A Potential Benefit of Quercetin in Preserving Tight Junction Integrity

Somrudee Chuenkitiyanon[a], Nontima Vardhanabhuti[b] and Suree Jianmongkol[c,*]

[a]*Interdisciplinary Program in Pharmacology, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand*

[b]*Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*

[c]*Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*

Abstract: Disruption of tight junction (TJ) integrity can occur from loss of TJ protein expression and/or disorganization. Consequently, epithelial/endothelial barriers lose their barrier functions in the control over paracellular transport, leading to the compromised defense mechanism of the organ. The TJ disruption has been related to multiple signal transduction pathways including mitogen-activated protein kinases (MAPK). Quercetin is one of the most widely studied flavonoids with broad spectrum of pharmacological activities. In addition to antioxidant capability, quercetin may exert its actions via the alteration of protein kinase activities and the sequential signaling processes. This may enable quercetin to prevent the disintegration of TJ structure and to enhance the expression, localization and interaction of TJ proteins. This review presents the information to corroborate the potential benefit of quercetin in preserving integrity and function of TJ complexes. The involvement of protein kinase C (PKC) and MAPK signaling pathway is emphasized.

Keywords: Quercetin, tight junction, protein kinases.

INTRODUCTION

Epithelial and endothelial tissues, which line up the cavities and surfaces of organ throughout our body, act as strong protective barriers against pathogens and chemical invasion. In these tissues, the epithelial/ endothelial cells are tightly connected with special intercellular bonding including gap junctions, desmosomes, adherence junctions and tight junctions (TJ) to seal the gap between them [1, 2]. The extracellular TJ architecture at the apical site regulates the paracellular movement of ions, solutes and immune cells across the epithelia and endothelia (barrier function). In addition, the TJ structure assists the cells in holding together as well as creates cell polarity to block the movement of integral membrane proteins between the apical and the basolateral sites (fence function) [3-6]. Disruption of TJ structure and function results in hyperpermeability and leakage of epithelial and endothelial barriers, which is a condition involved in a number of pathologic states such as inflammatory bowel disease (IBD), renal failure, edema jaundice, diarrhea, and blood-borne metastasis [7, 8]. Although the mechanisms of TJ assembly, maintenance and disruption are not fully elucidated, the potential benefits of several food components and compounds including quercetin, glutamine, epidermal growth factor (EGF) in enhancing and preserving TJ integrity have been identified [8-10].

*Address correspondence to this author at the Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand; Tel: +662 218 8318;
Fax: +662 218 8324; E-mail: sureejmk@yahoo.com

THE TIGHT JUNCTION: ITS COMPONENTS AND THE ROLES OF MAPKS

TJ complex is formed through interactions between a number of integral membrane proteins (e.g., occludin, claudin, junctional adhesion molecule or JAM), peripheral proteins (the zonular occludens (ZO) family including ZO-1, ZO-2, and ZO-3) and other junction-associated proteins (e.g., cingulin) [3, 4, 6, 11, 12]. Occludin and claudin are structural junction proteins that connect to the actin cytoskeleton through cytoplasmic ZO linkers [3, 4, 13]. The interactions between JAM, ZO-1, and other proteins such as cingulin provide the tightness of the junction.

Assembly, maintenance and function of TJ complexes involve TJ protein expression, phosphorylation, and protein-protein interactions [3, 14, 15]. These processes are related to multiple signaling transduction pathways including enzymes in the protein kinase C (PKC) and MAPKs families (e.g., extracellular signal-regulated kinase 1/2 or ERK1/2, and p38) [16-23]. In addition, a number of signaling molecules such as cyclic GMP (cGMP) [24, 25], calcium [23, 26, 27], NF-κB [28], nitric oxide (NO) [5, 29] and vascular endothelial growth factor (VEGF) [30, 31] also render their influence on the assembly and stability of the structure of TJ complexes.

