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Review

The intestinal ecosystem and probiotics

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Summary. The term "probiotic" comes from the Greek "*pro bios*" and means "pro-life". Nowadays, an increasing number of pharmaceutical preparations and functional foods are enriched with probiotics and for the patients it is increasingly important to receive the information needed to know how to orient in the choice. The benefits from probiotics are many and include the modulation of the intestinal microflora (stimulation of beneficial bacteria and inhibition of pathogens), the support of bowel function and the stimulation of the immune system. This broad spectrum of beneficial effects contributes to maintain efficient the intestinal ecosystem. Therefore, probiotics are an useful tool to prevent the formation of disorders and/or pathologies. The aim of this review is to describe the intestinal ecosystem and how probiotics could be effective in the treatment and prevention of possible alterations.

Key words: probiotics, intestinal ecosystem, intestinal microflora

Introduction

In recent years, the demand of health by healthy subjects increased in terms of quantity and quality. Patients increasingly informed and attentive to health and prevention issues go to their doctors for information and decision-making aspects. Hence the need of general practitioners to acquire new skills as they play a key role in the application and dissemination of appropriate prevention strategies.

The prevention and management of chronic diseases by General Medicine play a key role in population health. One of the most important topic in prevention is nutrition. Daily, general practitioners answer patients questions regarding their health and dispense nutrition advice.

In nutrition field, great attention has been paid to Mediterranean diet (1), but a new interesting topical is about "probiotics". This term was coined many years ago but its definition has recently reached an international consensus, replacing the old term of "lactic ferments" which has been used for a long time. The lactic ferments indicated the bacteria responsible of lactic fermentation and it corresponded to the British term "Lactic Acid Bacteria", which includes all bacteria that produce lactic acid from various substrates (eg. Lactobacilli, Streptococci, Lactococci). More than a century ago, the beneficial effects of lactic acid bacteria were proposed by Nobel laureate Elias Metchinkoff in his book entitled "The prolongation of life" (2). Metchinkoff, based on his observations on the longevity of the Balkan peoples who consumed large amounts of yoghurth, assumed a beneficial protective effect of the bacterial flora in humans, as well as the lactic fermentation stopped the putrefactive phenomena of food matrices (eg. milk, meat, vegetables) (2). This intuition found the scientific vali-

dation many years later and the small number of controlled clinical trials contributed to the persistence of a certain skepticism in the medical community about the clinical efficacy of probiotics, considered only placebo. In the last decade, many studies of molecular biology gave a better characterization of the bacterial species and their function; clinical trials of phase 2 and 3 in different clinical conditions have shown efficacy of strains with the characteristics required to be defined probiotics. It is increasingly clear that the action of probiotics should not be seen only in clinical terms but must be assessed from the point of view of the beneficial effects on people's health.

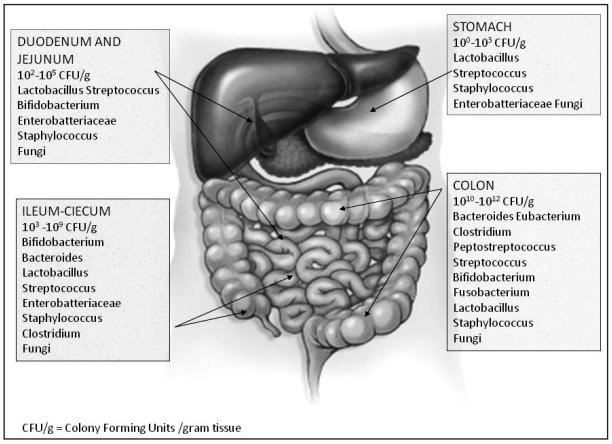

Figure 1. The intestinal Flora

The intestinal ecosystem

The term intestinal ecosystem has recently been introduced in the medical field. This term indicates the functions and interactions between the mucosal barrier, the local immune system and the intestinal microflora. The intestinal microflora is considered a real organ metabolically active and very important for the health of our body.

To understand the importance of the bacterial flora in the homeostasis of the organism it is necessary to know the extent and complexity of its composition, and the many activities and interactions that it performs. In the intestine, there were identified to date up to 500 different species of bacteria with a total luminal contents of microbial cells about ten times higher than the number of somatic cells. In one gram of feces they can be isolated 100-200 billion of bacteria, whose mass is physiologically about 60% of the weight of feces (3).

