PROTECTIVE EFFECT OF OLIGOMERIC PROANTHOCYANIDINS (OPCs) FROM THAI GRAPE SEEDS AGAINST H_2O_2-INDUCED TIGHT JUNCTION FUNCTION DISRUPTION IN HANMAN CACO-2 CELLS

Thanchanok Muangman[1], Prapaipat Klungsupya[1], Anawat Suwanagul[1], Akihiro Watari[2] and Kiyohito Yagi[2]

[1]*Thailand Institute of Scientific and Technological Research (TISTR), Techno Polis, Pathum Thani 12120, Thailand.*
[2]*Laboratory of Bio-Functional Molecular Chemistry, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan*

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INTRODUCTION

Oligomeric proanthocyanidins (OPCs) are polyphenolic compounds which occur abundant in red grape seed. They take the form of oligomers or polymers of polyhydroxy flavan-3-ol units, such as (+)-catechin and (-)-epicatechin. Many publications have been reported that OPCs is powerful antioxidant free radical scavenger suggesting that it can be effective in reducing the risk of cancer, cardiovascular disease and the number of chronic diseases. The extensive research both *in vitro* and *in vivo* studies shown OPCs can act as anti-carcinogenic agent by activities include reduced proliferation, increased apoptosis, cell cycle arrest in tumor cells.

Tight junction (TJ) is essential for the ‘‘tight’’ bonding between adjacent cells of the epithelial/endothelial protective barriers and regulating paracellular transport across cell layers. It is also known as occluding junctions or zonula occludens. The structure TJ is composed of integral membrane proteins such as occluding and claudin family connecting to actin cytoskeleton through zonular occudens (ZO) proteins. Disruption of TJ results in barrier leakage and compromises the defense mechanism of the body.

In 2008, Thailand Institute of Scientific and Technological Research (TISTR) was firstly developed method to extract the natural OPCs from Thai red grape seeds of *Vitis vinifera* cv. Ribier (Pok Dum). Furthermore, we previously reported the anti-oxidant, non-mutagenic, anti-mutagenic and anti-oxidative DNA damage of this Thai OPCs. The current study was carried out to further investigate the protective activity of Thai OPCs against H_2O_2-induced TJ function disruption and expression of claudin-4 in Caco-2 human intestinal epithelial cells.

MATERIALS AND METHODS

Extraction of oligomeric proanthocyanidins (OPCs) The natural OPCs were firstly extracted by the Thailand Institute of Scientific and Technological Research (TISTR) from grape seeds isolated from waste product of the winery and grape juice industry. The whole seeds were dried and subjected to extraction using suitable ratio of ethanol/water and temperature. The chemical composition and structure of OPCs were analyzed by high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy. For experimentation, OPCs stock solution (3,000µg/mL) was prepared by dissolving in distilled water and then stored at -25°C under dark condition until used.

Cell culture and maintenance The human colon adenocarcinoma Caco-2 cell line was employed. The cells were grown in DMEM medium (Nussui pharmaceutical, Tokyo, Japan) and supplement with 10% (v:v) of fetal bovine serum (Nichirei Biosciences, Tokyo, Japan), D-(+)-Glucose (Sigma, St.Louis, USA), MEM non-essential amino acids (Gibgo/Invitrogen, N.Y., USA), 1% of L-Glutamine, 1% of penicillin/streptomycin (Nacalai Tesque, Kyoto, Japan) in a humidified incubator at 37°C and 5% CO_2. Caco-2 cells were grown for 2-3 days to confluency of monolayers.

Determination of cytotoxicity The cytotoxic effect of MPCP#35 or Caco-2 cells was evaluated using Cell Count Reagent SF kit (Nacalai Tesque, Kyoto, Japan). Cells were seeded in 96 well plates and cultured for 24 h prior to treatment with various concentrations of OPCs. After treatment, the medium in each well was replaced with 100 µl of new medium and added 10 µL of Cell Count Reagent solution. Cells were incubated in a 5% CO_2 incubator at 37°C for 1-2 h. Cells viability was spectrophotometrically quantified at a measuring wavelength of 450 nm (Tristar LB 941, Berthold Technologies, Japan).

Reporter gene assay MPCP#35 cells were grown for 2-3 days to confluence. For luciferase assay cells at a density of 4 x 10^4 cells/ cm^2 were grown on 96-well plate. Confluent MCPC#35 cells were treated with OPCs for 24 h. Cells were washing twice by PBS after incubation time. Luciferase assay reagent (TOYO INK, Tokyo, Japan) was added to 100 µl/ well and shake for 30 min with aluminum foil cover. The light intensity was measure by the luminometer (TriStar LB 941, Berthold Technologies, Japan).

Measurement of trans-epithelial electrical resistance (TER) Caco-2 cells (4 x 10^4 cells) were seeded in 6.5 mm polycarbonate membrane inserted with 0.4 µm pore size (Corstar, Corning, USA) in culture medium. The TER across the monolayer was measured with a chopstick-like electrode connected to a Millicell-ERS (electrical resistance system, Millipore, USA) and the TER values were expressed as the measured resistance in ohms (Ω) multiplied by the surface are of the Transwell membrane (cm^2). CaCo-2 cells were grown until the TER was stable for 7-10 days in culture. Once stable resistance was obtain (>200 Ω cm^2), the cells were treated with protein samples. TER was measured just before adding protein samples and at 24 and 48 h.

