The effect of Prim-O-glucosylcimifugin on tryptase-induced intestinal barrier dysfunction in Caco-2 cells

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Abstract

The intestinal barrier dysfunction is a critical pathological change in irritable bowel syndrome (IBS). The objective of this study was to evaluate the effect of Prim-O-glucosylcimifugin (POG) on intestinal barrier dysfunction and reveal possible molecular mechanisms. Caco-2 cell monolayers induced by tryptase (TRYP) were used to establish an intestinal barrier dysfunction model. Caco-2 cell monolayers from both functional and dysfunctional samples were treated with POG (30, 60 and 120 µg/mL) for 2, 8, 24, 36, 48 and 72h. The Caco-2 cell monolayers were assessed by measurement of trans-epithelial electrical resistance (TEER) and percentage of fluorescein permeation (PFP). The expression of PAR-2 and MLCK mRNA was analyzed by RT-PCR and the level of ZO-1 protein expression was determined by western blot. In addition, the impact of POG on the distribution of the tight junction protein of Occludin was performed by immunofluorescence. Our results showed that POG elevated the TEER and decreased the PFP of the functional Caco-2 cell monolayers, as well as the dysfunctional Caco-2 cell monolayers. Furthermore, POG inhibited the expression of PAR-2 mRNA and MLCK mRNA and increased the level of ZO-1 protein expression in dysfunctional Caco-2 cells. The distribution of the Occludin proteins was ameliorated simultaneously. This study demonstrates that POG can enhance the intestinal barrier function of Caco-2 cell monolayers by inhibiting the expression of PAR-2 and MLCK and up-regulating the expression of ZO-1 protein, and ameliorated the distribution of Occludin protein.
Keywords

Prim-O-glucosyleimifugin (POG), Intestinal barrier, Caco-2 cells, irritable bowel syndrome, barrier dysfunction
**Introduction**

The intestinal barrier plays an important role in the maintenance of the body’s health. It not only protects the intestinal tract from harmful substances, but also improves the immune function of the gut\(^1,2\). Disorders of the intestinal barrier are associated with the pathogenesis of Irritable Bowel Syndrome (IBS), caused gastrointestinal tract symptoms, such as diarrhea and abdominal pain\(^3,4\). Moreover, IBS is a chronic relapsing disease in which symptoms may vary over time, bringing great inconvenience to the life of the patients.

TRYP, a serine protease, is released by mast cells upon degranulation. An increased release of TRYP has been reported in patients with diarrheic IBS (D-IBS)\(^5\). TRYP can activate the intestinal Protease Activated Receptor 2 (PAR-2), inducing intestinal barrier dysfunction and visceral pain sensitivity\(^6\). PAR-2 is a serine protease receptor that is highly expressed on intestinal epithelial cells. Then the activation of PAR-2 increases the expression of myosin light chain kinase (MLCK) and affects the tight junction proteins, such as ZO-1 protein and Occludin protein, that make up the intestinal epithelial barrier\(^7\). MLCK plays an important role in altering the intestinal epithelial tight junctions (TJs). MLCK transcription induces an increase in MLCK protein activity, which in turn promotes the phosphorylation of myosin light chain (MLC) and then changes the structure and distribution of TJ protein (ZO-1 protein and Occludin protein)\(^8-10\) which also plays a pivotal role in the tight junctions of the intestinal epithelial barrier\(^11\).

*Saposhnikovia divaricata* (Turcz.) Schischk (SD, Apiaceae), called “FangFeng” in Chinese, is widely distributed in northeast China. SD has been found to exert multiple
pharmacological effects, including analgesic, anti-diarrheal, anti-inflammatory, anti-oxidant, and anti-ulcerative effects, as well as to regulate intestinal motility\cite{12-15}. What attracted our attention was that there have been reported that SD exerts anti-diarrheal and analgesic effects in rats with IBS\cite{16-18}. And its therapeutic outcome have been attributed to the down-regulation of PAR-2 receptor expression and the reduction of the levels of SP, TNF-\textalpha{} and IL-6 in the colonic mucosa\cite{19}. The effective substance remains unknown. Several kind of compounds have been isolated from SD, including chromones, coumarins, mannitol, glycoside, and polyacetylenes\cite{20,21}. The major active constituents of SD are considered to be chromones and their glycosides\cite{20,22,23,24}. Prim-O-glucosylcimifugin (POG) [Fig. 1] is the highest content chromone of SD\cite{15}. Therefore, the present study was aimed to evaluate the effect of different doses of POG on intestinal barrier dysfunction and explore its possible molecular mechanisms.

