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Review

Probiotic as A Potential Gut Microbiome Modifier for Stroke Treatment: A Systematic Scoping Review of *in vitro* and *in vivo* Studies

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Abstract: Background: Pharmacologic and non-pharmacologic treatments for stroke are essential but could be costly or harmful whereas probiotic has been a promising alternative. This scoping review aimed to synthesize the *in vitro* and *in vivo* evidence of probiotics on stroke-related neurological, biochemical, and histochemical outcomes. Method: We searched in PubMed, Embase, and Cochrane Central Register of Clinical up to May 7, 2021, and screened by two independent reviewers. We included the use of probiotics, prebiotics, and symbiotics both in vitro and in vivo for the prevention or treatment of the stroke-related model. Result: Of 6,293 articles, 4,990 passed the initial screen, of which 36 theme-related full-texts were assessed and 13 were included in this review. Probiotics could ameliorate the neurological deficit and show their property as an anti-inflammation and anti-oxidative stress. Histopathologically decreased loss of cerebral volume and inhibition of neuronal apoptosis were found. Conclusion: There are potential cognitive benefits of probiotic supplementation, especially among animal models, on decreasing cerebral volume, increasing neurological score, and decreasing the inflammatory response. However, further investigation is needed to validate these conclusions in various populations.

Keywords: probiotic; stroke; gut microbiome; meta-analysis

1. Introduction

Stroke is a cerebrovascular disease that has been known to be a significant burden in low to middle-income countries and to be declining in high-income countries. In 2019, stroke was identified as the second leading cause of disability-adjusted life years (DALY) worldwide [1]. Numerous rehabilitation programs and interventions that require multimodality teams, both pharmacologic and nonpharmacologic, have been conducted. However, the outcomes have not met the needs of the burden of this dis-ease [2]. Several pharmacogenetic drugs have been investigated for use in stroke, including serotonergic drugs and dopaminergic drugs [3], but these drugs may cause a variety of side effects, including sexual dysfunction, weight gain, insomnia, or somnolence in SSRIs, and gastrointestinal side effects, motor disturbances, and others in dopaminergic drugs [4,5]. These potential side effects may have a significant impact on the patient's quality of life if taken for an extended period. Stroke patients require interventions with lower costs and fewer side effects, such as probiotics. Probiotics are live microorganisms found in a variety of foods that may benefit a variety of human organ systems and conditions, including the immune system, dysbiosis, and others [6]. A recent study discovered that the gut microbiota-brain axis, which is involved in multiple systems, including the endocrine, neurological, and digestive systems, had a direct effect on these systems and may be used cautiously in

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certain psychiatric diseases such as depression [7]. The gut microbiota-brain axis was also discussed in a study involving stroke and the microbiota, in which it was found that altering the gut microbiota could affect stroke outcome via bottom-up pathways [8]. These pathways are further investigated in other studies looking at the use of probiotics to alter the gut microbiota-brain axis, which found that probiotic use could improve the outcome of stroke model disease.[9] To our knowledge, few systematic reviews have been conducted on this topic and additional investigation is required. Therefore, the aim of this systematic review is to further investigate the outcome of probiotic use for the treatment of in vitro and in vivo study models of stroke disease and see if it could serve as part of an effective, low-cost treatment plan in some stroke patients.

2. Materials and Methods

2.1. Registration of protocol

This study was guided by the recommendation of The Preferred Reporting Items of Systematic reviews and Meta-Analyses Extension for scoping Review (PRISMA-ScR) statement.

2.2. Data sources and searches strategy

We used three databases including PubMed, Embase, and Cochrane Central Register of Clinical Trials to search for publications in the English language up to May 7, 2021. The terms "Probiotics", "Prebiotics, and "Symbiotic" were used in combination with "Ischemic Stroke", "Cerebrovascular Disorders", and "Brain Ischemia" as the keywords for a systematic literature search along with any synonyms. The detail of the search term is presented in the Supplementary Material. In addition, the reference lists of included articles were searched, as well as relevant citations from other journals via Google Scholar.

