber is not always a prebiotic [41].

Some non-starch polysaccharides are fiber, for example, cellulose, hemicellulose, pectin, latex, substances obtained from seaweed, as well as lactulose, soybean oligosaccharides, inulin, fructooligosaccharides, galactooligosaccharides, xylooligosaccharides, and isomaltoligosaccharides. Based on the number of monomers, prebiotics can be classified as disaccharides, oligosaccharides (3-10 monomers), and polysaccharides. The most satisfactory criteria for prebiotic classification as evidenced by *in vitro* and *in vivo* studies are oligosaccharides, including fructooligosaccharides (FOS), galactooligosaccharides (GOS), isomaltoligosaccharides (IMO), xylooligosaccharides (XOS), transgalactooligosaccharides (TOS), and soybean oligosaccharides (SBOS) [39, 40].

Furthermore, polysaccharides such as inulin, reflux starch, cellulose, hemicellulose, or pectin have the potential to be prebiotic. The use of glucooligosaccharides, glucooligosaccharides, lactitol, isomaltoligosaccharides, stachyose, raffinose, and saccharose as prebiotics requires further study [44].

Prebiotics are found in natural products, but they can also be added to foods. The purpose of this addition is to increase nutritional value and health. Some examples are inulin, fructooligosaccharide, lactulose, and galactose, and β -glucan derivatives. These substances can function as probiotic media for reproduction. Prebiotics are not digested by enzymes and reach the large intestine without change of shape, where they are fermented by saccharolytic bacteria (for example, genus *Bifidobacterium*). Prebiotic consumption greatly affects the composition of the gut microbiota and its metabolic activity [45]. This is due to modulation of lipid metabolism, increased calcium absorption, effects on the immunological system, and modification of bowel function [45]. The structure of the prebiotic molecule determines its physiological effects and the types of microorganisms that can utilize it as an energy source [23].

The mechanism of the beneficial effect of prebiotics on immunological function is unclear [46]:

1. Prebiotics are able to regulate the work of liver lipogenic enzymes by influencing the increased production of short-chain fatty acids (SCFA), such as propionic acid.

2. The production of SCFA (especially butyric acid) as a result of fermentation has been identified as a modulator of histone acetylation, thereby increasing the availability of many genes for transcription factors.

3. Modulation of mucin production.

4. FOS and some other prebiotics cause an increase in the number of lymphocytes and/or leukocytes in intestinal lymphoid tissue (GALT) and peripheral blood.

5. Increased secretion of IgA by GALT can stimulate intra-inflammatory macrophage phagocytic function.

The main purpose of prebiotics is to stimulate the growth and activity of beneficial bacteria in the digestive tract, which provides health benefits to the host. The end products of carbohydrate metabolism are mostly SCFA, namely acetic acid, butyric acid, and

propionic acid, which are then used by the host as an energy source [47]. As a result of carbohydrate fermentation, *Bifidobacterium* or *Lactobacillus* can produce several compounds that inhibit the development of gastrointestinal pathogens, as well as cause a decrease in intestinal pH [48]. Furthermore, bacteria of the genus *Bifidobacterium* showed tolerance to the resulting SCFA and decreased pH. Because of their beneficial effect on the development of commensal gut bacteria, the administration of prebiotics plays a role in inhibiting the development of pathogens. Studies on the inhibition of pathogen development by prebiotics are very limited. In 1997 and 2003, Bovee-Oudenhoven *et al.* studied the use of lactulose in the prevention of Salmonella Enteritidis in mice. The results show that a decrease in intestinal pH as a result of lactulose fermentation leads to decreased pathogen development and increased translocation of pathogens from the gut [49]. Administration of prebiotics can also increase the absorption of minerals, mostly magnesium and calcium [47, 48].