The effect of POG was examined on both a normal intestinal barrier and a dysfunctional intestinal barrier induced by TRYP. The possible molecular mechanisms of the effect of POG on the intestinal barrier were investigated. For this purpose, Caco-2 cell monolayers of both functional and dysfunctional were treated with POG, at doses of 30, 60 and 120 \(\mu\text{g/mL}\). The TEER and PFP were measured at 2, 8, 24, 36, 48, 72h after treatment POG. Additionally, the PAR-2 and MLCK mRNA expression levels in dysfunction Caco-2 cell monolayers were analyzed by RT-PCR, and the level of ZO-1 protein expression was measured by western blot. Simultaneously, the expression and distribution of the Occludin protein were examined using immunofluorescence.
Experimental

**Materials** Dulbecco’s modified Eagle medium (DMEM) and phosphate buffered saline (PBS) were obtained from Gibco. Fetal bovine serum (FBS) and TRIzol Reagent were purchased from Invitrogen. PAR-2 and MCLK PCR primers were synthesized by Sangon Biotech Co., Ltd. ZO-1, Occludin and β-actin antibodies were obtained from Abcam Inc. Tryptase, FSSRY-NH₂ and fluorescein were purchased from Sigma. PrimeScript 1ˢᵗ-Strand cDNA Synthesis Kit and Primer Taq were purchased from TaKaRa. The total protein assay kit was obtained from Nanjing Jiancheng Bioengineering Institute. MEM Non-Essential Amino Acids (NEAA) Solution was purchased from Thermo Scientific. prim-O-glucosylcimifugin (98% pure) were supplied by Sichuan Weikeqi Biological Technology Co, Ltd..

**Cell Line and Culture Conditions** Colonic adenocarcinoma cell line (Caco-2) was obtained from the American Type Culture Collection (HTB037). Caco-2 cells were cultured in DMEM supplemented with 10 % heat-inactivated FBS and 1 % NEAA at 37 °C in an atmosphere with 95 % air and 5 % CO₂ and utilized for experimentation at approximately 70-80% confluence. And the Caco-2 cell monolayers were formed after 20-22 day-cultures, until the TEER value was more than 300 Ω·cm².

**Cell viability** The POG toxicity was determined using an MTT (Sigma) assay. Caco-2 cells were seeded into 96-well plates at a density of 10000/cell, with each well containing 200 μL. After adherence, the cells were treated with various concentrations of POG (10, 50, 100, 250, 500, 1000, 5000 and 10000 µg/mL) or dimethyl sulfoxide (DMSO, Sigma; control cells).
for 24h before 10 µL MTT in medium was added to each well. the cells were incubated at 37°C for 4 hours. Then the absorbance was detected at 490nm using a microtiter plate reader (Bioteck Instruments).

**Culture of Caco-2 Cell Monolayers and Determination of Barrier Function** The Caco-2 cell monolayers were cultured, and the determination of barrier function was performed as previously described. Briefly, Caco-2 cells were transplanted on the apical compartment of collagen-coated polycarbonate membrane Transwell supports (transparent PET membrane: 1.0-cm² growth surface area, 0.4-μm pore size) in 24-well plates at 1*10⁵ cells/well. The apical and basolateral compartment respectively are 100 µL and 600 µL medium and incubated with appropriate culture conditions. The Caco-2 cells monolayers were grown on the Transwell, and the medium was replaced every 48 h. Recordings of TEER values were obtained every 24 h until they stabilized on 20-22 day using an Epithelial Voltohmmeter (World Precision Instruments) in each insert and multiplied by the membrane surface area, corrected by subtracting background resistance of the blank membrane (no cells). A 20-22-day model was used to study barrier function. It has been shown that after day 20-22 the cell monolayers were differentiated. The TEER values of the model Caco-2 cell monolayers was measured at 2, 8, 24, 36, 48 and 72 h after treatment with POG(30, 60 and 120 µg/mL). The same to the measurement of PFP values, 40 µg/mL fluorescein (dioxyfluoran, C₂₀H₁₂O₅) was added to the apical compartment, and the Transwells were incubated for 1 h. Then, 100 µL of medium from the apical and basal compartments was collected into 96-well plates, and the fluorescein was measured using a microplate reader at excitation and emission wavelengths of 485 and 540 nm, respectively. The monolayer
permeability of fluorescein was quantified as the PFP from the apical to the basal compartment.