2.3. Study selection

In this systematic scoping review, we worked with an information specialist to design an appropriate search strategy to identify original peer-reviewed articles that look at the use of probiotics, symbiotics, or prebiotics as a form of prevention or treatment for stroke. We defined that the population of each study could be conducted either in vitro (using components of an organism that have been isolated from their usual biological surroundings, ex. microorganisms, cells, or biological molecules) or in vivo (living organisms, ex. animals). The interventions were probiotics, symbiotics, or prebiotics compared with placebo. Probiotics were further categorized into food and non-food based. Food-based probiotics ranged from ginseng, and yam gruel to fermented soybeans, which have been traditionally used in Chinese traditional medicine [10]. Nonfood probiotics were composed of a variety of probiotics ranging from single-strain bacteria, such as *Clostridium butyricum* or lactobacillus, to a mixture of probiotics. *Clostridium butyricum* has been known to be a probiotic commonly used and studied in Asia with an effect on modulating immune processes and inflammatory processes in the intestinal tract [11]. Article screening for eligible studies that correlated with our inclusion and exclusion criteria was conducted by two independent reviewers (CSam and TN). Discrepancies between the two reviewers were resolved by consensus.

2.4. Data extraction

Data extraction was done by two independent reviewers (CS and CSam) for a published summary of probiotic effects in stroke animals and in vitro studies The following data was extracted: Study characteristics (authors, year of publication, study type, journal name, contact information, country, and funding); Intervention characteristics (probiotics, symbiotic, and prebiotics; food-based vs non-food based; the number of case and control); and outcome characteristics (neurological outcome, biochemical profile, dysbiosis index, and histopathology). We included all associated text, tables, and figures for extraction. We excluded non-original articles (review article, protocol, letter, comment, and guidelines);

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non-human studies; unpublished data or non-peer-reviewed data; and studies published in languages other than English.

2.5. Data synthesis and analysis

The primary outcomes included in the systematic review are neurological outcome, biochemical profile, dysbiosis index, and histopathology among in vitro and in vivo study models.

2.6. Patient and public involvement

There was no patient or public involvement in the study. However, the results of the studies included in this review (both in vivo and in vitro) could promote further research on this topic.

3. Results

3.1. Studies characteristics

The database search identified 6,293 potential records. After removing duplicates, 4,990 titles passed the initial screen, and 36 theme-related abstracts were selected as further full-text articles to be assessed for eligibility (Figure 1). A total of 23 articles were excluded for the following: 6 non-peer-reviewed, 4 duplicates, 4 protocols, 4 wrong interventions, 3 wrong outcomes, and 2 non-English languages. 13 studies were eligible for the data extraction and data synthesis.

There were 13 included studies with probiotic intervention which evaluated neurological outcome, biochemical, or histopathology. All studies were animal studies with 8 rats (61.5%), 4 mice (30.8%), and 1 gerbil (7.7%) as a study model. The study period was from 2004 to 2020. There was no in vitro study included. The setting took place in Northern America, Europe, and Asia. There was no study from Australia, Africa, and Southern America.

