Gut microbiota, probiotics and prebiotics in pediatrics

Črevesna mikrobiota, pro- in prebiotiki v pediatriji

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Abstract

Intestinal microbiota plays an important role in gut metabolism, defense against environmental pathogens, motility and immune system regulation. Microbiota is gradually aquired from the environment during and after delivery, with several factors, such as feeding habits, disease and antibiotic treatment, importantly influencing its composition. Growing awareness about the importance of the intestinal microbiota for the host physiologic processes and health has led to development of different strategies how to influence its composition and function. Probiotics, specific live microorganisms which when ingested in sufficient amount can promote health benefit to the host, and prebiotics, nondigestible food ingredients that benefit the host by selectively stimulating the favorable growth and/or activity of a limited number of health-promoting bacteria, have been increasingly used in the last decades. They exert their beneficial effects through three main mechanisms: by changing gut ecology, affecting the intestinal mucosal barrier, and by modulating the immune response. The efficacy of pro- and prebiotics in clinical practice has been extensively studied. The results of clinical studies with the specific strain or strains in a defined dose and vehicle should not be extrapolated to the use of other products. There is strong scientific evidence for the use of specific probiotics in acute gastroenteritis, antibiotic-associated diarrhea, prevention and treatment of atopic dermatitis, prevention of relapses of ulcerative colitis and pouchitis, as well as enhancing immune response, and some evidence of efficacy in prevention of necrotizing enterocolitis, treatment of *Clostridium difficile*-associated diarrhea, active ulcerative colitis, and functional gastrointestinal disorders.

Izvleček

Črevesna mikrobiota igra ključno vlogo pri presnovi v črevesju, zaščiti pred okoljskimi patogenimi mikroorganizmi, črevesni motiliteti in gibljivosti imunskega odziva. Razvoj mikrobiote po rojstvu poteka postopno; na njeno končno sestavo pa pomembno vplivajo dejavniki, kot so prehranjevalne navade, bolezni in zdravljenje z antibiotiki. Vse večje zavedanje o pomenu vpliva mikrobiote na fiziološke procese v telesu in na zdravje je pripeljalo do razvoja različnih strategij, s katerimi skušamo vplivati na sestavo in delovanje mikrobiote. Uporaba probiotikov, specifičnih živih mikroorganizmov, katerih uživanje v zadostni količini koristno vpliva na zdravje, in prebiotikov, neprebavljivih sestavin hrane, ki selektivno spodbujajo rast in/ali aktivnost omejenega števila zdravju koristnih bakterij, v zadnjih desetletjih strmo narašča. Za njihov učinek so ključni trije mehanizmi: sprememba črevesnega okolja, vpliv na črevesno sluznično pregrado in uravnavanje imunskega odziva. Intenzivno se raziskuje klinična učinkovitost pro- in prebiotikov. Pomembno je, da se ugotovitve kliničnih raziskav, v katerih so bili uporabljeni specifični sevi probiotikov v natančno določenem odmerku in obliki, ne posplošujejo. Obstajajo močni znanstveni dokazi o uporabnosti specifičnih probiotikov za zdravljenje akutnega infekcijskega gastroenteritisa, preprečevanje driske zaradi zdravljenja z antibiotiki, preprečevanje in zdravljenje atopičnega dermatitisa, preprečevanje aktivnih zagonov ulceroznega kolitisa in paučitisa ter splošno krepitev odpornosti organizma. Nekaj je tudi dokazov o njihovi učinkovitosti pri preprečevanju nekrotizantnega enterokolitisa, zdravljenju driske, povzročene z bakterijo Clostridium difficile, zdravljenju akutnih zagonov ulceroznega kolitisa in lajšanju funkcionalnih gastro-intestinalnih motenj.