Table 1: Several studies on the effects of prebiotics on inflammatory biomarkers associated with acute ischemic stroke

| Prebiotics | Dose | Subject | Findings | Reference |
|---------------------------------------|---|---|--|-----------|
| Inulin | 8% inulin = equal with 40 gr of fiber in human for 16 w | APOE4 model mouse (n=39) and old mouse (n=28) model APOE4 | Inulin increases the number of beneficial microbiota and decreases harmful microbiota in young mice feces. It also increases systemic metabolism as indicated by increased levels of SCFA, tryptophan-derived metabolites, bile acids, glycolytic metabolites, and scylloinositol. Inulin also reduces the expression of inflammatory genes in the hippocampus. | [52] |
| - Fructooligosaccharides (FOS) | - FOS (3 g/kg) | Mouse | - The number of <i>Bifidobacteria</i> after administration of FOS and GOS increased by 25% and 80%, respectively, compared to the number of <i>Bifidobacteria</i> in the control group | [53] |
| - Galactooligosaccharides (GOS) | - GOS (4 g/kg) for 5 w | | - BDNF protein levels in the hippocampus area increased significantly in the group of mice given FOS compared to the GOS and control groups - NR1 levels increased significantly in the front cortical GOS group and the hippocampal FOS group - NR2A levels increased significantly in the GOS group in the hippocampus - The concentration of amino acid D-alanine and D-serine increased significantly in the GOS group compared to the FOS and control groups - Plasma PYY concentrations increased significantly in the GOS group compared to the FOS and control groups - After the administration of prebiotic GOS, there is a positive correlation between the number of <i>Bifidobacteria</i> and the levels of NR1, plasma D-alanine, plasma L-alanine. There is also a positive correlation between D-serine concentrations and NR1 levels | |
| Chitosan oligosaccharides (COS) | COS is given orally in 200, 400, and 800 mg/kg for 14 d | Alzheimer Disease model mouse (<i>Sprague Dawley</i>) | COS given orally at doses of 200, 400, or 800 mg/kg is effective in improving learning ability and memory function. Moreover, it is also able to improve nerve apoptosis. The neuroprotective effect of COS is closely related to its ability to inhibit oxidative stress, which is shown by decreased levels of malondialdehyde (MDA) 8-8-hydroxy-2'-deoxyguanosine and increased levels of glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity. COS has also been shown to suppress the inflammatory response by decreasing the release of proinflammatory cytokines (e. g., IL-1 β and TNF- α). | [54] |
| Galactooligosaccharides (GOS) | 2% GOS in the amount of 8 ml/kg/day | ALS (<i>Amyotrophic lateral sclerosis</i>) model mouse | The administration of GOS and prebiotic yogurt GOS significantly slowed disease onset and prolonged life in the ALS (<i>Amyotrophic lateral sclerosis</i>) mice. Furthermore, this product increases the concentration of folate, Vitamin B12 and lowers homocysteine levels. GOS and yogurt prebiotics reduce the decrease in motor neurons, increase atrophy and mitochondrial activity in myocytes. GOS also suppresses the activation of astrocytes and microglia and regulates several factors associated with inflammation and apoptosis. | [55] |
| Bimuno-galactooligosaccharida (B-GOS) | 13 gram B-GOS powder for 3 w | mouse that is injected with lipopolisakarida (LPS) | Administration of bimuno-galactooligosaccharide (BGOS) reduces the proinflammatory cytokine IL-1 β and expression of the 5-HT2A cortical receptor | [56] |
| Bimuno-galactooligosaccharida (B-GOS) | 3% B-GOS solution for 3 w | Mouse (<i>Sprague Dawley</i>) | Cortical neuronal response to NMDA iontophoresis was greater (+30%) in mice treated with B-GOS compared to controls not given supplementation. The intake of B-GOS also inhibits the reduction of the NMDA response by the glycine site antagonist, HA-966. Mice fed B-GOS showed greater cognitive flexibility because B-GOS increased plasma acetate levels cortical GluN2B subunits and Acetyl Co-A Carboxylase mRNA. | [57] |
| Glucuronoligosaccharides (GMn) | GMn 10 mg/kg and 20 mg/kg for 7 d | Parkinson Disease model mouse | The result showed that GMn improved behavioral deficits in Parkinson's mice. Additionally, glucuronoligosaccharides contribute to regulating apoptotic signaling pathways through increased tyrosine hydroxylase (TH) expression in dopaminergic neurons. These results suggest that glucuronoligosaccharide protects dopaminergic neurons from apoptosis in Parkinson's mice. | [58] |
| Chitosan | 200, 500, 1.000 | mouse | COS can cross the blood-brain barrier very well both <i>in vivo</i> and <i>in vitro</i> via the | [59] |