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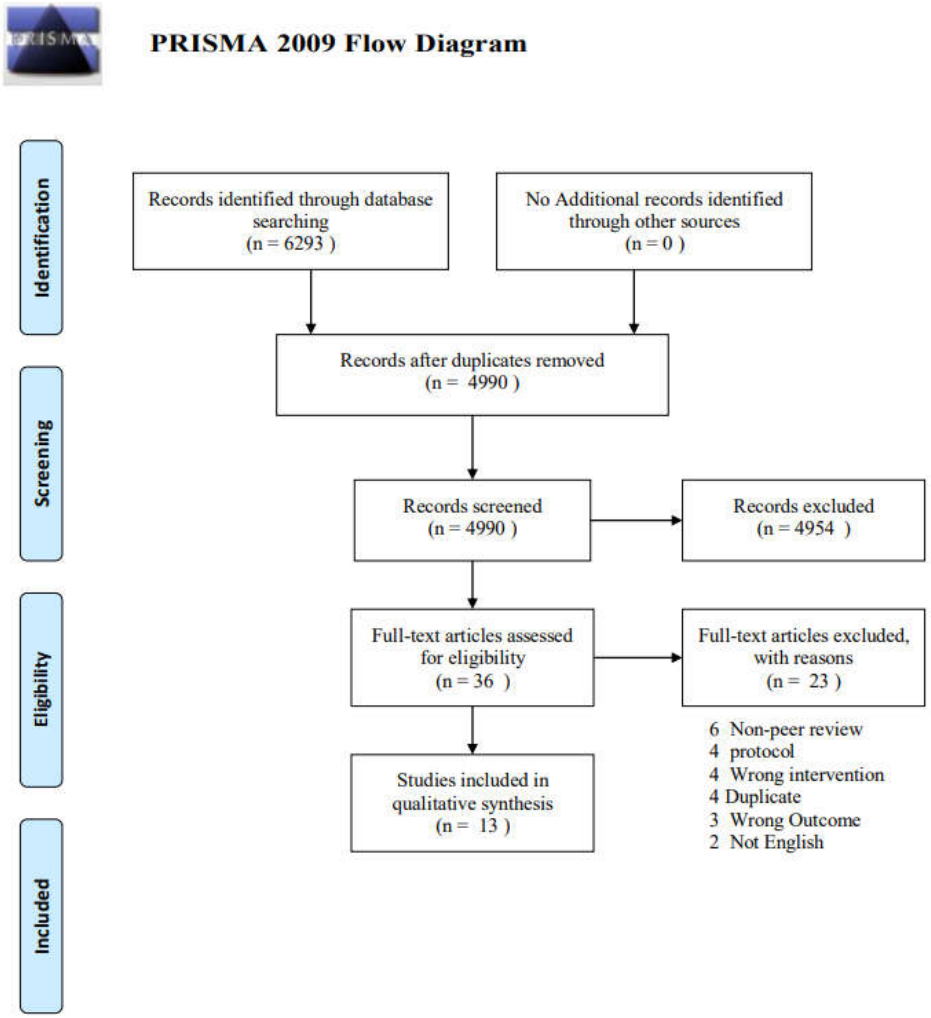

Figure 1. PRISMA Flow Diagram.

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3.2. Summary of probiotic impact in stroke-induced animal

There were multiple parameters used to evaluate the effect of probiotics in the animal model. Of the 13 included articles, both neurological outcomes and biochemical profiles were measured in 11 articles (84.6%). Similarly, histopathology was measured in 11 out of 13 studies (84.6%).

3.2.1. Neurological test

Most of the studies [9,12-20] showed that probiotic use could de-crease neurological deficits and some claimed that it can improve spatial learning ability and cognitive function. Tests used to measure cognitive functioning in the studies include Neurological Function Score [9,12-23], Morris Water Maze [15,22,23], and Open Field Test [23]. Morris water mazes were used to evaluate the capability of rodent spatial learning by navigating and locating the escape platform of the maze [24]. Open field tests were used in the observation of exploratory behavior by focusing on the movement activity of rodents, which could be related to motor power, emotional, and other instinctive behaviors such as fight and fear [25].

3.2.2. Biochemical level

The inflammatory response was also found to be impacted by probiotic use, including TNF alpha (26,28) and Interleukin 1 (IL-1) [26,28] beta, which show an anti-inflammatory effect of probiotics [14,15]. TNF alpha is secreted by macrophages and is responsible for acute inflammation processes that lead to cell necrosis or apoptosis [26]. IL-1 is known to be the regulator of inflammation and is involved in a variety of pathways, including the innate immune process which causes a range of functions from leukocytic pyrogen and fever to an activated immune system [27]. Other studies found that probiotics had been involved in decreasing multiple factors in metabolic pathways such as lipid profile, blood sugar and SCFAs [12,16,28]. Decreased oxidative stress has also been reported after using probiotics [15,19].

3.2.3. Histopathology

Some researchers have found that probiotics could be used to decrease cerebral infarction volume [19,20,24,26,32,33]. Moreover, probiotics have been found to inhibit neuronal apoptosis [30,31,32], which correlates with results from previous studies. Probiotics can also play a role in the immunologic pathway of the brain and decrease changes within the hippocampus [27,29,31].

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Table 1. The characteristics of the articles which probiotics are the intervention in stroke mice model.

