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The therapeutic management of gut barrier leaking: the emerging role for mucosal barrier protectors

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Abstract. – OBJECTIVE: Gut barrier is a functional unit organized as a multi-layer system and its multiple functions are crucial for maintaining gut homeostasis. Numerous scientific evidences showed a significant association between gut barrier leaking and gastro-intestinal/extra-intestinal diseases.

MATERIALS AND METHODS: In this review we focus on the relationship between gut barrier leaking and human health. At the same time we speculate on the possible new role of gut barrier protectors in enhancing and restoring gut barrier physiology with the final goal of promoting gut health.

RESULTS: The alteration of the equilibrium in gut barrier leads to the passage of the luminal contents to the underlying tissues and thus into the bloodstream, resulting in the activation of the immune response and in the induction of gut inflammation. This permeability alteration is the basis for the pathogenesis of many diseases, including infectious enterocolitis, inflammatory bowel diseases, irritable bowel syndrome, small intestinal bacterial overgrowth, celiac disease, hepatic fibrosis, food intolerances and also atopic manifestations. Many drugs or compounds used in the treatment of gastrointestinal disease are able to alter the permeability of the intestinal barrier. Recent data highlighted and introduced the possibility of using gelatin tannate, a mucosal barrier protector, for an innovative approach in the management of intestinal diseases, allowing an original therapeutic orientation with the aim of enhancing mucus barrier activity and restoring gut barrier.

CONCLUSIONS: These results suggest how the mucus layer recovering, beside the gut microbiota modulation, exerted by gut barrier protectors could be a useful weapon to re-establish the physiological intestinal homeostasis after an acute and chronic injury.

Key words:
Gut barrier, Mucus, Gut microbiota, Gelatin tannate, Mucosal protectors, Gut barrier enhancer.

Introduction

The gastrointestinal tract is the most exposed human habitat to the external environment with a surface area of 200 m^2. Every day, thousands of microorganisms and compounds derived from the digestion are in contact with it. In this condition, the role of the gut barrier is crucial. Indeed, it provides a complex defense system capable of separating the intestinal contents from the host tissues, modulating the absorption of nutrients and allowing the interaction between the resident microbial flora and the mucosal immune system, inhibiting the translocation of pathogens in underlying tissues[1].

In particular, the gut barrier is a functional unit organized as a multi-layer system. It is divided in two main parts: a physical barrier, which prevents bacterial adhesion and regulates paracellular diffusion, and a functional deeper barrier that is able to discriminate between pathogens and commensal microorganisms, by organizing an immunological tolerance towards commensals and an immune response towards pathogens. In addition, there are other mechanisms involved in maintaining the gut luminal homeostasis. For example, the low pH of gastric juice is bactericidal and therefore acts against infectious agents, while pancreatic enzymes are able to damage the bacterial cell.

The gut barrier starts from the resident microbiota. It competes with pathogens to gain space and energy resources, processes the molecules required for mucosal integrity and modulates the intestinal immunological response. The next level is represented by the mucus layer. It separates the luminal content from the deepest layers and contains antimicrobial products, and secretory IgA. Below the mucus, there is the monolayer of intestinal epithelial cells. This is able to form a physical barrier and contains the immunological cells. These cells

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can present pathogenic antigens to the cells of the functional barrier with the aim of developing an appropriate immune response[1-3].

The deep part of the gut barrier is represented by a complex network of immune cells, which are organized in a specialized and compartmentalized structure known as "gut associated lymphoid tissue" or GALT. The GALT consists of lymphoid follicles and is one of the major lymphoid organs, containing up to 70% of the body immune cells. It is responsible for the response to pathogenic microorganisms and the immune tolerance to commensal bacteria. This ability is explicated through a physiological relationships with the surface layer and the intimate contact with the external environment through specific immune cells: the dendritic cells and the M-cells within the Peyer's patches. These cells have the ability to acquire microorganisms and macromolecules and to present antigens to T lymphocytes, which produce cytokines activating the immune response.

The integrity of these structures is required for the maintenance of the normal intestinal permeability. The alteration of this equilibrium leads to the passage of the luminal contents to the underlying tissues and thus into the bloodstream (gut barrier leaking), resulting in the activation of the immune response and in the induction of gut inflammation. This permeability alteration is the basis for the pathogenesis of many diseases, including not only infectious enterocolitis and inflammatory bowel diseases (IBD), but also irritable bowel syndrome[4], small intestinal bacterial overgrowth (SIBO), celiac disease, hepatic fibrosis, food intolerances and also atopic manifestations[1,5,6].