Introduction

Intestinal microbiota (previously known as microflora) is a complex community of between 10^{13} to 10^{14} microorganisms, with over 1,000 different bacterial species, but also consisting of other microorganisms such as fungi, viruses and archaea, inhabiting the gastrointestinal tract. Many of these microorganisms are difficult to culture and only new culture-independent molecular genetic methods have provided a breakthrough in their recognition as well as a better insight into their metabolic function and interactions with host organism. Therefore, a new term "microbiome" is proposed to explain the whole catalogue of genes that intestinal microorganisms harbor.[1] In addition, a new term "metagenomics" is referred for studies of characterization of total DNA, microbial and host's, as in many cases they are both present in the same tissue samples, having a potential for the examination of complex microbial-host interactions. It is presumed that the number of microbial cells within the gut is ten times that of all cells in human body and microbiome contains 150 times more genes than human genome.[2]

Intestinal microorganisms are unevenly distributed along the gastrointestinal tract. The esophagus, stomach, and proximal parts of the small intestine contain relatively small numbers of bacteria due to intense motility and aggressive luminal chemicals such as gastric acid, bile and digestive enzymes. The jejunum contains predominantly aerobic bacteria, and the colon anaerobic ones, while the terminal ileum represents a transition zone between them. The highest bacterial concentrations are found in the proximal colon reaching up to 10^{12} bacteria/ml. Moreover, at any given level of the intestine, the bacterial microbiota composition varies along gut diameter, with some species tending to adhere to the mucosal surface while others predominate in the lumen.[2]

Microbiota has immense metabolic potential exceeding that of the liver, our biggest metabolic organ. Intestinal bacteria metabolize dietary components that escape digestion by human digestive enzymes, such as nondigestible carbohydrates (resistant starches, dietary fiber, oligosaccharides and plant cell wall material), and smaller quantities of proteins and fats, as well as secretions of our digestive system such as mucus and desquamated intestinal cells. In the proximal colon, where these substrates are abundantly available, the bacteria preferentially ferment carbohydrates, which are energetically more favorable than proteins, while in the distal parts of the colon where the quantity of carbohydrates progressively declines, more proteolytic then saccharolytic bacterial species can be found.[3]

The human gastrointestinal tract is presumed to be sterile before birth, and the first colonization takes place during the delivery. Studies revealed that soon after birth the microbiota of vaginally delivered newborns resembles their mothers' vaginal microbiota while in those delivered by Cesarean section it is composed of microbial species found on human skin.[4,5] Thereafter, microbiota is gradually aquired from the environment, with several factors such as feeding habits, disease and antibiotic treatment importantly influencing its composition. The composition of mother's milk rich with oligosaccharides and immune factors, in comparison with other mammalian species milk, is an important factor in establishing dominant intestinal flora in infants. Although *Bifidobacterum* is the most prevalent genus in both breastfed and formula-fed infants, it is much more abundant in the former group, and more adult-type composition of microbiota is typical for formula-fed infants.[6] In a case study infant's microbiota was monitored over the first 2.5 years of life. This study revealed that bacterial diversity increased linearly with time. Each dietary change was accompanied by changes in microbiota composition.[7] Although each person's microbiota composition remains relatively stable after initial intestinal colonization in the first years of life until old age, an immense interpersonal diversity has been found. Microbiome can be 80–90 % different from one person to another compared to our human genome, which is 99.9 % identical.[1]

However, despite great interpersonal variability, more than 90 % of intestinal bacteria in healthy humans are members of only

three bacterial phyla: the *Bacterioidetes*, the *Firmicutes* and the *Actinobacteria*.[8] Moreover, metagenomics data revealed that gut microbiota of nearly all analyzed samples can be separated into three robust clusters, named "enterotypes". Each of them is identifiable by variations in the quantity of three main genera: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2), and *Ruminococcus* (enterotype 3).[9] Enterotypes do not correlate with host properties such as nationality, age, and gender or body mass index.[9] However, enterotypes could be associated with long-term dietary patterns, particularly protein and animal fat (enterotype 1) and carbohydrates (enterotype 2).[10]

As mentioned before, intestinal microbiota, once established, is fairly stable over time. However, it is also relatively plastic, and different environmental factors can influence its short- and long-term composition throughout. For example, few days following treatment with broad-spectrum antibiotics, the gut microbiota experiences a decrease in taxonomic richness, diversity, and evenness.[11] Although the gut microbiota began to resemble its pretreatment state a week after treatment, the re-establishment of some species can be delayed for several years.[12]

The role of intestinal microbiota

Indigenous intestinal microbiota plays an important role in gut metabolism, defense against environmental pathogens, motility and immune system regulation.