| | | | | |
|--|--|---|---|------|
| oligosaccharides (COS) | µg/kg for 12 h | | GLUT1 transporter. COS reduces Aβ42-mediated cytotoxicity. COS significantly reduces amyloid-induced apoptosis, oxidative stress, and the release of inflammatory cytokines. | |
| Fructooligosaccharides from <i>Morinda officinalis</i> (OMO) | 50 and 100 mg/kg/d | Alzheimer Disease model mouse (<i>Sprague Dawley</i>) | OMO administration can significantly improve learning abilities and memory function. OMO administration not only improves oxidative stress and inflammatory disorders but also regulate neurotransmitter synthesis and secretion. Histological findings suggest that administration of OPT improves brain tissue swelling, neuronal apoptosis, and downregulation of expression of the intracellular markers of Alzheimer's Disease (Tau and Aβ1-42). The 16S rRNA sequencing of the intestinal microbiota showed that OMO administration maintained the diversity and stability of the number of microbes. Furthermore, OMO regulates the composition and metabolism of the gut microbiota in inflammatory bowel disease (IBD) mice given high doses of antibiotics, thus showing prebiotic potential. | [60] |
| Xylooligosaccharide (XOS) | 10% XOS in PBS (phosphate buffer saline) 1 ml/day for 12 w | <i>Wistar</i> Mouse that is given high fat diet | Prebiotics increased hippocampal plasticity and improved brain mitochondrial dysfunction in mice fed a high-fat diet. Oxidative stress and apoptosis in the hippocampus were significantly reduced and prebiotics also decreased microglial activation, thereby improving cognitive function recovery. | [61] |

SCFA (Short-chain fatty acid)

SCFA is a small organic monocarboxylic acid that has up to six carbon atoms chain lengths. It is the main product of anaerobic fermentation of indigestible polysaccharides, such as dietary fiber and resistant starch produced by microbiota in the large intestine [62]. Most of the SCFA consists of acetate (C2), propionate (C3), and butyrate [59, 60] in estimated molar flow rates of 60:20:2 [65], respectively. Approximately 500–600 mmol of SCFA is produced in the intestine per day, depending on dietary fiber content, microbiota composition, and intestinal transit time [62, 63]. Although the largest source of SCFA is anaerobic fermentation of fiber, acetate, propionate, and butyrate can also be produced from amino acid metabolism [68], where less than 1% of the large intestinal microbiota uses this metabolic pathway to produce SCFA [65, 66]. Protein fermentation usually occurs in the distal large intestine. When carbohydrates are completely fermented, they lead to the production of potentially toxic metabolites, such as ammonia, phenols, and sulfides, as well as branched-chain fatty acids (BCFA) [65, 67]. The acetate produced from acetyl-CoA derived from glycolysis in this case can also be converted into butyl-CoA enzymes: acetyl-CoA transferase [68, 69], and cow's milk fat also provide a source of butyrate [74].

SCFAs are absorbed by colonocytes, mainly via H⁺(MCT) or sodium (SMCT) dependent monocarboxylate transporter [75]. MCT shows different subtypes and expression patterns in different tissues. SCFA that are not metabolized in colonocytes are transported to the portal circulation and used as an energy substrate for hepatocytes [76], except for acetate, because these acetates are not oxidized in the liver [76]. Therefore, only a fraction of the acetate, propionate, and butyrate derived from the colon reaches the systemic circulation and other tissues [65]. Studies on SCFA in humans are mostly influenced by microbes and use fecal concentration as a parameter of SCFA production in the large intestine [77]. Although this is a valid approach, there are many potential sources of bias, such as intestinal transit and permeability, metabolite transport, and sample handling [78]. Thus, all these potential sources of bias should be considered when inferring the effect of SCFA, given that some experiments may be carried out under non-physiological conditions.