The assessment of the intestinal permeability and of the related molecular mechanisms may become an interesting parameter to consider in clinical practice for studying and, especially, treating these diseases. *In vitro* models (Caco2 and HT29-MTX cells) and *in vivo* non-invasive techniques (sugar tests and tests with radioisotopes) are effective methods for this type of evaluation. Finally, the modulation of the various barrier components is crucial for the control of intestinal permeability[1].

The gut barrier

Gut microbiota

The gut, which under physiological conditions also includes fungi and bacteriophages, harbors about 1 kg of bacteria that contain more than 3 million genes and have a mutualistic relationship with their host. Gut microbiota plays several functions: barrier, synthesis, immune stimulation, metabolism of nutrients, and metabolism of drugs and toxins. In particular, the microbiota is crucial for the digestion of energy substrates, the production of vitamins and hormones, and the protection of the host from the pathogenic species[2,7,8]. In fact, the microbiota is able to consume the nutrients necessary for survival of pathogens and can produce molecules, which inhibit the growth of pathogenic flora. Several studies showed that *Lactobacilli* and *Bifidobacteria* produce bactericidal acidic substances, such as lactic acid, bacteriocins and short chain fatty acids (SCFA). In animal models these products can suppress the growth of *Salmonella Typhimurium*. Recently, it has been shown that a compound formed by *Bacteroides thetaiotamicron* and *Eubacterium rectale* can induce the host production of mucosal glycans. These are exclusively used by these two bacterial strains and not by pathogens, which therefore are not able to proliferate[9-11].

Moreover, the enteric microflora produces nutrients for the mucosal cells. *Bacteroides thetaiotamicron* colonizes the outer mucus layer and can degrade peptides and glycans that constitute the mucus, producing SCFA, such as butyrate. The SCFA are involved in the stimulation of mucus production, in blocking the invasion and adhesion of *Escherichia coli*, and in increasing mucosal total and pathogen-specific IgA[12-14].

The microflora alteration is the basis of many gastrointestinal disorders such as gastritis, peptic ulcer, IBS, IBD and even gastric and colon cancer. In IBD, for example, the concentration of bacteria adhering to the intestinal enterocytes is higher compared to healthy controls and increases progressively with the severity of the pathology. In addition, there is a reduction in bacterial diversity with an increase in *Enterobacteriaceae*, including *E. coli*, and a marked reduction in *Bacteroides* and *Clostridium*. This condition leads to the reduction of enterocytes growth and maturation with a consequent increase in intestinal permeability[15-18].

Intestinal mucus

The mucus is the first physical barrier that bacteria encounter within the digestive tract. It acts as a shield for the epithelium, protecting it from harmful microorganisms and antigens, but also as a lubricant for intestinal motility. It consists of two layers: an inner layer, firmly adherent to the epithelial cells and approximately 50 μm thick,

and an outer layer, looser and less adherent, which is approximately 100 μm thick, according to the measurements made in animal models[19].

The inner mucosal layer is dense and does not allow bacteria to penetrate, thus making the epithelial cell surface free from bacteria. The inner layer turns into the outer layer, which is the habitat of commensal flora. Both layers are organized around the highly glycosylated mucin MUC2, which forms an amorphous polymer-like cover and is secreted by goblet cells. The MUC2 mucin is a molecule that has been preserved along the evolution since the first metazoans[20].

After secretion, the MUC2 mucin is organized in a hydrated and expanded network and forms, together with other secreted proteins, a well-organized mucus layer[21]. The protein composition is similar in the two mucosal layers since it is derived from a common cellular source. The physiological microbial flora resides in the outer mucus layer, while the inner layer is impervious to bacteria and acts as a protective barrier for the epithelial cell surface. This compartmentalization is essential for intestinal homeostasis within a highly colonized colon[22,23].

The importance of the mucosal barrier was further demonstrated in MUC2 deficient animals. In this model, the bacteria are in direct contact with the epithelial cells, but also with intestinal crypts. The increase in intestinal permeability, caused by the loss of mucus barrier, triggers an inflammatory reaction and, subsequently, can promote the development of colon cancer. Furthermore, mucus exerts not only a barrier function. Its content in glycans, linked to mucin MUC2, serves as nutrient for bacteria, but also as a binding site and, induces the selection of specific microbial species, which are essential for maintaining the integrity, homeostasis and intestinal function[24-26].

