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Review

Methods to determine intestinal permeability and bacterial translocation during liver disease ☆

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ABSTRACT

Liver disease is often times associated with increased intestinal permeability. A disruption of the gut barrier allows microbial products and viable bacteria to translocate from the intestinal lumen to extraintestinal organs. The majority of the venous blood from the intestinal tract is drained into the portal circulation, which is part of the dual hepatic blood supply. The liver is therefore the first organ in the body to encounter not only absorbed nutrients, but also gut-derived bacteria and pathogen associated molecular patterns (PAMPs). Chronic exposure to increased levels of PAMPs has been linked to disease progression during early stages and to infectious complications during late stages of liver disease (cirrhosis). It is therefore important to assess and monitor gut barrier dysfunction during hepatic disease. We review methods to assess intestinal barrier disruption and discuss advantages and disadvantages. We will in particular focus on methods that we have used to measure increased intestinal permeability and bacterial translocation in experimental liver disease models.

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Abbreviations: LPS, lipopolysaccharide; H&E, Hematoxylin & Eosin; PAMPs, pathogen associated molecular patterns; TLR, Toll-like receptor; FITC, fluorescein isothiocyanate-conjugated; PEG, polyethylene glycols; GFP, green fluorescent protein; MLN, mesenteric lymph nodes; BDL, bile duct ligation; BSA, bovine serum albumin; A1AT, Alpha-1-Antitrypsin; FABPs, fatty acid binding proteins; DAO, diamine oxidase.

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1. Introduction

The luminal side of the intestine is lined by epithelial cells, which promote water and nutrient absorption; the epithelium also provides a dynamic and semi-permeable barrier between the luminal microbiota and the host. The barrier is formed by individual epithelial cell membranes and tight junction proteins that seal the paracellular space between adjacent cells. Thus, the permeability of this barrier is regulated by the integrity of cellular plasma membranes and tight junctions, as well as by epithelial cell processes mediating secretion and absorption. Small molecules (<300 Da) and electrolytes passively cross the tight junction barrier (Sun et al., 1998). Both physiological and pathological stimuli change the barrier permeability. During homeostasis the intestinal epithelium absorbs nutrients while effectively preventing translocation of intraluminal bacteria. However, pathological conditions (e.g. toxins or intestinal inflammation) can increase the paracellular pathway and adversely affect barrier permeability, which poses the risk of an ineffective nutrient absorption and a failure to prevent the translocation of luminal bacteria and their products (also called pathogen associated molecular patterns or PAMPs). This can result in chronic intestinal diseases, but it might also affect other distant organs that drain and filter translocated bacteria and associated PAMPs (Sun et al., 1998; Turner, 2006; Marchiando et al., 2010a; Fouts et al., 2012).

The majority of the intestinal venous blood reaches the liver via the portal vein. Due to this unique blood supply system, the liver is vulnerable to exposure of bacterial products translocated from the gut lumen when intestinal epithelial barrier functions are disrupted (Seki and Schnabl, 2012). The liver represents therefore the first organ in the body that encounters not only nutrients from the diet, but also other molecules that are able to translocate from the intestinal lumen to the blood stream. The amount of translocated PAMPs is usually low during health (Bode et al., 1987; Fukui et al., 1991; Lin et al., 1995). However, liver diseases are associated with increased intestinal barrier permeability in humans (Bode et al., 1987; Fukui et al., 1991; Lin et al., 1995) and animal models (Yan et al., 2011; Hartmann et al., 2012; Hartmann et al., 2013). Increased levels of lipopolysaccharide (LPS or endotoxin) and bacterial DNA resulting from increased intestinal barrier permeability are elevated in the serum of patients with liver diseases (Fukui et al., 1991). Translocated bacterial products contribute to liver disease progression by binding to specific pathogen recognition receptors. In particular, Toll-like receptor 4 (TLR4), the major receptor for LPS has been implicated in the progression of many liver diseases and induces hepatic inflammation (Schnabl and Brenner, 2014). Increased translocation of microbial products due to a disrupted intestinal barrier will also lead to an activation of the mucosal immune system and secretion of inflammatory mediators, which in turn might increase barrier dysfunction. Such an inflammatory process might eventually also affect the quantity (overgrowth) and composition of the luminal microbiota (Marchiando et al., 2010a). Although intestinal permeability can increase with a rise in transcellular transport processes, the relevance and importance of transcytosis for liver disease have not been determined.

In addition, enhanced translocation of viable bacteria to mesenteric lymph nodes and extra-intestinal sites is commonly seen in patients with end-stage liver disease (cirrhosis) (Schnabl, 2013). The hepatic immune system might also be compromised in liver cirrhosis, so that translocated viable bacteria cannot be effectively cleared (Balmer et al., 2014). However, whether and how viable bacteria affect liver disease progression require further investigation.

Given the significance of monitoring intestinal permeability in the setting of acute and chronic liver diseases, we will review methods to assess gut permeability in mostly animal models.

2. Evaluation of intestinal integrity and mucosal tight junctions

Histology is important to initially evaluate the integrity of the intestinal barrier. Standard light microscopy of Hematoxylin & Eosin (H&E) stained intestinal sections is able to detect intestinal pathology including ulcerations of the mucosa and severe intestinal inflammation that will cause and contribute to increased intestinal permeability.

As mentioned above, tight junctions play an essential role in maintaining the integrity of the membrane barrier in the intestine. Tight junctions locate in the apical end of the lateral membrane and are composed of couples of transmembrane proteins such as occludins and claudins interacting with intracellular anchor proteins such as zonula occludens proteins which in turn are connected to the actin cytoskeleton. Tight junctions are rate-limiting for the paracellular leakage pathway (Menard et al., 2010).

Electron microscopy led to the discovery of tight junctions in epithelial barriers. The zonula occludens (tight junction) is characterized by fusion of the adjacent cell membranes with a dense outer leaflet of the adjoining cell membranes, which converge to form a single intermediate line. A diffuse band of dense cytoplasmic material is often associated with this junction (Farquhar and Palade, 1963). In addition, many reports have employed mostly immunofluorescent staining methods for visualization using specific antibodies directed against various tight junction proteins (Hartmann et al., 2012; Chen et al., 2014; Chen et al., 2015). Protein and mRNA transcript levels of tight junction molecules can be assessed with western blotting and quantitative PCR, respectively (Chen et al., 2015). Tight junction complexes are composed of multiple proteins. Because the importance of single molecules for the integrity and function of tight junctions is rather obscure at this moment, functional assays might be necessary to elucidate their role.

An example for the importance of evaluating gut barrier integrity in culture and in vivo is alcoholic disease. A direct cytotoxic effect of high concentrations of ethanol (>40%) increases intestinal permeability by causing vascular and mucosal damage, which can be best seen on H&E stained slides (Szabo et al., 1985). However, subsequent Caco-2 monolayer cell based studies showed that even lower, non-cytotoxic doses of ethanol may alter the structure and function of tight junctions through activating myosin light chain kinase (MLCK) (Ma et al., 1999). Differentiated intestinal epithelial cells such as Caco-2 cells are commonly used to functionally analyze tight junction dynamics. Although ethanol has been reported to disrupt tight junctions in Caco-2 cells, acetaldehyde, a product of ethanol metabolism, is a much stronger inducer of tight junction dysfunction. Detailed protocols using acetaldehyde

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