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Cell culture techniques for the study of drug transport

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SUMMARY

The growth of differentiated cell monolayers on microporous filters is providing powerful new techniques for investigating the transport of drug and delivery systems across defined cellular barriers, and for discriminating between different routes and mechanisms. The growth, characterization and potential use of these systems is illustrated by studies on the human Caco-2 cell system which provides an in vitro model of the intestinal epithelial barrier. This system, still in the early stages of characterization and development, displays a number of carrier-mediated and vesicular transport systems found in the intestine in vivo, and is thus providing a useful system for studying the intestinal transport of drugs including peptides and proteins.

INTRODUCTION

Recent advances in cell and tissue culture methodologies, particularly in the growth of differentiated human cells, are providing new and powerful tools for cellular and molecular biology studies on the process involved in differentiation and in the intracellular sorting of molecules and membranes. These new techniques are also beginning to be used for investigating the transport of drug and delivery systems into specific cells and across specific biological barriers (1). Of particular relevance and focus is the need to have in vitro systems that can be employed to devise new strategies for the absorption and delivery of new drug classes arising either from rational drug design or through recombinant DNA technology.

New cell culture systems provide the potential for rapidly evaluating the permeability and metabolism of a drug, for defining the mechanisms of transport of drugs and delivery systems, and for testing novel strategies for enhancing drug transport and drug targeting. In addition they provide the opportunity to use human rather than animal tissues with the potential of minimizing time-consuming and expensive animal studies.

Cell culture systems that display many of the morphological and functional properties of in vivo cell layers that act as barriers to the absorption and site-specific delivery of drugs have been established. These include epithelial barriers that form the intestinal, rectal, buccal, sublingual and nasal mucosae, cells that form the epidermis of the skin, and vascular endothelial barriers, e.g. brain capillary endothelial cells. This review will discuss the growth and characterization of cell culture systems and their application to studies on the transport of drugs. A human intestinal cell (Caco-2) system will be used to illustrate some of the major issues as this system is the best characterized of all the in vivo cell culture systems being employed to study drug transport.

GROWTH AND CHARACTERIZATION OF CELL CULTURE SYSTEMS

A major impetus for the use of cell culture systems for studying the transport of drugs and drug delivery systems has been the development of filter-chamber cultures based on the design of mini-marbrook chambers (2). In these systems cell monolayers can be grown on microporous filters contained within a filter chamber (Fig. 1). Separation of the culture

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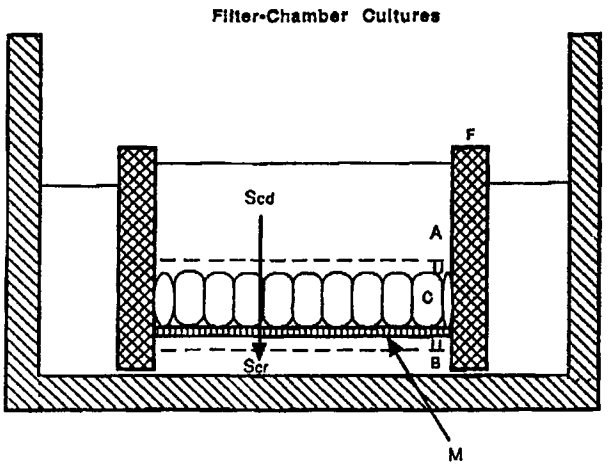

Fig. 1 : Monolayers of cells grown on microporous membranes in chambers. A, apical culture medium; B, basolateral cultural medium; C, cell monolayer; M, microporous membrane; U, unstirred water layer; Scd, solute concentration donor side; Scr, solute concentration receiver side; F, filter chamber

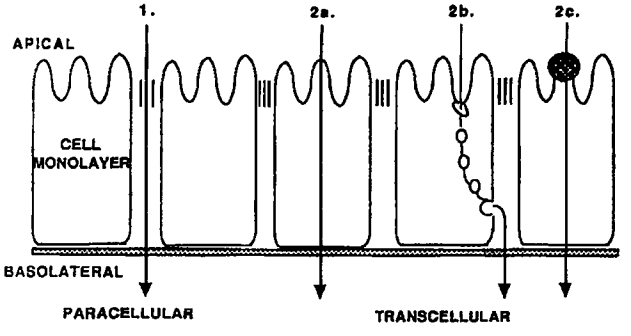

Fig. 2 : Diagram of routes and mechanisms for the transport of drugs and delivery systems across cellular barriers. 1, Paracellular; 2, Transcellular (2a diffusion; 2b transcytosis; 2c, carrier-mediated)

fluids on the apical and basolateral sides of the cell layer allows transport studies to be performed in either direction, i.e. from the apical to the basolateral side and vice versa. A number of variations on the basic theme (shown in Fig. 1), which differ in the composition of the microporous membrane, the design of the filter chamber, and whether the system is stagnant or stirred, have been used (3). The growth of cell monolayers in these systems has provided new and powerful techniques for exploring the fundamental aspects of the transport of drugs and delivery systems across defined cellular barriers, and for discriminating between different routes and mechanisms (Fig. 2).