Epithelial cells

The intestinal epithelium is organized in a monolayer of cells, with a thickness of only 20 μm and composed by 5 different cell types: enterocytes, endocrine cells, M cells, G cells and Paneth cells. The enterocytes are the most represented cell type. They act as a physical barrier that inhibits the translocation of luminal contents in the inner tissues. They are connected by intercellular structures called tight junctions calls. These are characterized by trans-membrane proteins, which interact with adjacent cells and with intracellular proteins associated with the cytoskeleton[27]. Together, these components form a complex and homogeneous network. In the intestinal epithelium there are two main types of junctions: the adherens junctions (AJs) and the tight junctions (TJs). Both types are formed by in different concentrations of cadherin, claudin, and zonuline.

The modulation of these junctions, and therefore of the epithelial barrier permeability, takes place through the myosin phosphorylation and the actin-myosin complex contraction. An alteration of this balance increases the mucosal permeability, causing the passage of endoluminal molecules in the deeper layers, determining the activation of the adaptive immune response and leading to the inflammatory state[28-31]. This condition is observed especially during the infectious enterocolitis and IBD. In the first condition, the *Entero- hemorrhagic E. coli* (EHEC), and *Entero-pathogenic E. coli* (EPEC) have the ability to adhere to intestinal epithelial cells and to break the integrity of the barrier through the alteration of tight junctions. Moreover, a dysfunction of the AJ protein has been described in IBD. This is due to a reduction of E-cadherin. This alteration leads to the weakening of intercellular adhesion and to an inflammatory response. Recent studies showed that high concentrations of IFN-γ and TNF-α, typical found in UC and CD, can cause a reorganization of numerous proteins that constitute the tight junctions, such as zonulin-1, JAM-A, occludin, claudin-1 and claudin-4. Even in this case, this leads to an increased intestinal permeability, which is also found in IBS, especially in the subgroup of IBS with diarrhea (IBS-D)[32,33].

Gut barrier leaking and related diseases

The ability of the various intestinal barrier components to ensure the physiological permeability even in presence of pathogenic factors is essential for health maintenance. In fact, when the protection exerted by the intestinal barrier fails, the immune cells come in direct contact with antigens in the intestinal lumen. This results in the impairment of normal physiological barrier functions, such as the immune response to pathogens (bacteria, viruses, fungi and parasites), the recognition of "self" antigens, the tolerance towards the commensal flora and the desensitization to food antigens. Numerous scientific evidences showed a significant association between gastro-intestinal and extra-intestinal diseases and

permeability alteration. Among these, autoimmune diseases, including celiac disease, IBD, diabetes mellitus type I, multiple sclerosis and rheumatoid arthritis, but also liver cirrhosis, acute pancreatitis, infectious gastroenteritis, and small intestinal bacterial growth (SIBO) are included. Even diseases not directly related to the mucosal barrier function, such as heart failure or autism, can cause an increase in intestinal permeability. In some cases, such as in IBD and celiac disease, the permeability alteration could be the primary pathogenic cause. In others, such as in liver cirrhosis, it would lead to the translocation of microbial antigens into the entero-hepatic circulation with the consequent exacerbation of liver fibrosis and of portal hypertension, and a further increase in permeability[1].

Whether the alteration of the barrier function is an epiphenomenon, an early event, or an essential step in the pathogenesis of these diseases, needs to be clarified. However, it is well known that an increase in intestinal permeability contributes to the exacerbation of these pathologies. In many cases, the same drugs that lead to disease resolution, even temporarily, also lead to a recovery of the permeability and therefore to an adequate physiological intestinal homeostasis[24].

IBD are an example of increased intestinal permeability. It plays a central role in the pathogenesis of this disease. In this case, the altered intestinal permeability precedes the development of the disease. The exact pathogenesis remains unknown, but it certainly involves genetic, immunological and environmental factors that initiate the autoimmune process. In Crohn's disease clinically asymptomatic patients, an increase in intestinal epithelial permeability anticipates of about 1 year the recurrence of the disease. This suggests that a defect in intestinal permeability is an early event and is predictive for the clinical exacerbation of the disease[34]. While the primary defect of the intestinal barrier seems to be involved in the early stages of IBD pathogenesis, the production of cytokines, including IFN-γ and TNF-α, secondary to the inflammatory process, would serve to perpetuate the increase in intestinal permeability following the reorganization of the proteins that constitute the intercellular junctions. In this manner a vicious cycle is created. The primary dysfunction of the barrier would cause an initial passage of luminal contents, which in turn triggers an immune response with the maintenance of an altered permeability and the exacerbation of the disease[35].

