CacoGoblet, a ready-to-use intestinal cell-based model to screen compounds' anti-inflammatory capabilities

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INTRODUCTION

Identification of active molecules with gastrointestinal anti-inflammatory properties mainly relies on time-consuming methods that quantify the effect of these compounds on the expression and/or release of cytokines (e.g., IL-8). To speed up this process, a 21-day differentiated co-culture of human colon carcinoma cell lines (Caco-2 and HT-29) grown in 24-insert permeable supports was used to screen compound gastrointestinal (GI) anti-inflammatory properties. The approach is based on the correlation between gut inflammation and the evaluation of two indicators of cell barrier integrity, the Transepithelial Electrical Resistance (TEER) and the Lucifer Yellow Paracellular Permeability (LY P_{app}/Flux).

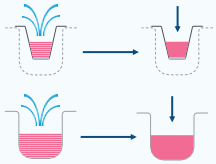
OBJECTIVES

The main objective of this study is to evaluate the effectiveness of measuring two indicators of cell barrier integrity (TEER and LY P_{app}/Flux) in a co-culture of Caco-2 and HT-29 cells to assess compound anti-inflammatory properties.

MATERIALS AND METHODS

LIQUEFACTION AND EXCHANGE OF SHIPPING MEDIUM (SM)

1. Liquefy SM for 4h at 37°C.

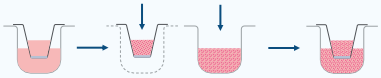


2. Aspirate SM and add fresh culture medium.

3. Allow the cells recover for 72h at 37°C.

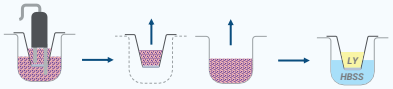
TESTING ANTI-INFLAMMATORY ACTIVITY

4. Remove the culture medium and subject cells to serum deprivation for 24h.



5. Pre-incubate cells for 3h with baicalein, the reference anti-inflammatory compound.

6. Cytokine induction in the absence/presence of baicalein up to 72h.



7. Evaluate TEER, LY P_{app}/Flux, and collect samples to assay IL-8 secretion by ELISA.

RESULTS

EVALUATION OF TEER AND LY P_{app}/Flux

Quantifying **TEER and LY Papp/Flux** in cytokine-induced cells not exposed to baicalein **reduced TEER values by 35% and increased LY P_{app}/Flux by 50%.** Conversely, **baicalein-treated cells normalized cell barrier integrity** in a dose-dependent manner.

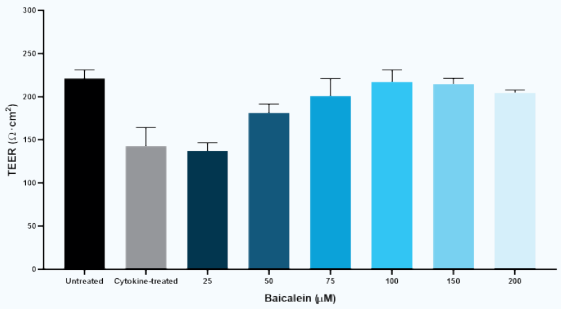


Figure 1. TEER measurements in 72h cytokine-induced Caco-2/HT-29 cells incubated in the absence/presence of increasing concentrations of baicalein, a reference anti-inflammatory compound.

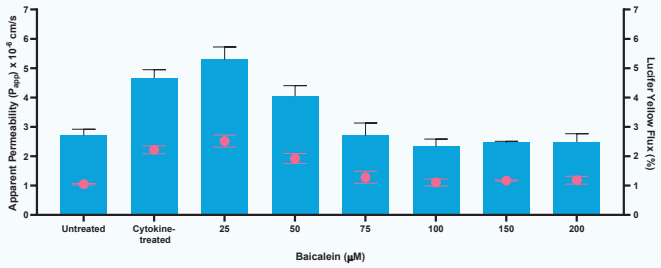


Figure 2. LY P_{app}/Flux in 72h cytokine-induced Caco-2/HT-29 cells incubated in the absence/presence of increasing concentrations of baicalein, a reference anti-inflammatory compound. Bars indicate LY P_{app} and dots indicate LY Flux values.

INTERLEUKIN-8 (IL-8) SECRETION

The disruption and recovery of cell barrier integrity was correlated with the **release of IL-8**. Results indicated that **IL-8 release decreased with increasing concentrations of baicalein, correlated with the evolution of TEER and LY P_{app}/Flux values** in a healthy barrier.

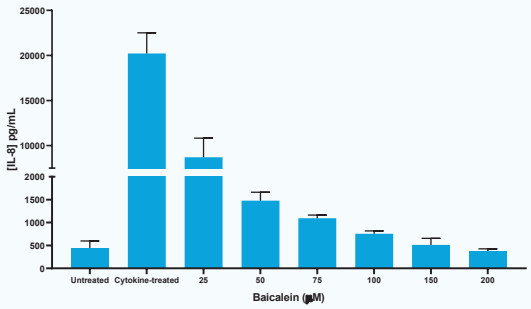


Figure 3. Measurement of IL-8 secretion by enzyme-immunoabsorbent assay (ELISA) after 72h of Caco-2/HT-29 exposure to a cocktail of cytokines in the absence/presence of increasing concentrations of baicalein.

REPRODUCIBILITY BETWEEN ASSAYS

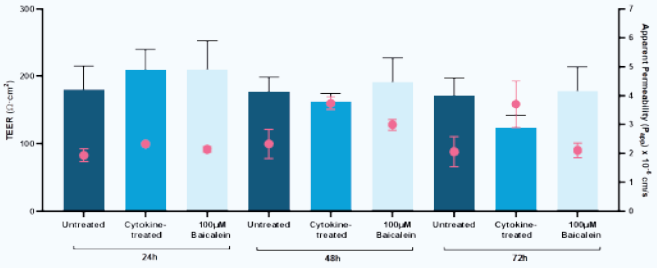


Figure 4. TEER and LY P_{app} values up to 72h incubation of cytokine-induced cells in the absence/presence of 100µM baicalein. Bars indicate TEER and dots indicate LY P_{app} values.

Measurement of two indicators of cell barrier integrity (TEER and LY P_{app}/Flux) in a co-culture of Caco-2 and HT-29 cells can be used to replace IL-8 secretion for screening compounds with anti-inflammatory activity at the early stages of drug development.

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