

Assess acute toxicity in the gastrointestinal tract with CacoReady, an *in vitro* cell-based model

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INTRODUCTION

Compound intestinal toxicity is conventionally performed in non-differentiated human adenocarcinoma-derived cells (Caco-2) plated in 96-well plates. However, under these growing conditions, only late endpoints such as cell membrane disruption can be used to assess cellular viability. **CacoReady, a ready-to-use Caco-2 cell-based model, emerges as an alternative to standard toxicity assays.** These 21-day differentiated Caco-2 cells grown on permeable supports that separate an apical and a basal compartment allow evaluating two early-stage indicators of cell barrier integrity (transepithelial electrical resistance (TEER)) and Lucifer Yellow (LY) paracellular flux (PF) which may have a better predictive value than conventional tests.

OBJECTIVES

The main objective of this study is to compare the predictive value of early- and late-stage indicators to assess potential compound toxicity in the gastrointestinal tract.

MATERIALS AND METHODS

Quinidine, verapamil, and gemfibrozil, known to cause diarrhea as a side effect with a known grade of toxicity incidence, and nadolol, its non-toxic counterpart, were used as reference compounds. Stock solutions were prepared in DMSO and subsequently diluted in HBSS for further use. Caco-2 cells either seeded in 96-well plates or on transwell inserts (CacoReady) were exposed to increasing concentrations of the compounds for a 24 h period in the cell incubator. At the end of this period, Alamar Blue, a resazurin-based solution, was employed to measure membrane disruption in Caco-2 cells seeded in the 96-well plate. TEER and LY paracellular permeability were the parameters evaluated in CacoReady as indicators of cell barrier integrity.

CacoReady and its two early-stage indicators (TEER and LY PF) of cell barrier integrity assess compound intestinal toxicity more accurately than the conventional Caco-2 cell toxicity assay. Furthermore, CacoReady enables compound stratification as low, moderate and highly toxic.

RESULTS

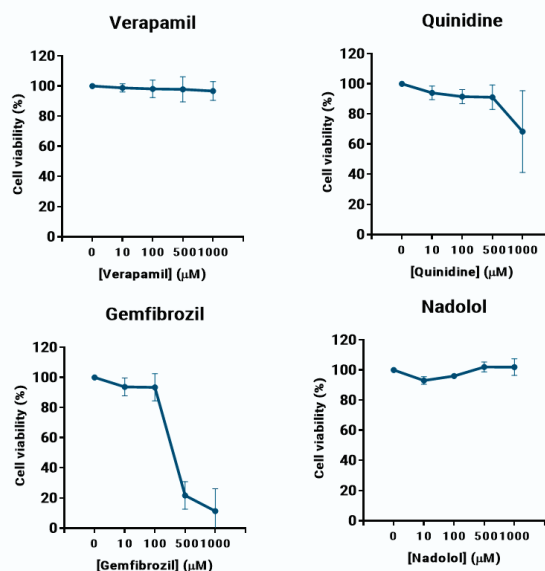


Figure 1. Predictive value of late-stage indicators to evaluate compounds toxicity in the gastrointestinal tract. Caco-2 cells seeded in 96-wells microplates were exposed to increasing concentrations of Quinidine (Panel A), Verapamil (Panel B), Gemfibrozil (Panel C) and Nadolol (Panel D). At the end of a 24 h incubation period, the number of viable cells was assessed by the AlamarBlue cell viability assay.

Results (mean \pm SD) are expressed as the percentage of viable cells over control (untreated cells). Data is representative of three independent experiments.

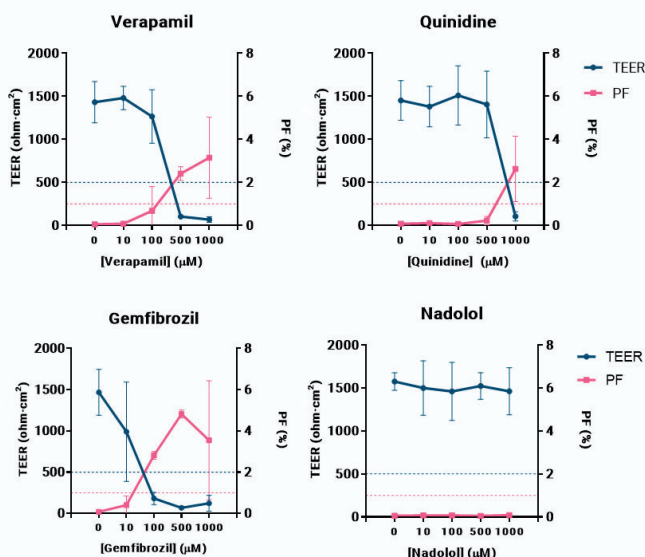


Figure 2. Predictive value of early-stage indicators to evaluate compounds toxicity in the gastrointestinal tract. CacoReady 96-well plates format were exposed to increasing concentrations of Quinidine (Panel A), Verapamil (Panel B), Gemfibrozil (Panel C) and Nadolol (Panel D). At the end of a 24 h incubation period, barrier disruption was assessed by measuring TEERs and the LY paracellular flux (PF).

TEER values and PF (mean \pm SD) are expressed in ohms·cm² and as a percentage of LY fluorescence at C0, respectively. Blue and pink striped lines indicate reference values for TEER (500 ohms·cm²) and LY PF (0.7%), respectively. Data is representative of three independent experiments.



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REFERENCES:
Peters et al, *Toxicol Sci*, 168:3-17, 2019.
J.J. Xu et al, *Chemico-Biological Interactions* 150:115-128, 2004.
R. Konsoula, F.A. Barile, *Toxicology in Vitro* 19:675-684, 2005.