Glucose-mediated cytoprotection in the gut epithelium under ischemic and hypoxic stress

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Review

Summary. Single-layered intestinal epithelia play key roles in the maintenance of gut homeostasis and barrier integrity. Various types of epithelial cell death, including apoptosis, necrosis, and necroptosis, have been detected in ischemic and hypoxic stress conditions, thus resulting in bacterial translocation and gut-derived septic complications. Cytoprotective strategies, such as enteral glucose uptake, rescue intestinal epithelium from cell death after ischemic and hypoxic injury. Although glucose metabolism and energy production are generally considered to be the key factors in cytoprotection, the precise modes and sites of action have not been clarified. Our recent studies have demonstrated that energy restoration promotes crypt hyperplasia but does not prevent epithelial cell death under ischemic stress. On the other hand, glycolytic pyruvate prevents epithelial cells from undergoing apoptosis and necroptosis by scavenging free radicals in an ATP-independent manner. Distinct gut protective mechanisms involving ATP, pyruvate, glucose metabolic enzymes, and sodium-dependent glucose transporter activation are discussed here. Overall, glucose-mediated cytoprotection may be a universal mechanism that has evolved in epithelial cells for the maintenance of intestinal homeostasis. Enteral glucose supplementation is beneficial as a perioperative supportive therapy for the protection of gut barrier integrity.

Key words: Ischemia/reperfusion, Hypoxia, Intestinal epithelium, Barrier function, Glucose metabolism, Cell death

Histophysiology of intestinal epithelium

Intestinal epithelial cells undergo rapid turnover in a state of dynamic equilibrium and play a crucial role in maintaining homeostasis. Stem cells in the crypt divide and migrate upward and become differentiated epithelial cells with digestive, absorptive, and secretive functions. The fully differentiated cells develop a long brush border with extensive expression of membranous enzymes and transporters, which facilitate nutrient uptake. On the villus tip or surface extrusion zone, the epithelial cells are shed and undergo apoptosis (Yen and Wright, 2006; Yu et al., 2012). Although surface cell apoptosis occurs, an intact epithelial barrier is sustained under physiological conditions (Madara, 1990; Guan et al., 2011).

Because they serve as a portal to the external environment, epithelial cells are often exposed to noxious agents and infectious pathogens. High levels of commensal bacteria are also present in the gut lumen. A favorable symbiotic relationship between the host and microbes is dependent on the integrity of the single-layered epithelial barrier (Ley et al., 2006; Yu et al., 2012). With a dynamic turnover rate of 5-7 days, cytoprotective mechanisms evolved by epithelial cells represent the basis of gut homeostasis. A number of cytoprotective mechanisms have been proposed, including nutrient uptake and transcriptional adaptation.
Glucose transport and metabolism in intestinal epithelial cells

Glucose transporters are ubiquitously expressed on all cell types in the body, including three subtypes of sodium-dependent glucose transporters (SGLTs) and fourteen members of glucose transporters (GLUTs). In the small intestinal epithelial cells (enterocytes), the major transporter expressed on the apical membrane for active glucose uptake is SGLT1, whereas GLUT2 is responsible for the basolateral diffusion of glucose into the bloodstream (Ferraris and Diamond, 1997; Daniel and Zietek, 2015). Glucose binding to SGLT1 activates downstream signaling for insertion of GLUT2 into the apical membrane for facilitative diffusion of enteral glucose (Kellett and Helliwell, 2000; Mace et al., 2007). Aside from glucose transport, enterocytes also express apical GLUT5 for uptake of dietary fructose (Ferraris and Diamond, 1997). On the other hand, large intestinal epithelial cells (colonocytes) do not normally express SGLT1 or GLUT2 but are equipped with GLUT5 and GLUT6 (Godoy et al., 2006).

The metabolic pathways in enterocytes and colonocytes are different. Preferential energy sources for enterocytes include glucose and glutamine; the former predominantly undergoes anaerobic glycolysis, and the latter predominantly undergoes mitochondrial respiration for energy production (Kight and Fleming, 1995; Fleming et al., 1997). Owing to the presence of anaerobic bacteria in the large intestine, short chain fatty acids (e.g., butyrate) fermented from dietary fiber after diffusion through the lipid bilayer membrane are the major energy source for colonocytes (Roediger, 1982; Wong et al., 2006). The utilization of butyrate for mitochondrial oxidative phosphorylation in colonocytes generates more than half of the energy output, even in the presence of glucose (Roediger, 1982; Wong et al., 2006).