Intestinal bacteria produce several vitamins, especially vitamin K and several vitamins from the B group.[13] The main products of bacterial carbohydrate fermentation are short-chain fatty acids (SCFAs): acetate, propionate and butyrate.[14] While propionate is predominantly transported to the liver via portal blood and used for gluconeogenesis, acetate can be oxidized by many different tissues. Butyrate can be used directly from the intestinal lumen by colonocytes and represents major energy source to the colonic mucosa. Beside their energy value, SCFAs may affect satiety hormones and play a role in insulin resistance.[3] SCFAs lower intestinal pH, which results in the inhibition of growth and survival of pathogenic bacteria,[15] in the reduction of harmful secondary bile acids formation,[16] and in the decrease of bacterial proteolytic activity leading to the production of potentially toxic substances such as ammonia, amines, phenols, thiols and indoles.[3,17] All three major SCFAs have trophic effect on the intestinal mucosa. They stimulate epithelial cell proliferation and differentiation.[18] Moreover, SCFAs may also possess anti-inflammatory properties.[3]

Gut microbiota establishes its protective effect against environmental pathogens entering gastrointestinal tract through several direct and indirect mechanisms. Commensal microorganisms themselves provide the first line of defense by inhibiting gut colonization with pathogens by competition for food and micronutrients, blocking adhesion sites on the gut epithelium and production of bacteriostatic or bactericidic substances such as hydrogen peroxide, hydrogen sulphide, lactic acid and specific bacteriocins which can be active against pathogenic bacteria, yeast, fungi, protozoa and viruses.[19] However, the most efficient defense against environmental pathogens is achieved indirectly, through the complex network of cooperation between intestinal epithelium and mucosal immune system orchestrated by commensal microbiota.[20]

The intestinal epithelium is a selective barrier that separates intestinal luminal contents from the internal body tissues. It has a bimodal function, maximizing nutrient absorption while preventing the passage of "non-self" luminal components such as bacteria and food components.[21] The epithelium consists of a single layer of epithelial cells, which include enterocytes, mucus-secreting goblet cells, enteroendocrine cells, and Paneth cells secreting antimicrobial peptides and proteins such as defensins. In addition, the mucus, tight junctions between the cells and immune functions of gut epithelium (expression of co-stimulatory molecules, secretion of cytokines, and regulation of immune response) play an important role in assuring selective permeability of the intestinal epithelial barrier.[20] The in-

testinal microbiota enhances epithelial barrier function by modulation of mucus and defensin secretion, increasing tight junction function and by regulating its immune response.[21]

As the gastrointestinal tract represents the major entry of foreign substances (microorganisms, food ingredients) into our body, it is understandable that it must be equipped with the most efficient immune protection. Therefore, GI tract is a home for more than a half of a whole body's immune cells. The so-called gut-associated lymphoid tissue consists of a variety of cells such as macrophages, dendritic cells, B lymphocytes, and different subgroups of effectors and regulatory T lymphocytes. They are often grouped in organized structures, such as Peyer's patches, lymphoid follicles and mesenteric lymphoid nodes, but are also scattered throughout the intestinal mucosa, especially in lamina propria.[20] The intestinal immune response, both innate and acquired antigen-specific, should be precisely regulated to perform its duty properly. Namely, it should clearly discriminate between potentially harmful components such as pathogenic microorganisms that must be destroyed and eliminated, and harmless food ingredients and members of commensal microbiota that should be tolerated not inducing self--destructive intestinal inflammation.

The intestinal immune homeostasis is achieved through a complex interaction between the epithelium, macrophages and dendritic cells in response to the gut microbiota, and the resulting T cell differentiation and ratios.[21] The main way of communication between intestinal microorganisms and host organism's cells is established through pattern-recognition receptors expressed on both cellular surface and in cytoplasm (Toll-like receptors and Nod-like receptors). These receptors are responsible for the initial recognition of specific microbial components (pathogen-associated molecular patterns) and the discrimination between pathogenic and commensal microbes.[22,23] It has been well established that initial colonization with intestinal microorganisms plays a crucial role in the development of both intestinal and systemic immune system, and that perturbations of microbiota composition can lead to immune dysfunctions resulting in diminished host protection against environmental pathogens as well as adverse immune reactions such as allergic, autoimmune and auto-inflammatory conditions.