The dysfunctional Caco-2 cell monolayers from the intestinal barrier were induced by TRYP(Sigma) as previously described\textsuperscript{26}. Briefly, the model Caco-2 cell monolayers were incubated with TRYP (1.3 µmol/L) from the basal side for 72 h. To assess the effect of POG on intestinal barrier function, dysfunctional Caco-2 cell monolayers were incubated with POG (30, 60 and 120 µg/mL). The TEER and the PFP of the cell monolayers were determined as previously described.

**RT-PCR Analysis of PAR-2 and MLCK mRNA Expression** The total RNA from the Caco-2 cells was extracted using the TRIzol reagent dissolved in DEPC-treated water according to the manufacturer’s instructions. RNA was reverse-transcribed to cDNA using oligo (dT) primers by PrimeScript 1st-Strand cDNA Synthesis Kit in a final volume of 20 µL under the conditions recommended by the supplier (TaKaRa). For polymerase chain reaction (PCR) amplification of PAR-2 and β-actin, 1 µL of the cDNA template and the following specific primers were used: PAR-2 F, 5'-CCC TTT GTA TGT CGT GAA GCA GAC-3'; R, 5'-TTC CTG GAG TGT TTC TTT GAG GTG-3' (452 bp); β-actin F, 5'-TTG TAA CCA ACT GGG ACG ATA TGG-3'; R, 5'-GAT CTT GAT CTT CAT GGT GCT AGG-3' (754 bp). The reaction cycle conditions were the following: 5 min at 95 °C and 35 amplification cycles consisting of denaturation at 95 °C for 40 s, annealing at 60 °C for 40 s, and extension at 72 °C for 60 s. And for MLCK and β-actin, 1 µL of cDNA template and the following specific primers were used: MLCK F, 5'-TCT GAA CTC ACA ACG GTA GG-3'; R, 5'-GCT CAA AGT CCT CGT CAA-3' (376 bp); β-actin F, 5'-TCA CCC ACA CTG TGC CCA TCT
ACG A-3'; R, 5'-CAG CGG AAC CGC TCA TTG CCA ATG G 3' (300 bp). The reaction cycle conditions: 5 min at 94 °C and 30 amplification cycles consisting of denaturation at 94 °C for 30 s, annealing at 54 °C for 45 s, and extension at 72 °C for 60 s.

The PCR products were resolved via 2% agarose gel electrophoresis and visualized through ethidium bromide staining. The PCR products of predicted size were stained using ethidium bromide and visualized by an ultraviolet transilluminator. Quantification of each band was performed using a Scion Image densitometry analysis software package.

**Western Blot Analysis of Expression of ZO-1** Briefly, the cells were washed with pre-cooled PBS and subsequently lysed with RIPA lysis buffer. The protein concentration was determined through the bicinchoninic acid (BCA) assay reagent with the Bradford method. For western blotting, protein samples (20 µg) of Caco-2 cells were separated via 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The samples were then transferred onto PVDF membrane and incubated with primary antibody (anti-ZO-1 with dilution: 1:500 or anti-β-actin with dilution: 1:1200) overnight at 4°C and then with the secondary antibody (horseradish peroxidase-conjugated anti-rabbit IgG) for 2 h. The intensity of the immunoblot signal was determined by the ECL Western blotting detection reagent according to the manufacturer’s instructions and the expression of ZO-1 was analyzed quantitatively using the GeneTools software from Syngene.

