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Effect of Capsaicin on the Tight Junctional Permeability of the Human Intestinal Cells

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Abstract: In arid and semi-arid lands, people eats many kinds of spices. Capsaicin is one effect active component of spices. Previously we demonstrated that capsaicin induced tight junction (TJ) opening in human intestinal Caco-2 cells. We showed that the increase of TJ permeability was involved in actin polymerization. However the underlying mechanisms are unclear. The major aims of this study were to determine the effect of capsaicin on intestinal epithelial TJ permeability and to elucidate the mechanisms involved in this process using an established in vitro intestinal epithelial model system consisting of Caco-2 intestinal epithelial monolayer. Treatment Caco-2 cells with capsaicin increased TJ permeability as measured by transepithelial electrical residence (TER). However, TJ was recovered soon. Hence, in this study, we tested our hypothesis that influence on Caco-2 cells by the capsaicin is different depending on time moment. Our data suggest that capsaicin induced different influence depending on the time moment.

Keywords: Capsaicin, Claudin-1, Occludin, Tight Junction (TJ), Transepithelial electrical residence (TER)

1. Introduction

Capsaicin is a major constituent of red pepper, may affect the function and permeability of the human intestinal epithelial Caco-2 cells in vitro. In previous studies, we showed that TJ permeability increased in capsaicin-treated human intestinal epithelial Caco-2 cells, and this mechanism involved actin polymerization (Han *et al*, 2002). There is many evidence that member of the small GTPase protein family of molecular "switches" such as Ras, Rac, RhoA, have functional roles in adhesions and tight junction assembly and maintenance (Braga, 2002; Rubenstein *et al*., 2005; Giesemann *et al*., 2008).

Caco-2 cells are often used as an in vitro model of the intestinal epithelium. In these cells the most apical cell-cell junction is TJ, which permeability is regulated by various factors, such as food factors and chemicals. Measurement TER value of TJ is known to be correlated with change in the paracellular permeability of the cell monolayer (Hashimoto et al., 1997). The TJ forms a paracellular barrier on the lateral membranes of adjacent cells and acts as a structural barrier to the paracellular passage of water-soluble molecules bacteria (Tsukura *et al*., 2007). Disruption of the TJ barrier allows paracelllular penetration of toxic luminal substances such as anti-inflammatory drugs, which promote gastrointestinal mucosal injury. A number of proteins associated with the cytoplasmic surface of TJ have been identified, including ZO-1, ZO-2, as well as two transmembrane proteins, occludin and claudin-1 which has been suggested to function in creating the intercellular seal (Christina *et al*., 1997).

In this study, we have measured the TER and paracellular flux analysis to elucidate TJ opening. The level of polymerized actin was determined by flow cytometry. We also have performed western blot analysis to determine the possible mechanism behind the TJ opening induced by capsaicin in Caco-2 cells. We found that capsaicin effectively induces change expression of occludin, claudin-1 and RhoA, Rac1. In short time treatment, capsaicin induced the same change of occludin and claudin-1 protein expression. Although in long time treatment, capsaicin down regulated only occludin protein expression level.

2. Materials and Methods

2.1. Cell culture

Human intestinal epithelial Caco-2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplement with 10% fetal bovine serum, 1% non-essential amino acid, 1% penicillin streptomycin. They were incubated in an atmosphere of 5% CO_2 at 37˚C. Cells from each flask were

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detached trypsin, resuspended in fresh medium, and cultured on collagen coated dish for TER assay and determination of F-actin and western blot assay. The cells were seeded 1 x 105cells/ml for every assay.

2.2. TER measurement

To study the time-course effect of 100 μM capsaicin on TJ opening, TER measurement was performed. For the TER experiments, cells were seeded in 24 well transwell coated with collagen. The cells were cultured about 10 days to establish monolayer integrity. Medium of the cells were changed every other day. And the cells were treated with 100 μM capsaicin for varying time periods. Then TER measurements were performed following the methods of Han *et al*. (2002).