Therefore, in this context new therapeutic strategies focused on the recovery of the physiological function of the intestinal barrier may offer an innovative approach for the clinical improvement of these highly debilitating chronic diseases.

The pharmacological modulation of the intestinal barrier

Despite clear evidence, the measurement of intestinal permeability is still far from becoming a laboratoristic parameter able to drive the clinical practice. However, many drugs or compounds used in the treatment of gastrointestinal disease are able to alter the permeability of the intestinal barrier. The main substances used in gastroenterology clinical practice are steroids, aminosalicylates, biologics (*i.e.*, anti-TNF-α), probiotics and mucosal protectors. While corticosteroids, salicylates and anti-TNF-α act in the modulation of intestinal permeability by reducing intestinal inflammation, probiotics appear to act through the modulation of mucin production and of epithelial junctions strength. Finally, emerging evidences suggest an important role for the mucosal protectors, such as the gelatin tannate, a medical device recently introduced also in Italy for the treatment of acute diarrhea.

Corticosteroids are the mainstay therapy for the induction of clinical remission in mild to severe Crohn's disease. Among these, prednisone is able to reduce intestinal permeability in 50% of patients, measured through the analysis of the relationship lactulose/mannitol[36]. Even prednisolone is able to reduce the permeability in children and adolescents with active Crohn's disease and ulcerative colitis[37]. This effect is due to the anti-inflammatory properties of corticosteroid, including the ability to inhibit the expression of pro-inflammatory cytokines such as TNF-α and NF-κB[38], although high concentrations of steroids (prednisone and budesonide) are related to a reduced mucosal healing[39].

The use of 5-aminosalicylic acid (5-ASA) is one of the leading treatments in the treatment of uncomplicated mild to moderate IBD. These substances, such as mesalazine, promote the rapid recovery of mucosal integrity through TGF-mediated signaling, thereby reducing intestinal permeability[40,41].

Biological drugs, including anti-TNF-α, are the most recently used drugs in the treatment of IBD. They act by blocking the action of TNF-α, reducing inflammation and restoring mucosal in-

tegrity. Some studies with ^{51}CrEDTA, showed a significant reduction in intestinal mucosal permeability after treatment with anti-TNF-α and the reduction is proportional to disease activity and mucosal healing[42].

Probiotics are frequently used in functional gastrointestinal disorders and in recent years have been tested for the treatment of IBD, SIBO and infectious diarrhea. Many studies highlighted the role of probiotics in the modulation and reduction of intestinal permeability. In a double-blind, cross-over, placebo-controlled trial, a mixture of *Lactobacillus rhamnosus* and *Lactobacillus reuteri* was administered for 6 weeks in 41 children with atopic dermatitis with increased intestinal permeability. After the treatment it was assessed a significant reduction in intestinal permeability, as documented by the test lactulose/mannitol[43]. Furthermore, *Lactobacillus rhamnosus GG* is able to accelerate the maturation of the intestinal barrier and tp induce the expression of claudin 3 in animal models, while Lactobacillus casei can increase the genes expression coding for zonulin in Caco2 cells[44,45]. *Saccharomyces boulardii*, in combination with the standard therapy, improves intestinal permeability in Crohn's disease patients in remission, with a clear normalization of the relationship lactulose/mannitol[46].

The mucosal protectors, such as sucralfate and bismuth, are normally used for a prolonged period in the treatment of peptic ulcer disease. These compounds protect the epithelial cells from the acidic gastric juices and digestive enzymes. They seem to be effective also in reducing the inflammation in infectious diarrhea. Among the mucosal protectors, the gelatin tannate is emerging between the most promising intestinal barrier modulator drugs. It is a combination of tannic acid (penta-m-digallolyl glucose) and gelatine, and could be able to create a protective film, forming bonds with the mucin, thus protecting the gut from the aggressive penetration of commensal bacteria (barrier protector). Tannins are widely distributed throughout the plant kingdom. Since tannic acid is one of the principal tannins, the term tannin is ordinarily used as a synonym for tannic acid. Gelatin is a collagen derivate, which is ingested as a powder insoluble at gastric acidic pH, and which becomes a gelatin with the increase of pH over 5.5[47]. Gelatin tannate is commonly employed as an intestinal astringent and present in the market with various name, like tanagel, gelenterum or tasectan[48]. This complex passes through the stomach unaltered, and once in the intestine it may exert its action by restoring its physiologic barrier function. Furthermore, the well-known astringent properties of tannins allow the precipitation of pro-inflammatory mucoproteins from the intestinal mucus and their elimination through the feces[49,50]. Recent studies presented at international conferences but not yet published, showed that at intestinal mucosal level, gelatin tannate acts in a non-dissociated form as a muco-adhesive film, with a protective effect on the intestinal barrier (gut barrier enhancer) and an indirect anti-inflammatory effect.

