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The importance of intestinal dysbiosis in mood disorders

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ABSTRACT

Intestinal bacteria and the microbiota-brain-intestin axis have been the subject of intense research in the last decade. Until recently, it seemed unlikely to combine fecal microflora with mood disorders. The growing amount of animal research shows that one of the etiological factors of mood disorders may be irregularities in this area. Evidence indicates the existence of an extremely important two-way relationship between bacteria, intestines and the brain, and that this interaction is complex and takes place on many levels. Understanding and analyzing this dependency gives new possibilities in the therapy of mood disorders, such as the use of psychobiotics, prebiotics or drugs which selectively eliminate specific bacterial strains (antimicrobials). Psychobiotics are „good" bacteria which, when consumed in appropriate doses, have a positive effect on the intestinal axis and on the condition of patients with mental disorders. Studies on their use show good results in the treatment of diseases such as depression. In people suffering from depression, significant differences in the composition of the intestinal microflora were observed, which speaks for its essential role in this disease. Even greater role of bacteria in mood disorders favors research that proves that fecal microbiota transplant entails consequences in the form of behavioral changes.

***Keywords*:** dysbiosis, depression, psychobiotics, mood disorders

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1. INTRODUCTION

The relationship between mental health and the condition of the intestinal microflora is an area of intense research over the last decade. The bi-directional interaction between the human brain and the intestines has been known for many years. However, in recent years, an important role of the intestinal flora has been noticed, both commensal and pathogenic in this relation [1]. Currently, it is believed that the intestinal microflora may be necessary for the proper functioning of the brain-intestinal axis [2]. The relationship between dysbiosis and the occurrence of not only diseases such as ulcerative colitis and irritable bowel syndrome, but also depression was noted [3,4].

400-1000 different species of bacteria are present in the human intestine. There are 1011 bacterial cells per gram of colon content and the mass of human body microbiota is approximately 2 kilograms [1,5]. The microbiome contains mainly two bacterial filotypes, Bacteroidetes, and Firmicutes, and to a lesser e tent Proteobacteria, Actinomyces, Fusobacterium and Verruvomicrobia [6].

Intestinal microflora through direct and indirect pathways remains in the bi-directional relationship with the central nervous system. Microbiome affects the brain not only through the intestinal nervous system (microbiota-gut-brain axis) but also through the endocrine system (hypothalamic-pituitary-adrenal axis), the immune system (chemokines, cytokines), the autonomic nervous system and the metabolic changes [6,7]. The individual composition of the intestinal microflora depends on many factors — the manner of delivery (by natural or cesarean section), genetic predisposition, age, diet, physical activity, environment, stress, infections, chronic diseases or antibiotic therapy [8].

2. THE ROLE OF THE INTESTINAL MICROBIOTA

Microbiota has many functions. First, it creates the intestinal barrier, stimulates the regeneration of the intestinal epithelium, produces mucus and nourishes the mucous membrane by producing short-chain fatty acids [6]. Secondly, it is assumed that it is vital for brain processes, such as myelination, neurogenesis, microglia activation, modeling behavior and affecting psychological processes [2]. Maintaining the proper activation of microglia may prevent neurodevelopmental and neurodegenerative disorders [9,10]. Thirdly, it participates in the maturation of the immune system by stimulating the innate immune system early in life and the local and systemic immune response, provides nutrients, hormones and vitamins, and performs detox functions [6].

Studies on mice indicate that mice devoid of intestinal flora have impaired social behavior, wrong stress response and a major level of anxiety [2,11,12]. A lot of research indicates that physical and psychological stress can affect the composition of intestinal microflora in rodents [12]. In studies in which antibiotics were used to disturb the balance in the colon microenvironment, memory impairment, recognition of new objects and changes in levels of metabolites and expression of molecules important for brain function have been reported [13].

It was also observed that fecal microflora transplantation may be accompanied by the transfer of behavioral traits [2].

3. THE MICROBIOTA-INTESTINE-BRAIN AXIS

To describe the processes mediating the central nervous system (CNS) on the functions of restriction, the term 'emotional motor system' has been introduced [1]. The term refers to several systems:

- ✓ branches of sympathetic and parasympathetic nervous system
- ✓ hypothalamic-pituitary-adrenal axis
- ✓ endogenous pathways which modulate pain and discomfort

They mediate in affecting many emotional states, including gastro-intestinal functions. Separate activation of one or more systems at a time has a direct and indirect effect on the intestinal microflora. Most studies refer to bacteria contained in stool samples. The influence of these systems on bacteria which bind biofilms which adhere to the intestinal mucosa is unknown. Bacteria forming biofilm seem to be less sensitive to environmental changes, but may be more involved in bi-directional interaction with the host [1,14].