Author	Country	Type of intervention		Animal type	Number of case	Number of control	Measure outcome	Result of outcome
		Non-food based	food-based					
Akhoundzadeh, 2018[9]	Iran	Combination of 4 viable probiotic bacteria strains, namely Bifidobacterium breve, Lactobacillus casei, Lactobacillus bulgaricus (Lactobacillus delbrueckii subsp. bulgaricus), and Lactobacillus acidophilus		Mice	5	5 mice received saline, 5 mice were sham operated	Infarct size, neurological outcome, biochemical markers	Reduced infarct size 52%, could not improve neurological function, decrease the malondialdehyde content and TNF alpha level
Bae, 2004[34]	South Korea	Red ginseng and fermented red ginseng		Rat	Ginseng 5 , red ginseng 5 , fermented red ginseng 5	10	Infarction area, volume	Fermented red ginseng treated group reduction of the infarction area in all regions and total infarction volume
Chen R, 2019[35]	United States		Puerariae Lobatae Radix(PLR)+Chuanxiong Rhizoma(CXR)	Rat	Not found	Not found	Neurological function score, body weight gain, cerebral infarction area, serum level of LDL/HDL/TG/TCHO/ blood viscosity/fibrinogen level/ platelet aggregation rate	Repair neurological impairment, reduce the cerebral infarction, reverse the dyslipidemia, reduce the blood viscosity and thrombotic risk
Li, 2018[13]	United States		Panax Notoginsenoside extract(PNE)	Germ free rat	Not found	Not found	Neurological evaluation, TTC assessment of infarct size, pro inflammatory cytokine/BDNF/GABA in rat hippocampus	Decrease neurological deficit scores, Decrease cerebral infarct volume, Upregulate the expression of GABA receptor in hippocampus
Liu, 2015[23]	China	Clostridium butyricum		Mice	12	12	behavioral tests , Open field test Morris water maze	Improves Spatial Learning Ability, Ameliorated the Morphological Changes in the Hippocampus, increase butyrate in the brain

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Mei, 2017[14]	China	Shuan tong ling	Rat	Not found	Not found	Neurological deficit, infarct volume, inflammatory cytokines (TNF alpha, IL 1beta)	Reduce infarct volume, increase neurological scores, decrease TNF alpha and IL 1beta
Nagao, 2019[22]	Japan	fermented ginseng	Rat	Not found	Not found	Spatial memory evaluate using Morris water maze (MWM), use neuronal nuclei positive cells to assess hippocampus neuron loss, protein expression of caspase3/Iba1/glial fibrillary acidic protein	Shortened the extended time to reach the platform in the MWM, ameliorated loss of hippocampus cornu ammonis neurons and increase caspase3 / Iba1
Pang, 2020[15]	United Kingdom	yam gruel	Rat	9	18	Neurological deficits, MWM test (spatial learning and memory function), SOD and MDA measurement, TNF alpha and IL 1beta and LPS measurement, characteristic of gut microbiota	Improve cognitive function : increase relative content of probiotic bacteria and SCFAs in intestinal tract , cerebral cortex reduced oxidative stress and inflammatory response, promote the expression of neurotransmitters and brain derived neurotrophic factor
Park, 2016[16]	Korea	Chungkookjang (fermented soybean)	Gerbil	Not found	Not found	Neuronal cell death and cytokine expression in hippocampus, neurological deficit, serum cytokine levels, glucose metabolism	Prevent the neuronal cell death and symptom such as dropped eyelid/bristling hair/reduced muscle tone and flexor reflex/abnormal posture, suppress cytokine expression, prevent the impairment of glucose metabolism

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Rahmati, 2019[17]	Netherlands	Commercial probiotics (LactoCare capsule, 109 CFU, ZIST TAKHMIR, Tehran, Iran), which are a mixture of seven probiotic bacteria strains, including Lactobacillus caseiZT-Lca.106, Lactobacillus acidophilus ZT-Lac.51, Lactobacillus rhamnosus ZT-Lrh.54, Lactobacillus bulgaricus ZT-Lbu.90, Bifidobacterium breve ZT-Bbr.22, Bifidobacteriumlongum ZT-Lca.106, and Streptococcus thermophilus ZT-Sth.20	Mouse	30	20	Spatial and learning memory, histological damage and apoptosis, malondialdehyde (MDA) content and brain-derived neurotrophic factor (BDNF) level	reduced spatial memory impairment and neurological dysfunction, MDA and BDNF change was not significant
Sun, 2016 [18]	Netherlands	Clostridium butyricum	Diabetic mice	Not found	Not found	Cognitive impairment, blood glucose level, neuronal injury, apoptosis, expression of Akt/p-Akt/caspase3 level	Ameliorate cognitive impairment, ameliorate histopathologic change in the hippocampus, Decrease blood glucose level, increase p-Atk expression and decreased caspase3 expression = inhibit neuronal apoptosis
Wanchao, 2018[19]	China	Inactivated lactobacillus	Rat	24 (Divide into 4 groups with varies concentration)	6	Cerebral infarction volume, neurolobehavioral scores, SOD + MDA levels ( ), tunnel and TLR4/ IkB/A20 (cell apoptosis)	ILA ; neurobehavioral scores improve/ cerebral infarction volume decrease/neural cells apoptosis decrease/ MDA level decrease / SOD activity increase/ inhibit expression of TLR4 and

