Opening Pandora’s Box in the Tight Junction

Daniel F. Balkovetz

Birmingham Veterans Affairs Medical Center, and Departments of Medicine, Cell Biology, and Microbiology, University of Alabama at Birmingham, Birmingham, Alabama


The epithelial tight junction (TJ) is one of the epithelial cell–cell junctional complexes and is critical for the maintenance of epithelial cell polarity and control of paracellular transport across epithelial tissues. In many renal physiology and nephrology textbooks, the renal epithelial TJ is simplistically depicted in cartoons as a box between epithelial cells with an arrow going through to illustrate the role of the TJ in paracellular transport (Figure 1). The understanding of the TJ protein composition and function is a rapidly advancing field. Major breakthroughs in the characterization of the TJ architecture came from the laboratory of the late Shoichiro Tsukita (1). Tsukita’s group identified the first transmembrane protein components of the TJ: Occludin and a family of proteins named claudins. The first claudins were reported in 1998 (2). To date, 24 claudin isoforms have been identified, and the composition of particular claudin isoforms within a TJ seems to determine its paracellular transport properties. In the kidney, the different claudin isoforms are expressed in a nephron segment–specific pattern (3). The paracellular transport properties vary in these different segments of the nephron and correlate with the composition of claudin isoforms in the TJ of the different nephron segments (4). For example, claudin-16 (also known as paracel-lin) is expressed in the TJ in the thick ascending limb and distal convoluted tubule and seems to mediate paracellular resorption of both Mg²⁺ and Ca²⁺. Mutations in claudin-16 are a cause of familial hypomagnesemia-hypercalciuria syndrome in humans (5).

In this issue of JASN, Feldman et al. (6) provide evidence for a unique and unappreciated effect of cyclosporin on the modulation of epithelial TJ barrier function. They report that cyclosporin increases TGF-β production and secretion and the expression of the TGF-β receptor II. TGF-β activates extracellular signal–regulated kinase 1/2 (ERK1/2) and increases TJ trans-epithelial resistance (TER), a surrogate marker of TJ “tightness.” The increase in TJ TER correlates with increases in TJ-associated proteins occludin, claudin-1, claudin-3, and zona occludens-2 (ZO-2) (although the investigators looked only at TJ-associated proteins occludin, claudins 1 to 4, claudin-16, ZO-1, and ZO-2). Collectively, this study clearly demonstrates that cyclosporin modifies the composition of proteins in the TJ as well as TJ function.

The cellular mechanism by which cyclosporin increases TER of the TJ involves the TGF-β/ERK1/2 signaling cascade, but the exact structural changes in the TJ protein composition that lead to the increase in the TER remain to be defined. Claudin-2 is an important determinant of TER in epithelia, including two strains of MDCK cells. MDCK strain II cells have a low TER (approximately 100 Ω/cm²) and abundantly express claudin-2 in the TJ. Conversely, MDCK strain I cells have a high TER (approximately 4000 to 10,000 Ω/cm²) and do not express claudin-2. Furuse et al. (7) showed that transfection of claudin-2 into MDCK strain I cells results in a TER value very similar to that observed in MDCK strain II cells. Two recent studies showed that activation of ERK1/2 by either epidermal growth factor (8) or hepatocyte growth factor (9) results in a significant rise in TER accompanied by a dramatic loss of claudin-2 expression in MDCK strain II cells. Curiously, the study by Feldman et al. in this issue of JASN demonstrates activation of ERK1/2 by cyclosporin, a dramatic increase in TER, but no changes in expression of claudin-2 (6). Their data suggest that changes in other TJ-associated proteins, other than claudin-2, can also increase TJ barrier function as measured by TER. For example, a recent report from the Yu laboratory showed that introduction of claudin-19 into the renal epithelial TJ increases the TER of renal epithelial TJ (10). Clearly, the regulation of TJ composition and function by cytokines and drugs will prove to be complex. Additional work is needed to clarify the role of ERK1/2 signaling in the regulation of both the expression of various claudin isoforms in the TJ and the paracellular permeability of the TJ in various epithelial tissues.

How could cyclosporin-specific modification of the renal epithelial TJ contribute to the adverse effect of nephrotoxicity? The study of Feldman et al. (6) demonstrates an increase in TER; no effect in paracellular flux of dextran; and increases in occludin, claudin-1, claudin-3, and ZO-1 in MDCK cells during a 72-h period. From a pathophysiologic standpoint, acutely increasing the TJ TER along the nephron would not provide an obvious cause of cyclosporin-related nephrotoxicity, such as a reduction in glomerular filtration and/or hyperkalemia. However, the long-term consequences of cyclosporin exposure in vitro and in vivo on the renal TJ composition and function are not known. In the rat liver, cyclosporin acutely increases the paracellular permeability across the TJ (11). If cyclosporin also increases in vivo TJ paracellular permeability in renal tubular
epithelial cells, then a reduction of GFR could occur from increased tubular backleak of glomerular filtrate. In addition, increasing paracellular permeability to potassium ions in the distal nephron would cause cyclosporin-induced hyperkalemia. The mechanism of tubular potassium backleak that leads to cyclosporin-induced hyperkalemia is supported by studies that showed a reduction in the transtubular potassium gradient in patients who took cyclosporin (12). Another way by which cyclosporin-induced alteration in TJ permeability could contribute to reduced renal function may occur at the level of ultrafiltration formation within the glomerulus. It has been shown that cyclosporin alters the expression of ZO-1 in the glomerular podocyte slit diaphragm (13). Alteration of TJ composition and function in the glomerular podocytes is likely to modulate the ultrafiltration barrier in the glomerulus and subsequently change GFR.

Much work is needed to facilitate our conceptual understanding of the regulation of epithelial TJ composition and function during normal physiologic as well as pathologic conditions. Recent advances in the identification of the architectural protein composition of the TJ provide us with needed information about what is in the TJ box and how to study TJ function/regulation. As with other discoveries, opening the TJ box will reveal the formidable complexities of this fascinating organelle and potentially release apparent evils, such as confusion and controversy regarding the function and regulation of the TJ. Like Pandora’s box, the opening of the TJ box will also provide humankind with hope that we will ultimately understand how the TJ works and is regulated. The light to study TJ structure, function, and regulation is becoming brighter!

Disclosures
None.

References

See the related article, “A Role for TGF-β in Cyclosporine-Induced Modulation of Renal Epithelial Barrier Function,” on pages 1662–1671.