The autonomic nervous system (ANS) is an intermediary element in the communication between the CNS and the digestive system. It plays an important role in the control of intestinal functions such as peristalsis, secretion of acid, bicarbonates, mucus and modulate mucosal immune response [1,6]. Changes in this range may affect the supply of nutrients, important for the proper functioning of the intestinal microbiota, maintaining the correct pH and general environment in the intestinal lumen [1]. Intestinal motility disorders can cause serious changes in microbiota. This leads to the overgrowth of intestinal bacteria (SBBO) and bacterial translocation (BT) [15]. Mucus secretion provides an appropriate environment for the development of biofilm. ANS directly affects the immune response of the gut by activating macrophages and mast cells [1]. Stress can affect each of these mechanisms. Stress-induced changes in intestinal motility, mucus production, ion development, and increased permeability to macromolecules have been demonstrated. This increase in permeability may allow bacterial antigens to penetrate the intestinal epithelium and induce an immune response in the intestinal mucosa [16,17].

The CNS probably plays a superior role in the release of signaling molecules such as catecholamines, serotonin, GABA, dynorphin, and cytokines into the lumen of the intestine (through neurons, immune and enterochromaffin cells) [18]. In one of the studies, serotonin secretion was detected in the light of the stomach in response to intrathecal administration of thyrotropin releasing hormone analogue (it is a mediator secreted in response to stress at low temperatures) [19]. Different types of stressors can increase noradrenaline levels in the gut. Noradrenaline has been shown to stimulate the proliferation of some intestinal pathogens and to increase the virulence of Campylobacter jejuni [18].

Not only signal molecules can affect the bacteria present in the gut, but also the compounds produced by the bacteria can affect the host. Molecules discharged by bacteria to communicate with each other can be recognized by intestinal and immune cells as well as nerve endings. The metabolites produced by bacteria include but are not limited to short-chain fatty acids (SCFA), bile acids, GABA, serotonin, noradrenaline and tryptophan [18]. The research suggests that a large proportion of the metabolites identified in the circulation are derived from intestinal bacteria, for example fluctuations in serotonin plasma levels may indirectly result

from the host's interaction with bacteria [20]. Various types of receptors for SCFA have been identified on enteroendocrine cells, submucosal neurons and cerebral ganglia [18].

Studies show that intestinal microflora can modulate intestinal motility and affect the mental state of a person. For example, Bifidobacterium bifudum and Lactobacillus acidophilus may stimulate motor activity, while Escherichia coli may suppress it. Also in a randomized study of healthy men and women after the use of a probiotic containing Lactobacillus and Bifidobacterium, psychological stress and anxiety appeared, although another study using the same probiotics did not confirm these results [1,18].

Specific cells are the enterochromaffin cells present in the intestine. They express serotonin, signal molecules and are responsible for communication between the lumen of the intestine and the CNS. On the one hand, they are in contact with the intestinal microflora, and on the other hand, with efferent and afferent nerve endings, located in the lamina propria of the mucous membrane [1]. In the vicinity of these cells, there are vagal afferent ends which receive information about the environment of the lumen. Under normal conditions, they are indirectly activated by compounds such as serotonin, cholecystokinin, histamine, somatostatin, melatonin, secretin and others. All of these compounds can be released by neuroendocrine cells in the intestinal mucosa [1,18].

4. INTESTINAL MICROBIOTA AND NEUROPSYCHIATRIC DISEASES

The linking of microbiota with the functioning of CNS opens new paths in the search for causes of neuropsychiatric diseases. The etiology of these diseases is multifactorial, the one example is the influence of various environmental factors, which may initiate the development of the disease in genetically predisposed individuals [21]. However, it should be taken into account that neuropsychiatric diseases can develop only in one of the monozygotic twins, which suggests that genetic factors are not decisive [21]. Only recently, the impact of microbiota on the functioning of the brain, and thus also on the psyche, began to be interesting. Research carried out in this direction provides us with more and more information on the relationships between them [22,23]. Neuropsychiatric diseases are a serious and common problem. Most of them are associated with symptoms of a greater or lesser degree lasting a lifetime, leading to behavioural disorders, and thus economic, emotional and psychological problems not only for people affected by the disease but also for their relatives [24]. Studies show that intestinal microbiota is able to form the physiology of the brain and thus also human behaviour, and suggest that microbiota is a key factor that causes many neuropsychiatric diseases [22].

