P18 - Intestinal Permeability Studies using a more realistic barrier: performance of co-cultures of Caco-2/HT29-MTX cells

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The intestinal barrier, essential for overall health, can have its permeability affected by certain food compounds and additives. Among various models, *in vitro* cellular monolayers are the most commonly used to study this process. Among these, Caco-2 cells—representing enterocytes—are commonly used, though they lack complexity to mimic some properties of the intestinal barrier. This limitation can be overwhelmed by co-culturing it with HT29-MTX cells, which allows the secretion of mucus and mimics goblet cell functioning.

This study aimed to evaluate the intestinal permeability by assessing the paracellular and transcellular transport of lucifer yellow (LY) and propranolol (PR), two intestinal permeability markers, respectively, using a 9:1 co-culture of Caco-2/HT29-MTX cells.

Cells were cultivated separately in complete medium. Functional monolayer formation was monitored over 28 days using transepithelial electrical resistance (TEER) measurements in triplicate plates, with values ranging from 450.6 to 1287.3 Ω·cm². Transport assays were conducted on day 21 by applying LY and PR to the apical compartment and measuring their passage to the basolateral side. Apparent permeability coefficients (Papp) and basal recovery values were estimated by fluorescence quantification.

Statistical analyses were conducted to evaluate variability in TEER, Papp, and basal recovery results. The Shapiro-Wilk test was used to assess normality, and comparisons used one-way ANOVA followed by Tukey's test or the Kruskal-Wallis test followed by Dunn's test when requisites of ANOVA were not met. Significant differences in TEER values were observed between days 8–20 ($p = 5.8e-11$), 22–28 ($p = 2.3e-09$), and before vs. after transport on day 21 ($p < 2.2e-16$). For day 21 transport assays, a significant difference was found in LY basal recovery for two out of three plates ($p = 0.03$). As for the PR Papp values, there is a significant difference between duplicate plates ($p = 0.005$), as one plate was excluded due to a poor calibration curve fit.

These findings will inform improvements to the protocol for assessing intestinal permeability using co-culture models. Thus, Caco-2/HT29-MTX co-culture appears to be a promising model for evaluating the impact of food components and additives on the intestinal barrier.