

## CACO-2 CELLULAR SYSTEM : AN OVERVIEW

### INTRODUCTION

The gastrointestinal tract remains the most popular and acceptable route of administration for drugs. It offers the great advantage of convenience and many compounds are well absorbed and thereby provide acceptable plasma concentration-time profiles.

Currently there is considerable interest from the pharmaceutical industry in development of cell culture systems that would mimic the intestinal mucosa in order to evaluate strategies for investigating and/or enhancing drug absorption. The intestinal epithelial cells of primary interest, from the standpoint of drug absorption and metabolism, are the villus cells, which are fully differentiated cells. *In vitro* cell culture system consisting of a monolayer of viable, polarized and fully differentiated villus cells, similar to that found in the small intestine, represents a valuable tool in the study of drug and nutrient transport and metabolism.

### IN VITRO METHODS

Various *in vitro* methods are listed in United States FDA guidelines, acceptable to evaluate the permeability of a drug substance, includes monolayer of suitable epithelial cells. One such epithelial cell line that has been widely used as a model system of intestinal permeability is the Caco-2 cell line. Since most drugs are known to absorb via intestines without using cellular pumps, passive permeability models have come into the limelight.

In the 1990s membrane-based drug assays led to the passage of drugs through the intestinal mucosa and an important

Caco-2 assay emerged in pharmaceutical research (Hilgars, A.R., Conradi, R.A., Burton, P.S.; Pharm. Res. 7, 902-910, 1990 and Karlsson, J., Artursson, P; Int. J. Pharmaceutics 71, 55-64, 1991).

### THE PERMEABILITY OF THE MONOLAYERS

In a typical Caco-2 experiment, a monolayer of cells is grown on a filter separating two stacked micro well plates. The permeability of drugs through the cells is determined after the introduction of a drug on one side of the filter. The entire process can be automated, and when used in conjunction with chromatography and/or mass spectroscopy detection, it enables any drug's permeability to be determined.

The Caco-2 cell line, which exhibits a well-differentiated brush border on the apical surface and tight junctions, and which expresses typical small-intestinal microvillus hydrolases and nutrient transporters, has proven to be the better *in vitro* model for the following reasons : (a) to rapidly assess the cellular permeability of potential drug candidates (b) to elucidate pathways of drug transport (e.g., passive versus carrier mediated) (c) to assess formulation strategies designed to enhance membrane permeability (d) to determine the optimal physicochemi-

cal characteristics for passive diffusion of drugs, (e) to assess potential toxic effects of drug candidates or formulation components on this biological barrier.

Since differentiated Caco-2 cells express various cytochrome P450 isoforms and phase II enzymes such as UDP-glucuronosyltransferases, sulfotransferases and glutathione-S-transferases, this model could also allow the study of presystemic drug metabolism.

The Caco-2 cell model has the advantages of simplicity and reproducibility. US FDA recognizes Caco-2 to measure permeability as part of the bioequivalence waiver process.

A drug substance with respect to solubility and permeability can be classified in 4 categories:

- Category-I: high solubility and high permeability
- Category-II: low solubility and high permeability
- Category-III: high solubility and low permeability
- Category-IV: low solubility and low permeability.

### TRANSPORT STUDIES

Caco-2 cultures are considered accep-

table for transport studies if they meet the following criteria:

TEER > 1000 ohm cm<sup>2</sup>.

- Mannitol < 20 nm/s.
- Propranolol > 5x the permeability of mannitol.
- Pgp Expression > 1.5x vinblastine permeability in the presence of verapamil.

For transport studies, Caco-2 cells are seeded on polycarbonate membranes inside Transwell cell culture chambers. The six-well culture plates with culture medium are incubated at 37 °C (5% CO<sub>2</sub>) with medium change every second day. The confluent monolayers are used between the 21th and 25th day after seeding. The integrity of the monolayers is checked at the end of the culture period. Permeation measurements are performed using the support membrane with and without cell monolayer (negative control).

For the assays, 2.6 mL of fresh culture medium is placed under each Transwell cell culture chamber (receiver chamber). 1.5 mL of product or reference is applied directly on the apical side of the monolayer. 1.5 mL of fresh culture medium is applied as control. The monolayers are then incubated at 37 °C (5% CO<sub>2</sub>) for 0.5 - 1 - 2 - 3 - 4 and 5 hours. At each incubation time, the culture medium is withdrawn from the receiver chamber for quantification of the molecule to be tested and immediately replaced by 2.6 mL of fresh culture medium. Each product is tested on 6 separate monolayers.

## STEADY-STATE FLUXES

Steady-state fluxes ( $J_{ss}$ ) are determined from graphs in which the cumulative amount of penetrated substance is plotted against time. Fluxes are calculated from the slope of the steady-state portion of the curves. Drug permeability is calculated using the equation :

$$K_p = (V / A \times C_d) \times (dC_r / dt)_{ss}$$

$K_p$  is the permeability coefficient (cm/s),  $A$  is the surface area of the culture,  $C_d$  and  $C_r$  are the concentrations in the donor and the receiver compartments,  $V$  is the volume of the receiver compartment and  $dC_r/dt$  is the steady state slope of the concentration versus the time profile in the receiver compartment.

In order to compare the *in vitro* absorption of a molecule from e.g. different formulations, the cumulative concentrations are expressed as the function of the square root of time and the slope of the curve is calculated for each replication. The slopes are compared using a nonparametric confidence interval method based on the Wilcoxon Rank Sum/ Mann Whitney Rank test. If the 90% confidence interval for the ratio of the average slope for the unknown over the average slope of the reference falls within the limits of 75% to 133.33%, the tested formulation and the reference are considered as equivalent.

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## CONTACT INFORMATION

### EUROPE

+ 33 1 53 78 18 79  
clinicalresearch@sgs.com

### NORTH AMERICA

+ 1 877 677 2667  
clinicalresearch@sgs.com

[WWW.SGS.COM/CRO](http://WWW.SGS.COM/CRO)

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