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ORIGINAL PAPERS

Animal Models in the Microbiota vs. Irritable Bowel Syndrome Manifestations - Preliminary Aspects on the Probiotic Therapy in Irritable Bowel Syndrome

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Abstract

Irritable bowel syndrome (IBS) is a common functional disorder that affects the digestive system and especially the large intestine, expressed mainly through symptoms including diarrhea, constipation, abdominal pain, bloating and cramping. It could be associated with mood disorders including depression and anxiety. Additionally one of the causes of IBS could be a change in gut microflora. Also, could exert a significant role in this context and their potential benefits in maintaining a healthy gut. Here we discussed the possible role of therapy with probiotics in IBS, as well as some important animal models regarding this topic.

Keywords: Irritable bowel syndrome (IBS), Microbiome, Gut-brain axis, Probiotics

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Introduction

In the 1680s, Antonie van Leeuwenhoek pioneered the study of the relationships that might exist between microbial communities and humans by comparing his own oral and fecal microbiota. He discovered the presence of protozoa inside his stool samples and further expanded its investigations towards other individuals in states of health or disease [1].

However, only in 2008 is the so-called Human Microbiome Project (HMP) officially launched. First or „Jumpstart" phase had as its main objectives to develop new algorithms (libraries with reference sequences), technologies and tools for computational analysis dedicated for exploring the variability of intestinal flora [2].

Moreover, Integrative Human Microbiome Project (iHMP) or phase two emerges about six years later, the main goal being to create complete characterizations of the human „microbiome" (term coined by Joshua Lederberg in the early 2000s). Three different perspectives defined iHMP: (I) pregnancy, delivery mode and premature births, (II) IBS's underlying mechanisms and potential triggers and (III) how diverse stressors affect patients with prediabetes [3].

All microorganisms which colonize the human body are grouped in four major ecosystems, mainly large populations of protozoa, archaea, bacteria, fungi and viruses, exceeding the total number of germ and somatic cells by a factor of ten. Thus, it possesses over one hundred and fifty times more bacterial genes compared to the total number of those involved in the structure of our DNA, having a biomass production closely to the overall weigh the of the human brain [4].

To further deepen this subject, the most numerous associations are gathered at the level of the digestive tract, with a density of 10^{14} who are subsequently divided into three enterotypes : *Ruminococcus, Prevotella* and *Bacteroides* [5].

Microbiota = the second brain

Every healthy and pathological human gastrointestinal (GI) microbiota harbors an immense number of microscopic entities alongside whom we have evolved throughout millennia [6]. The most abundant associations belong to the phyla *Firmicutes, Bacteroidetes, Actinobacteria* and *Verrucomicrobia*, [7] and presently unifying over 1000 species who have been already cultured and analyzed phylogenetically; 92 *Eukarya*, 8 *Archaea* and 957 for *Bacteria* [8].

Gut-brain axis (GBA) is a dense network which reunite a number of fundamental elements such as the central nervous system (CNS), respectively the neuro-endocrine and -immune systems, sympathetic and parasympathetic

components of the autonomic nervous system (ANS) and the enteric nervous system (ENS) [9].

In the beginning was believed that gut microbiome (GM) fulfill key roles such as (1) direct inhibition of pathogens overgrowth, reflected by a fortification of host's immune defenses, (2) developing and maintaining the integrity of intestinal epithelium barrier by secretion of immunoglobulin A to limit bacteria entry into tissues, (3) to facilitate nutrient absorption and a more recent trend has focused on its role (4) in guiding maturation of immune system [10].

The hypothesis according to which GI flora is identical has led to several controversies, Turnbaugh et al., [11], revealing that monozygotic (MZ) pairs have a distinctive signatures with a α-diversity indicating approximately 800 [12]. These arguments apply in a much smaller percentage for brothers. In a study conducted by Schloss et al., [13], a metagenomic shotgun analysis was conducted with the aim of distinguishing each microbial community of the family members (two parents with six children ranging in age from two months to ten years) from normal individuals who live in the same geographic region as reference. *Bifidobacterium* and *Escherichia* were the most dominant strains encountered inside all siblings with the mention that the microbiota of the two-year-old was more similar to her weaned brethren. Twelve operational taxonomic units (OTUs) were identified within the family from which four were location specific belonging to the genus *Bacteroides,* family *Lachnospiraceae* and *Subdoligranulum* genus. This inter-individual variation it appears to be the result of a combination amidst temporal and spatial factors.

Animal models in the microbiota vs. IBS interactions

Although the structure of DNA in humans and murine models share a similarity of approximately 100%, the reproduction of neuroanatomical and chemical characteristics in order to mimick the particularities that define neurodegeneration sphere has its limitations [14].

At the moment, an ideal model does not exist. Radu et al. [15], emphasizes the particularities by which the voluntary administration of agonists, antagonists or by physical manipulation could be induced symptoms that mimick a CNS disorder.

In order to test the most reliable technique used to reproduce IBS, according to the results obtained by Vannucchi and Evangelista [16] and by other researchers, both chronic stress induced by either maternal separation (MS) or by water avoidance stress (WAS) are the most conclusive compared with acute stressors such as Wrap Restrain Stress test (WRS).

