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PERSPECTIVE

Loosening the Ties That Bind—Novel Strategy to Enhance Oral Bioavailability

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The vast majority of the gastrointestinal tract is lined by a columnar epithelium that presents a formidable barrier to the delivery of hydrophilic drugs and absorption of other similar substances, including nutrients (Burton et al., 2002; Houin and Woodley, 2002). Such substances can only traverse the lipid barriers inherent in the transcellular route across this polar epithelium at physiologically meaningful rates if specific membrane transporters exist to facilitate their diffusion or, in some cases, to provide for their active or secondary active transport. Although such transport proteins can be exploited to permit the uptake of certain drug classes [e.g., the peptidomimetic agents, including several antibiotics, that are absorbed via the proton-coupled peptide transporter, PepT1 (Inui et al., 2000)], many other desirable hydrophilic agents are essentially limited to intestinal uptake via the paracellular route. Paracellular permeability across the gastrointestinal tract, as in other tissues lined by columnar epithelia, including the kidneys, biliary tract and gallbladder, is largely determined by the barrier and transport properties of a specialized class of homotypic cell-cell adhesions known as tight junctions. In this issue, Tavelin et al. (2003) report on a novel strategy to decrease the effectiveness of such adhesions, an approach that significantly increased paracellular permeability for small molecules in a model intestinal epithelium. Moreover, they describe a means to modify the stability of their active reagent such that this substance, or derivatives thereof, may ultimately prove usable in vivo. If the present work can be extrapolated to the in vivo situation, it may provide a means to revolutionize oral delivery of drugs that previously permeated the intestinal epithelium only poorly, if at all. Given the reluctance of most patients to comply with medication regimens that involve anything other than oral dosing, the insights provided here are likely to improve the benefits to be derived from agents already known as well as providing more options for the design of future orally available drug structures (Watts and Fasano, 2000).

Tight junctions were long considered to be static structures that simply fulfilled their fence and barrier functions in an unregulated fashion. However, recent molecular advances as well as studies of cellular physiology in model epithelia have instead revealed that both the permeability and selectivity of tight junctions can be modulated dynamically by a variety of signals (Mitic et al., 2000). Much of the progress in this field has rested on a significantly enhanced understanding of the proteins that make up the junction itself, as well as those components of the junction on its cytoplasmic face that link the junctional region both to the cellular cytoskeleton and to signal transduction modules (Gonzalez-Mariscal et al., 2003). Junctional sealing is accomplished via the interactions of two classes of integral membrane proteins: the claudins and occludin. Occludin was the first protein to be identified as an actual junction molecule, and each molecule of this membrane-spanning protein interacts with a partner on the neighboring cell via the binding of two extracellular loops (Furuse et al., 1993). However, the precise role of occludin in junction sealing, particularly in the gastrointestinal tract, was brought into question when mice genetically targeted to lack this molecule were found to have no overt defects in intestinal permeability (Saitou et al., 2000). This led to the search for additional tight junctional components and ultimately to the discovery of the claudins (Furuse et al., 1998). The claudins are a large family of proteins that also interact with partners on neighboring cells to effect junctional adhesions via extracellular loops. However, unlike occludin, the large diversity of claudins [more than 20 have now been described (Tsukita et al., 2001)] allows for a significant expansion of the heterogeneity of junctional properties. Each individual claudin seems to display specific properties with respect to the efficiency, or "tightness", of the junction it produces and, in some cases, is able to serve as a selective pore that permits the passage of some ionic species (e.g., divalent cations for claudin-16) but not others (Simon et al., 1999; Colegio et al., 2002, 2003; Gonzalez-Mariscal et al.,

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2003). The complexity of the system is increased further by the knowledge that epithelia may express several members of the claudin family simultaneously, and also that claudin members may form heterodimers with other family members on adjacent cells, further expanding junctional diversity (Rahner et al., 2001; Gonzalez-Mariscal et al., 2003). Finally, the few claudin knock-outs that have been constructed to date can be demonstrated to have specific, albeit sometimes subtle, changes in epithelial barrier properties in a variety of organs (Mitic et al., 2000). Thus, overall, there is both redundancy and complexity in the make-up of intercellular tight junctions in both the intestine and elsewhere.