We now know that both the human genome and microbiome are necessary to maintain normal health, due to the microbiome's impact on the host's physiology, including the development and functioning of the brain [23].

Going further, one of the most interesting issues is also determining what affects the composition of microbiota. Factors which form the composition include: cesarian birth, gestational age, formula feeding, environment, host genetics, exposure to infection (including maternal) and the use of antibiotics. In addition, stress, especially during childhood and in prenatal life [22]. The original composition of microbes depends on the method of delivery. In neonates born via the vaginal route, fecal bacteria and bacteria present in maternal genital tracts dominate, e.g. Lactobacillus spp. For comparison, in neonates born via caesarean section, the composition of microbiota is more similar to the microbiota composition of their mothers' skin.

Staphylococcus, Corynebacterium Propionibacterium and the presence of Bifidobacteria spp. is less marked [25]. The method of feeding a newborn is also an important determinant of his early microbiota. Only the presence of certain species of Bifidobacterium spp. has been observed in newborn babies who are exclusively breastfed. Their domination decreases in favor of increasing the diversity of bacteria after discontinuation of breastfeeding and introduction of solid foods. However, in the composition of microbiota of newborns fed with artificial food, a lower presence of Bifidobacteria and more frequent presence of bacteria from the group of Coli, Bacteroides and Clostridium difficile was noticed. However, microbiota is also a flexible element in adults and the diet has a strong influence on its composition [25].

The discovery that the diverse microbiological composition is associated with behavior and cognitive processes has had a significant contribution in establishing the microbiota-intestine-brain axis as a development of the known concept of the intestine-brain [23]. This issue gives us a huge field to study the possibility of modifying our behavior and mood through bacteria. There are many studies carried out to determine the role of the microbiota-intestine-brain axis in maintaining our health and etiology of stress-related diseases such as depression, anxiety or irritable bowel syndrome but also neurodevelopmental diseases such as autism. In addition, attention is paid to the role of epigenetic mechanisms in shaping brain functions and behavior [23].

Free of germs (germ-free (GF)) mice (mice that have never been exposed to any bacteria) have been used to show how important microbiota is for the functioning of the hypothalamic-pituitary-adrenal axis. These mice have extensive neurodevelopmental changes in the brain, including changes in monoaminergic neurotransmission and anxiety-related behavioural changes. Recently, it has been noticed that they also show features similar to autism, such as deficits in sociability, social cognition and increasing repetitive behaviours. Interestingly, as in autism and other neurodevelopmental diseases, these changes are more pronounced in men than in women. In addition, studies on germ-free mice can be extended to allow researchers to "humanize" intestinal microbiota, i.e. transplantation of fecal microflora from a particular person or animal model. Indeed, it has been shown that behavioral traits can be transmitted between mice [22].

Serotonin (5-HT) is a neurotransmitter involved in the modulation of many physiological processes, including mood, sleep, aggressiveness and sexual behavior. Serotonergic neurons are one of the earliest developed and widespread neuronal systems in the mammalian brain [26]. Serotonin is a key neurotransmitter in the intestinal axis [25]. The lack of 5-HT in the CNS leads to a decrease in body growth and affects proper brain wiring which may predispose to the appearance of neurodevelopmental disorders. Support for the role of 5-HT in brain development is based on the assumption that dysregulation of serotonergic signaling is the cause of the disorders that were thought to have a developmental basis, such as schizophrenia, affective disorder, anxiety, autism and mental retardation [26].

Research also proves another interesting phenomenon, which is the modification of the host epigenome through the activity of microbiota, affecting the expression of genes [2]. Because changes in epigenetic status are often inherited by daughter cells, the accumulation of epigenetic changes can trigger lifelong inflammatory response, autoimmunity and allergic diseases [27]. It is now known that epigenetic mechanisms are involved in neurogenesis, neural plasticity, learning and memory, and in the etiology of disorders such as depression, addictions, schizophrenia and cognitive dysfunctions.