The establishment of in vitro cell culture systems that mimic normal biological barriers in vivo requires careful selection of the source of cells that will produce a relevant system when growth under defined

Table I : Factors affecting the growth of cellular barriers in culture

Cell Related	*Culture System Related*
Primary or cell line	Media components
Normal or transformed	Seeding density
Differentiation potential	Microporous membrane properties
Passage number	Hydrodynamic forces
Cell heterogeneity	Extracellular matrix
Viability	Feeding regimen
Phenotypic stability	

Table II : Parameters for the characterization of differentiated cell cultures

Parameter	*Measurement*
Morphology	Polarity, ultrastructure
Tight-junctions	Presence, permeability
Electrical properties	Ssc, Rt
Metabolic properties	e.g. Glycolysis, peptidases
Cell surface markers	Properties & polarity of enzymes, receptors, lipids
Biosynthesis	Structure & fidelity of sorting of secretory, cytoplasmic & membrane proteins, lipoproteins & lipids
Endocytosis	Efficiency & fidelity of intracellular routing of different vesicular pathways
Carrier-mediated transport	Rate, polarity & specificity of transport
Transepithelial water flux	Dimensions of paracellular pathway
Solute diffusion	Dimensions of unstirred water layers
Permeability	Diffusion of low molecular weight compounds & macromolecules

culture conditions, and rigorous characterization of the morphological and functional properties of the cell layers obtained. The transport and metabolic properties of cultured cells can be greatly influenced by a number of variables pertaining to the cells themselves or the environment of the cell culture

system (Table I). For example the source of cells can be primary cultures, passage lines, or transformed lines. The number of times the cells have been passaged, the phenotypic stability of the cell line, the heterogeneity of the cell line, and the ability of the cell line to undergo differentiation can also have profound effects on the properties of the final culture system. In addition the properties of cultured cells can also depend on a number of components in the cell culture system, and the presence of other cell types.

After selection of an appropriate cell source a number of criteria (Table II) can be used to compare the morphological, biochemical and transport properties of the in vitro biological barrier with those of the barrier in vivo. Most of these properties arise from the establishment of cell polarity and the presence of tight junctions between adjacent cells (4). An important consideration in selecting routine criteria for characterizing an epithelial barrier is to have well defined in vivo criteria for comparison. A number of the criteria listed in Table II, e.g. aspects of the biosynthetic and endocytic pathways of proteins have not yet been well characterized in vivo, and are currently the focus of basic cell and molecular biological research. Thus, the functioning of these pathways cannot yet be used as rigorous criteria for characterizing a cellular barrier in culture. It also follows that the most appropriate criteria should be selected for the proposed series of experiments. For example, the transcellular transport of cobalamin across human Caco-2 cells is optimal at a later stage in culture than the development of maximal electrical resistance across the monolayers (12), therefore in this case electrical resistance measurements are not a good predictor of cobalamin transport.

As the growth and characterization of cells will be specific to the cell type, the discussion will focus on one specific cell culture system that mimics the intestinal epithelium.

TRANSPORT AND PERMEABILITY PROPERTIES OF HUMAN Caco-2 CELLS: An in vitro model of the intestinal epithelial cell barrier

The majority of in vitro methodologies (5) that have been used to study the transport of drugs across the intestinal epithelium do not have the morphological and functional properties of normal adult human epithelial layers. In addition they lack the viability and versatility required for quantitive measurements of transepithelial drug transport and for the examination of transport mechanisms. The use of isolated human intestinal epithelial cells has been slow to progress because these cells are difficult to culture and have limited viability (6). Recently attention has turned to human adenocarcinoma cell lines that reproducibly display a number of properties characteristic of differentiated intestinal cells (7–10). The HT-29 and Caco-2 cell lines (4) have been widely used to study intestinal epithelial differentiation and function because of their ability to express morphological and biochemical features of adult differentiated enterocytes and goblet cells (11). As the Caco-2 cell line displays the most highly differentiated properties under standard culture conditions (10), it appears to be the most relevant in vitro system for investigating transepithelial transport processes and as such has become the focus of attention for such studies.

A number of laboratories (10, 12, 13) have demonstrated that Caco-2 cells can be routinely grown as confluent monolayers on microporous filters. The monolayers develop an enterocytic morphology typical of villus cells and a polarity of a number of brush-border enzymes (10, 12, 13). Depending upon the exact experimental conditions used, full expression of these properties is achieved between approximately 15–20 days in culture (12, 13). Establishment of the barrier function of the monolayers can be demonstrated by lack of passage of a number of permeability markers (12, 13). The inability of horseradish peroxidase (mol. wt. 40,000) to cross the tight junctions developed between adjacent Caco-2 cells demonstrates that the barrier properties of the in vitro system to this macromolecular probe are similar to those of the small intestine in vivo (14).

The small amount of transcellular transport of horseradish peroxidase reflects its transport through cells in endocytic vesicles similar to that described in vivo (15). The integrity of the monolayers has also been routinely demonstrated by measuring the transepithelial electrical resistance (16). A range of values between 150–400 ohms.cm^2 have been reported indicating that in different laboratories, under different culture conditions, Caco-2- monolayers can display the electrical properties of either small intestinal or colonic enterocytes (12, 13, 16).