SCFAs play a role in improving gut health by maintaining the integrity of the intestinal barrier, producing mucus, and providing protection against inflammation and thus reducing the risk of colorectal cancer [81–84]. Although a comprehensive understanding of SCFA-induced signaling is still very limited, it is well known that SCFA binds to G protein-coupled receptors (GPCR). The most studied SCFA-related receptors are GPR43 and GPR41. These two receptors were later renamed free fatty acid receptors (FFAR2 and FFAR3), as well as GPR109a/HCAR2 (hydrocarboxylic acid receptors) and GPR164, which are expressed in various cells, starting from the mucosa of the digestive tract to the immune and nervous systems [85, 86]. The effect of activating these receptors is very different and depends on the cell where the receptor is expressed. For example, binding of SCFA to its receptors on enteroendocrine cells results in the secretion of glucagon-like peptide 1 (GLP-1) and peptide YY

(PYY) [85], while signaling on β-pancreatic cells also contributes to increased insulin secretion [86].

Another mechanism of SCFA in regulating systemic function is through inhibition of histone deacetylase (HDAC) activity, thereby promoting acetylation of lysine residues present in nucleosome histones in various cell populations [87]. This intracellular signaling mechanism has been found, both in the gut and associated immune tissue [88], as well as in the peripheral nervous system and central nervous system [87].

The effect of SCFA on different organs and systems has now been widely described, although only a small fraction of SCFA in the large intestine reaches the systemic and tissue circulation. One of the effects of SCFA on the immune system is butyrate can induce Treg differentiation and control inflammation [73, 91-93]. Effects on activation of brown adipose tissue [93], regulation of liver mitochondrial function [94], body-wide energy homeostasis [95], and control of appetite [93] and sleep [96] have been associated with all types of SCFA.

SCFA and the brain

SCFA plays an important role in the microbiota-gut-brain communication process. Mobilization of SCFA in the microbiota-gut-brain communication process is aided by the expression of MCTs in endothelial cells [71, 98]. SCFA can be detected in human cerebrospinal fluid (CSF) in the range 0–171 µM for acetate, 0–6 µM for propionate, and 0–2.8 µM for butyrate [98]. Butyrate levels in the brains of rats supplemented with *Clostridium butyricum* reached a range of 0.4 to 0.7 µmol/g, where these levels were found to be higher than levels in peripheral blood [26, 100]. SCFA are also important in maintaining the integrity of the blood-brain barrier related to the distribution of molecules and nutrients from the circulatory system to the brain. In addition, other functions of SCFA are related to brain development and central nervous system homeostasis. Evidence from research in experimental animals reports that SCFA can regulate the function of the blood-brain barrier. Germ-free mice have shown decreased expression of tight junction proteins, such as claudin and occludin, resulting in increased permeability of the blood-brain barrier from intrauterine life to adulthood [100]. The germ-free mice then undergo recolonization with a complex microbiota or monocolonization with butyrate-producing bacterial strains. The results obtained were that butyrate can improve the integrity of the blood-brain barrier [100]. Likewise, an *in vitro* study of propionate-treated cerebrovascular endothelial cells showed that propionate can reduce permeability caused by exposure to lipopolysaccharide (LPS) [101].

Several studies have shown that SCFAs that enter the central nervous system have neuroactive properties, although the exact mechanism is unclear. Many animal studies have found that SCFA exerts a major influence on neurological function and behavior as well as improvements in neurodevelopmental and neurodegenerative disorders [73, 13, 74, 78, 103].

The formation of a healthy nervous system is essential for cognitive, emotional, and social functioning. Glial cells, especially microglia

cells, play an important role in the maturation and completion of the communication process between nerve cells [104, 105]. Therefore, the control of the innate immune function in the central nervous system is essential for brain development where the gut microbiota plays an important role in the immunological function of the central nervous system. One study explained how the microbiota could influence microglial maturation and function [32]. Microglia from mice colonized with SCFA-producing microbiota showed normal maturation and function, whereas non-colonized mice (given antibiotics) showed delayed development of microglia. Oral administration of a mixture of three types of SCFA consisting of acetate, propionate and butyrate plays a significant role in the maturation of microglia [32], although the mechanisms involved in the control of maturation and function of microglia by SCFA have yet to be investigated further. It is possible that FFAR2 receptor activation may be instrumental in this process because mouse microglia lacking the FFAR2 receptor were observed to be similar to microglia in non-colonized mice [107].

