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THE ROLE OF THE MICROBIOTA IN ALS PATHOGENESIS AND PROGRESSION: A LITERATURE REVIEW

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ABSTRACT

Introduction

In recent years there has been a great interest in investigating the intestinal microbiota and its role both in healthy people and in various pathologies. This has given rise to the concept of the gut-brain axis, where the relationship between the microbiota and neurological pathologies, such as amyotrophic lateral sclerosis, whose pathogenesis is still unclear, is studied

Development

This review provides an overview of the role of the gut microbiota in the pathogenesis and progression of amyotrophic lateral sclerosis.

Conclusions

There is evidence of the role of the modulation exerted by the microbiota on the immune state as well as its direct effects on the CNS.

Keywords: Amyotrophic Lateral Sclerosis; Microbiota; Neurodegenerative diseases

Introduction

Since the beginning of this century, interest in the intestinal microbiota has emerged because of the theory of holobiont evolution and the advent of metagenomics. From there, the scientific production related to the microbiota is increasing, in which its role in different pathologies has been discovered, including amyotrophic lateral sclerosis [1].

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease of unknown cause, characterized by the simultaneous involvement of upper and lower motor neurons, producing motor conditions such as muscle weakness [2,3].

Methodology

We reviewed the role of the intestinal microbiota by performing a bibliographic search in Pubmed, Scielo and Google Scholar with the terms "Microbiota" "Amyotrophic lateral sclerosis". 37 were selected to make up this manuscript. All available articles written in Spanish or English were found and duplicate articles were excluded.

Definition and etiopathogenesis of ALS

Amyotrophic lateral sclerosis (ALS) is a progressive, fatal, and multifactorial neurodegenerative disease that affects the motor system, causing selective degeneration of upper and lower motor neurons, producing muscle weakness, as well as cognitive or behavioral alterations [4].

Its global incidence corresponds to 1.7 per 100,000 inhabitants and its prevalence is 4.1 to 8.4 people per 100,000 inhabitants. Its incidence is higher in European countries (2.1 to 3.8) compared to Asian (0.8 to 1.2) and American (1.75 to 3.4) countries (1.2).

Its age of presentation is variable, registering a higher incidence in patients between 45 and 70 years.

The pathogenesis of ALS includes aggregation of insoluble proteins within cells and abnormal activation of immune cells. In almost all patients, there is an accumulation of tar DNA-binding protein 43 (TDP-43) plus other proteins, but a small group have accumulations of superoxide dismutase 1 (SOD1). Evidence supports that some of the aggregated proteins can be transferred from cell to cell in a prion-like manner, which could explain the distinctive spread of weakness from the initiation site [5,6].

ALS can be sporadic (SALS) or familial (FALS) due to genetic mutations, since certain genes have been identified as susceptibility markers, but there is no consensus on this aspect in large-scale studies [2,3]. ALS is generally considered to arise from a combination of genetic susceptibility and environmental exposure [6]. The identified risk factors are advanced age, family history and male gender [2,3].

Life expectancy for ALS is 1 to 3 years after diagnosis and 2 to 5 years after disease onset, often due to respiratory muscle failure [5].

Gut-brain axis

During the last decade, the study of the relationship between the intestine, the development of the central nervous system and the microbiota, a system known as the “gut-brain axis”, which includes neural, immune, endocrine and metabolic pathways, has intensified [7].

It could then be defined that the intestine-brain axis is a bidirectional communication pathway between the Central Nervous System, the gastrointestinal tract and the Intestinal Microbiota, mediated by products generated by bacteria that act at the systemic level, as well as by endocrine and neuronal mechanisms [8].

Different studies in germ-free animals and in animals exposed to pathogenic microbial infections, antibiotics, probiotics, or fecal microbiota transplantation suggest a role for the gut microbiota in host cognition or in the pathogenesis of different neurodegenerative diseases. In fact, it is known that

microbiotadysbiosis-induced increase in intestinal and blood-brain barrier permeability can mediate or affect the pathogenesis of several neurodegenerative disorders, especially those associated with aging [3,7,10].

