Protection of epithelial tight junction : a new therapeutic approach in the treatment of infectious diarrhea

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To the Editor,

Infectious diarrhea is a major gastrointestinal disease associated with morbidity and/or mortality in many countries. It is now well demonstrated that besides various modes of single bacterial penetration of epithelial cell or membrane receptor activation the bacterial invasion is linked to tight junction (intercellular) opening giving rise to a massive bacterial invasion of cells and submucosal layer (1). This invasion triggers mucosal defense mechanisms including water secretion (diarrhea), stimulation of the local resident immune system and attraction of circulating immune cells (neutrophils, T-lymphocytes, macrophages) and phenotypic changes of epithelial cells corresponding to an inflammatory reaction (2).

Until now, only very few investigations have taken into consideration the bacterial mechanisms involved in TJ opening (3-4). Interestingly, whatever the pathogens or the pathogenic toxin considered, they always activate the epithelial cell (EC) cytoskeleton contraction through a phosphorylation of actin-myosin filaments by activating myosin light chain kinase (MLCK) (Table 1). Membrane receptors activate several intracellular pathways to activate MLCK such as MAPkinase, Rhokinase favoring the intracellular entry of calcium. One future strategy to prevent the massive passage of bacteria through TJ should be to reduce the EC cytoskeleton contraction and TJ opening. Direct blockade of MLCK activation by selective inhibitors as well as blockade of membrane receptors triggering MLCK acitvation may be useful to prevent bacterial invasion. This hypothesis is supported by experimental data showing that substances susceptible to block zonulin (eukaryotic analogue of the V. cholera-derived zonula occludens toxin (ZOT) receptor such as AT1001 or to prevent cytoskeleton contraction and mucosal immune response in animal models of celiac disease or lung allergy have efficacy (11). Data have also been accumulated showing that L-glutamine prevents TJ leakiness in several experimental conditions and particularly in *E. coli*-induced increase of TJ permeability in weaned piglets (12) but the mechanism of action remains obscure. In contrast, the unique role of MLCK activation in MLC phosphorylation has been extensively validated using MLCK inhibitors such as ML7, ML-9 or PAR-2 antagonists that are fully active to prevent TJ opening and bacterial translocation at colonic level in models of IBD and IBS (13-14). However, since the TJ entry of commensal and pathogenic bacteria is the most important factor triggering symptoms, inflammation, secretion and pain, it is tempting to speculate that any mechanical device such as “biofilm” able to prevent or to limit the passage of pathogens and toxins should be of therapeutic interest in the absence of any available drug to prevent

Table 1. — **Infectious diarrhea and gut paracellular permeability**

Influence of various intestinal pathogens on epithelial cell cytoskeleton contraction giving rise to increase in intestinal intercellular permeability triggering bacterial translocation and mucosal invasion. For all of them, activation of epithelial cell cytoskeleton contraction depends upon myosin light chain kinase (MLCK) activation

	Barrier disruption	MLC Phosphoryl.	Bacteria, Mmol. translocation
Enteropathogen E. coli (EPEC) (5)	TJ + AJ (T84, Caco2)	MLCK +	yes
Enterohemorhagic E. coli (5)	TJ (T84)	MLCK + (PKC)	yes
Vibrio cholerae (9)	TJ (in vivo)	MLCK + (Zon. Occlud.)	yes
Yersinia (10)	TJ (MDCK-1)	F-actin, ZO1 (Occludin)	yes
Clostridium difficile (8)	TJ	MLCK + (RhoA)	yes
Salmonella enterica (6)	TJ + AJ	MLCK (?) (ZO1, Occlud.)	yes
Shigella flexneri (7)	TJ (in vivo)	MLCK + (spread)	yes

TJ : tight junction ; AJ : adherens junction ; MLCK : myosin light chain kinase.

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cytoskeleton contractions. We have recently accumulate experimental data confirming that film-forming substances such as gelatin tannate (GT) acting as a mucosal barrier may prevent the increase of TJ permeability limiting the passage of macromolecules and bacteria in several models of bacterial infection with a reduction of mucosal inflammation (15). Unfortunately, until now, the current clinical evidence is only based on small open Spanish and Italian studies that have to be confirmed in higher quality and larger clinical trials (16).

Nevertheless, all these data confirm the importance of the TJ opening in the pathogenesis of intestinal infection and that pharmacological prevention of TJ opening or limiting the passage of bacteria and toxin by a mucosal film-forming barrier like GT has to be considered of paramount importance to cure water secretion and mucosal inflammation in intestinal bacterial infections limiting the long-term consequences of mucosal immune stimulation responsible for post-infectious Irritable Bowel Syndrome (PI-IBS).

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