The concentration of the bacterial flora varies along the digestive tract increasing exponentially in the oral-fecal-route. In the stomach and in the first section of the small intestine there is a low concentration of bacteria (101-104 per gram of content) mainly due to the presence of acid, bile and pancreatic juice which is not favorable for their development and propulsive motor activity that prevents stable colonization and pushes the contents of the lumen to the ileum. The bacterial concentration increases gradually along the intestine to reach 1011-1012 microorganisms/g in the colon (Figure 1). The colonization of the intestinal lumen occurs during the birth and the initial pattern of bacteria is influenced by the type of childbirth, nutrition and social-environmental conditions. A few hours after birth aerobic bacteria begin to develop (coliforms, streptococci, lactobacilli, enterococci) and at 10-11 days of age Bacteroides appear. The latter are the main constituents of the bacterial flora which is outlined in 3-4 weeks of life and does not change significantly in normal conditions. So the initial colonization is crucial in determining the final composition of the intestinal microflora of the adult, which varies from individual to individual and remains constant during life even if the concentration of the various species may temporarily vary in different pathological conditions. The completed flora consists mainly of anaerobic microorganisms (eg. Bacteroides, Bifidobacterium, Eubacterium, Clostridium, Peptostreptococcus, Streptococcus) that are 100-1000 times more numerous than the aerobic (Escherichia, Enterobacter, Enterococcus, Klebsiella, Lactobacillus, Proteus, etc.).

Functions of the intestinal microflora

Numerous studies, performed primarily on animals sterile intestinal contents (germ-free), demonstrated the multiple activities of the intestinal flora, which are grouped in metabolic, trophic and protective functions. The most important metabolic function is the digestion (fermentation) of carbohydrates non-digestible by humans (cellulose, hemicellulose, pectins, gums, starch) by the bacterial enzymes, resulting in the production

of short chain fatty acids (SCFA) and gases (H_2, CO_2, methane, hydrogen sulphide) (4). The SCFA are an energy source for the bacteria, for colonocytes and, once absorbed, for the somatic cells; moreover, they are involved in the metabolism of sugars by improving insulin sensitivity, they acidify the intestinal environment (5) by preventing the proliferation of pathogenic germs (6), they increase the blood flow and the intestinal motility and promote the re-absorption of water and ions. The digestion of peptides and proteins (7), such as elastin, food collagen, pancreatic enzymes, mucin, epithelial cells and bacteria lysates, leads also to the production of SCFA, but also potentially toxic substances, such as ammonia, amines, phenols, thiols, indoles and gases. Fermentation takes place primarily in the cecum and ascending colon where the environment is more acidic for the presence of saccarolitic flora in rapid growth. Rot is instead prevalent in the distal colon, where the flora is more static and the pH is closer to neutrality. Another metabolic activity of intestinal bacteria is the production of vitamins (pantothenic acid, biotin, pyridoxine, riboflavin), but it is still unknown how they are utilized by the body. The trophic function of the intestinal microflora (8) occurr through the proliferation and differentiation of epithelial cells (by SCFA) and through maturation and stimulation of the intestinal immune system (gut associated lymphoid tissue, GALT - about 25% of the intestinal mucosa). Many studies highlighted the complex interaction between the bacterial flora and the GALT that is fundamentally important, already in the early stages of life, for the development of our immuno-regulatory system (6). The protective function operates mainly through a physical barrier that prevents the adesion and the tissue penetration of pathogens and injurious substances. The protection mechanisms may include the competitive binding with intestinal epithelial cell receptors, the competition in the use of substrates (space and food), the production of antimicrobial substances (bacteriocins, ammonium, H_2O_2) and the lowering of the luminal pH through the production of SCFA (9). The protective function is also expressed through immunomodulation with increase in the specific antibody response and the regulation of the production of pro- and anti-inflammatory cytokines. The immuno-surveillance monitors infections and immune-tolerance prevent the development of allergies (10).