**Immunofluorescence analysis of the distribution of occludin proteins** The distribution occludin protein was analyzed with immunofluorescence microscopy. Cells (3 × 10^6 cells/well) in 6-well plates were fixed with 4%paraformaldehyde at room temperature for
30 min after POG treatment for 36 h. Then cells were permeabilized with PBS containing 0.1% Triton and then blocked with blocking buffer containing 0.3% bovine serum albumin (BSA) for 30 min at room temperature. Cells were incubated with Occludin antibody (1:1000) overnight at 4 degree and then washed with PBS three times for 15 minutes and incubated with FITC marker Goat anti rabbit IgG (1:100) at room temperature for another 1 h. Observe and record the result as soon as possible with fluorescence microscopy at the condition of excitation wavelength 492nm and emission wavelength 520nm.

**Statistical Analysis** All experimental data has been presented as the mean ± standard deviation (SD), and the analyses of the significance of the differences were performed via analysis of variance (one-way ANOVA) followed by Student-Newman-Keuls test (S-N-K) using SPSS 17.0. Differences were considered significant if P < 0.05.

**Results**

The POG toxicity was illustrated in Fig. 2. The viability of Caco-2 cell incubated with POG (250, 500, 1000, 5000 and 10000 µg/mL) for 24 h was decreased compared with the control cells (P<0.05). In the range of POG concentration 10~250 µg/mL, the viability of Caco-2 cell was above 80%.

The TEER value of the normal Caco-2 cell monolayers that were incubated with POG (30, 60 and 120 µg/mL) for 8, 24, 36, and 48 h was significantly elevated compared with the control cells (P<0.05), contrariety to the PFP (Fig. 3A-3B). It can be seen in Fig. 4A-4B that
the TEER and PFP value of the dysfunctional Caco-2 cell monolayers induced by TRYP was obviously decreased and increased respectively compared with the control cells (P<0.05). And POG (30, 60 and 120 µg/mL) for 2, 8, 24, 36, and 48 h significantly increased the TEER and decreased PFP compared with the dysfunctional cell monolayers without treatment with POG (P<0.05). Our data showed that the addition of varying doses of POG produced a dose-dependent increase in the TEER and a decrease in the PFP on both functional and dysfunctional cell monolayers, suggesting that POG can promote the barrier function of Caco-2 cell monolayers in a dose-dependent manner.

As shown in Fig. 5, the PAR-2 mRNA expression of the dysfunctional Caco-2 cell monolayers induced by TRYP was much more than the control cells (P<0.05). POG (30, 60 and 120 µg/mL) reduced the expression of PAR-2 mRNA compared with the dysfunctional cell monolayers (P<0.05). FSSRY-NH₂, which is the PAR-2 antagonist and was used as a positive control drug, distinctly affected the inhibition of the expression of PAR-2 mRNA compared with the dysfunctional cell monolayers (P<0.05).

The MLCK mRNA expression of the dysfunctional Caco-2 cell monolayers induced by TRYP was increased compared with the control cells (P<0.05) in Fig. 6. After treatment with POG (30, 60 and 120 µg/mL), the MLCK mRNA expression was decreased compared with the dysfunctional cell monolayers (P<0.05).

The ZO-1 protein expression in the dysfunctional Caco-2 cell monolayers induced by TRYP was decreased compared with the control cells (P<0.05). After treatment with POG (30, 60 and 120 µg/mL), the ZO-1 protein expression was significantly elevated compared with the dysfunctional cell monolayers (P<0.05). FSSRY-NH₂, applied as a positive control drug,
significantly increased the expression of ZO-1 protein compared with the dysfunctional cell monolayers (P<0.05). (Fig. 7).

In Fig. 8. There was no significant difference in fluorescence intensity between each group. However, in the dysfunctional Caco-2 cell monolayers induced by TRYP, the occludin protein was loose, apparently serrated, or notched and fissured(Fig. 8B). Apparently, in POG(30, 60 and 120µg/mL) treatment groups, the green fluorescence distribution showed obvious integrity and clear boundary. And the higher the dose, the more obvious the effect.

