Childhood gastrointestinal dysfunction and protection mechanism of intestinal mucosal barrier

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Childhood gastrointestinal dysfunction has been recognized as the triggering elements of multiple organ failure. The causes of gastrointestinal dysfunction include: injury of intestinal barrier, release of inflammatory mediators, translocation of intestinal bacteria, gastrointestinal mucosal ischemia and anoxia injury caused by abnormal intestinal blood flow perfusion, and apoptosis of gastrointestinal epithelial cells. To prevent and treat this disease, the mechanism of gastrointestinal mucosal protection should be clarified in various aspects. Prostaglandins (PGs) and nitric oxide (NO) have a protective effect on gastrointestinal mucosa by inhibiting acid secretion, increasing mucus and bicarbonate ion secretion and by increasing mucosal blood flow as well as inhibiting mast cell and leukocyte adhesion. Many peptides participate in the process of gastrointestinal mucosal protection and injury repair differently with transforming growth factor, pancreatic secretory trypsin inhibitor (PSTI), glucagon-like peptides-2 (GLPs-2), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and epithelial growth factor (EGF). Phase II enzymes form a super-family of detoxification enzymes involving in the protection of cells from potentially harmful compounds and oxidation injury. In the trifoil peptide family, the stable structure of each peptide gives a characteristic three-loop shape and provides for exceptional resistance to proteolytic degradation. The peptides participate in mucosal repair by epithelial restitution and reconstitute the integrity of mucosa barrier. Intestinal trifoil factor (ITF) is also an endogenous peptide which could inhibit the apoptosis of epithelial cells through the NF-κB cascade regulation. Glutamine protects mucosa via three aspects: maintenance of mucosal barrier, regulation of the enteric immune function, and protection of mucosa from oxidization injury. Clinical manifestations of the disease and possible intervention approaches are also described.

Key words: gastrointestinal dysfunctions; children; pathogenesis; mucosal protection; clinical feature; diagnosis

Childhood gastrointestinal disorder is a clinical syndrome resulting from any abnormalities in the process of digesting food, absorbing nutrition and excreting trash. Its common symptoms include vomiting, abdominal pain, diarrhea, and constipation. Hematemesis, bloody stool, and toxic intestinal paralysis can be seen in severe cases. The causes of this disease vary despite gastrointestinal diseases themselves, gastrointestinal dysfunction is common in patients with severe illness such as infection, trauma, shock, and asphyxia. Currently, gastrointestinal disorders in severe cases have been recognized as a "motor of multiple organ failure".[1] But the mechanism of childhood gastrointestinal dysfunction is not clear yet, and the disease is somewhat difficult to manage. In the late period, the mortality of the disease is extremely high. Thus it is necessary to study the pathogenesis and seek the effective therapy and preventive approach.

Gastrointestinal dysfunction

Gastrointestinal dysfunction usually occurs in the process of critical illness induced by infectious or non-infectious factors. Severe infection, sepsis, shock, asphyxia, trauma, severe encephalic hypertension, and severe hypoxic/ischemic encephalopathy are the common causes of the disease. It is also related to dysfunction of gastrointestinal motility, illness of digestive organs, and maladjustment of intestinal bacteria. Other systemic diseases including acute pulmonary, hepatic, kidney, encephalic and immune dysfunction also induce or aggravate gastrointestinal dysfunction.
Pathogenesis

**Injury of intestinal barrier**

Damaged function of intestinal barrier is the pathologic basis of gastrointestinal dysfunction, and the pathophysiologic process of severe diseases and multiple organ injury. Gastrointestinal barrier is composed of mucus, gastric acid, bile salt, digestive enzyme, gastrointestinal epithelia and their tight junctions, secreting immunity global protein, and local cell immunity. Among these factors, the integrity of the gastrointestinal epithelium is fundamental for maintaining the morphology and function of the barrier. The permeability of intestinal barrier is dependent on the tightness and integrity of the intestinal epithelial structure. When the body is in stress, systemic immunity and hepatic macrophage function tend to reduce. A large quantity of enteric bacteria and endotoxin invade into the systemic circulation and tissues through the portal vein and mesentry lymph system, leading to bacterial translocation and endotoxinemia, which aggravate the impairment of the intestinal barrier. Thus more and more enteric bacteria invade into the blood and accelerate the course of the disease.

