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Title page

Drugs and tight junctions: side effects and opportunities for new therapeutic approaches

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Alteration in the intestinal permeability is involved in the pathogenesis of several diseases (1). Intestinal permeability is continually modified in response to physiological and pathological factors (1,2). Epithelial cells, tight junctions (TJs), adherens junctions, and luminal secretions represent the elements by which depends intestinal permeability (1,2). The TJs and adherens junctions are collectively referred to as the apical junctional complexes (AJCs) that is involved in multiple functions (1-5). They are dynamic structures which normally regulates the trafficking of nutrients, compounds and fluids between intestinal lumen and submucosa. These structures act also as a boundary within the plasma membrane itself, separating the apical and basolateral cell surface domains. They play an important role in the intestinal morphogenesis, differentiation and wound healing (1,2). Finally, AJCs participate in signal transduction across epithelial cells in both directions and in regulation of many cytoskeleton functions (1,2). Major proteins in the AJCs are transmembrane proteins, occludins, claudins, junctional adhesion molecules, coxsackie adenovirus receptor, cytoplasmic plaque proteins, zonula occludens (ZOs), zonulin receptors, cingulin, cytoplasmic TJ-associated protein (7H6) and E-cadherin (2,6). The ZOs are the best characterized proteins of the AJC. The ZO-1 and ZO-2 are involved in the organization of proteins within the tight junctional plaque so that signaling events can be propagated (8). Occludin is a transmembrane protein that serve as cell-to-cell adhesion molecule and it participates to maintain the cellular polarization and intramembrane fence that restricts the diffusion of lipids in the outer leaflet of the plasma membrane (7). Claudins interact with ZO-1, ZO-2, and ZO-3 and they are able to polymerize and form TJ strands in the absence of ZO binding region (8). Components of AJCs interact with each other and their function can be modulated by intrinsic or extrinsic stimuli with consequent modification of intestinal permeability (5).

In this issue of *JPGN*, Youmba SB et al. reported a dose-dependent effect of MTX on intestinal permeability (9). Results of this study showed as MTX regulates expression and cellular distribution of several TJ proteins such as occludins, claudins, and ZO-1. In this paper, the Authors

demonstrated that the intracellular effects of MTX involves MAPK and NF-kB, according to recent studies that have suggested an important role of these pathways in the regulation of intestinal barrier functions and wound healing (10). Methotrexate (MTX) is an inhibitor of dihydrofolate reductase and DNA synthesis, that is widely used, at high doses, in cancer chemotherapy, and, at lower doses, as anti-inflammatory agent, in chronic inflammatory diseases, like inflammatory bowel diseases (11,12). MTX may determines intestinal injury by inducing apoptosis, hypoproliferation, inflammation, and bacteria colonization (10,11). A further effect of MTX on intestinal TJs increases the concerns regarding the use of this chemotherapeutic agent in children. The effect of MTX on intestinal permeability might results in an increased transport of xenobiotics and pathogens across the epithelial barrier with further risk of infections in immunocompromised subjects (13). In addition, MTX may have potential effects also on brain barrier (14). At the same time, the manipulation of intestinal permeability could represents an interesting therapeutic opportunity to limit side effects of MTX and other chemotherapeutic agents. In the last decades many drugs have been evaluated for their ability in modifying intestinal permeability, acting on TJ. A new molecule, namely AT1001, entered clinical trial in humans (15,16). AT1001 is a zonulin peptide inhibitor that competitively blocks the apical zonulin receptor and prevents the opening of TJ (15,16). This peptide has been tested in subjects with celiac disease (15). More recently in animal models of inflammatory bowel disease the AT1001 significantly attenuates colitis reducing intestinal permeability (16). In the next future, the use of this new molecule may help to minimize the deleterious consequences of chemotherapeutic on intestinal permeability. On the other hand, an increasing number of molecules has been proposed as enhancer of drugs paracellular-delivery via TJs modulation (8,17-19). Manipulation of paracellular transport represents an interesting field of research with the aims to increase oral drug delivery and to replace parenteral with enteral route for the administration of therapeutic compounds. In contrast to strategies targeting transepithelial/transendothelial pathways, modulators acting on TJs could enhance paracellular permeability of biological barriers for a large number and variety of drugs, including hydrophylic

compounds, biopharmaceuticals, like peptides, proteins, nucleic acids, and viral vectors without the need to modify the drugs (8). Unfortunately, the use of the absorption enhancers proposed so far is very limited because of scarce selectivity and potential toxicity (8). However, interesting consequences may derive from recent trials with PN159 and YY_{3-36} used in patients with type 2 diabetes to enhance insulin absorption (8). In this scenario, it is reasonable to speculate that MTX could be responsible of the enhanced intestinal absorption of itself and of other chemotherapeutic or immunosuppressant drugs (20). In this way, MTX may improves clinical efficacy of therapeutic strategy based on the use of multiple drugs simultaneously (10-21). Thus, a careful analysis of the effects on intestinal permeability should be included in the pharmacodynamic assessment of old and new chemotherapeutic and immunosuppressant drugs (22).

Controlled and reversible opening of the TJs by safe and effective molecules is a fascinating challenge of pediatric gastroenterology in the next decade (23). While new TJ regulators are under development, many of drugs currently used in children with specific indications may have further effects on intestinal permeability that remain to be defined (24). Thus, aspects that were considered as “dark side” of such therapy so far, could represent an opportunity to improve efficacy of several therapeutic strategy in the next future.

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