Therefore, it was suggested that intestinal microflora may be also involved in pathogenesis and be a risk factor for the development of neuropsychiatric disorders through epigenetic modifications, which are highly dynamic and reversible [2]. Epigenetic modifications associated with many diseases were recognized as a missing element in the puzzle connecting the development of the human genome, environment and phenotype [27].

Behavioral studies conducted on animals that have had specific infections and have taken specific antibiotics and probiotics show reproducible and largely consistent effects of different microbic states on mouse behavior. The most frequently noted was the changed behavior associated with anxiety. Specific behavioral (anxiety) and biochemical parameters (related to the metabolism of tryptophan) are reversible. Others, such as 5-HT levels or social cognitive skills, could not be reversed by restoring normal microbiota. In fact, the reversibility of these changes in a GF mouse is only possible if recolonization occurs at a crucial time during adulthood [23]. In addition, strong changes in gene expression have been found within the cerebellum and hippocampus, whereas in the hypothalamus, which is the brain region responsible for regulation of the stress level, almost no differentiating changes were found [23].

More and more research on animal models show the relationship between the microbiota and the level of stress, anxiety and depression and its role in the pathogenesis of these conditions. Currently, research is also focused on the positive behavioral effects of various bacterial strains, mainly Bifidiobacteria and Lactobacillus but also Mycobacterium vaccae [23]. Intestinal probiotics are associated with increased resistance to stress and reduced basic level of anxiety. Animal germ-free models show an increased basal level of corticosteroids with increased stress response, hyperlocomotion, reduced levels of brain-derived neurotrophic factor (BDNF), reduced levels of anxiety and reduced social activity. It may seem contradictory that endocrine stress parameters are elevated when anxiety is reduced at the same time. However, these parameters do not necessarily correlate with each other and microbiota may have different effects on them. On the other hand, dysbiosis of the intestinal environment is marked by increased anxiety, depressive behavior and memory disorders together with a reduced level of key neurotrophic factors such as BDNF [23].

Most living organisms are equipped with mechanisms to deal with stress. In response to stress, the hypothalamic-pituitary-adrenal axis (HPA) is activated [2]. As a result, there is an increase of cortisol in humans' blood. This axis is subjected to programming in the early stages of life [12]. The most consistently demonstrated irregularity in patients with depression is the dysregulation in this hypothalamic-pituitary-adrenal axis (HPA), manifested by elevated levels of cortisol [28]. In one study involving the germ-free mouse that grew up in a sterile environment, an excessive HPA axis response to an acute stressor was noted. It is noteworthy that this increased responsiveness of the HPA axis can be reversed by monosociation with a single bacterial strain, in this case Bifidobacterium infantis [12].

Depression is a mood disorder associated with stress, and thus also with a disturbed HPA axis. Evidence suggests that intestinal microflora plays a key role in modulating depression [2]. It is believed that Bifidobacterium and Lactobacillus in the gut may have a beneficial effect on the body's response to stress and therefore depressive disorders. A study was conducted to compare the amount of these bacteria in patients with severe depression (MDD) and in healthy ones as a control group. Sick patients had significantly lower Bifidobacterium numbers and showed a lower Lactobacillus count than the control group. Individuals whose bacterial count was below the optimal cut-off point were significantly more frequent in patients with severe depression than in the control group, both for Bifidobacterium and Lactobacillus [29].

Other studies conducted on a group of people with severe depression (MDD) also showed changes in the composition of microbiota compared to the control group. Patients with acute disorders had higher levels of Bacteroidetes, Proteobacteria and Actinobacteria, while the Firmicutes level was significantly reduced. Despite the high inter-individual variability, the levels of several predominant types of bacteria were significantly different between the group of patients and the control group. First of all, MDD groups had elevated levels of Enterobacteriaceae and Bacteroidetes but decreased levels of Faecalibacterium. Negative correlation was observed between the amount of Faecalibacterium and the severity of depressive symptoms [30].

5. PSYCHOBIOTICS

Numerous studies on the relationship between intestinal microbiota and the functioning of the brain and the occurrence of psychiatric disorders have opened new therapeutic possibilities. Psychobiotics are beneficial bacteria, which when eaten in appropriate doses have a positive effect on the intestine-brain axis and on the state of patients with mental disorders [5,31]. Some authors extend this concept by prebiotics that favor the development of beneficial intestinal bacteria [32,33]. When applied properly, they can exert anxiolytic and antidepressant effects. These activities take place thanks to the bacteria-brain relationship, mainly through the intestinal nervous system and the immune system [32].