It has also been shown that the imbalance of the intestinal microbiota can induce inflammation associated with the pathogenesis of obesity, type 2 diabetes mellitus, Alzheimer's disease, Parkinson's disease, and ALS, through the modulation of protein misfolding, aggregation, and transmission from the periphery to the CNS [3,7,9].

It is known that patients with different psychiatric disorders such as depression, bipolar disorder and autism, as well as patients with different types of allergies, have significant differences in their microbiota compared to healthy people [3,10,11].

Microbiota and ALS pathogenesis

The term “microbiome” alludes to all the microorganisms living on the body, while the term “microbiota” refers to the microorganisms living in specific locations, such as the gut. Many studies have shown that gut microbiota is implicated in neurodegenerative diseases such as Parkinson's and Alzheimer's diseases. In ALS, this information is limited [6].

The gut acts as a barrier to harmful substances. Dysbiosis can lead to increased intestinal permeability, which facilitates dietary toxins entrance, and consequential damage. Another function is the immune system modulation. Both the systemic and gut lymphoid cells in the gut play a role in the dysregulation of the peripheral immune system in ALS, because reduced levels of regulatory T cells have been associated with disease advancement. Monocytes are activated and dendritic cells also play a role [6].

In the brain and spinal cord of an ALS patient, microglia are activated, leading to neuroinflammation, which contributes to disease pathogenesis through several mechanisms, including the production of reactive oxygen species (ROS) and

cytokines inflammation, activation of the innate immune system, and infiltration of immune cells [12].

The gut microbiota has the ability to synthesize neurotransmitters (such as -amino butyric acid (GABA), norepinephrine, and dopamine) that modulate immune system activation [13], along with its ability to regulate the protein TLR2, which has been found to it has the ability to modulate neurotransmitter levels [14], therefore, it could potentially influence microglial activation and disease regulation.

On the other hand, protein fermentation occurs in the large intestine, where carbohydrates are already broken down and promote the synthesis of toxic metabolites, such as phenols, ammonia, and sulfides. Acetate molecules from acetyl-CoA derived from glycolysis could also be converted to butyrate molecules [15]. Short-chain fatty acids (SCFAs), in addition to exerting local influences in the colon, have an important role in microbiota-gut-brain crosstalk [16]. Zhang et al. [17] showed that treatment with 2% water-butyrate in an ALS mouse model restored gut integrity and survival, proposing that SCFA butyrate can be used as a powerful therapeutic agent for ALS.

Preclinical and clinical studies involving gut microbiota and ALS demonstrated a distinct microbial signature in ALS. Dysbiosis was identified before motor dysfunction onset and immune cell activation by using the mutant SOD1G93A mouse model, exhibiting a leaky gut, increased number of abnormal intestinal Paneth cells and decreased butyrate-producing microbial communities [5]. Mice treated with butyrate restored intestinal microbial homeostasis and decelerated ALS progression [17]. Moreover, enhanced intestinal permeability and diminished butyrate-producing bacteria (BPB) has been identified in ALS pathogenesis [18]. Gut bacterial dysbiosis and reduced formation of tight intestinal junctions were surpassed by butyrate oral administration. In addition, mice life spam was also prolonged by butyrate action [17].

Thus, microbiota may influence neuronal damage pathogenesis through the synthesis of neuroactive metabolites and toxins or via modulation of immune response, dietary compounds or drug metabolism [19].

Although the composition of the human microbiome varies in each individual due to different factors such as diet and age, similar proportions of bacterial families remain. In the first stage of ALS, there is dysbiosis, which has been characterized by the significant reduction of *Butyrivibrio fibrisolvens* and *Firmicutes* (butyrate producers), as well as a decrease in the expression of proteins involved in tight and adherens junctions. Additionally, a significant increase in the genera Dorea (commonly harmful microorganisms) and Anaerostipes has been observed, as well as a significant decrease in the genera *Prevotella* and *Lachnospiraceae*, beneficial microorganisms for ALS patients. Similarly, an increase in the *Bacteroidetes phylum* over *Firmicutes* has been recognized in ALS patients, as well as a low abundance of yeasts and a high abundance of *Escherichia coli* and *Enterobacteriaceae* [20].

