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Caco-2 monolayers in experimental and theoretical predictions of drug transport ☆

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ABSTRACT

This review examines the use of Caco-2 monolayers in the prediction of intestinal drug absorption. First, the different routes of drug transport in Caco-2 monolayers are compared with those seen in vivo. Second, the prediction of drug absorption in vivo from transport experiments in cell monolayers is discussed for different classes of drugs. Finally, the use of Caco-2 monolayers as a reference model in physico-chemical and theoretical predictions of drug absorption is discussed. We conclude that Caco-2 monolayers can be used to identify drugs with potential absorption problems, and possibly also to select drugs with optimal passive absorption characteristics from series of pharmacologically active molecules generated in drug discovery programs.

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1. Introduction

During the last few years, the use of intestinal epithelial cell lines such as Caco-2 and HT29 has increased dramatically in many research fields including the pharmaceutical sciences (Fig. 1). The cell lines are now routinely cultivated as monolayers on permeable filters for studies of the transepithelial transport of drugs (for reviews, see [1, 2]; Fig. 2). Most studies of drug transport in cell monolayers have been performed using Caco-2 cells and are of a mechanistic nature. In general, the aim has been to investigate whether a drug is actively or passively transported across the intestinal epithelium and, if the transport is active, to identify the relevant carrier. Using such studies, new and sometimes unexpected drug transport routes have been identified [3–5]. Most studies on active drug transport in Caco-2 monolayers have investigated two transport systems, the dipeptide carrier [6, 7] and P-glycoprotein [8, 9]. Fewer studies have been published on the passive transport of drugs. These studies have shown that cell monolayers can be used to identify drugs with potential absorption problems and possibly also to predict drug absorption in vivo [10–12]. Since drug transport studies in cell monolayers are easy to perform and require only small quantities of drugs, they have been suggested for screening of drug absorption at an early stage in the drug development process (see the review by Bailey in this volume). Recently, Caco-2 monolayers were used to screen permeability of a synthetic peptide library containing 375 000 discrete tripeptides, divided into 150 pools [13]. Automated procedures for screening of drug transport in Caco-2 monolayers using robotics have been reported [14].

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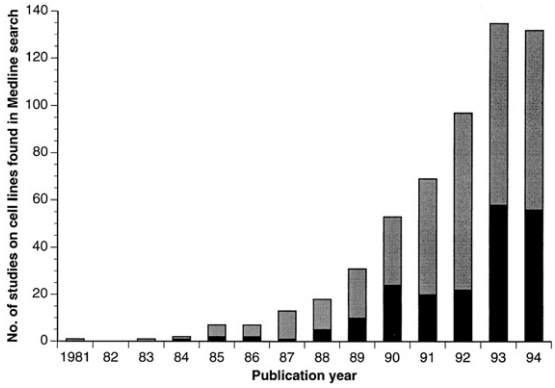

Fig. 1. Increase in the number of papers per year dealing with Caco-2 cells. The dark parts of the staples show papers dealing with absorption, transport and/or permeability. Source: Medline.

In this review, results of studies investigating the use of Caco-2 monolayers in the prediction of intestinal drug absorption are summarised. Data from a recently introduced theoretical model for prediction of passive transcellular drug absorption are also presented. For reasons of simplicity, the review deals exclusively with epithelial permeability and, therefore, other factors that may influence the extent of drug absorption and bioavailability such as solubility; formulation factors (e.g. absorption enhancers) and presystemic and systemic drug metabolism will generally not be considered. The basic characteristics of intestinal epithelial cell lines are discussed in detail in the review by Quaroni and Hochman elsewhere in this volume.

2. Transport of drugs in Caco-2 monolayers and intestinal tissues

The transport of drugs across the intestinal epithelium may occur by one or more of four different routes: the passive transcellular and paracellular routes, the carrier mediated route and by transcytosis (Fig. 2). Caco-2 monolayers have been used to study drug transport by all four routes. In this section, we will first consider how different classes of drugs are transported by these routes in the intestinal epithelium. We will then compare drug transport in Caco-2 monolayers with that in vivo.