Discussion

This study demonstrated that POG can promote intestinal barrier function and ameliorate intestinal barrier dysfunction in Caco-2 cells. These effects are possibly mediated through down-regulating the expression of PAR-2 and MLCK mRNA and enhance the level of ZO-1 protein expression, ameliorate the distribution of Occludin protein.

The intestinal barrier is a crucial component of gut homeostasis that defends against many pathogens, such as bacteria, toxins and food antigens. Caco-2 cells, which exhibit a well-differentiated brush border, tight junctions and intestinal proteins, have been widely used to investigate the drug’ effect on paracellular permeability and as an intestinal barrier model in vitro. In the present study, our results demonstrated that POG can increase the TEER and reduce the PFP in Caco-2 cells, which shows that POG promoted epithelial barrier function in a dose-dependent manner (Fig. 3). In agreement with our present study, Elamin et
al. used Caco-2 cells as an intestinal barrier model and found that short-chain fatty acids (SCFAs) can reinforce the barrier function in Caco-2 cells by measuring the TEER and paracellular permeability\(^2\). Clinical data suggest that a high level of TRYP released by mast cells upon degranulation in D-IBS, is associated with alteration in intestinal barrier function\(^3\). TRYP increases the permeability of colon tissue in diarrhea-predominant IBS patients\(^4\). A study conducted by Ludidi et al. revealed that TRYP can increase the permeation flux of 4-kDa fluorescein isothiocyanate-labelled dextran (FD4) in Caco-2 cells, indicating an effect of enhancing paracellular permeability and intestinal barrier dysfunction\(^5\). In our study, we induced Caco-2 cell monolayers with TRYP, and this treatment reduced the TEER and increased the PFP. After treatment with POG, the TEER obviously increased, and the PFP decreased compared with the values obtained in the TRYP-induced intestinal barrier dysfunction Caco-2 cells (Fig. 4). The current evidence of the effect of POG on the intestinal barrier is limited. Two similar studies conducted showed that the active ingredients of SD affect the intestinal permeability and decrease the level of NO and DPPH free radicals\(^6\).

The intestinal barrier dysfunction in IBS is associated with the disturbance of gut sensory and motor functions. PAR-2 activation is responsible for intestinal barrier dysfunction and the sensitization of sensory neurons in patients with IBS\(^7\). PAR-2 belongs to the PARs family and is distributed throughout the gastrointestinal tract, localized in epithelial cells, myocytes and enteric neurons\(^8\). The activation of PAR-2 on the intestinal epithelial barrier directly affects cytoskeletal contraction by triggering relevant paracellular pathways with subsequent changes in tight junction permeability. PAR-2 is activated by
serine-proteases, such as trypsin and tryptase, which in turn brings great impact on epithelial migration, integrity, and barrier function\textsuperscript{36}. A study conducted by Piche et al. demonstrated that Caco-2 cells exposed to tryptase in the plasma of IBS-D patients elevates the expression of PAR-2 mRNA, leading to an increase in paracellular permeability and a reduction in TEER\textsuperscript{37}. In the present study, the results showed that the level of PAR-2 mRNA significantly elevated in dysfunctional Caco-2 cells and POG would down-regulated the expression of PAR-2 mRNA (Fig. 5).