Alterations of the intestinal ecosystem

The maintenance of the intestinal ecosystem is based on the integrity and stable cooperation between the microflora, the immune system and the barrier formed by the intestinal mucosa. Any event that intervene to alter each of these components creates an imbalance with the consequent onset of local and/or systemic diseases (11,12). It is very important the balance between the different species of the bacterial flora in which there are harmful bacteria (*Pseudomonas aeruginosa,* bacteria belonging to the genera Staphylococcus, Clostridium, Proteus, Veillonella), protective bacteria that become harmful in particular conditions (*Escherichia coli*, enterococci, streptococci, Bacteroides) and bacteria with protective action (belonging to the genera Lactobacillus, Bifidobacterium, Eubacterium) (13). The pathogenic strains are characterized by their ability to produce toxins, they could be invasive and produce substances with carcinogenic action; in health conditions their growth and their metabolic activities are inhibited by the protective flora. Table 1 lists some conditions that alter the composition of the intestinal microflora

Table 1. Main causes of altered intestinal flora

Anatomical alterations
Diverticula
Stenosis and obstruction
Motility alterations
Progressive systemic sclerosis
Diabetic neuropathy
Pseudo intestinal obstruction
Accelerated gastric emptying
Incontinence of ileocecal valve
Post-surgical alterations
Ansa blind
Gastric and intestinal resections
Resection of ileocecal valve
Jejunum-ileal bypass
Other conditions
Malnutrition
Immunodeficiency
Aging
Prolonged therapy with antisecretory (H2 blockers or PPIs)
Antibiotic therapy

(10). Many of these conditions can also cause bacterial overgrowth in the first sections of the small intestine (duodenum and jejunum) with a clinical profile that is currently defined by the acronym SIBO (Small Intestinal Bacterial Overgrowth). Alterations of the intestinal flora could also occur during antibiotic therapies, with aging and with nutritional modifications. It is well known that there is a relationship between diet and the incidence of certain types of cancer, particularly colon cancer. A diet high in fat and red meat seems to be associated with a high risk of colorectal cancer and this carcinogenic effect could be mediated by the modification of the intestinal microflora resulting in the selection of germs producing carcinogens (co-carcinogens or pro-carcinogens). Therefore, some bacteria can induce alterations in colonocytes DNA by producing molecules such as heterocyclic amines, which instead may be removed by other bacteria (14). Some studies in animal models showed that bacteria belonging to the genera Bacteroides and Clostridium increase the incidence and growth of tumors of the colon, whereas lactobacilli and bifido-bacteria, seem to prevent tumorigenesis (15). Lesions of the intestinal barrier may allow the passage of bacteria contained in the lumen through the mucosa (generally aerobic gram-negative as Escherichia, Proteus, Klebsiella), a phenomenon called translocation. After passing through the mucosa, viable bacteria can reach the mesenteric lymph nodes, spleen and liver through the lymphatic vessels and then disseminate in the organism causing sepsis.

In healthy subjects, it is believed that the positivity of the culture of the mesenteric lymph nodes does not exceed 5%, while it rises to 15-40% in diseases such as multiple organ failure, severe acute pancreatitis, advanced liver cirrhosis, intestinal obstruction, chronic inflammatory bowel diseases (16-18).

Translocation was described in patients undergoing laparotomy, in the post-operative sepsis, in spontaneous bacterial peritonitis in patients with liver cirrhosis. The lesions of the intestinal wall are a frequent feature of inflammatory bowel disease (such as ulcerative colitis, Crohn's disease), whose etiology to date is still unknown. Although the infectious origin of these diseases remains for now just one of the hypotheses, numerous studies showed that the intestinal flora contributes to the development and maintenance of inflammation; for this reason, current studies aim to evaluate the effect of probiotics in the treatment of these diseases (18).

Probiotics: definitions and properties

Probiotics are defined as "live and vital microorganisms that confer health benefits when consumed in adequate amounts, as part of a food or a supplement". It is important to distinguish them from "prebiotics", which indicate the non-absorbed food components that stimulate the growth of one or more protective bacteria of the intestinal flora and, in this way, it makes a positive effect on human health. In addiction,"symbiotic" means the combined administration of specific probiotics with prebiotics to determine benefits through a synergic action of the two components (19). The authorization for the use of probiotics is now based on the respect of the criteria defined by the international guidelines (20) which include identification, safety, efficacy. It is important to consider that the probiotic properties are the characteristics of the single strain studied and it can not be extended to other bacteria, even if they belong to the same species. Regarding the other criteria, the in vitro and in vivo tests are needed to determinate the safety and functional characteristics required for a probiotic: resistance to gastric acid and bile, adhesion to mucus and/or intestinal cells, antimicrobial activity against pathogenic strains, reduction of the adesion of pathogens, hydrolysis of bile salts, resistance to spermicides (for probiotics used in the vagina).