**Release of inflammatory mediators**

Bacteria and endotoxin continuously invade into the circulation, stimulate the systemic immune system and trigger the "cascade effect", leading to overwhelming production and release of inflammatory mediators such as tumor necrosis factor-α (TNF-α), IL-1, IL-4, IL-6, IL-8, IL-10, IL-13 and platelet-activating factor. These cytokines play the roles of cell signal communication in many physiological and pathological processes. The complement system could also be activated to have a systemic inflammatory response. More severe re-assault initiates or accelerates multiple organ dysfunction syndrome, eventually resulting in multiple organ failure because of loss of compensation.

**Intestinal bacteria translocation**

In certain conditions, bacteria in the intestine penetrate the epithelia and invade into the mesentery lymph node (MLN) and other remote organs (liver, spleen, lung and kidney) as well as blood, resulting in intestine-originated infection. This process is defined as the bacterial translocation of the intestine. The promoting factors are as follows: 1) intestinal mucosal injury such as mucus erosion, ischemia and atrophy; 2) alteration or shift balance of intestinal flora because of the long-term abuse of wide-spectrum antibiotics, and destroyed biologic function of barrier of the beneficial enteric flora; in most cases, G- bacilli are more potentially invasive than G+ bacteria, and the anaerobic predominates; 3) systemic immunity dysfunction such as damaged systemic and intestinal immune barrier, which may be caused by systemic immune disease, and long-term administration of steroids.

There are three routes for bacteria translocation of the intestine: 1) direct route: the enteric bacteria directly invading into the abdomen through the damaged intestinal mucosa; 2) blood route: bacteria arriving at the liver and other organs through the portal vein circulation; and 3) lymph route: bacteria going to MLN through the lymph system, then to the circulation. The third route is more important among them.

**Abnormalities of intestinal blood flow perfusion**

When the body is in stress and severe illness, the sympathetic nerve became excited. In order to guarantee oxygen supply to essential organs such as the brain, heart, liver and kidney, blood is redistributed. The contraction of gastrointestinal vessels reduces the blood stream volume to 50%-80%, which results in a hypoperfusion state that causes gastrointestinal mucosal ischemia and anoxemic injury including cell dropsy, inflammatory response, increased vascular permeability and metabolic abnormities of intestinal membrane, eventually resulting in tissue damage. At the same time, the intestine became the conglomeration pool of polymorphonuclear neutrophils (PMNs). Interaction of the intestine PMN and vessel endothelia can cause the activation of PMN and induce the injury and inflammatory response of distant organs during the second attack. Intestinal mucosa cells suffer from anoxia and acidosis under hypo-perfusion and hypoxemia, thus leading to hypoxanthine accumulation. A large amount of oxygen free radicals generating after reperfusion serve as mediators of "reperfusion injury", which aggravate mucosal erosion again, and eventually lead to irreversible pathologic changes.

**Apoptosis of gastrointestinal cells**

The damage of the cell structure is the pathologic foundation of gastrointestinal dysfunction. Gastrointestinal epithelial necrosis was once thought to induce structure breakage of the mucus, but recent research has indicated that cell apoptosis plays an important role in some diseases associated with injury to the gastrointestinal barrier. Some gastrointestinal diseases such as inflammatory bowel disease, intestinal Salmonella infection, and gastroenteritis tumor are due to the apoptosis out of control. Other factors also contribute to gastrointestinal apoptosis. For example, more apoptotic cells can be found in the rat model of short bowel syndrome than in the normal control. The apoptosis of microvascular endothelial cells is essential to intestinal stem cell injury in radioactivity-
induced gastrointestinal disorder. Endotoxin and fasting can increase the activity of caspase-3 and -6, cell apoptosis and vascular permeability, thus resulting in gastrointestinal dysfunction and remote organ-pulmonary infection. Mucosal cell apoptosis in the ileal extremity significantly increases in the rat model of obstructive jaundice, then proceeds to destroy the intestinal barrier and promote bacterial translocation. Other researchers found that non-steroid anti-inflammation drugs have anti-tumor effect by inducing the cell apoptosis of the oesophagus, stomach and colon. Abdominal injection of Adriamycin was used to study the intestinal barrier of rats; the result suggested that the increased apoptosis of intestinal epithelial cells is the cause of the increased biphasic permeability of the intestinal barrier. Moreover, chemotherapy induced enteritis results from increased apoptosis of intestine gland cells. Apoptosis of intestinal cells is significantly increased in hemorrhage shock rats because of ischemia and anoxia, leading to the damage of the intestinal barrier which can be alleviated by IL-6 administration. Increased apoptosis of intestinal cells was observed in extensively burned rats, suggesting that it may be one of the factors leading to gastrointestinal dysfunction.