The activation of myosin light chain kinase (MLCK) is vital to the enhancement of paracellular permeability and is essential for tight junction barrier regulation in response to Na+-nutrient cotransport, inflammatory cytokines, or pathogenic bacteria\textsuperscript{38, 39}. MLCK can change the junctional actomyosin ring structure and tight junction morphology\textsuperscript{40}. The integrity of tight junctions depends on many tight junction proteins, which make up the normal intestinal architecture. ZO-1 and occludin proteins are essential for cytoskeletonally-mediated barrier regulation\textsuperscript{41}. Disruption and redistribution of the ZO-1 and occludin proteins could lead to tight junction barrier dysfunction. These changes in ZO-1 and occludin proteins are the result of the activation of MLCK\textsuperscript{10}. In the dysfunctional Caco-2 cells, our data suggested that MLCK mRNA expression was significantly increased and ZO-1 protein expression was remarkably reduced. Although the expression level of occludin did not decrease obviously, the distribution of occludin protein changed remarkable. After treatment with POG, the expression of MLCK mRNA were down-regulated and the level of ZO-1 were enhanced. Beyond that, the POG would ameliorate the distribution of Occludin proteins.(Fig. 6-Fig. 8).
In conclusion, our research shows that POG has potential therapeutic efficacy in the treatment of IBS. Down-regulation of PAR-2 and MLCK expression, up-regulation of ZO-1 protein expression and ameliorated the distribution of Occludin proteins may be the possible molecular mechanism. Therefore, the beneficial effects of POG on intestinal barrier dysfunction suggest that POG may be a promising therapeutic drug for some diseases associated with intestinal barrier dysfunction, such as IBS.

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Conflicts of Interest
The authors declare no conflict of interest.
References


Legends for Figures

Fig. 1 The structural formula of the prim-O-glucosylcimifugin

Fig. 2 The viability of Caco-2 cell incubated with POG (10, 50, 100, 250, 500, 1000, 5000 and 10000 µg/mL) for 24 h (n=6). Control: 0 µg/mL. * P<0.05 compared with the control cells; Δ P<0.01 compared to the control cells.

Fig. 3 Effects of POG on barrier function in normal Caco-2 cells (n=9). (A) Effect of POG on transepithelial electrical resistance (TEER) in Caco-2 cell monolayers. Control: 0 µg/mL POG; POG-30 µg/mL: 30 µg/mL POG; POG-60 µg/mL: 60 µg/mL POG; POG-120 µg/mL: 120 µg/mL POG. * P<0.05 compared with the control cells; Δ P<0.01 compared to the control cells. (B) Effect of POG on the percentage of fluorescein permeation (PFP) in Caco-2 cell monolayers. Control: 0 µg/mL POG; POG-30 µg/mL: 30 µg/mL POG; POG-60 µg/mL: 60 µg/mL POG; POG-120 µg/mL: 120 µg/mL POG. * P<0.05 compared with the control cells.

Fig. 4 Effect of POG on the dysfunctional Caco-2 cell monolayer induced by TRYP (n=9). (A) Effect of POG on the transepithelial electrical resistance (TEER) in Caco-2 cell monolayers. Control: 0 µg/mL POG and 0 µmol/L tryptase; Tryptase: 0 µg/mL POG and 1.3 µmol/L tryptase; POG-30 µg/mL: 30 µg/mL POG and 1.3 µmol/L tryptase; POG-60 µg/mL: 60 µg/mL POG and 1.3 µmol/L tryptase; POG-120 µg/mL: 120 µg/mL POG and 1.3 µmol/L tryptase. □ P<0.01 compared with the control cells; * P<0.05 compared with the cells induced with tryptase; Δ P<0.01 compared with the cells induced with Tryptase. (B) Effect of POG on the percentage of fluorescein permeation (PFP) in Caco-2 cell monolayers. Control: 0 µg/mL
POG and 0 µmol/L tryptase; Tryptase: 0 µg/mL POG and 1.3 µmol/L tryptase; POG-30 µg/mL: 30 µg/mL POG and 1.3 µmol/L tryptase; POG-60 µg/mL: 60 µg/mL POG and 1.3 µmol/L tryptase; POG-120 µg/mL: 120 µg/mL POG and 1.3 µmol/L tryptase. □ P<0.01 compared with the control cells; * P<0.05 compared with the cells induced with tryptase; \(^\) P<0.01 compared with the cells induced with tryptase.