The certification of the safety of probiotics is necessary to prevent the occurrence of any systemic infections, harmful metabolic activities, excessive immune stimulation, gene transfer. The bacterial genera most used and studied (Lactobacillus and Bifidobacterium) are the prrof of safety as they are common commensals of human microflora and they are used for the fermentation of many foods. There are very few reported cases so far, in which it can be assumed that the probiotic, added to the diet, has given rise to intestinal infections, and they are all described in debilitated patients exposed to extensive antibiotic therapies (20).

Probiotics are those bacteria that regulate actions and processes that take place in the bowel (digestion,

immune defense, defense bacteriological etc.) and action of welfare. The word was coined about 50 years ago and it has had several changes over the years, but in this context it seems appropriate to refer to the one adopted in December 2005 by the Ministry of Health: "probiotics are live microorganisms and vital that confer health benefits when consumed in adequate amounts, as part of a food or a nutritional supplement. This definition is similar to that provided by FAO/WHO in 2002 and then also reflected in the world (20) .

The guidelines that define what probiotics are, taken by international organizations and by Italian Ministry of Health, consider the correct identification of the bacterial strain, the effective dose, the safety and efficacy. Regarding identification, bacteria are so many and they are different from each other; even those they belong to the same "bacteria family". The bacteria species are identified by the use of Latin and the two names genre + species (for example in the name Lactobacillus casei, the first term indicates the bacterial genus, while the second specify which the specie is). We could compare the bacterial species to the family and the genre to the clan. The ability to be probiotic is a property of the strain and within a specie, not all the strains are probiotics. Therefore it is crucial, both in scientific studies and in commercial products, that it is indicated the probiotic strain to which it refers, and not just the specie (eg Lactobacillus casei Shirota, or Bifidobacterium lactis BB12). The effective dose, that is the amount of live and vital bacterial cells for administration to an individual needed to express probiotic properties, is definitely an essential point to be determined. Safety must be demonstrated in vitro and in vivo in animals. Most of the currently known probiotic organisms are included in the GRAS list (Generally Recognized As Safe) and therefore their use is considered safe. The effectiveness of a probiotic strain is assessed by in vivo tests, during the volunteers consume the product under medical supervision and it is then subjected to specific tests (for example, the profile of immune cells) (20).

Functional probiotic foods

A functional food can thus be a natural food or it can be a food that has been modified to have a functional influence on the health and well-being of the consumer through the addition, removal, or modification of specific components (21).

A typical characteristic of probiotic bacteria is their ability to arrive alive and active in the colon in high quantities. As far as probiotics are in foods, it necessarily implies that they have to overcome the gastric barrier and tolerate the presence of bile acids in such a way as to arrive alive in the intestine in much greater amounts than those found for the bacteria normally used in the production of yoghurt. To be considered probiotic, a bacterium must therefore be able to have scientific studies which demonstrate the vitality in the human gut and the ability to play a positive activity to the consumer (22).

Dairy-related functional foods include yogurt and other fermented dairy products. Various species of lactobacilli and bifidobacteria are used in the production of fermented milk products.

These live microorganisms have been reported to have several beneficial effects, including aiding the prevention of cancer and hypertension, as well as therapeutic effects on intestinal tract function, immune function, and stomach health (23). The cancer-preventing mechanism of probiotics is reportedly related to their byoproducts, which might decrease cancer cell proliferation (24) Furthermore, probiotics might improve symptoms of lactose intolerance and reduce serum cholesterol (25).

The strain *Lactobacillus casei Shirota* (LcS) has been the subject of scientific research to prove its probiotic properties and safety of its use for more than 70 years.

In vitro it has been observed that LcS is able to tolerate the gastric fluids and the presence of sodium deoxycholate better than other strains used for the production of yoghurth (26). A recent study reports the presence of the strain LcS in the faeces of healthy volunteers after three weeks of daily intake of fermented milk containing 6.5 billion this bacterium (27). This result shows that this strain can tolerate the passage through the stomach and it can reach the intestines alive and active, critical prerequisite for defining a bacterial strain probiotic . It was noted that LcS does not adhere permanently to the intestinal walls and therefore, 2-3 weeks after the last dose, it is no

longer isolated from feces (28). This suggests the need to continue restoring the probiotic every day in order to keep it in sufficiently high amounts within the gut.

