An Exploratory Study of Two Caco-2 Cell Models for Oral Absorption: A Report on Their Within-laboratory and Between-laboratory Variability, and Their Predictive Capacity

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Summary — In 2005, the European Centre for the Validation of Alternative Methods (ECVAM) sponsored a study aimed at evaluating the reproducibility (between-laboratory and within-laboratory variability) and the predictive capacity of two in vitro cellular systems — the Caco-2/ATCC parental cell line and the Caco-2/TC7 clone — for estimating the oral fraction absorbed (Fa) in humans. Two laboratories, both of which had experience with Caco-2 cultures, participated in the study. Ten test chemicals with documented in vivo oral absorption data were selected. Atenolol, cimetidine and propranolol were included as reference compounds for low, medium and high intestinal absorption, respectively. Transport experiments were independently carried out in the two laboratories, according to an agreed protocol. The apparent permeability coefficient ($P_{\text{app}}$) was calculated in either the apical to basolateral (absorption) or the basolateral to apical (efflux) direction. To investigate the involvement of possible active transport processes, experiments were also performed in the presence of sodium azide plus 2-deoxy-D-glucose in the donor compartment. Before performing the permeability experiments, the highest concentration that did not impair barrier integrity was identified for each test chemical in both cell models, by applying the chemicals together with a marker of the paracellular pathway. In addition, barrier integrity was assessed by measuring the trans-epithelial electrical resistance. All the permeability data obtained were independently analysed. Reproducibility was assessed for the seven substances for which sufficient data were available. Within-laboratory variability was based on coefficient of variation (CV) values. Median CV values of 10.4% and 14.7% were found for the two laboratories. Concerning between-laboratory reproducibility, comparable response levels were obtained for the three reference compounds and for paracetamol, while, for the other chemicals, the results were less reproducible — in particular, for compounds known to be actively transported. The $P_{\text{app}}$ values obtained for both cell lines were comparable for identical experimental conditions. Despite the limited number of substances tested, the predictive capacity was investigated by using two mathematical models available in the literature. Good estimations of the human Fa were obtained for five well-absorbed compounds, while moderately and poorly absorbed compounds were overestimated. It is proposed that a confirmatory study addressing the main results, including power considerations, would now be useful.

Key words: Caco-2, Caco-2/TC7, intestinal absorption, prediction model, prevalidation.

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Introduction

Intestinal absorption plays a key role in the evaluation of the toxicity of xenobiotics, mainly in oral toxicity studies, but also in drug permeability studies, since oral delivery is one of the most common routes of drug administration (1). Intestinal absorption is influenced by several factors, of which the physicochemical properties of the substance (e.g. solubility, lipophilicity) and the complex biological processes involved (e.g. metabolism, active transport) are the most important. From this point of view, the Caco-2 cell line, which retains some of the functional and morphological properties of the human intestine, is a very popular in vitro model for intestinal absorption, and is used as a screening tool in drug discovery programmes, for the prediction of intestinal permeability (2, 3). Permeability values estimated by using this model correlate well with human permeability values, allowing good correlations with the fraction absorbed (Fa) in humans for many drugs (4–6). When cultured for about three weeks on a