Intestinal absorption of glucose is remarkably adaptive, and the uptake level changes according to diet, age, energetics, and stress. After ingestion of dietary carbohydrate, glucose transporter expression on enterocytes is observed within minutes to several hours (Diamond and Karasov, 1987; Ferraris and Diamond, 1992). In contrast, total parenteral nutrition or fasting for a prolonged period may lead to decreased epithelial glucose uptake in the small intestine (Kotler et al., 1980). Hormones that regulate mucosal growth and transporter activity and consequently increase glucose uptake include gastrin, neurotensin, glucagon-like peptides, and epidermal growth factor (Ferraris and Diamond, 1997). Recent evidence has shown that luminal contents, such as the presence of glucose and non-metabolized sugars (such as artificial sweeteners), activate taste receptors and result in upregulation of the expression of SGLT1 (Moran et al., 2010; Stearns et al., 2010). In addition, bacterial lipopolysaccharide binding also stimulates translocation of intracellular vesicular SGLT1 to the apical membrane (Yu et al., 2006).

Cells that undergo hypoxic stress respond by adapting to increased glucose uptake. Previous studies have demonstrated transcriptional upregulation of GLUT-1 by binding of hypoxia-inducible factor (HIF)-1 to a hypoxia-responsive element in the promoter region of the glucose transporter (Ouiddir et al., 1999; Hayashi et al., 2004). A recent study has shown an increase in GLUT1 and GLUT4 transcript and protein levels of colonic epithelial cell lines after hypoxic challenge (Huang et al., 2013). Overall, the fluctuations of glucose transporter expression may reflect the fundamental metabolic requirements for cell survival.

Epithelial cell death and barrier damage in response to stress

Owing to the anatomical structure and vascular distribution, surface epithelia are the most fragile cell types in the gut tissues after exposure to ischemic and pathogenic stress. Surface cell death and mucosal ulceration are repaired by crypt hyperplasia through epithelial restitution and wound healing. Various types of epithelial cell death, including apoptosis, necrosis, and necroptosis, have been documented in the gut. Apoptosis and necroptosis are programmed cell death processes that are regulated by a cascade of signaling molecules. Apoptosis is characterized by caspase activation and DNA fragmentation. Necroptosis is executed by receptor-interacting protein (RIP)1/3 signaling and mitochondrial generation of reactive oxygen species (ROS), thus eventually resulting in necrotic features of the cells. In contrast, necrosis is an uncontrolled, unregulated form of cell death. Detailed information on the types of cell death, has been described in other articles (Vandenabeele et al., 2010; Gunther et al., 2013; Linkermann and Green, 2014; Huang and Yu, 2015).

Excessive epithelial cell death and gut barrier dysfunction have been observed after exposure to metabolic, inflammatory, oxidative, and heat stress. Metabolic stress in the gut is primarily caused by depletion of oxygen and nutrients, such as during mesenteric ischemia, trauma, hemorrhagic shock, necrotizing enterocolitis, and major abdominal and vascular surgery (Sreenarasimhaiah, 2005; Jilling et al., 2006; Zou et al., 2009; Yu, 2010; McEltroy et al., 2013). An increase in apoptosis, necroptosis, and necrosis in gut epithelia has been observed in animal models of mesenteric ischemia/reperfusion (I/R) (Azuara et al., 2005; Chang et al., 2005; Huang et al., 2011, 2016). Mucosal ulceration and epithelial cell death (i.e., apoptosis, necroptosis, and necrosis) have been found in patients and animal models with inflammatory bowel disease (Heller et al., 2008; Qiu et al., 2011; Welz et al., 2011; Su et al., 2013; Pierdomenico et al., 2014). Various factors, such as inflammatory hypoxia, free radicals, immune factors (e.g., natural killer cells, cytotoxic T cells, and tumor necrosis factor α), or thermal stress as a physiological equivalent to fever, are all possible causes of induction of cell apoptosis and
necrosis (Kinoshita et al., 2002; Merger et al., 2002; Sun et al., 2002; Haynes et al., 2009; Sakiyama et al., 2009; Kallweit et al., 2012). With such a wide range of potential death stimuli, efficient cytoprotective mechanisms are crucial for the maintenance of epithelial survival and gut barrier integrity.

**Rescue strategies against epithelial cell death by glucose**

Protection against I/R injury by supplementation of glucose has been documented in various organs, including the kidney, heart and intestine (Kehrer et al., 1990; Ramasamy et al., 2001; Kozar et al., 2002). Glucose-mediated cytoprotection in gut epithelium has been reported after exposure to ischemic and inflammatory stress (Kozar et al., 2002; Huang et al., 2011, 2016). Enteral glucose not only prevents epithelial apoptosis and necroptosis but also restores crypt proliferative functions after ischemic insult (Huang et al., 2011, 2016). The attenuation of ischemic injury by glucose supplementation had previously been mistaken for an absence of stress stimuli. However, this hypothesis has recently been overturned by novel findings demonstrating that ischemia-induced cell death remains present despite replenishment of ATP (Huang et al., 2016). Here, the distinct cytoprotective roles of ATP, pyruvate, glucose metabolic enzymes, SGLT1-mediated signaling pathways, and the BAD/glucokinase axis will be highlighted (Fig. 1).