Neuroinflammation is also an important process that affects brain function. Based on the results of observations in antibiotic-induced mice, it was found that systemic disruption of the gut microbiota by antibiotics resulted in changes in immune response, especially to the pro-inflammatory profile [32]. Neuroinflammation is also an important process that affects brain function. Based on observations in antibiotic-induced mice, systemic disruption of the gut microbiota by antibiotics results in altered immune responses, particularly to the pro-inflammatory profile [108]. This causes the central nervous system to become more susceptible to increased intensity of the inflammatory response when antibiotics eliminate all microbiota [108–110]. This suggests that antibiotic-induced disruption of the diversity of gut microbes affects inflammation of the nerve cells characterized by changes in microglial morphology [111–113]. In addition, several studies have reported that sodium butyrate is able to decrease microglial activation and secretion of pro-inflammatory cytokines [114]. Likewise, acetate administration in microglia primary cultures has been shown to reduce inflammation through

decreased expression of IL-1 β , IL-6, and TNF- α as well as phosphorylation of p38 MAPK, JNK, and NF- κ B [115]. Likewise, acetate, which is also capable of modulating inflammatory cytokines and signaling pathways in the primary culture of astrocytes [116]. Although the mechanism of the effect of SCFA on microglia still requires further study, the inhibition of HDAC which epigenetically results in gene expression has been considered as the main effector mechanism induced by SCFA [117]. Thus, the role of acetylation in modulating glial cells is by means of anti-inflammatory and as neuroprotective. By considering the role of microglia in influencing the formation of neural tissue as well as the influence of microbiota on this process, SCFA may provide a new way to modulate impaired immunity in the brain that causes neurodevelopmental and neurodegenerative disorders.

In addition to providing energy for cells and influencing the maturation of microglia, SCFA also affects neuron function. It has been described that SCFAs can modulate levels of neurotransmitters and neurotrophic factors. Acetate has previously been shown to alter levels of the neurotransmitters glutamate, glutamine and GABA in the hypothalamus and increase expression of anorexigenic neuropeptides [118]. Propionate and butyrate exert influence on intracellular potassium levels as evidence of the involvement of SCFA in the cell signaling system [119]. SCFA specifically regulates the expression levels of tryptophan 5-hydroxylase 1, an enzyme involved in serotonin synthesis, and tyrosine hydroxylase, an enzyme involved in the biosynthesis of dopamine, noradrenaline and adrenaline; therefore, it produces effects on brain chemical components [13, 119–122]. Elimination of the microbiota by antibiotics can result in hippocampal neurogenesis and memory impairment, which can be partly reversed with the administration of a microbiodator and can be fully recovered with probiotic treatment or exercise [124]. The existence of this cognitive deficit is related to changes in the expression of signaling molecules that are related to cognition, such as brain-derived neurotrophic factor (BDNF), N-methyl-D-aspartate receptor subunit 2B, serotonin transporter, and neuropeptide Y system [125].