The efficacy of this test is confirmed by the stimulation of fecal excretion in rats immobilized for 2h where it was noted an increased activity of a G-protein coupled receptors, corticotropin releasing factory type 1 receptor (CRF1r) [17],

their data fitting with the role of this receptor in mediating stress states. Neurokinin 1 (NK_1), antagonist with antidepressant and anxiolytic effects was found to be inhibited after a partial restraint for also 2 hours [18], similar to 5-HT3R [19], role played by ovarian hormone in female Sprague-Dawley rats.

Early separation of the offspring from their mother in the first week of life influence HPA substance P induced histamine axis, cognitive and emotional functions [20]. Visceral hypersensitivity and cell hyperplasia are two typical signs for IBS and common reactions following maternal separation [21,22]. Interestingly, females are much more predisposed as sensitivity than males after maternal separation [23].

After 6h per day during one week in plastic containers, Wistar rats displayed IBS-like symptoms due to relative prolonged exposure to chronic stress. In Y-maze test, stress exerted is positively associated with the increasing number of errors. Also, in the radial-arm maze, alterations occurred create a cognitive „loop" for a short amount of time, as well as in elevated plus maze. To a lesser extent, in the forced swimming test was present a significantly lower mobility, and in the open field test, the symptoms were those characteristic of IBS [24].

In a similar manner, the same author combine water avoidance with randomized mild stressors as a new approach and after 7 days, they observed a replicative behavioural sphere similar to IBS [25]. However, the most efficient IBS animal models mainly address the psychological factor rather than molecular pathogenesis. It can be concluded that the current IBS animal models consist in psychological and physiological responses to acute or chronic stress factors.

Preliminary aspects on the probiotic therapy in IBS

Probiotics are considered the most powerful alternative intended for the reconstruction of the gut flora and it has been show to improve, in adequate doses gut epithelial integrity by diminishing IBS symptomatology [26]. In order to exert their activity, it must meet four conditions: (1) it must survive in GI until they reach the colon, (2) it must not have adverse effects, (3) it must be genetically stable, and (4) to be hostile to pathogens which reside in the gut. In contrast, prebiotics have the ability to stimulate the growth and/or activity of those that already are found in the gut [27], while synbiotics are a mixture of pro- and prebiotics helping to cross through the upper GI tract and facilitate theirs establishment in the colon [28].

Although their potential is already well known, psychobiotics constitute a new class of supplements, which act in a manner similar to probiotics, their effects being interconnected [29]. The results were not delayed, but although little is known, it seems that the potential has a beneficial effect against triggering IBS [30].

Under normal circumstances, any dysbiosis could be easily overcome. Although these reliable alternatives have proven their effectiveness, a number of other variables must be considered (see below).

One of the earliest interactions of the fetus with microbes occurs once it passes into the birth channel where bacteria from the level of urogenital tract shape infants microbiota [31]. Presently, the most paradigms spin around processes such as colonization, preterm birth or how birth mode influences the further development of the infant.

Collado et al., [32] collected meconium, faeces, placenta, amniotic fluid and colostrum samples from fifteen pairs. They showed that placenta and amniotic fluid have a low richness and diversity, with the predominance of *Proteobacteria*, while meconium samples suggest a foeto-maternal transfer. After almost one week, infants microbiota resembled with that found in colostrum, this suggesting that the colonization might start in fact *in utero*.

By quantifying total's 16S rRNA through q-PCR, Lauder et al., [33] revealed no significant differences between placenta and negative controls in copies number, but vaginal and oral swab samples presented a higher number. PCR-based methods determine that microbial invasion of the amniotic cavity (MIAC) is around 30-50%. Among the common anaerobic species, also were identified *Sneathia sanguinegens*, and *Leptotrichia* spp. [34]. However, if placenta possesses beneficial microorganisms is still under question because a newly published article contradicts most of the arguments and supports the idea of favorable conditions for pathogens [35].

In the last decades, the number of caesarean sections (C-section) have reached a critical point because it creates imbalances between beneficial and harmful bacteria. *Enterococcus, Streptococcus, Staphylococcus*, or *Propionibacterium* were predominant in all inoculated agar plates from umbilical cord blood C-section neonates samples [36], while in preterm borns, in the amniotic fluid, species such as *Sneathia sanguinegens*, *Leptotrichia amnionii* and an uncharacterized bacteria were reported [34].

Longitudinal and whole-genome shotgun metagenomic analysis revealed a disrupted transmission of maternal *Bacteroides* to the detriment of *Enterococcus*, *Enterobacter* and *Klebsiella* species [37]. Naturally, the digestive tract is a gigantic tank of Antibiotic Resistance Determinants - ARDs, and usage of antibiotics in order to treat infections caused by pathogens have long time repercussions upon overall health of the baby [38]. This is due to the fact that the human resistome is altered preformed and the administration of the same drug in the future will have no effect. All these aspects cumulated indicates that every individuals have a unique microbiome [39]. To sum it up, C-sections create disruptions and lead to various disorders like obesity and autoimmune diseases [40].

Conclusions

Certainly, intestinal microflora remains one of the most controversial topics presently. Not only does it possess the ability to induce a multitude of gastrointestinal deficiencies, IBS and the transition to IBD, but also in modulating psychiatric and neurodegenerative disorders. All the conventional alternatives synthesized so far restore to some extent the host's eubiosis and diminish the severity of the symptomatology. Based on these considerations, we believe that it is absolutely necessary to deepen these studies in order to establish with approximation the role of the "forgotten organ".

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