**Roles of ATP**

An energy decrease has been assumed to be the major cause of epithelial cell death under ischemia. In support of this hypothesis, numerous studies have shown that intestinal I/R-induced histopathological injury and barrier defects are alleviated by supplementation with enteral nutrients, such as glucose and glutamine (Adhieh et al., 1998; Blikslager et al., 1999; Kozar et al., 2002; Sukhotnik et al., 2007). The beneficial effects of glucose and glutamine have been attributed to energy...
metabolism after sodium-dependent transporter uptake. Glutamine protection against I/R-induced epithelial barrier dysfunction is also partly dependent on its role as an immune-enhancing agent (Kozar et al., 2004b; Sato et al., 2005). In contrast, alanine and arginine (as non-metabolizable solutes in the gut) cannot prevent ischemia-induced barrier damage (Kozar et al., 2004a; Sato et al., 2005). These results suggest that glucose and glutamine are preferable fuels for epithelial cells and confer protection against ischemic damage.

ATP is generally considered to be the predominant metabolite providing glucose protection in I/R damage. However, the exact modes and sites of action of ATP had not been elucidated until a recent study used liposomal delivery of ATP in place of enteral glucose. Enteral glucose has been found to prevent epithelial apoptosis and necroptosis and to restore crypt proliferation in the ischemic gut (Huang et al., 2011, 2016). However, replenishment of ATP also prevents crypt dysfunction but does not diminish the villous epithelial cell death or correct the histopathological damage in the ischemic intestine (Huang et al., 2016). These results are consistent with the hypothesis that ATP is crucial for active crypt proliferation and possibly for epithelial restitution and healing after blood reperfusion. Nevertheless, it challenges the traditional view that energy depletion is the initiating factor triggering epithelial cell death under ischemic stress and also suggests that an alternative metabolite might be responsible for the anti-death mechanisms.

Roles of pyruvate

Pyruvate is the end product of glycolytic metabolism. Under aerobic conditions, pyruvate is transported by the mitochondrial pyruvate carrier (MPC) and converted to acetyl-CoA, which enters into the tricarboxylic acid (TCA) cycle; this cycle produces nicotinamide adenine dinucleotide (NADH), which enters the electron transport chain (ETC) and results in ATP synthesis. In anaerobic conditions, pyruvate is reduced by NADH, thus forming lactate and the oxidized form of the co-factor NAD+. In addition to its metabolic role, pyruvate is also an endogenous scavenger for ROS, including superoxide and hydrogen peroxide (Brand and Hermfisse, 1997; Kao and Fink, 2010).

Abundant studies have shown that intraluminal or intravenous administration of pyruvate derivatives ameliorates free radical production and prevents intestinal I/R-induced mucosal injury and barrier damage (Cicalese et al., 1996; Cruz et al., 2011; Petrat et al., 2011). However, the source of free radicals, either from infiltrating phagocytes after blood reperfusion or from the stressed epithelia, has been unclear. To further investigate epithelial death, we have assessed the protective effect of pyruvate in intestines subjected to ischemic stress alone (Huang et al., 2016). Enteral instillation of pyruvate mimics the protective effect by glucose in terms of reduction of epithelial apoptosis and necroptosis but does not reverse the crypt dysfunction in the ischemic gut (Huang et al., 2016). These findings suggest that glucose-mediated epithelial cell death resistance is dependent on glycolytic pyruvate but not ATP (Huang et al., 2016). In addition to direct scavenging of free radicals, pyruvate also partially limits subsequent reperfusion injury by preventing epithelial death-dependent bacterial translocation and infiltration of phagocytes.