Numerous scientific studies, in vitro, in animals but also in clinical studies attest the ability of this strain to bring many benefits to the body, by the modulation of the intestinal microflora, the functionality of the intestine and the stimulation of certain immune system activity (eg. Natural Killer cells activity) (29-35).

Omega-3 fatty acids, which are an important functional component and essential fatty acid of animal origin, are mainly found in fatty fish. The two primary n-3 fatty acids are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA),26 and these fatty acids have been reported to reduce the risk of coronary heart disease (36), cardiac deaths, and fatal and non-fatal myocardial infarction, as well as to reduce triglycerides (37). In addition to the natural sources of n-3 fatty acids, such as fatty fishes like salmon, tuna, and sardines, several commercial food products are now fortified with n-3 fatty acids. Some examples of commercially available n-3-fortified foods include eggs, milk, mayonnaises, margarines, and salad dressings (38).

Red wine contains phenolic compounds, and it could be considered as a functional food. Phenolic compounds have been reported to have antioxidant effects and might prevent the oxidation of low-density lipoprotein (LDL) (39). Wine drinking has also been linked to the reduction of certain cancers (40).

Soy protein has many significant health benefits for humans, such as lowering the levels of total and LDL cholesterol in the blood and reducing the risk of certain cancers. The US Food and Drug Administration (FDA) has also reported that daily consumption of 25 g of soy protein, coupled with a low-cholesterol diet,might reduce the risk of heart disease (37).

Coronary heart disease (CHD) is related with the level of cholesterol in the serum (41). Plant sterols (phytosterols) influence the serum cholesterol level by inhibiting absorption of cholesterol and LDL cholesterol (42).

Plant sterols can be obtained from fruits and vegetables; however, such foods contain only small amounts of phytosterols. 54 Nuts and vegetable oils contain higher amounts of phytosterols than fruits and vegetables (43). In addition to these foods, food markets offer plant sterolenriched

foods, like spreads or drinks. The FDA has reported that a daily intake of at least 1.3 g of plant sterols might reduce the risk of heart disease (37).

Consumption of whole grains and cereals are known to improve digestion due to their high fiber content. In addition, the endosperm of whole grains contains bran and germs that are rich in micronutrients and phytochemicals (44). It has been reported that consumption of whole grains might reduce the risk of heart disease, type 2 diabetes mellitus, and various types of cancer (45).

Another fiber-rich food group is fruits and vegetables – a group that also contains many vitamins and nutrients beneficial for health. The FDA has reported that consumption of fiber-rich

foods, like grains or fruits and vegetables, when coupled with a low-fat diet,might reduce the risk of some types of cancer, and intake of soluble fiber in which grains, vegetables, and fruits are rich, might reduce the risk of heart disease (37).

Clinical applications

Data from in vitro studies should be followed by the evaluation of effectiveness and usefulness in vivo. Also in vivo, in healthy volunteers, the effective dose can be determined, the quantity of live and vital bacterial cells for administration to an individual so that they can express probiotic properties. The works published in the last five years show a number of beneficial effects on the human body provided by probiotics (Table 2).

Proven benefits of probiotics include the treatment of acute (46) and antibiotic-associated diarrhea (47); applications with substantial evidence include the prevention of atopic eczema and traveler's diarrhea (48); promising applications include the prevention of respiratory infections in children, prevention of dental caries (49,50), elimination of nasal pathogen carriage, prevention of relapsing C. difficile–induced gastroenteritis, and treatment of inflammatory bowel disease (51); and proposed future applications include the treatment of rheumatoid arthritis (52), treatment of irritable bowel syndrome, cancer prevention, pre-

Table 2. Mechanisms of probiotics beneficial effects in humans

Bacterial pathogens growth inhibition:
Reduction of luminal pH
Production of bacteriocins
Resistance to colonization
Block of epithelial adhesion
Increase protective barrier effect:
Production of short-chain fatty acids (SCFA)
Increased production of mucus
Stimulus to the production of zonulin (component of tight junctions)
Adjusting the mucosa permeability
Competition with patogen adhesion sites and with toxins receptors
Modification of immunoregolation:
Increase of IL-10 and TGF-β and decrease of TNF
Increased secretion of IgA

vention of ethanol-induced liver disease, treatment of diabetes, and prevention or treatment of graft-versus-host disease. The use of probiotics in medical practice is rapidly increasing, as are studies that demonstrate the efficacy of probiotics (51).

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