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

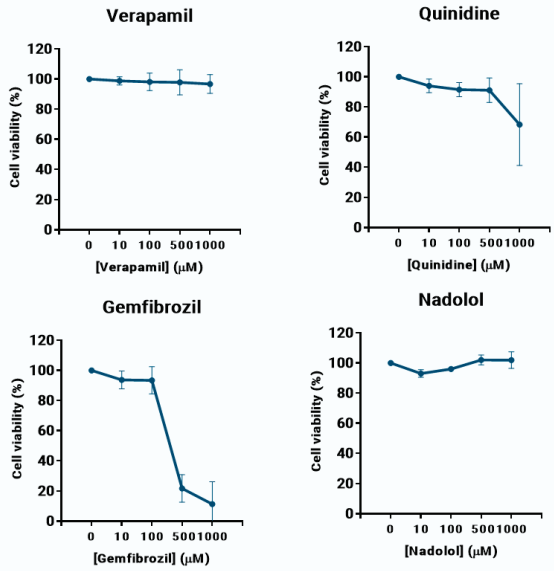

Figure 1. Predictive value of late-stage indicators to evaluate compounds toxicity in the gastrointestinal tract. *Caco-2 cells seeded in 96-wells microplates were exposed to increasing concentrations of Quinidine (Panel A), Verapamil (Panel B), Gemfibrozil (Panel C) and Nadolol (Panel D). At the end of a 24 h incubation period, the number of viable cells was assessed by the AlamarBlue cell viability assay.*
Results (mean ± SD) are expressed as the percentage of viable cells over control (untreated cells). Data is representative of three independent experiments.

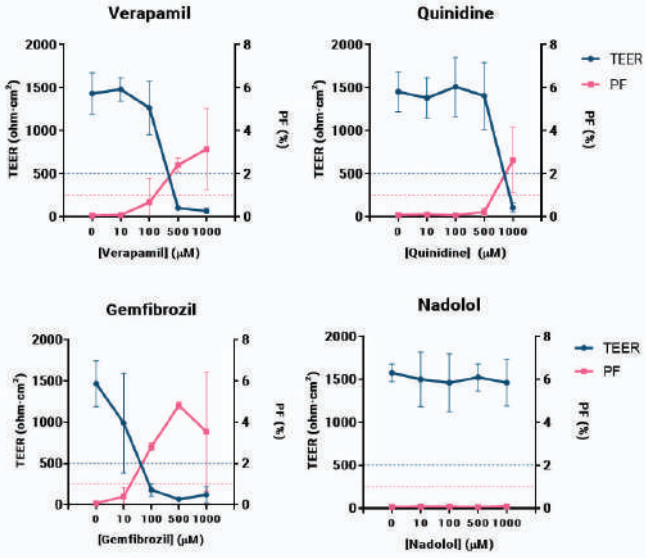

Figure 2. Predictive value of early-stage indicators to evaluate compounds toxicity in the gastrointestinal tract. *CacoReady 96-well plates format were exposed to increasing concentrations of Quinidine (Panel A), Verapamil (Panel B), Gemfibrozil (Panel C) and Nadolol (Panel D). At the end of a 24 h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