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							promote the expression of IkB and A20 = reduce oxidative stress
Zhang, 2019[20]	Netherlands	Chamomile	Rat	Not found	Not found	Neurological score following MCAO; infarction size and neurological deficits, protein levels of Nrf2/Keap1/HO1/ERK	Decrease in both infarct volume and neurological scores following MCAO, significantly increase the activity of HO1 and Nrf2

4. Discussion

This systematic review showed that a variety of probiotic types have been used in interventions for stroke treatment, which makes it difficult to compare results between the studies. Despite these differences, we found similar results in multiple parameters including neurological score and brain volume, which suggests that probiotic supplementation could be a beneficial component of rehabilitation programs in post-stroke patients. For example, a systematic review of probiotic use in human stroke patients in other modalities was also published and found that probiotics in enteral nutrition can improve clinical parameters including, infection events, intestinal dysbiosis, gastrointestinal complications, and nutritional status. [29] However, more research is needed due to the lack of literature available on probiotic supplementation in human specimens and its effect on neurological modalities.

4.1. Probiotic and inflammatory effects

There has been growing evidence that supports beneficial effects of inflammatory processes in various aspects of ischemic cerebral disease. To illustrate a bidirectional process that involved peripheral immune response and the cerebral ischemic brain model. Many pathways were found in the inflammatory process including: interleukin 1 as protection of hippocampal CA1 in gerbil, T-reg in postischemic anti-inflammatory effect through use of IL-10 secretion in ischemic brain tissue, and the complex role of neutrophils that could destabilize the blood-brain barrier by enzyme secretion and create inflammation by blocking vascular supply. [30] These pathways are linked to the essential role of inflammation in ischemic brain disease.

Although not fully understood, the gut-brain axis has been known to be the novel bidirectional pathway involving the gut and brain. More-over, it is thought to involve the vagus nerve in multiple complex pathways including the enteric nervous system, neuronal-glial endothelial interaction, and immune and inflammatory cell response involving DAMPS and cytokine. Previous associations have also been found to suggest gut dysbiosis or an altered strain of gut microbiota to be preset in stroke patients.[31] Thus, some studies have included probiotic supplementation as a primary form of intervention found probiotic use to improve the neurological outcome by using the bidirectional pathways [32].

4.2. The use of the hippocampus in measured outcome

The hippocampus is a known part of the limbic system and plays a critical role in many neurological diseases, including Alzheimer's disease. This part of the brain has various functions, NB ,including memory, mood, emotional drive, and spatial navigation. Thus, much of the research presented in our study used many components from the hippocampus, such as neuron loss as the neurological outcome, to evaluate the effect of probiotics in stroke animal models [33].

4.3. Low-quality paper with no control or vague parameter

We found that many studies didn't use case and control comparison, which prevented us from being able to qualitatively interpret the results, and thus acts as a limitation of this review. Moreover, the variety of parameters used to evaluate the outcome of probiotic use made it difficult to form a conclusion from this review.

4.4. Further research

This systematic review suggests that the lack of standardization among probiotic use in the studies poses a major obstacle in interpreting the role of probiotics on stroke treatment. Therefore, more in-depth studies of each probiotic are needed to consider changes to the the mainstay of treatment in stroke patients. Moreover, the scarcity of in vivo studies further emphasizes the need to conduct more research on this topic. Investi-gating the effects of probiotic supplementation on stroke patients in great-er detail could ultimately lead us to a cheaper and nonpharmacologic treatment option for post-stroke patients.

5. Conclusions

Current literature suggests that there are potential cognitive benefits of probiotic supplementation, especially among animal models, on decreasing cerebral volume, increasing neurological score, and decreasing the inflammatory response. However, more research is needed to confirm these suggestions among other populations.

Supplementary Materials: Table S1: Probiotic Search Term.

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