2.1. Transport pathways across the intestinal epithelium

Rapidly and completely absorbed drugs are generally lipophilic and distribute readily into the cell membranes of the intestinal epithelium. Since the surface area of the brush border membranes is >1000-fold larger than the paracellular surface area [15], it can be assumed that these drugs are transported exclusively by the passive transcellular route. Most approved drug products which are rapidly and completely absorbed following oral administration are transported by the passive transcellular route (Fig. 2).

Drugs that are slowly and incompletely passively absorbed, such as hydrophilic drugs and peptides, distribute poorly into cell membranes. It is therefore generally assumed that these drugs are transported

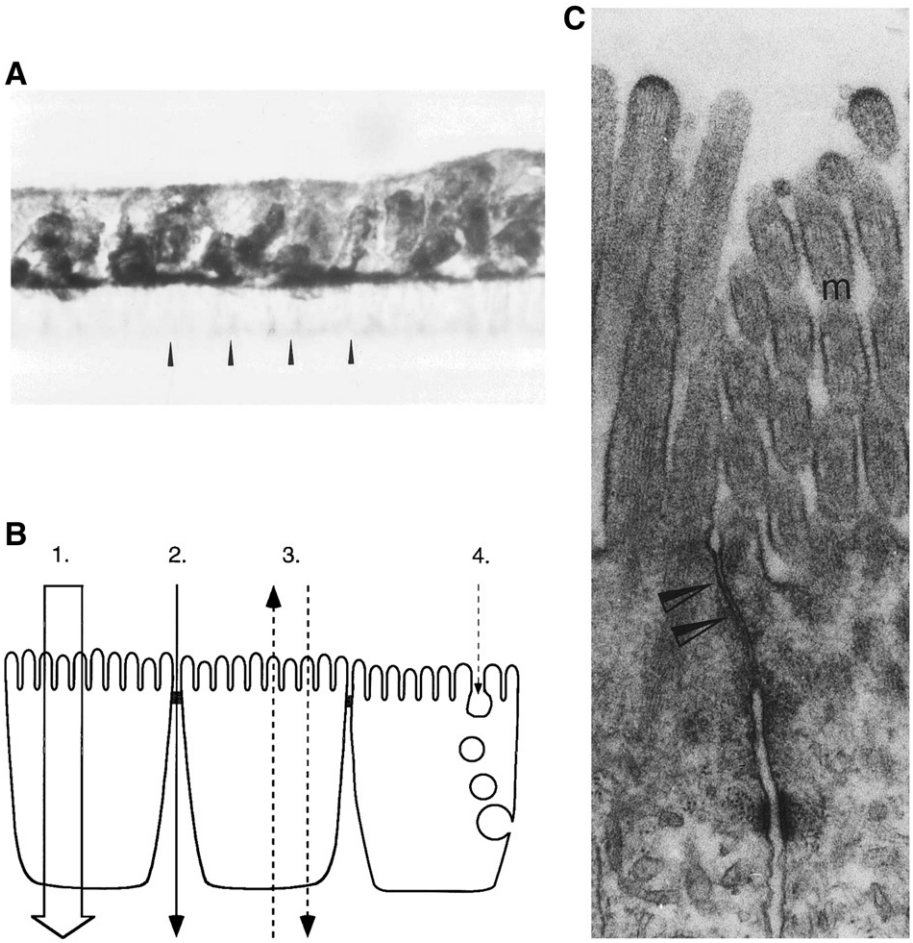

Fig. 2. A. Cryosection (4 μm) of an intestinal epithelial cell monolayer grown on a polycarbonate filter. The cells where stained with hematoxylin/eosin and fixed with formalin before sectioning with a Leica Jung CM3000 cryostat (courtesy by Dr Göran Ocklind). The arrowheads indicate the border of the premeable support. B. Schematic drawing of an intestinal epithelium. The arrows indicate the four different drug transport routes: 1, the passive transcellular; 2, the passive paracellular; 3, the active carrier-mediated transcellular and 4, the transcytosis routes. C. Transmission electron micrograph of the apical part of two Caco-2 cells with microvilli (m) and a tight junction (arrowheads).