2.3. Paracellular flux

To assess paracellular permeability, cell monolayer was established on 12 well transwell coated with collagen. The cells were cultured under the same condition as for TER measurement condition. And the cells were incubated with 100 μM capsaicin for varying time periods in the presence of Lucifer yellow CH dilithium salt. At given time, sample from basal side were taken and placed in 96 well plates. Fluorescence was measured with plate reader (POWERSCAN HT; Dainippon pharmaceutical) for excitation at 485 nm and emission at 530 nm.

2.4. Detemination of cellular F-actin-

Determination the levels of polymerized actin was performed as described. Briefly Caco-2 cell monolayers that had been incubated on the dish for 5 days were rinsed with phosphate-buffered saline (PBS) (-) and then incubated for varying time periods with or without capsaicin (100 μM). After incubation, the cell monolayers were detached by trypsin and cell-suspension was made. The cell lysates were centrifuged to yield a clear lysate. Supernatant was removed, and 3.7% formealdehyde was added, with incubatation for 15 min, vortexed and centrifuged at 8000 x g, 4˚C for 5 min. The supernatant was removed, and then washed with PBS (-). The cells were fixed with 0.2% TritonX and incubated for 5 min, centrifuged at 8000 x g, 4˚C for 5min and the supernatant was removed. The cells were washed by PBS (-), stained with 70 nM rhodamine-phalloidin, and washed by PBS (-). Stained cells were measured by flow cytometry (GE Healthcare).

2.5. Western blot analysis

To study the time-course effect of 100 μM capsaicin on TJ proteins and small GTPases protein expression, Caco-2 monolayers were treated with 100 μM capsaicin for varying time periods. After capsaicin treatment, Caco-2 monolayers were immediately rinsed with ice-cold PBS, and then lysed in 1 ml of cold RIPA buffer (50 mM Tris-HCl, pH 8.0, with 150 mM sodium chloride, 1.0% lgepal CA-630 (NP-40), 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) according to the manufacture's instruction. The cell lysates were centrifuged to yield a clear lysate. Supernatant was collected and protein measurement was performed using 2-D Quant Kit from GE Healthcare. Loading buffer was added to the lysate containing 15 μg of protein and boiled for 5 min, after which proteins were separated on a SDS-PAGE gel. Proteins from the gel were transferred on a membrane (Nitrocellulose membrane). The membrane was incubated with blocking solution (5% skimmilk, Brock ace) for 1 hour. The membrane was incubated with appropriate primary anti-bodies in blocking solution. After being washed in TBS-1% Tween 20 buffer, the membrane was incubated in appropriate secondary anti-bodies and developed using the ECL plus Western Blotting Detection System (GE Healthcare) on Polaroid 667 film (Polaroid) using ECL TM Mini-camera (GE Healthcare).

3. Results and Discussion

Capsaicin was not cytotoxic at concentration up to 100 μM as determined by the MTT assay (data not shown). The permeability of Caco-2 cells was assessed by measuring TER and the flux of the Lucifer yellow. TER is direction indicator to check whether TJ is tightness of the paracellular seal or not. TER value was decreased, and after increased significantly. This indicated that TJ permeability was recovered. The result of paracellular flux measurement also confirmed the same effect. However after TJ recovery,

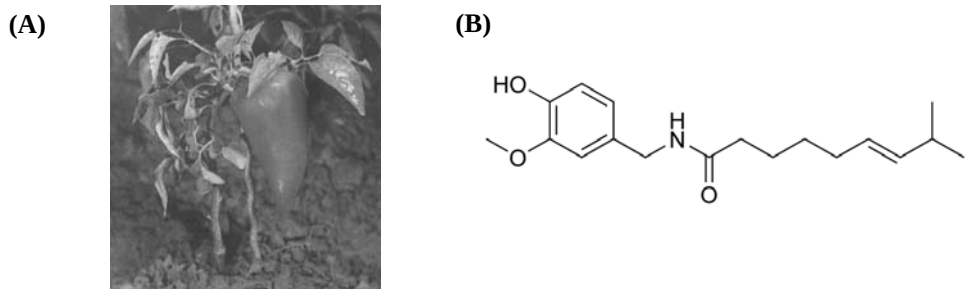

Fig.1. (A) Capsicum annum: local name is chill, red pepper, paprika, capsicum, (B) Structure of capsaicin.