Beneficial microorganisms of the genera *Faecalibacterium* and *Bacteroides* are reduced in ALS patients. It must be considered that the increase in nicotinamide, a product of Akkermansia muciniphila, has been related to motor and functional improvements in patients with ALS [20].

Therapeutic approaches aimed at restoring intestinal barrier function, such as curcumin or atorvastatin, are known to be related to neuroprotection, thanks to their anti-inflammatory properties [21,22].

Microbial taxa and ALS

Current evidence has shown a difference between gut microbiota composition in ALS diagnosed patients and healthy individuals. Important variations at taxonomic categories, from phylum to species have been identified. Microbiota modification is not limited to composition percentages of the same microbial taxon. Phylogenetic analyzes have shown the existence of 133 unique operational taxonomic units (OTUs) in ALS patients and 65 in healthy volunteers, in addition to 1,196 OTUs common to both [23]. In the light of recent studies, gut microbiota seems to be more diverse in ALS patients, in terms of species richness and evenness [23,24]. Nevertheless, other results indicate that as the disease

progresses the diversity of gut microbiota declines, probably due to a gradual disability causing limitations for usual dietary habits [25].

Microbial taxa decreased in ALS

Mouse models revealed a decrease of butyrate-producing bacteria (BPB) in ALS [26]. A significant intestinal reduction of BPB (*Lachnospiraceae*, *Anaerostipes Eubacterium rectale*, *Roseburia intestinalis*) in ALS patients was also reported [27]. Also, microbes belonging to the phylum *Firmicutes,* and to the genus *Megamonas* were scarce [28].

Microbial taxa increased in ALS

Zeng et al. found a significant increase of microbes from the phylum *Bacteroidetes,*the genus *Kineothrix, Parabacteroides, Odoribacter, Sporobacter, Eisenbergiella, Mannheimia, Anaerotruncus* and *Porphyromonadaceae* [28]. Furthermore, the class *Bacteroidia,* the order *Bacteroidales,* and the family *Porpyromonadaceae* were also present in a higher number in ALS cases [29].

Microbial metabolites

Microbe species are able to survive using similar or even the same enzymatic systems, then studying a specific taxa variation is not complete without exploring its functional characteristics [29].

By analyzing the integrity of metabolic pathways in which gut microbiota is involved, Hetzberg et al. [24] described lower levels of enzymes required for carbon metabolism and regulation of histidine kinase response, in addition to a complete lack of butyrate production pathway enzymes.

Role of microbiota metabolites in ALS

Microbiota-gut-brain axis metabolites

Microbiota metabolites may be involved in ALS pathogenesis and progression by reaching the CNS through the bloodstream. Such is the case of nicotinamide. Preclinical and clinical studies found systemic and CSF reduced levels of nicotinamide in individuals affected by ALS. It appears nicotinamide levels are

inversely proportional to disease progression [4,30]. Nevertheless, a cause-effect relationship has been found only in murine models, while human studies remain in a descriptive stage [4]. Similarly, several studies about the importance of microbiota-derived SCFA in neurodegenerative diseases have confirmed a low production of butyrate in ALS individuals compared to healthy controls [15,24,31]. SCFA, involved in regulatory T lymphocytes homeostasis, could be associated with the microbiota-gut-brain axis (MGBA) and the neuroimmune-endocrine axis [32].

Microbes and metabolites correlation was assessed by metabolomics and metagenomics. Results suggest higher levels of coproporphyrinogen and 4-hydroxybenzoylcholine in ALS patients. Also, lower levels of acylcarnitine, 2-(1-ethoxyethoxy) propanoic acid and 3,7-dihydroxy-12-oxocholanoic acid were found [28]. These findings contrast with those presented by Chen et al. [33] where a sample of 160 patients was compared with healthy controls and with their spouses. This study identified that carnitine and betaine levels were higher in ALS patients, while choline, trimethylamine N-oxide and butyrobetaine levels were lower for the comparison with age and gender matched healthy controls. The comparison with ALS patients' spouses only showed a significantly higher concentration of carnitine in ALS individuals.