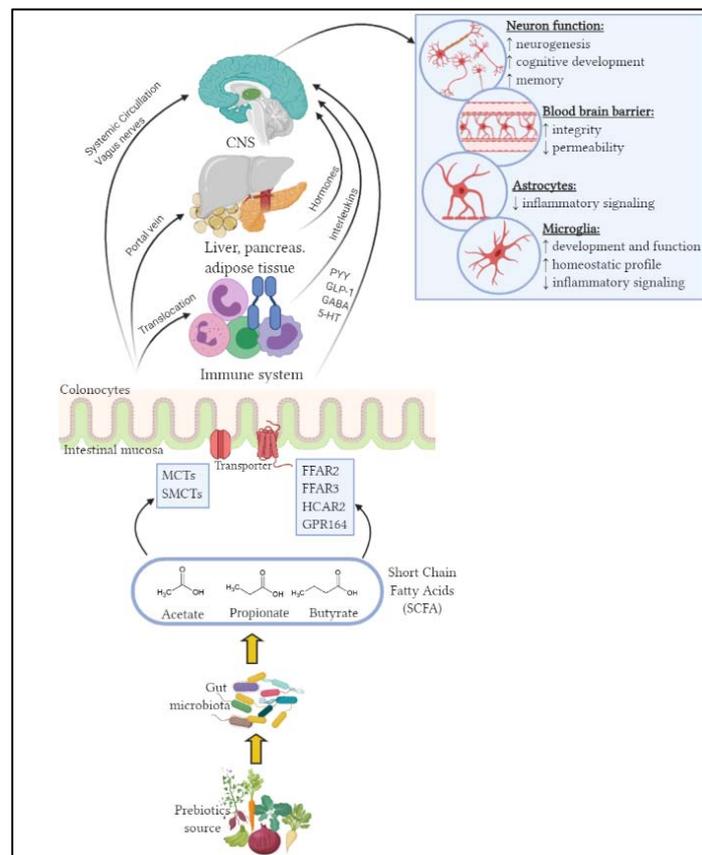


Fig. 3. Mechanism of SCFA in influencing gut and brain communication [98]

Neurotrophic factors, including nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), and BDNF which regulate growth, survival and differentiation of neurons and synapses in the central nervous system, also play an important role in thinking and memory processes in various brain disorders. These factors have been shown to be modulated by SCFA [50, 125–127]. BDNF expression, neurogenesis, nerve proliferation in mice [128–130], as well as long-term memory consolidation can be stimulated by sodium butyrate [132]. Furthermore, all three types of SCFA have been shown to increase the growth rate of human progenitor nerve cells and encourage more cells to undertake mitosis [133]. This provides some clues as to how SCFA may regulate early nervous system development. SCFA exhibits effects on several neuronal functions, such as improving sleep cycles [96], suppressing orexigenic activity in neurons expressing neuropeptide Y in the hypothalamus [93], modulating signaling processes triggered by ghrelin receptors [134], as well as contributing to circadian rhythm and appetite control. The mechanisms involved in the modulation of nerve function by SCFA have revealed that some of these effects may be mediated by activation of the GPR41/GPR43 receptor. The effects of other SCFAs, particularly propionate and butyrate, are mediated through HDAC inhibitory activity [111, 121].

SCFAs may directly influence the brain by strengthening BBB integrity, modulating neurotransmission, influencing levels of neurotrophic factors and increasing memory consolidation. However, more research is needed to understand the exact mechanisms involved in these neuroactive effects.

CONCLUSION

In recent years, research related to the gut microbiota has been highly developed in the biomedical field. Recent research has found that an imbalance in the number of probiotic and pathogenic gut bacteria contributes to several neurological disorders, particularly acute ischemic stroke. In order for probiotic bacteria to survive and multiply in the digestive tract, it is necessary to use prebiotics. Prebiotics cannot be digested by the digestive tract enzymatically so fermentation by probiotic bacteria in the large intestine is required to produce metabolites, one of which is short-chain fatty acids (SCFA). SCFA (short-chain fatty acids), which are the end products of fermentation by the intestinal microbiota, play a role in gastrointestinal physiology, immune function, metabolism, and even in the development and homeostasis of the central nervous system.

The two-way communication that occurs between the microbiota and the brain can be mediated through various immunological mechanisms. The mechanism of microbiota-gut-brain communication process still needs further study, although there are many explanations about it. Most of the studies are still being carried out on experimental animals so readers should be careful when attributing the effects of SCFA to humans. SCFA (short-chain fatty acids) metabolites can modulate the *microbiota-gut-brain* axis process and have a positive impact on the brain in direct and indirect ways. From this process, it can further assist in developing new therapies to treat neurological disorders, especially acute ischemic stroke.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Author declares no conflict of interest

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