Our group studied the effect of gelatin tannate in Dextran Sodium Sulphate (DSS)-induced model of murine colitis[24-26]. DSS-induced colitis represents a T-cell independent, chemically-induced model of epithelial damage and acute inflammation, primarily driven by innate immune responses. It has been recently shown that DSS directly affects gut epithelial cells of the basal crypts, disturbing the integrity of the mucosal barrier[51]. It exerts its action by causing a fast alteration in the mucus permeability and a disruption of the mucus biophysical structure. Thus, it allows bacteria to enter and penetrate the inner mucus layer. Once bacteria come in contact with the epithelial cells, enter the crypts and are taken up by epithelial cells, GALT is triggered to react against also relatively harmless commensal bacteria and to cause an inflammation reaction[22]. Several studies focused on the clinical improvement of acute DSS-induced colitis by using probiotics and antibiotics in order to modulate the commensal microflora[52-57]. On the other hand, this model is a useful way to evaluate those factors that can modulate the anatomical and functional health of the gut barrier, by acting not only on the gut microbiota, but also on the recovering of the mucus layer. In this model we showed that gelatin tannate is able to significantly reduce the clinical activity of acute colitis compared to placebo. In particular, confocal microscopy showed the polymeric protective layer that gelatin tannate forms on the ulcerated mucosa, thus preventing the activation of the immune response[58]. In addition to these findings we assessed a modulation of the microflora composition, a restoring of mucus layer and, consequently, of gut permeability. Furthermore, another group showed that gelatin tannate reduces the proinflammatory effects of lipopolysaccharide (LPS) in human intestinal epithelial cells[59]. It is able to inhibit the intercellular adhesion molecule-1 (ICAM-1) expression