Some investigations revealed that intestinal bacteria may also produce neurotransmitters and neuromodulators, which can influence gut sensation and motility.[2] Therefore, it is not surprising that perturbations in gut microbiota can be an important factor in the pathogenesis of functional gastrointestinal disorders such as functional constipation, functional abdominal pain and irritable bowel syndrome.

Probiotics and prebiotics

Growing awareness about the importance of the intestinal microbiota for the host physiologic processes and health has led to the development of different strategies how to influence its composition and function. Antimicrobials, including antibiotics, although being the most potent weapon against pathogens, are seldom the method of choice since they kill not only harmful, but also commensal microbes and can cause immense "collateral damage" resulting in gut dysbiosis leading to numerous adverse consequences for the host organism. Therefore, more "soft-tuning" strategies, such as use of probiotics and prebiotics are advocated to achieve the desired effect.

Probiotics are defined as specific live microorganisms which, when ingested in a sufficient amount can promote health benefit to the host.[24] In order to qualify as a probiotic, microorganisms should fulfill most, if not all, of the following criteria:[25] they should be strictly specified at the genus, species and strain level, and specific strains should be registered and disposed in an international culture collection. It has been well established that specific beneficial effects are apparently strain-specific and therefore, generalization of the efficacy to the whole species or even genus could be misleading. Although the vast majority of probiotics belong to *Lactobacillus* and *Bifidobacterium* genera, not all lactobacilli or bifidobacteria

harbor probiotic properties. Moreover, in contrast to expectations, even the nonpathogenic strain of *Echerichia coli* (Nissle 1917) and certain yeasts, such as *Saccharomyces boulardii*, are qualified as probiotics. It is always emphasized, that probiotics should be extremely safe. The safety of probiotics is supported by the fact that many strains are of human origin and have a long history of safe use. Consequently, many probiotics and their applications have been granted GRAS (generally regarded as safe). However, this classification should not be generalized and does not warrant permanent surveillance for potential risks such as invasiveness and potential for transfer of antibiotic resistance to other microorganisms.[26,27] Because the effects of probiotic microorganisms are generally dependent on their viability, both their stability during the processing and storage and their ability to survive intestinal transit through the stomach and proximal small bowel to finally adhere to mucosa and colonize the intestine should be demonstrated.[25] Last, but maybe one of the most important criteria for a specific microorganism to be qualified as probiotic, is its scientifically proven effect on the promotion of health or prevention and treatment of a specific disease.[25]

Prebiotics are nondigestible food ingredients that benefit the host by selectively stimulating the favorable growth and/or activity of a limited number of health-promoting bacteria.[28,29] These bacteria, especially but not exclusively bifidobacteria and lactobacilli, are usually already residing in the gut as part of indigenous microbiota. However, prebiotics can also be applied to enhance the survival and action of ingested probiotic bacteria. When probiotics and prebiotics are combined in one product to achieve synergistic effects they are usually addressed as synbiotics. The vast majority of prebiotic substances are carbohydrates that are indigestible for human digestive enzymes but can be fermented by beneficial bacterial genera in colon and serve as a substrate for their metabolisms. Some of them may occur in natural foods, like human milk oligosaccharides in mother's milk, while the others are added to different foods to provide their health benefit. Good examples of prebiotics are fructo-oligosaccharides (FOS), inulin, galacto-oligosaccharides (GOS), soybean oligosaccharides, but also some complex polysaccharides that constitute dietary fiber.[28]

Although viability of probiotics seems to be crucial for their efficacy, some small molecular metabolic products of probiotic bacteria (e.g. SCFAs) may also exert beneficial influence on host biological functions. These substances are sometimes referred to as postbiotics.[28]

Mechanisms of action of probiotics

Probiotics exert their beneficial effects through three main mechanisms: by changing gut ecology, affecting the intestinal mucosal barrier, and by modulating the immune response.[19,30]

Probiotics can inhibit the growth of enteric pathogens by decreasing luminal pH because of production of lactic acid and SCFAs, changing redox potential, production of non-specific inhibitors such as hydrogen peroxide and hydrogen sulphide, and specific bactericidal peptides and proteins such as bacteriocins.[19,31] They compete with other microorganisms for limited sources of macro- and micronutrients and for binding sides on the gut epithelium, not allowing free space for pathogens to adhere. The anti-adhesive properties of probiotics may result from competition for the same receptor, by the induction of increased production of mucin and biosurfactants, by the degradation of receptors or even by simulation of receptor sites utilized by pathogens. Moreover, some probiotic bacteria can directly inhibit the production of toxins by pathogenic bacteria,[32] or cause their destruction by secretion of specific proteases.[19,30]