RESULTS

Cell Viability OPCs had no significant effects on the viability of MPCP#35 and Caco-2 cells at the concentration of 0.01- 0.1 mg/mL.

Effect of OPCs on claudin-4 promoter activity Luciferase reporter gene assays were performed to study effects of OPCs on the promoter of claudin-4. The claudin-4 promoter was cloned into a PGVCL4P vector bearing a luciferase gene for quantitative measurement of promoter activity. It was found that OPCs at concentration of 0.2 mg/mL increased the promoter activity to 291 ± 14% expressed as percentage of control as demonstrated in Figure 1.

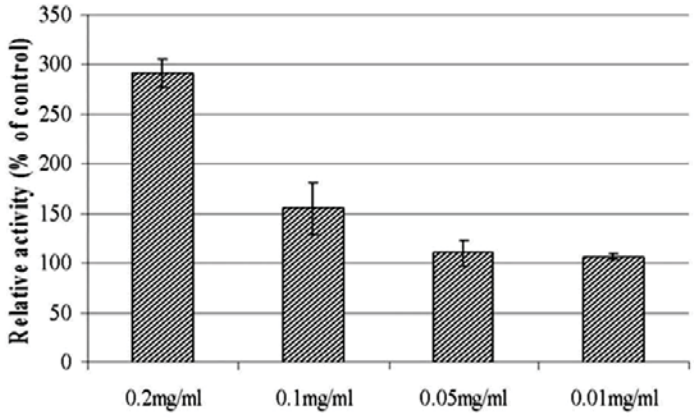

Figure 1 Effect of OPCs on claudin 4-promoter activity (luciferase)

Effects of OPCs on TER values The ability of OPCs to preserve the function of tight junction structure was tested using TER measurement. Tightness of the junction would result in high membrane electrical resistance. The effects of OPCs on TER were measured in Caco-2 monolayers over 48 h. In comparison with the control, OPCs (0.2 mg/mL) slightly increased the TER values at 24 h and 48h as shown in Figure 2.

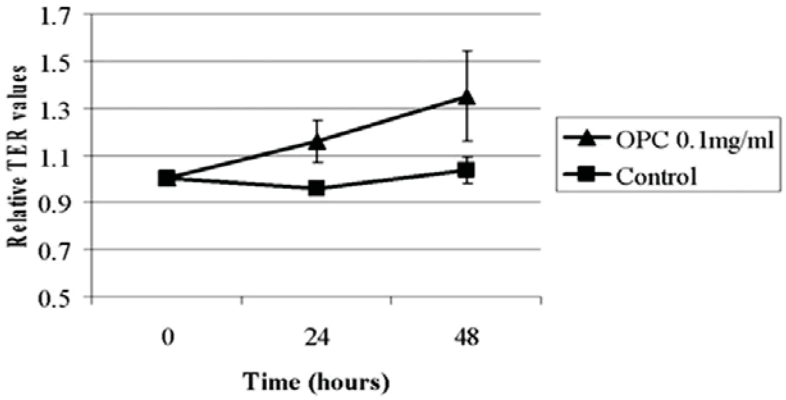

Figure 2 Effects of OPCs on trans-epithelium electrical resistance (TER) values of Caco-2 cells.

DISCUSSION

A significant body of evidence has demonstrated that inflammatory bowel disease has been associated with a disorder of the intestinal TJ barrier function [1-2]. A large number of studies have reported that various food components provide beneficial of anti-inflammatory, anti-mutagenic effects in the intestines and also intestinal TJ barrier functions [3-5]. OPCs are the most abundant of flavonoids found in the seed of grape (*Vitis vinifera*) especially from red grape seed extract. In our study, OPCs induced claudin-4 expression at a transcriptional and protein level including of TER values improvement. Regarding results obtained from this study, OPCs enhanced barrier properties in Caco-2 cells used for the study of epithelial functions. This effect ranks among a large number of beneficial effects reported for dietary proanthocyanidins, which include suppression of carcinogenesis and prevent of cardiovascular disease.

Our present study illustrates similar results to other flavonoids such as genistein, quercetin, myricetin and EGCG [6]. The two compounds had been reported their protective and promotive effects on intestinal TJ barrier function. Study in colonic epithelial HT-29/B6 cells shown that genistein was completely blocked the decreasing of TER induced by inflammatory cytokine [7]. Moreover, genistein also exhibited to suppress decrease TER induced by enteric bacterial in Caco-2 cells [8]. The results of the two flavonols; quercetin and myricetin demonstrated a decreased permeability of lucifer yellow and increased the TER correlated with overexpression of claudin-4 in Caco-2 cells [9]. Furthermore, on the basis of report gene assay, that quercetin stimulates claudin-4 promoter activity via enhanced the claudin-4 expression at a transcriptional level in Caco-2 cells similarly as resulted in OPCs [10].

CONCLUSION

Regarding the present results, we found and concluded that OPCs enhanced tight junction (TJ) barrier properties in Caco-2 cells via claudin-4 protein. The improvement of TJ function might represent an important protective effect of this substance against barrier disturbance in intestinal inflammation. However, the molecular mechanisms underlying this OPCs-mediated effect remain unclear. Thus, further studies are suggested.

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