**Clinical manifestations**

**Abdominal distension**

Abdominal distension is due to gastrointestinal gas and digestive maladjustment induced by enteric automatic nerve disorder and gastrointestinal ischemia. It also can be seen in the paralytic intestine and dysfunction of gaseous absorption caused by peritonitis and abdominal injury. Abdominal distention is always associated with abdominal pain, vomiting, failure to pass gas or stool, and decreased or absent bowel sound. Abdominal distention and silent bowel sound can predict the deterioration of illness and irreversible illness with poor prognosis.

**Diarrhea**

Mucosal injury, decreased digestive area, and secondary lactose deficiency and intolerance cause diarrhea in some children.

**Stress ulceration (acute gastric mucosal injury)**

Stress ulceration is characterized by vomiting of blood or coffee-like material and passing of dark stool. Initially these symptoms are mild, only a minority of children experience abdominal pain, bloating, nausea and vomiting. A large volume of blood was found in the vomit and stool, even the perforation of gastrointestinal mucosa may occur during the deteriorative stage.

**Laboratory examination (still under investigation)**

**Gastric intramucosal pH (pHi) measurement**

After emptying the balloon gas, a tube for gastric tonometry is inserted into the stomach, and 4 ml normal saline is infused into the balloon. After 30-90 minutes, the saline is drawn out. The first 1.5 ml is abandoned while the last 2.5 ml is used for blood gas analysis. At the same time, arterial blood sample is collected for blood gas analysis, and calculate the measurement with the following formula:

\[ \text{pH} = 6.1 + \lg \left( \frac{\text{arterial HCO}_3^-}{0.03 \times \text{PaCO}_2} \right) \]

(normal range: 7.35-7.45).

**Measurement of arterial lactic acid**

The normal value of arterial lactic acid is about 1 mmol/L, and it can be increased to 2 mmol/L under critical conditions. Stress, shock and hypoperfusion can lead to anaerobic metabolism and hyperlactacidemia.

**Diamine oxidase (DAO) measurement**

Ischemia and hypoxemia can induce increased release of DAO from mucosa, consequently increase the DAO concentration in blood.

**Others**

When gastrointestinal bleeding occurs, occult blood test of feces is positive with decreased blood level of hemoglobin. Cytokine monitoring indicates the conditions of systemic inflammatory reaction and release of inflammatory mediators. Whether the balance of the systemic internal environment can be maintained is investigated through measurement of serum electrolyte, blood glucose and blood gas, as well as blood osmotic pressure analysis. Hepatic and renal function and serum myocardium zymogram may reflect the degree of multiple organ injury.

**Diagnosis**

The following vital signs indicate the presence of gastrointestinal dysfunction: sudden appearance or gradual aggravation of abdominal distension, reduction or absence of bowel sound, vomiting of coffee-like material or hemafecia. However, if stress ulceration demands blood transfusion or toxic enteroparalysis appears with abdominal distension, gastrointestinal failure must be considered. Critical symptoms are fundamental to gastrointestinal dysfunction or failure. Meanwhile, acute abdomen, mechanical ileus and intestinal perforation should be excluded.

**Interventional approaches**

Etiological treatment includes active control of primary
diseases, correction of functional impairment of important organs and improvement of circulation. Sepsis control and removal of the infection focus, and proper use of antibiotics should be emphasized while fluid therapy and calorie supply are given.

Symptomatic therapy is geared to relieve abdominal distension, protect the mucosa of the gastrointestinal tract by reducing hydrogen ion concentration with H2-receptor blockers such as cimetidin, ranitidine and proton pump inhibitors as omeprazol. Hemostasis and correction of anemia are also essential.

Protection mechanism of gastrointestinal mucosa and clinical agents

Mucosal protection refer to the various factors, which can maintain the integrity of gastrointestinal mucosa without injury in long-term exposure to lumen pH, and changes of osmotic pressure and temperature, and counteracting local or systemic inflammatory response. It is a dynamic process rather than a static barrier. When the exogenous and endogenous aggressive factors increase, the function of mucosal protection also increases. Even if the mucosa is destroyed, it can be repaired rapidly. The regulating mechanism of mucosal protection is of extremely importance. For example, the nerve, body fluid, blood, growth factors and immune regulation play important roles in the counteraction of mucosal injury.