Fig. 5 Effect of POG on PAR-2 mRNA expression in the dysfunctional Caco-2 cell monolayer induced by TRYP. The Caco-2 cells were incubated with POG (30, 60 and 120 µg/mL) for 36h. The expression of PAR-2 mRNA was detected via RT-PCR as described in the text. The figure represents one of four experiments with similar results. Control: control group; Tryptase: 1.3 µmol/L tryptase; POG-30: 30 µg/mL POG and 1.3 µmol/L tryptase; POG-60: 60 µg/mL POG and 1.3 µmol/L tryptase; POG-120: 120 µg/mL POG and 1.3 µmol/L tryptase; FSSRY-NH\(_2\): 30 µmol/L FSSRY-NH\(_2\) and 1.3 µmol/L tryptase. # P<0.05 compared with the control cells; * P<0.05 compared with the cells induced with tryptase.

Fig. 6 Effect of POG on MLCK mRNA expression in the dysfunctional Caco-2 cell monolayer induced by TRYP. The Caco-2 cells were incubated with POG (30, 60 and 120 µg/mL) for 36h. The expression of MLCK mRNA was detected via RT-PCR as described in the text. The figure represents one of four experiments with similar results. Control: control group; Tryptase: 1.3 µmol/L tryptase; POG-30: 30 µg/mL POG and 1.3 µmol/L tryptase; POG-60: 60 µg/mL POG and 1.3 µmol/L tryptase; POG-120: 120 µg/mL POG and 1.3 µmol/L tryptase; FSSRY-NH\(_2\): 30 µmol/L FSSRY-NH\(_2\) and 1.3 µmol/L tryptase. # P<0.05 compared with the control cells; * P<0.05 compared with the cells induced with tryptase.

Fig. 7 Effect of POG on ZO-1 protein expression in the dysfunctional Caco-2 cell monolayer
induced by TRYP. The Caco-2 cells were incubated with POG (30, 60 and 120 µg/mL) for 36h. The protein expression of ZO-1 was detected via western blotting as described in the text. The figure represents one of four experiments with similar results. Control: control group; Tryptase: 1.3 µmol/L tryptase; FSSRY-NH₂: 30 µmol/L FSSRY-NH₂ and 1.3 µmol/L tryptase; POG-120: 120 µg/mL POG and 1.3 µmol/L tryptase; POG-60: 60 µg/mL POG and 1.3 µmol/L tryptase; POG-30: 30 µg/mL POG and 1.3 µmol/L tryptase. # P<0.05 compared with the control cells; * P<0.05 compared with the cells induced with tryptase.

Fig. 8 The distribution of tight junction protein Occludin in the dysfunctional Caco-2 cell monolayer induced by TRYP (100*). The Caco-2 cells were incubated with POG (30, 60 and 120 µg/mL) for 36h. The protein location of Occludin was detected via Immunofluorescence as described in the text. A: control; B: 1.3 µmol/L tryptase; C: 30 µg/mL POG and 1.3 µmol/L tryptase; D: 60 µg/mL POG and 1.3 µmol/L tryptase; E: 120 µg/mL POG and 1.3 µmol/L tryptase; F: 30 µmol/LFSSRY-NH₂ and 1.3 µmol/L tryptase.

(Color figure can be accessed in the online version.)
Fig. 1
Fig. 2

Cell viability (%) vs. concentration (μg/mL) for different treatments.
Fig. 3

Graph A: TEER (Ω cm²) over time (h)
- Control
- POG-30 ug/mL
- POG-60 ug/mL
- POG-120 ug/mL

Graph B: PFP (%) over time (h)
- Control
- POG-30 ug/mL
- POG-60 ug/mL
- POG-120 ug/mL
Fig. 4

Graph A: TEER (Ω-cm²) over time (h) for different treatments:
- Control
- Tryptase
- POG-30 ug/mL
- POG-60 ug/mL
- POG-120 ug/mL

Graph B: PFP (%) over time (h) for different treatments:
- Control
- Tryptase
- POG-30 ug/mL
- POG-60 ug/mL
- POG-120 ug/mL
Fig. 5

![Figure 5](image-url)

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Fig. 6

**MLCK**

**β-actin**

![Image](image_url)

**Graph:**

- **Y-axis:** Relative RNA expression of MLCK
- **X-axis:** Control, Tryptase, POG-30, POG-60, POG-120, FSSRY-NH2

- Bars marked with `*` indicate significant differences.
- Bar marked with `#` indicates a different comparison.

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