Caco-2 monolayers are being widely used to study the transepithelial transport pathways shown in Figure 2. A number of specific transport systems that absorb nutrients and macromolecules from the small intestine are functional in the Caco-2 systems (Table III). While detailed kinetic and molecular comparisons between these systems in vitro and in vivo are not yet

Table III : Transport properties of human Caco-2 cells grown in filter culture

	Transport Route	*Polarity*	*Reference*
1.	Carrier-mediated		
	a) Bile acids	Apical	(12, 18)
	b) Amino acids	Apical	(19, 20)
2.	Endocytic		
	a) IF-Cbl	Apical	(12, 21)
	b) EGF	Basolateral	(28)
	c) Transferrin	Basolateral	(29
3.	Passive diffusion		
	a) Beta blockers	None	(12)
	b) Peptides		(23)
4.	Permeability to macromolecules		(12, 13)

available the systems involved in the carrier-mediated transport of bile acids (12, 18) and large neutral amino acids (19) show many of the basic properties (i.e. specificity, saturability, compatibility, unidirectionality) found in the small intestine in vivo. The development of these systems is also a function of the time in culture. The functioning of these carrier-mediated systems and the electrical properties of the cell monolayer can be reproducibly achieved and maintained for several weeks in culture (12, 13, 18, 19).

A number of receptor-mediated endocytic systems that are involved in the transport of proteins and protein bound ligands in vivo are also functional and show the expected polarity in filter grown Caco-2 cells (Table III). The transcellular transport of cobalamin (vitamin B12) mediated via a specific receptor that binds and internalizes intrinsic-factor-cobalamin and secretes transcobalamin 2 (12, 21, 25) is of particular interest following reports that cobalamin-drug conjugates cross the intestinal epithelium in vivo (22). Caco-2 is the only human cell line reported to transcellularly transport cobalamin via the intrinsic factor receptor. In the absence of many details on the mechanism of these endocytic pathways, Caco-2 cells have become an important in vitro system for studying the basic cellular and molecular biology of these processes.

A number of studies on the use of Caco-2 cell culture to study drug transport have been reported. Studies on a series of beta-blockers (12; Table IV) and peptides (23) have indicated that the Caco-2 system may be useful for predicting the in vivo absorbability of a range of drugs, and for distinguishing the relative

Table IV : Passive transport of a series of beta-blockers across Caco-2 cells[1]

Drug	*Relative lipid solubility*[2]	*Transport across Caco-2 cells (Kapp/h)*[3]	*Oral dose absorption*[4]
Atenolol	0.003	0.00168	50
Metoprolol	0.15	0.222	95
Propranolol	5.4	0.329	90

[1]Data taken from Wilson et al. (12)
[2]Distribution coefficients *n*-octanol/buffer, pH 7.0, 20°C
[3]Appearance rate in the basolateral fluid
[4]Percentage administered dose

contributions of the paracellular and transcellular pathways, although many more examples are required to test the extent of such correlations between transport across the human cell system and oral absorption in vivo. The use and merits of a stirred system for determining the contribution of the unstirred water layer has recently been reported (3). The HT-29 system has been used to study the uptake of the orally active antibiotic, cefalexin by a dipeptide transport system (24). The Caco-2 system may also be useful for studying intestinal metabolism considering the known presence of brush border hydrolases and phenylsulfotransferase (10, 26); however, much work will need to be done to prove that the specificity, kinetics and mechanism of action of these enzymes is identical to their in vivo counterparts.

While it is clear that Caco-2 cells in filter culture display many of the features of normal small intestinal enterocytes it is equally clear that they also show a number of abnormal metabolic biosynthetic properties (27) probably reflecting their transformed phenotype. Other studies (12) indicate that Caco-2 cell layers consist of morphologically (and probably biochemically) heterogeneous enterocytes. In addition a number of cell types present in the normal intestinal epithelium, e.g. M-cells, goblet cells, have not been detected in these cultures. Thus there are many features of the intact adult intestinal epithelium that are not present in Caco-2 cultures. A challenge for future developments in cell culture methodologies will be to reconstitute an intestinal epithelial barrier containing the majority of cell types.

CONCLUSIONS

The use of novel cell culture techniques to study the transport of drugs and drug delivery systems across specific cellular barriers is at an early stage in its

development. However, it is already clear that these techniques have enormous potential for investigating transport mechanisms to determine their relevance for enhancing the delivery of drugs either through drug modification or through the use of a novel drug delivery system.

The discussion has focused on the intestinal epithelium since this is the most advanced of the in vitro cell barrier systems, however significant progress is also occuring on the growth and characterization of other barriers, and in particular the epidermis and the endothelium from brain capillaries (1). The increasing use of these techniques by scientists in pharmaceutical R&D is being paralleled by their use in basic research in cellular and molecular biology aimed towards elucidating transport pathways and mechanisms. Of crucial important will be the development of in vitro–in vivo correlations that not only validate the cell culture systems but provide information on the scope of their potential for predicting in vivo absorption.

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