Prostaglandins (PGs) and nitric oxide (NO) exert protective effect on gastrointestinal mucosa by inhibiting secretion of acid, increasing mucus and bicarbonate ion, increasing mucosal blood flow, and controlling mast cells and leukocyte adhesion. PGs and NO also serve as immune regulators. Cyclooxygenase (COX), a key enzyme for synthesis of prostaglandins, exists with two isoforms: COX-1 and COX-2. COX-1 is constitutively expressed in the gastrointestinal tract in large quantities and has been suggested to maintain mucosal integrity through continuous generation of prostaglandins. COX-2 is not commonly expressed in normal gastric mucosa, the increased expression of COX-2 was only induced by certain cytokines, growth factors, mitogenic agents or endotoxin. Thus COX-2 is thought to be related to the generation of PGs in the inflammatory response.

There are two kinds of NO synthase (NOS) with three isoforms: configuration NOS (cNOS) and inducible NOS (iNOS). cNOS can be divided into neuronal NOS (nNOS) and endothelial NOS (eNOS) according to the different generating locations. eNOS also can be found in the epithelium and colon matrix. cNOS can be constitutively expressed in normal conditions, but the expression of iNOS is increased only by introduction of certain cytokines such as lipopolysaccharide and TNF-α. NO generated from cNOS plays an important role in maintaining mucosal integrity, while protecting the mucosa from the injury of ethanol, stress, ischemia and reperfusion. It is generally accepted that NO generated from iNOS may participate in the pathological process of the disease and exert a cytotoxin effect.

NO and PGs, not self-independent, can affect or cooperate with each other. NO can stimulate the activation of COX, and exerts mucosal protection combined with PGs against the damage of the harmful substances.

Studies indicated that many peptides participate in gastrointestinal mucosal protection and injury repair.

The principal role of transforming growth factor is to stimulate cell differentiation and migration and prevent drug-induced mucosal injury. However, its effect on mitosis is not prominent.

Pancreatic secretory trypsin inhibitor (PSTI) can strongly inhibit the activity of trypsin and other proteases. It was also found in mucus-secreting cells of the gastrointestinal tract, which suggest that PSTI may protect mucus from excessive destruction by proteases and maintain the normal function of mucus layer.

Glucagon-like peptides-2 (GLPs-2) is secreted from endocrine cells in the gastrointestinal tract when they absorb the nutrition. By inhibiting cell apoptosis, GLPs-2 can reduce the severity of chemical-induced enteritis, vascular reperfusion injury, incidence and injury of dextran sulfate induced colitis in a murine model. Moreover, GLPs-2 also alleviates "mucositis" induced by chemotherapeutic agents. Thus, this enteric peptide has become a potential therapy agent.

A recent study showed that intravenous administration of basic fibroblast growth factor (bFGF) can inhibit cell apoptosis of the microvascular endothelium, and prevent radiotherapy-induced damage to murine intestinal gland and consequent gastrointestinal dysfunction. bFGF can also counter the inhibition effect of acid on the reconstruction of mucosa. Currently, it is also recognized as a mucosal protection factor.

Platelet-derived growth factor (PDGF) can stimulate the proliferation of fibroblasts, epithelial and endothelial cells. PDGF is characterized by aggregation of monocytes, neutrophils and some smooth muscle cells, which are necessary for tissue repair.

The role of the epithelial growth factor (EGF) in the maintenance of mucosa integrity is not very clear. As a strong stimulator of mitosis, EGF promotes the proliferation and differentiation of the epithelium, modulates the genesis and growth of the embryo, as well as tissue repair and regeneration. Evidence has supported the hypothesis that EGF and its receptor can stimulate...
epithelial reconstitution, inhibit gastric acid secretion, increase mucosal flow, and promote healing of acute and chronic injury. However, some studies indicate that EGF does not affect the cell proliferation and gastric acid secretion when the mucosa is normal. Once the mucosal barrier is damaged, EGF may promote cell proliferation and mucosal repair.[29] Recent studies indicated that pre-administration of EGF can decrease the severity of acute and chronic gastrointestinal injury, which provides a new strategy for prevention and treatment of digestive system injury.