Probiotics enhance intestinal barrier function by inducing the production of mucin,[33] and defensins,[34] by preserving epithelial cell layer integrity because of reinforcement and induction of synthesis of tight junction proteins[35,36] and prevention of cytokine-induced apoptosis of epithelial

cells,[37] and by accelerating epithelial repair after injury.[19,38]

Gut microbiota is an important mediator of immune-regulation in the gastrointestinal environment, hence many probiotics act upon modulation of immune responses. The effect on the immune response differs in relation to the specific probiotic strain and they may involve both the innate and adaptive immune responses.[19] Immunoregulatory role is an important factor for the elimination of intestinal pathogens on one side, and for tempering inflammatory response when it is redundant and harmful for the host like in case of inflammatory bowel diseases, on the other. While some probiotics may induce increased production of secretory IgA, enhance activity of natural killer cells,[39] and up-regulate pro-inflammatory cytokines through activation of the transcription factors, such as nuclear factor κB, and activating protein-1, the others may produce suppressive effects dominated by immune tolerance through the induction of anti-inflammatory cytokines, such as IL-10 and TGF-β, and decreasing expression of pro-inflammatory cytokines such as TNF and interferon-γ.[19,31] It seems that many of the immunoregulatory properties of probiotics depend on their ability to induce the maturation of antigen presenting cells like dendritic cells via different pattern-recognition receptors that finally results in T-cell differentiation into Th1, Th2 or regulatory T cells and consequent production of different sets of pro- or anti-inflammatory cytokines.[20,25]

Clinical use of probiotics and prebiotics

Probiotics and prebiotics are available in a variety of forms, such as food products, infant formulas, enteral nutrition, food supplements, and even drugs. However, they contain a broad spectrum of different bacterial strains and prebiotic substances in very different concentrations. The efficacy of pro- and prebiotics in clinical practice has been extensively studied during the last two decades. The results of good quality clinical trials led to numerous reviews and recommendations.[28,40-44] It is worth stressing once again that thinking of probiotics and prebiotics as one common group of microorganisms or substances sharing similar properties and efficacy is completely wrong. The results of clinical studies with specific strain or strains in a defined dose and vehicle should not be extrapolated to the use of other products. Moreover, as the mechanisms and area of action are specific for each strain or prebiotic substance, the clinicians should be aware that no probiotic or prebiotic is efficient for all indications. Therefore, the choice of an appropriate preparation with evidence-based clinical efficacy for a specific indication in clinical practice is crucial.

This paper is focused only on general recommendations, and it is far beyond its scope to go into details which specific probiotics and prebiotics have been scientifically proven for efficacy in different diseases. Unfortunately, the number of high quality clinical studies with prebiotics is scanty. The recommendations for the use of probiotics are usually based on the fact how strong is the evidence that a specific probiotic efficiently prevents or treats certain disease. According to this, there is a very strong scientific evidence for the use of specific probiotics in the treatment of acute gastroenteritis, the prevention of antibiotic-associated diarrhea, the prevention and treatment of atopic dermatitis and the prevention of relapses of ulcerative colitis and pouchitis, as well as enhancing immune response for the prevention of common community acquired infections of gastrointestinal and respiratory systems. Quite strong is the evidence of specific probiotics' efficacy in the prevention of necrotizing enterocolitis, treatment of *Clostridium difficile* associated diarrhea, treatment of active ulcerative colitis, and alleviation of the symptoms of functional gastrointestinal disorders such as irritable bowel syndrome. However, for several other diseases, such as Crohn's disease, and radiation colitis, the evidence of probiotic efficacy is too scarce or the results of clinical trials too conflicting to recommend their use.

According to growing evidence about the importance of intestinal microbiota in health and disease, both probiotics and

prebiotics possess an immense potential for clinical use. The number of clinical trials with probiotics and prebiotics increases from year to year. Therefore, we can expect that new probiotics and prebiotics as well as new indications for their use will emerge over the next few years, with two of them, i.e. the prevention and treatment of metabolic disorders and cancer already being under intensive research.[45-48]

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