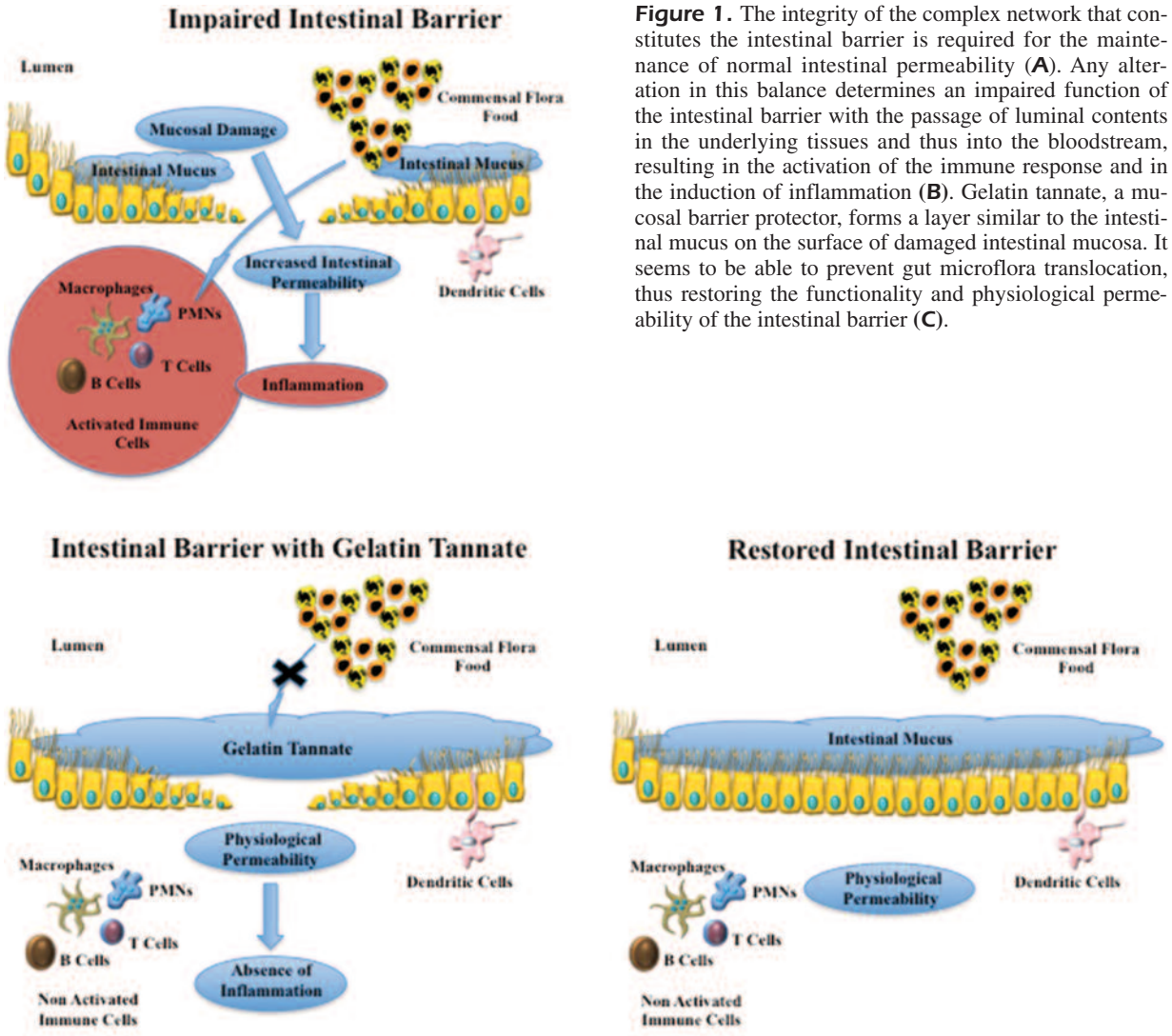

Figure 1. The integrity of the complex network that constitutes the intestinal barrier is required for the maintenance of normal intestinal permeability (**A**). Any alteration in this balance determines an impaired function of the intestinal barrier with the passage of luminal contents in the underlying tissues and thus into the bloodstream, resulting in the activation of the immune response and in the induction of inflammation (**B**). Gelatin tannate, a mucosal barrier protector, forms a layer similar to the intestinal mucus on the surface of damaged intestinal mucosa. It seems to be able to prevent gut microflora translocation, thus restoring the functionality and physiological permeability of the intestinal barrier (**C**).

in LPS-stimulated Caco-2 cells[59]. ICAM-1 is induced on a wide variety of cells by inflammatory stimuli such as LPS. Together with this, adding gelatin tannate at different concentrations induces a dose-dependent inhibition of IL-8 and TNF-α released by LPS-stimulated Caco-2 cells[59]. Finally, tannic acid is a polyphenolic compound, which includes Gallic acid that has been shown to exert potent antioxidant effects. We can speculate that the observed evidence could depend also on this important characteristic of gelatin tannate.

In this scenario, gelatin tannate, thanks to its chemical structure, seems to substitute mucus function impairment and reestablish the gut barrier permeability and homeostasis, by acting as a gut barrier enhancer and modulating gut microbiota (Figure 1). Taken together, our data suggest that not only the microbiota modulation, but also the recovering of gut mucus layer in course of acute colitis, can decrease the clinical disease severity in mice. The translational therapeutic implications of these concepts are obvious and open new horizons to novel, targeted, and more effective options for patients with colitis and impaired gut permeability[58].

Conclusions

The intestinal barrier is a functional unit whose integrity is necessary for the maintenance of normal intestinal permeability. The ability of its vari-

ous components to ensure the physiological permeability in presence of pathogenic noxae from the external environment is essential for health maintenance. When the protection exerted by intestinal barrier fails, the immune cells come in direct contact with luminal antigens. This results in the impairment of normal physiological barrier functions. The alteration of this delicate balance is the basis for the pathogenesis of many intestinal and extra-intestinal diseases, including infectious enterocolitis, inflammatory bowel disease, irritable bowel syndrome, small intestinal bacterial overgrowth, celiac disease, hepatic fibrosis, food intolerances and also allergies. Many drugs or compounds used in the treatment of gastrointestinal diseases are able to alter the intestinal barrier permeability. The most important are steroids, aminosalicylates, biologics and probiotics. Recent data highlighted and introduced the possibility of using gelatin tannate, a mucosal barrier protector, for an innovative approach in the management of intestinal diseases, allowing an original therapeutic orientation with the aim of enhancing mucus barrier activity and restoring gut barrier. These results suggest how the mucus layer recovering, beside the gut microbiota modulation, could be a useful weapon to re-establish the physiological intestinal homeostasis after an acute and chronic injury.

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Conflict of interest

Franco Scaldaferri and Antonio Gasbarrini were consultants for Acraft-Angelini, Ancona, Italy.

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