Oxygen free radicals play an important role in the pathogenesis of mucosal injury. Thus, it is necessary to develop mucosal protection agents that could eliminate oxygen radicals. Quinone reductase, glutathione S-transferase, and UDP-glucuronosyltransferase are the representatives of phase II enzymes, a superfamily of detoxification enzymes involving in the protection of cells from harmful compounds and oxidation injury. Butylated hydroxyanisole (BHA), an antioxidant, was found to have significant protective effect on ethanol-induced mucosal injury while reducing the production of malondialdehyde. The mechanism of BHA is related to the increased activity of the phase II enzymes. In addition, BHA can induce the activity of glutathione reductase, leading to the bioconversion of GSSG to GSH, which account for maintaining mucosal integrity and preventing lipid peroxidation injury. Moreover, GSH as an important defending factor plays an important role in eliminating oxygen radicals, sustaining the concentration of cell calcium and maintaining the activity of Na⁺-K⁺-ATP enzyme.

The trifoil peptide family is composed of three peptides: spasmylytic polypeptide (SP), breast cancer related peptide (pS2), and intestinal trifoil factor (ITF). They share a common feature—the trefoil structure of six cysteine residues held together by three pairs of disulfide bonds. This stable structure gives the peptide a characteristic three-loop shape and provides for exceptional resistance to proteolytic degradation. Increased expression of pS2 and SP can be observed at the time of gastrointestinal tract infection and ulceration and acts earlier than the expression of EGF.[28] Gastrointestinal mucosa is prone to injury in pS2 and ITF gene knockout mice. A recent study showed that oral administration of SP can significantly alleviate the gastrointestinal mucosal injury induced by ethanol, indomethacin and aspirin. Trefoil peptides and mucin glycoproteins form a continuous gel on the mucosal surface for the resistance to proteolytic degradation and mechanical pressure. In addition, it can also protect the monolayer epithelium from the damage of various exogenous substances such as bacteria toxin, bezoar, choler acid and oleic acid. The mucosal barrier is destroyed after acute mucosal injury, rapid repair of the mucosa is needed to protect the sub-mucosa tissue from proteolytic degradation and gastric acid. Cell migration is one of the key processes of the early repair, i.e., trifoil peptide family (TPF) participates in mucosal repair by stimulating the migration of surviving cells from the edge of the damaged region to the denuded area, a process called epithelial restitution or reconstitution of the integrity of mucosa barrier. ITF is also an endogenous peptide characterized by anti-apoptosis or epithelial cell apoptosis inhibited through the NF-κB cascade regulation. In a necrotizing enterocolitis (NEC) animal model induced by abdominal and subcutaneous injection of ITF, inflammatory response and oxidation injury were reduced.

Glutamine (Gln) is a kind of amino acid abundant in bloodstream and inner-tissue free pools. It not only can provide nitrogen to amino acid, protein and nucleic acid, but also can be oxidized to produce energy. The principal fuel of the intestinal mucosa and other rapidly growth cells is Gln, rather than glucose. Gln provides 70% of the total fuel in normal diet, glucose only provides less than 20%. However, intestinal mucosa itself can not produce Gln, nor can store it. The source of Gln has to depend on endogenous and exogenous route, and the endogenous route is more important. When severe stress occurs, glutamine requirement could exceed the level normally produced in the body. It will lead to relatively Gln deficiency if it can not be supplied from the diet. Many animal experiments and clinical studies showed that Gln protects mucosa through three aspects:[30] maintenance of mucosal barrier, regulation of the enteric immune function, and protection of mucosa from oxidation injury. Total parenteral nutrition and enteric nutrition with proper amount of Gln can increase the height of villus, enlarge the surface of mucosa, and increase the depth of the cavity. In addition, it also increases the mitosis of the cavity cells, accelerates the regeneration of epithelial cells, increases the repair capacity, and promotes the synthesis of enteric mucin. In fact, Gln helps to increase the tight junction of mucosa cells, reduce the apoptosis of epithelial cells, and prevent permeability incensement caused by mucosa atrophy and infection. Besides, Gln can decrease the release of pro-inflammatory cytokines, which can aggravate systemic inflammatory response, and reinforce cell immunity. Supplement of Gln can also normalize the expression of ICAM-1, participate in synthesis of the antioxidant—glutathione, and alleviate oxidant mediated reperfusion injury. It has been well accepted that Gln can improve gastrointestinal mucosa repair in various kinds of pathological conditions including burn, sepsis, post-operation, and chemotherapy and radiotherapy for cancer patients.
Because Gln solution is not stable, an artificial analogue has been developed with high solubility and tolerance to high temperature. The agent can be separated into free Gln and alanine by digestion of dipeptide enzyme in human body. Gln dipeptide has proved to be a safe and effective Gln source in total parenteral nutrition.

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