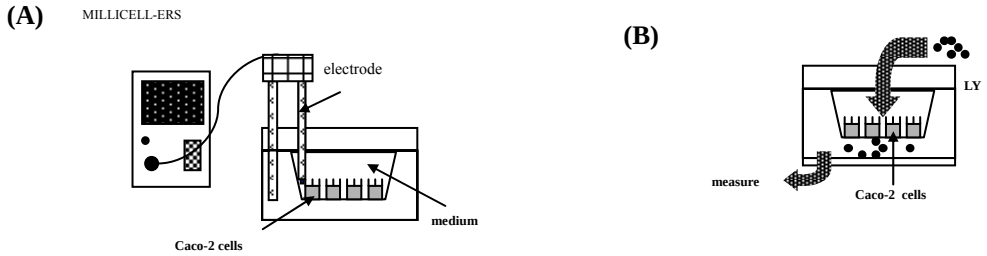

Fig.2. (A) Illustration of TER measurement. To evaluate the state of TJ is measuring the electrical resistance value of the cell layer by an electrode. TER is decided on a cell part and permeability of the intercellular space part by the ion. However, TER value expresses intercellular space namely a state of TJ because the latter is much bigger. **(B) Illustration of measuring of paracellular flux.** This supports the TER measurement.

TER decreased slowly which indicated that TJ is open. And we confirmed that actin polymerization regulated TJ opening.

To examine the expression of TJ proteins and small GTPases involved in TJ opening induced by capsaicin, we performed a western blot analysis on capsaicin-treated Caco-2 cells. Since we observed TJ opening happened two time course (long and short time) during capsaicin treatment we investigated these proteins expression in both condition.

The intestinal epithelial TJ plays a very important role in absorption and barrier function. However, the defective intestinal epithelial TJ barrier has been proposed as an important pathogenic factor leading to the intestinal inflammation (Welcker *et al*, 2004). In previous study, we demonstrated that capsaicin-induced increase in Caco-2 cells TJ permeability recovery (Isoda *et al*., 2001; Han *et al*., 2005). Clarification of the mechanism may give possiblity to regulate of absorbance and barrier function without inflammations. The time course of capsaicin effect on Caco-2 TJ permeability suggested that the capsaicin effect was due to acute intracellular signaling process by capsaicin binding to capsaicin receptor TRPV1. In previous study, we clarified that capsaicin-induced increase of capsaicin receptor TRPV1 protein level and increase of Caco-2 TJ permeability (Isoda *et al*., 2001). The initial increase in Caco-2 TJ permeability occurred within 30min following capsaicin exposure with subsequent recovery. Moreover, after recovery TER slowly decreased over time. In this study, we examined some of the expression of proteins that mediated the capsaicin modulation of Caco-2 TJ barrier, TJ proteins and small GTPases. In previous study, we indicated that capsaicin-induced increase in TJ permeability is associated with the cytoskeleton reorganization of the actin filaments by determining the cellular F-actin amount in Caco-2 cells (Han *et al*., 2002). Cofilin dephosphorylation can directly induce actin reorganization, which can result in TJ opening (Nagumo *et al*., 2007). We investigated protein expression of Rac1, α-actinin and RhoA, because they were well-known interaction with actin. On the other hand, the precise role of TJ proteins in the modulation of intestinal TJ barrier function remains unclear. Reference suggested a correlation between occludin expression and TJ barrier function. Increase expression of intestinal occludin and claudins cooperate in the regulation of selective paracellular permeability in MDCK

cells (Balda et al., 2000). Moreover, a number of studies indicated a correlation between an increase in epithelial TJ permeability and a decrease in occludin expression.

4. Conclusions

In this study for the first time, we showed that 100μM capsaicin concentration increased the permeability in Caco-2 cells. We confirmed different changes of the expression of TJ proteins and small GTPases protein expression.

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