With respect to cellular components responsible for junction regulation, a host of signaling proteins cluster tightly with occludin and the claudins at the level of the tight junction and can be demonstrated to alter the junction's properties in a dynamic fashion (Gonzalez-Mariscal et al., 2003; Matter and Balda, 2003). For example, many proteins that associate with the cytoplasmic face of the junction function as protein kinases and/or their substrates, or contain regions in their sequences (such as postsynaptic density 95/disc-large/ZO-1 and membrane-associated guanylate kinase domains) that allow the recruitment of additional kinases and other signaling molecules. These findings have led to the speculation that specific post-translational modifications, such as phosphorylation and dephosphorylation, of junction-associated proteins may be responsible for altering junction protein conformations, thereby leading to changes in junctional permeability and/or selectivity (Mitic et al., 2000; Gonzalez-Mariscal et al., 2003). For example, more highly phosphorylated forms of occludin are selectively concentrated at the tight junction, whereas nonphosphorylated molecules seem to be free to migrate to the cell cytoplasm (Andreeva et al., 2001). However, the wealth of proteins associated with the junction [an excellent and comprehensive review of this area was recently published (Gonzalez-Mariscal et al., 2003)] means that our understanding of this area is somewhat in its infancy, particularly with respect to the role of junctional regulatory pathways in normal intestinal physiology. For example, conflicting data are available regarding the role of ZO-1 phosphorylation in its localization and/or stability (Gonzalez-Mariscal et al., 2003). Nevertheless, it is apparent that some intestinal microorganisms exploit the regulation of tight junction components as part of their pathophysiology (Hecht, 1995; Fasano et al., 1997; Clatworthy and Subramanian, 2001).

Tight junctional proteins also serve to link the junctional region to other cellular components, with perhaps the most important of these being the actin cytoskeleton. In fact, in columnar intestinal epithelial cells, a perjunctional actin ring is seen in a circumferential distribution at the level of the tight junction, and several junctional components, including ZO-1, contain actin binding sites that allow them to act as linkers between cytoskeleton and ultimately the sealing proteins (Fanning et al., 2002; Gonzalez-Mariscal et al., 2003). The significance of this linkage seems to lie in the contractile properties of the cytoskeleton; contraction of the perijunctional ring is proposed to exert a force on the junctions that results in their physical separation (Mitic and Anderson, 1998). This may underlie normal physiology in some cases; it has been proposed that signaling occurring during the process of transcellular glucose absorption results in the activation of myosin light chain kinase and a subsequent increase in cytoskeletal tension. The resulting increase in paracellular permeability may enhance nutrient absorption via this route in the fed state to optimize the uptake of luminal nutrients during periods of maximal availability (Berglund et al., 2001). Conversely, evidence exists to suggest that the cytoskeleton is also a mediator of inappropriate junction dysfunction in the setting of intestinal infection with specific microorganisms or in the case of mucosal injury secondary to inflammation (Resta-Lenert and Barrett, 2002; Zolotarevsky et al., 2002). In the latter cases, the unregulated decrement in barrier function may lead to a perpetuation of disease as luminal toxins and antigenic material gain unimpeded access to the lamina propria (Zolotarevsky et al., 2002).

Therefore, it is clear that those who are interested in enhancing drug delivery via the oral route have a long list of potential targets at the level of the tight junction at their disposal. Indeed, many substances have been described that can increase paracellular permeability, although this has usually been associated with irreversible changes and/or changes of uncontrollable magnitude and with the risk of cellular toxicity (van Hoogdalem et al., 1990; Swenson et al., 1994; Yamamoto et al., 1996). Tavelin et al. (2003) reasoned that an extracellular target might carry a lower risk of cell injury, because permeation of a presumably hydrophobic modulatory substance across the cell membrane would not be required. Moreover, given the molecular heterogeneity of claudin-based junctional sealing versus the ubiquitous presence of occludin in intestinal tight junctions, they chose to explore whether agents targeted to this latter molecule might form prototypes of tight junction modulators that ultimately would prove clinically useful. Thus, based on the knowledge that occludin molecules interact with their partners on neighboring cells via their extracellular loops, they designed peptides homologous to these sequences and applied them to a model intestinal epithelium, the Caco-2 cell line. In fact, they showed that a sequence corresponding to the N-terminal portion of the first extracellular loop of occludin increased monolayer permeability without an effect on cell viability. However, the clinical usefulness of this initial approach was limited by the fact that these peptides were only effective when applied to the basolateral side of the monolayer, a circumstance that would be difficult to reproduce in vivo. They went on to show that the lack of apical efficacy was most probably caused by a combination of two factors: self-aggregation of the peptides in solution before they could bind to the junction-associated occludin and destruction of the peptide by apical peptidases expressed by these epithelial cells.