We base the effects of psychobiotics mainly on rodent studies and distinguish three levels on which psychobiotics operate:

- ✓ Psychological impact on emotional and cognitive processes
- ✓ Effects on the hypothalamic-pituitary-adrenal axis, on glucocorticosteroid stress and inflammation, which is characterized by abnormal cytokine levels. Pro-inflammatory cytokines show a positive correlation with the occurrence of mental disorders such as depression
- ✓ Effects on neurotransmitters and proteins [5,32,34]

The basic microorganisms used are gram-positive Bifidobacterium and Lactobacillus. These bacteria do not have pro-inflammatory lipopolysaccharide chains and do not cause an immune response [33]. In studies on mice after administration of Lactobacillus rhamnosus, the symptoms of anxiety and depression were clearly reduced and also there were changes on the level of GABA in the central nervous system. The same results were not observed in mice previously subject to vagotomy [35].

In healthy female volunteers who consume a fermented milk product with a mixture of probiotics Bifidobacterium animalis, Streptococcus thermophilus, Lactobacillus bulgaricus and Lactococcus lactis for four weeks, the effect on the activity of brain emotional centres was shown [36]. In healthy volunteers who consumed for 30 days a mixture containing Lactobacilus helveticus and Bifidobacterium longum, a beneficial effect on the symptoms of anxiety and depression as well as decreased cortisol levels were observed [36]. In another study noted improve mood in healthy subjects lasting three weeks after consuming milk drink containing Lactobacillus casei [37]. Administration of Bifidobacterium infantis to rats that were exposed to stress factors showed a decrease in IFN-gamma, TFN-alpha, IL, IL-10 concentrations and an

increase in tryptophan and its metabolite concentration in the central nervous system in relation to the control group. In subsequent studies, also on rats there was a similar antidepressant effect when using Bifidobacterium infantis as when administering citalopram [5,38]. 28-day treatment with Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus, and Lactobacillus brevis probiotics by 40 healthy volunteers showed a reduction in the level of aggression and a reduction in depression symptoms in comparison with placebo [5]. Mood disorders and anxiety disorders often co-exist with other conditions. In the conducted studies, administration of a probiotic containing Lactobacillus casei reduced anxiety in patients with chronic fatigue syndrome [36].

Research on healthy people and animals show a clear relationship between intestinal microflora and emotional state. Differences in the composition of intestinal microbiota between healthy individuals and patients with depression were demonstrated. In studies carried out on animals differences in microbiota were found in depressive models in relation to control models. In addition, the microflora of animals was similar to the one occurring in patients with depression - the number of Bacteroidetes increases and the amount of Firmicutes and Lactobacillus decreases. Further evidence that depression can be caused by changes in the intestinal microbiota are fecal microbiota transplants. Transplants were carried out from people with depression and from healthy people to germ-free mice. Mice that received a transplant from depressed patients showed more symptoms of depression. In addition, the differences in the microflora of mice were similar to differences between human donors [39].

In 2016, Schnorr and Bachner combined psychotherapy with dietotherapy to treat panic attacks. They eliminated from the diet products that cause a prolonged glucose increase and increased the amount of food rich in probiotics. Such treatment alleviated the symptoms of anxiety and insomnia and increased the amount of Lactobacillus in the stool sample [39].

An alternative to psychobiotics may be drugs selectively eliminating specific bacterial strains (antimicrobials), which have an adverse effect on mental health [28]. Anti-inflammatory drugs have been shown to have beneficial effects in the treatment of major depressive disorder. One study investigated the effects of minocycline on the mood of patients suffering from depression [40].

6. SUMMARY

The combination of intestinal microbiota with psychiatric disorders gives new therapeutic options. Therapy for depressive disorders is aimed at brain abnormalities, while other disorders are secondary. These findings may give a new look at how to combat such disorders [39]. Research carried out on animal models provides us with a lot of new information on the functioning and impact of the intestinal microbiota on our bodies.

However, this topic is still new. The possibility of affecting the psyche through modulation of the composition of microbiota is a very tempting vision of therapy but only through long-term research it will be possible to predict whether it is the right therapeutic goal.

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