ANTIDIARRHOEAL AGENTS AND PARACELLULAR PERMEABILITY OF E COLI-INFECTED CACO-GOBLET INTESTINAL MODEL

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Background:
Diarrhoea continues to be an important cause of morbidity and mortality worldwide, in spite of the advances in health technology, improved management, and increased use of oral rehydration solutions in recent decades, persisting as a major cause of death in children under five years of age. It has been demonstrated that gelatine tannate protects intestinal cells from damage induced by *Escherichia coli* whilst also preventing *E coli* adhesion in Caco-Goblet® intestinal epithelium model. The present study aims to evaluate this dual-inhibitory effect upon *E coli* for a new product combining gelatine tannate with a mixture of probiotics (TSC Duo) as compared to other antidiarrhoeal agents already available in the market.

Aims:
We assessed the efficacy of TSC Duo (5 mg/mL) in counteracting the increase in paracellular permeability induced by *E coli* to diosmectite (5 mg/mL), a probiotic mixture (12.5 mg/mL), and *Saccharomyces boulardii* (12.5 mg/mL) using an *in vitro* intestinal epithelial model (Caco-Goblet) pre-infected with *E coli*.

Study design and methods:
The Caco-2 monolayer is a relevant, well-established model that recreates *in vitro* the intestinal mucosa deprived of mucous cells; the modified model Caco-Goblet, including mucus-secreting goblet cells (HT 29-MTX), represents a more predictive model to study paracellular permeability and product interaction with mucus. After pre-inoculation with *E coli* at the concentration of 1e+08 CFU/mL for 30 minutes, we tested the effect of the above antidiarrhoeal agents applied for either 1h or 24h onto to the Caco-Goblet cells. Cell permeability was measured as percent change in transepithelial electrical resistance (TEER) as well as passage of Lucifer Yellow (LY).

Results:
TSC Duo was able to counteract the negative effect of E coli on TEER (reduction) by as much as 123.08% and 149.54% of recovery after 1h or 24h of treatment, respectively; the probiotic mixture was effective at inducing a TEER recovery of 107.17% at 1h but only 17.92% at 24h. These values were significantly lower for diosmectite and *S boulardii* (1h: 68.81% and 16.05%, respectively; 24h: -4.50% and 11.42%, respectively). Similarly, the passage of LY was significantly reduced following treatment with TSC duo for 1h and 24 h (0.41±0.00 and 1.34±0.34, respectively) as compared to diosmectite (1.90±0.32 and 2.30±0.14, respectively), probiotic mixture alone (0.54±0.02 and 6.44±0.42, respectively) and *S boulardii* (1.70±0.04 and 4.16±1.20, respectively).

Conclusions
The most significant recovery of barrier integrity and fence properties as shown by TEER and LY results, respectively, for gelatine tannate combined with probiotics could be explained by its positive interaction with the mucous proteins in the epithelial surface. These findings obtained on a biologically relevant and well-established *in vitro* model are of utmost importance, contributing to the increasing body of evidence that gelatine tannate, either alone or combined with probiotics, protects intestinal cells from *E coli* infection by inhibiting the adhesion and internalisation of bacteria, preventing the increase of tight junction permeability and modulating cytokine gene expression.