The MAPK signaling pathway has been linked to a broad spectrum of cellular responses to extracellular stimuli such as growth factors and stress [32, 33]. In addition to the roles in cellular function, growth and survival, MAPK proteins have been linked to formation and structural integrity of the

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TJ [10, 17, 18, 20, 21]. However, there are discrepancies among scientific reports on MAPK activities, which may be related to the cell culture system, the culture conditions and the timing of experimentation. For example, ERK1 has been found to interact with occludin in epithelial cells and is required for proper distribution and organization of the TJ proteins and actin cytoskeleton [10, 17]. Inhibition of MEK-1/2 (MAPK/ERK-1/2) can prevent barrier formation and upregulation of the TJ protein claudin-2 in epithelial cells [34]. By contrast, activation of ERK1/2 in the transfected MDCK cells with an activated Ras mutant has been reported to increase in the transepithelial permeability of mannitol by six-fold, along with a disappearance of occludin from cell-cell contact sites [17]. In addition, the ERK1/2 inhibitor PD98059 could prevent the disruption in barrier function that was induced by cyclosporine A via activation of ERK1/2 MAP signaling cascade in the MDCK monolayers [35]. Thus, the roles of MAPK activities in TJ regulation are quite complicated and need further elucidation.

DISRUPTION OF TIGHT JUNCTIONS

Disruption of TJ assembly and function can be resulted from a number of conditions such as oxidative stress, inflammation and alcohol exposure [7, 8, 10, 35-38]. Oxidative stress interferes with several cellular homeostasis and functions, leading to the pathological stress such as increase in intracellular Ca^{2+} and activation of the protein kinase family [18, 20, 27, 39]. Hence, oxidative stress has been linked to several degenerative diseases and pathological states such as Alzheimer's disease, Parkinson's disease, ischemia/reperfusion injuries, inflammation, seizures, stroke, and trauma. In epithelial and endothelial tissues, oxidative stress destabilizes the TJ complexes, leading to an increase in paracellular permeability and barrier leakage [17, 18, 20, 21, 40, 41]. For example, H_2O_2 increases the permeability of several epithelial and endothelial barrier models through disruption of the TJ structure [18, 42-44]. The mechanisms of oxidative stress-induced barrier disruption are still incompletely understood. However, several lines of evidence indicate that oxidative damages affect the expression, localization and organization of TJ regulatory proteins, in particular occludin, ZO-1 and claudins [14, 15, 30, 34, 38, 45]. It was demonstrated that a decrease in occludin protein was responsible for a barrier leakage and loss of transepithelial resistant (TER) values in bovine pulmonary artery endothelial cells treated with H_2O_2 [18, 46]. H_2O_2-induced hyperpermeability of epithelial monolayers has been linked to a decrease in expression and localization of occludin and ZO-1 proteins. This was evidenced by a reduction in their amounts from the western blot analysis along with the discontinuous pattern at the circumferential cell border from immunofluorescent staining of these two junctional proteins [44].

Oxidative stress is able to trigger signaling pathways in TJs regulation involving MAPKs (especially ERK1/2, p38, and c-Jun NH2-terminal kinase or JNK), PKC, phosphodiesterase, small G protein Rho, and intracellular Ca^{2+} [17, 19-21, 26, 37, 38, 47, 48]. Oxidative stress-induced hyperpermeability is related to phosphorylation of occludin and ZO-1 at the tyrosine residues, downregulation of occludin and activation of MAPK signaling pathways [17, 18]. Consequently, the dissociation of these junctional proteins from the actin cytoskeleton along with redistribution from the junctional area takes place [14, 15]. These processes can be protected with VEGF and EGF through the alteration of ERK and ERK1/2 activity [10]. Kevil *et al*. (2000) demonstrated that HUVEC treated with H_2O_2 at the concentration of 500 μM for 3 hrs resulted in hyperpermeability corresponding to ERK1/2 activation and disorganization of occludin and ZO-1 at the cell-cell contact sites [18]. Huot *et al*. (1997) also showed that H_2O_2 administration caused an early increase in ERK1/2 activity, and a more prolonged increase in p38 MAPK activity [49]. Application of the specific ERK1/2 inhibitor (PD98059) can inhibit the oxidative stress-induced hyperpermeability and affects the redistribution of occludin [18]. Treatment with the p38 inhibitor (SB202190) attenuates an increase in solute permeability in the model of human endothelial cells exposed to H_2O_2 [50]. Furthermore, the permeability increasing effects of H_2O_2 may be related to an activation of PKC along with a rising of intracellular Ca^{2+} [48, 51]. The increase in intracellular Ca^{2+} activates Ca^{2+}/calmudulin kinases, which subsequently activates all three MAPKs (ERK, JNK, and p38) [52].

QUERCETIN AND ITS POTENTIAL TO MAINTAIN THE INTEGRITY OF TIGHT JUNCTION

Recently, there are a number of investigations searching for compounds with barrier protective activities. Growing evidence suggests that certain food components such as glutamine, polyunsaturated fatty acid (PUFA) and flavonoids may be TJ modulators that maintain or enhance TJ integrity and function [8, 9].