To overcome the limitations of the native peptides as junction modulators, the authors took their work one step further by synthesizing lipopeptide derivatives in which the peptide sequence was conjugated to a medium-chain lipoamino acid. This modification would be expected to protect the peptide somewhat from degradation by amino-ectopeptidases, perhaps allowing it to concentrate in the apical plasma membrane via insertion of the lipophilic tail into the membrane bilayer. Moreover, this lipid modification conferred an additional advantage in that it seemed to reduce significantly the propensity of the peptide to form homodimers. When the lipopeptide was applied to Caco-2 monolayers, it increased paracellular permeability significantly even with apical addition, an effect that was also accompanied,

as expected, by a decrease in transepithelial electrical resistance. The modulatory effect on tight junctions required release of the peptide from the lipoamino acid moiety, because peptidase inhibitors could block it. Finally, they also made the serendipitous finding that the kinetics of junctional modulation varied for the two stereoisomers of their lipopeptide derivative; the L-isomer exerted a sustained effect that increased with time whereas the D-isomer had an effect that was more transient. The differences seen apparently relate, at least in part, to the rate at which the derivative releases free peptide, and they may well be useful clinically under specific circumstances in which it might be desirable to provide only a limited enhancement of barrier permeability as a drug is delivered to the lumen.

Thus, Tavelin et al. (2003) have developed novel reagents that might ultimately lead to improved oral bioavailability for both existing and novel therapeutics. However, despite the intriguing nature of their results, several questions remain. From a basic science perspective, the results reported were perhaps surprising given the fact that genetic ablation of occludin expression has no discernible effect on tight junction integrity in the murine gastrointestinal system (Saitou et al., 2000). One possible explanation for this discrepancy, proffered by the authors, is that loss of occludin during development may result in adaptive changes in the claudin system such that barrier integrity can be sustained. On the other hand, short-term modulation of occludin adhesion, such as that putatively achieved here, would be expected to illustrate the incremental contribution of this molecule to overall junctional properties in the physiological state. Furthermore, the authors examined only the impact of their reagent on mannitol permeability and electrical properties of the tight junctions in their cell monolayers and did not assess whether their peptides could cause changes in macromolecular permeability. Were this to occur, it would entail the risk of stimulating inappropriate mucosal immune responses to dietary and other luminal antigens, perhaps thereby resulting in inflammation. Likewise, the possible permeation of luminal toxins and other microbial products capable of activating basolateral pattern recognition receptors on the epithelium, such as the toll-like receptors, could also activate a proinflammatory cascade that might ultimately result in mucosal damage (Cario et al., 2002). Thus, it will be important to document the extent of the barrier defect, no matter how transient, that is produced by the agents studied here or by novel agents based on their structure. Finally, in a practical sense, the types of reagents studied here may require significant additional modifications before they can effectively be used clinically. For example, even if lipoamino acid modification of the N terminus protects the occludin loop peptides from rapid degradation by membrane-delimited ectopeptidases expressed on the apical surface of the epithelium, there is no guarantee that these derivatives would similarly be protected from the action of gastric and pancreatic endopeptidases and proteinases and, indeed, from the damaging effects of gastric acid. It may be necessary to deliver such agents in a setting where gastric acid secretion is suppressed (a maneuver that entails its own risks for host defense) or to formulate them with the drug of interest in a controlled release form that delivers appreciable quantities of the active ingredients only at or near the site of absorption. Nevertheless, it seems likely that many, if not all, of these limitations can be overcome with the creative application of medicinal and formulation chemistries. If this can be accomplished, the principles developed by Tavelin et al. (2003) seem likely to allow for the oral delivery of a wide variety of agents for which this previously was not possible and thus to the development of new and more effective therapeutic options for a wide range of human diseases.

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