A comparative study between ALS patients and healthy individuals, despite finding no significant differences, reported an increase trend of NO2-N/NO3-N and γ-aminobutyric acid, as well as a decrease trend of human endotoxin [34].

Zhang et al. [35] described the potential harmful effect of gamma-glutamyl amino acids, like gamma-glutamyl phenylalanine, 1-arachidonoyl-GPI and 3-methyl-2-oxobutyrate, which may increase ALS risk. The metabolites related to these molecules, produced by the action of the microbiota, are permeable through the blood-brain barrier and it has been described that they could induce brain disease. On the other hand, 4-acetamidobutanoate could lower ALS risk.

Microbiota and ALS progression

Multiple relevant studies have shown that microbiome has a contribution to ALS progression.

Intestinal motility governs absorption of nutrients through the enteric nervous system. Before ALS onset, 2-month-old mice that had lower intestinal motility, decreased grip strength and reduced time in the rotarod. Another study found that transgenic TDP43A315T mice had degeneration of nitric oxide synthase neurons in the enteric nervous system. Since nitric oxide synthase regulates intestinal peristalsis, there was inhibitory control that led to dysmotility, pseudo-obstruction and sudden death, it also showed downregulation of endogenous TDP-43 in spinal cord and brain prior to neurodegeneration [36]. A 2-sample Mendelian randomization study of 98 genera of the human gut proved a relationship with the ALS, showing that, through inverse variance-weighted method, OTU10032 unclassified *Enterobacteriaceae* species-level OTU and unclassified *Acidaminococcaceae* were associated with higher risk of ALS. Gamma-Glu-Phe statistically showed a potential deleterious effect [35].

Therapeutic alternatives for ALS based on microbiota modification

Polyphenols are bioactive substances with multiple health benefits . Among them, their neuroprotective effect prevents inflammation. It contributes to the cognitive functions maintenance and reduces pathogenic bacteria while increasing beneficial microorganisms [20].

This wide group of compounds resulting from vegetal secondary metabolism comprises nearly 8,000 different molecular structures belonging to two main types: flavonoids and non-flavonoids [37].

Resveratrol and curcumin, from the non-flavonoids group, and epigallocatechin gallate (EGCG), from the flavonoids type group, have been investigated as therapeutic alternatives compounds with potential beneficial compounds with relevance for ALS treatment patients [38].

The neuroprotective effect of resveratrol lies in the activation of the protein deacetylase SIRT1, that leads to an improvement of the altered energy metabolism and helps to avoid the aggregation of mutant proteins [39]. Curcumin impedes reduced SOD1 aggregation. Due to its low oral bioavailability, some analogues like dimethoxy curcumin and monocarbonyl dimethoxicurcumin C has been tried, and showed the property of restoring mitochondrial damage, improving high excitability, preventing aggregation of mutant TDP-43 and reducing oxidative stress. EGCG also acts by protecting motor neurons from oxidative stress and mitochondrial damage. In mice, EGCG produced a delay in the onset of symptoms, with better motor function and increased life expectancy [38].

Moreover, polyphenols are metabolized by gut microbiota depending on the initial characteristics of bacterial populations. individuals with high levels of *Bacteroides* responded to anthocyanins and flavonoids by decreasing this bacterial genus, and increasing levels of *Lachnospiraceae*, *Ruminococcus* and *Collinsella*. On the other hand, patients with low *Bacteroides* levels showed the opposite response and exhibited an increase of *Bacteroides* along with a decrease of *Lachnospiraceae, Ruminococcus* and *Collinsella* [40].

The most recent studies, both *in vitro* and *in vivo* studies support the strong probiotic character of polyphenols, suggesting them as substances with potential therapeutic effects [41].

Conclusions

At present, the interest towards the investigation of the intestinal microbiota in neurological diseases, especially ALS, has increased considerably, not only due to the increase in the prevalence of ALS, but also due to the evidence of the role of modulation that the microbiota exerts on the immune state such as its direct effects on the CNS.

It is necessary to advance in clinical trials to later establish clinical guidelines and protocols that include the management of dysfunctional microbiota, since training in this field in multidisciplinary professionals will impact actions towards patients with ALS.

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