Quercetin is one of the most widely distributed flavonoids in plants. This polyphenolic compound is found abundantly in fruits and vegetables including apples, onions, berries, beans as well as in food products and beverages derived from plants such as olive oil, tea, and red wine [53-55]. It is also found as a major component in several herbal medicines including *Ginkgo biloba, Hypericum perforatum* (St. John's Wort), *Sambucus canadensis* (elder), *Vaccinium macrocarpon* (cranberry) and *Oenothera biennis* (evening primrose). Similar to other flavonoids, quercetin contains a broad spectrum of pharmacological and clinically relevant activities including carcinostatic, anti-inflammatory, and antioxidant actions [53, 55-57]. It has been found to suppress cell proliferation, modify eicosanoid synthesis, prevent platelet aggregation, stabilize immune cells, and promote relaxation of vascular smooth muscle [55]. As an antioxidant, quercetin is reported to protect oxidative injuries as well as inflammatory-related injuries [58-60]. The action of quercetin has been linked to a number of enzymes involved in proliferation and signal transduction pathways including PKC, tyrosine kinase, PI-3 kinase, NF-κB, and the MAPK family [55, 61-64]. As abovementioned, alteration in the activity of these kinases (PKC and MAPKs in particular) and their sequential signaling pathways significantly influence the assembly and integrity of TJ structure. Thus, it can be hypothesized that quercetin can be a barrier protective agent through its capability to preserve TJ integrity and function.

Although the reported actions of quercetin on barrier functions are quite limited, a growing body of evidence suggests that quercetin can influence the epithelial barrier integrity and function via modulation of structural TJ proteins [44, 65, 66]. Treatment of Caco-2 monolayers, which is a

known model of the intestinal epithelium, with quercetin for 48 hrs enhanced barrier functions, as evidenced by an increase in TER values [65]. These findings are related to an increase in claudin-4 expression [65, 66]. The study of Suzuki and Hara (2009) further demonstrated that the influence of quercetin during the 48-hr exposure on TJ assembly was biphasic and time-dependent. In the early phase, quercetin promotes TJ assembly and interaction with actin cytoskeleton through distribution of TJ proteins (ZO-2, claudin-1, and occludin) [66]. During the later phase, expression of claudin-4 increases [66]. This promotive effect of quercetin on TJ assembly and barrier functions may be related to the inhibitory action on PKC and its signal transduction pathway, but not to the antioxidant action [65]. Myricetin, which is a more potent antioxidant than quercetin, does not have intestinal barrier enhancing effect in the model of Caco-2 monolayers [65].

Our laboratory is interested in the protective ability of quercetin against oxidative stress-induced breakdown of epithelial and endothelial barriers. Recently, we demonstrated that quercetin was able to prevent the breakdown of barrier functions and the disintegration of TJ complexes in the ECV304 monolayers upon exposure to non-lethal concentrations of H_2O_2 (100 μM; 4 hrs) [44]. Pretreatment the cells with quercetin (10 μM; 30 min) prior to H_2O_2 could preserve the normal levels of ZO-1 and occludin expression as well as their localization at the cell border. The integrity of TJ complexes in the quercetin pretreated group was also maintained as evidenced by an increase in TER values and a decrease in phenol red permeability in comparison with the group treated with only H_2O_2. In addition, quercetin could suppress H_2O_2–mediated activation of p38 MAPK whereas it potentiated the effect of H_2O_2 on ERK1/2 activities. Although the molecular targets of quercetin were not yet identified, our findings suggested that the protective effects of quercetin might involve the altered MAPK activities, in particular the decrease in p38 MAP signaling.

CONCLUSION

Endothelium/epithelium barriers can be primary targets of oxidative assaults, leading to functional abnormalities associated with the collapse of TJ structure. As a result, changes in paracellular solute permeability can be observed in correlation with the loss of expression and disorganization of TJ proteins. Quercetin, an edible flavonoid, has been demonstrated its benefit in enhancing TJ assembly and in preserving TJ integrity and function against H_2O_2-mediated TJ disruption that leads to hyperpermeability of epithelial barriers. The TJ modulating effects of quercetin involves with PKC and MAPK activities and their subsequent signaling cascades. The TJ protective activities of quercetin might be clinically beneficial in reducing the potential threat to the organ system and to preserve a normal physiological state of the blood-organ barrier.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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None declared.

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