Roles of glucose metabolic enzymes

Under low-oxygen conditions in ischemia or inflammation, HIF-1 is translocated into the nuclei of intestinal epithelial cells (Koury et al., 2004). HIF-1 is a transcription factor that upregulates various glucose metabolic enzymes (Denko, 2008; Lu et al., 2008). A cytoprotective role of HIF-1 related to its transcriptional regulation has been documented in models of mesenteric ischemia and experimental colitis and in ileal loop models with exposure to bacterial toxins (Karhausen et al., 2004; Hart et al., 2011; Mones et al., 2011; Grenz et al., 2012; Keely et al., 2014). Moreover, HIF-1 activation is also linked to the resolution of intestinal ischemic injury as well as to adaptive protection by ischemic/hypoxic preconditioning (Koury et al., 2004; Chen et al., 2014). The glucose metabolic enzymes upregulated by HIF-1 include hexokinase (HK), glyceraldehyde phosphate dehydrogenase (GAPDH), and pyruvate dehydrogenase kinase (PDK) (Denko, 2008; Lu et al., 2008). However, most studies showing hypoxic adaptation for anaerobic glycolysis have been performed in colon adenocarcinoma cell lines (Denko, 2008; Lu et al., 2008; Marin-Hernandez et al., 2009). It therefore remains unclear whether findings in cancer cell lines can be translated to normal epithelium. Further studies on whether chronic or long-term HIF1-mediated glucose death resistance can drive genetic mutation and contribute to tumor transition from normal epithelium are needed. Metabolic reprogramming related to glycolytic enzymes in colon cancer, has been described in recent reviews (Marin-Hernandez et al., 2009; Semenza, 2010; Huang and Yu, 2015).

Roles of SGLT1-mediated signaling pathways

Several lines of evidence indicate that phosphatidylinositol 3-kinase (PI3K)/Akt and inhibitor of kappa b (IxB)/nuclear factor kappa b (NFxB) pathways are involved in non-nutritive mechanisms of SGLT1-mediated glucose cytoprotection. Previous studies have shown that SGLT1-dependent glucose uptake inhibits epithelial cell apoptosis caused by mesenteric I/R and microbial products, and activation of the PI3K/Akt pathways partly contributes to the anti-apoptosis response (Yu et al., 2005, 2006, 2008). Cotransport of sodium and glucose triggers the activation of Akt and results in recruitment of the
sodium-hydrogen exchanger to the apical membrane (Shiue et al., 2005), thus decreasing cellular acidosis. Others have demonstrated that SGLT1 activation by glucose or by non-metabolizable sugars suppresses proinflammatory NFκB signaling and IL-8 production in intestinal epithelial cells after endotoxemia (Palazzo et al., 2008). NFκB signaling has been linked to anti-apoptotic and pro-proliferative events in gastrointestinal epithelial cells (Potoka et al., 2000; Egan et al., 2004; Li et al., 2005; Liu et al., 2012).

Roles of glucokinase and pro-apoptotic regulator

An elegant study has demonstrated that glucose deprivation in hepatocytes results in cell apoptosis through a mechanism involving the dephosphorylation of BAD (a pro-apoptotic Bcl2 family member), which is associated with the suppression of glucokinase activity (Danial et al., 2003; Danial, 2008). Functionally similar to HK, which exists in all cell types, glucokinase is a glycolytic enzyme specific to hepatocytes and pancreatic β cells and acts in the first metabolic step, catalyzing glucose to D-glucose-6-phosphate. During cell apoptosis, oligomerization of BAK/BAD (in a dephosphorylated form) neutralizes the pro-survival Bcl-2, Bcl-XL, and Bcl-2 proteins and causes mitochondrial outer membrane permeabilization and cytochrome c release (Shimizu et al., 1999; Tsujimoto and Shimizu, 2000). BAD normally resides in a mitochondrial complex with glucokinase, and the phosphorylation of BAD is necessary for maximal glucokinase activity for glycolysis in hepatocytes (Danial et al., 2003).

The finding demonstrates that the BAD-glucokinase interaction is a key component of the glucose sensing machinery in normal hepatocytes. However, under cell death stimuli (e.g., glucose deprivation), dephosphorylated BAD coordinates the apoptotic and glycolytic pathways by simultaneously driving cell death and diminishing glucose utilization and metabolism (Danial et al., 2003; Gimenez-Cassina and Danial, 2015). Cells undergoing apoptosis also show decreased glycolytic ATP generation. This finding, together with our finding that restoration of ATP in the ischemic gut does not prevent epithelial apoptosis (Huang et al., 2016), suggests that an energy decrease may not be the initiating factor but instead may be a consequence of cell death. More importantly, the pro-proliferation ATP is not the major metabolite responsible for death resistance conferred by glucose in gut ischemia.

Concluding remarks

Novel findings challenge the traditional view of energy depletion as the major underlying cause of ischemic cell death and also argue against ATP being the principal protective glucose metabolite. The current understanding is that ATP is crucial for maintenance of crypt proliferation, whereas glycolytic pyruvate is the predominant metabolite for inhibition of villous epithelial death under ischemic stress. Overall, enteral glucose uptake (uncoupled with energy production) confers death resistance in ischemic epithelium, probably through multiple mechanisms that involve pyruvate-mediated free radical scavenging and SGLT1-mediated anti-apoptotic signaling.

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References

Glucose protection against gut ischemic stress

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