GLIADIN PEPTIDES INDUCE INCREASED CACO2 MONOLAYER PERMEABILITY AND TIGHT JUNCTION PROTEIN ZO-1 REDISTRIBUTION

Ramzi El Asmar, Lucia Zampini, Tiziana Galeazzi, University of Ancona, Manjusha Takar, Sandro Drago, Center for Celiac Research University of Maryland, Giovanni Valentino Coppa, University of Ancona, Alessio Fasano, Center for Celiac Research University of Maryland, Carlo Catassi, Center for Celiac Research University of Maryland and University of Ancona

Background: Celiac disease (CD) is an immuno-mediated enteropathy triggered by the ingestion of gliadin and other gluten proteins. While the cascade of immunological reactions occurring in the lamina propria has been partially elucidated, the interaction between gliadin and the epithelial barrier is still unknown.

Aim: to investigate the changes induced by gliadin peptides on Caco2 monolayers permeability.

Material and methods: Human intestinal cells (Caco2) were grown on polarized filters. Gliadin or casein pepsyn-trypsin digests were added to the luminal culture medium for a 3-hour incubation. The intestinal barrier function was assessed by evaluation of: (1) trans-epithelial electrical resistance (TEER), (2) release of zonulin, a modulator of intercellular tight junctions (tj), measured by sandwich-ELISA, (3) luminal to serosal lactulose transport measured by high performance anion exchange chromatography and, (4) localization/migration of zonula occludens protein (ZO-1), by direct immunofluorescence.

Results: Caco2 monolayers exposed to gliadin showed a significant decrease in TEER (Δ changes, mean ± SE: -197.0 ± 10.9 Ω.cm2; n = 18) after 3 hours exposure compared to unexposed controls (ΔTEER: -16.3 ± 1.63, n = 10). Conversely, monolayers exposed to casein did not show any significant TEER changes (-37.6 ± 4.7, n = 8). The gliadin-mediated TEER decrease was mirrored by zonulin release into the apical (1.45 ± 0.24 ng/mg protein, n=6; controls 0.102 ± 0.017, n=6) but not basolateral (0.001 ± 0.002 ng/mg protein) monolayers media. After 3-hour incubation with the gliadin, serosal lactulose was 0.32 ± 0.028 mg/ml (n=5) compared to unexposed controls (0.029 ± 0.002 mg/ml; n=5) and to casein-exposed monolayers (0.023 ± 0.002 mg/ml, n=5). Unexposed and casein-exposed Caco2 monolayers showed the typical immunofluorescence pattern of ZO-1 localization at the cell periphery, after both 1- and 3-hour incubation. Conversely, monolayers exposed to gliadin showed a redistribution of ZO-1 protein after 1 and 3 hours, with a decreased staining at the edge of the cells.

Conclusions: in a model of human differentiated enterocyte monolayers, gliadin PT-digest induced a polarized (luminal) zonulin release, an increased monolayer permeability as established by both TEER changes and increased paracellular lactulose transport, and ZO-1 re-distribution from the tj complex. This gliadin-specific effects may represent early events in CD pathogenesis in genetically susceptible individuals.