JPRHC

Review Article

CACO-2 CELLS: AN OVERVIEW

A. LAKSHMANA RAO*, G. G. SANKAR**

For author affiliations, see end of text

This paper is available online at www.jprhc.com

ABSTRACT

The Caco-2 cell line is an immortalized line of heterogeneous human epithelial colorectal adenocarcinoma cells. Human colonic adenocarcinoma cells that are able to express differentiation features characteristic of mature intestinal cells, such as enterocytes or mucus cells. These cells are valuable in vitro tools for studies related to intestinal cell function and differentiation. The Caco-2 cell line is widely used with in vitro assays to predict the absorption rate of candidate drug compounds across the intestinal epithelial cell barrier. Caco-2 may also refer to a cell monolayer absorption model. Cell-based functional assays, such as the Caco-2 drug transport model for assessing intestinal transport, are extremely valuable for screening lead compounds in drug discovery.

Keywords: Caco-2 cells, Passive transport, Apoptosis.

INTRODUCTION:

Human colon adenocarcinoma (Caco-2) cells have been widely used as in vitro models to evaluate the transport of drug candidates across the intestinal epithelial barrier. Caco-2 cells when grown on semi permeable filters[1], spontaneously differentiate in culture to form confluent monolayers which both structurally and functionally resemble the small intestinal epithelium. These cells resemble various biological membrane properties; including enzymatic and transporter systems. In addition to its usefulness as an absorption model, the Caco-2 cells are useful for studying the metabolism of drugs. Caco-2 cells form monolayers of differentiated epithelial cells joined by intercellular tight junctions, which prevent the paracellular diffusion of solutes. Thus, this system provides a selective barrier for modeling structure transport relationships for both passive and carrier-mediated transport. In addition, Caco-2 cells express apical efflux mechanisms, which may play a major role in restricting oral drug absorption. Caco-2 cells may be used for screening of drug absorption at an early stage in the drug development process.

Transport mechanisms

The transport of drugs[2-4] across the intestinal epithelium may occur by one or more of four different routes:

Passive transcellular route:

Rapidly and completely absorbed drugs are generally lipophilic and distribute readily into the cell membranes of the intestinal epithelium. It can be assumed that these drugs are transported exclusively by the passive transcellular[5] route.

Passive paracellular route:

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 through the water filled pores of the paracellular[6] pathway across the intestinal epithelium. But it is not finally established, as it is possible that even very hydrophilic drugs may be transported partly by the transcellular route.

Carrier-mediated route:

The low efficiency of the paracellular pathway has stimulated investigations into ways to enhance the permeability by this route. Many of these studies have been performed in Caco-2 cells and have provided new insight into the regulation of tight junctions-the rate limiting barrier of the paracellular pathway. Some hydrophilic drugs whose chemical structures mimic those of various nutrients can be transported across the intestinal epithelium by active carrier-mediated[7] transport.

Transcytosis:

The low capacity of transcytosis route from the mucosal to the serosal side of the intestinal epithelium makes this route less attractive for the transport of drugs. It has

therefore mainly been considered as a route for highly potent drugs, such as peptide[8] antigens which are excluded from other transport pathways due to their size.

Passive drug transport through Caco-2 cells

A biological membrane, consisting of an aqueous (paracellular) and lipoidal[9] (transcellular) pathway in parallel, can be characterized by its lipophilic character, the presence of water pores and the existence of stagnant diffusion layers. Passive diffusion[10] is the most significant transport mechanism for the majority of drugs, with the physicochemical properties of both the drug and the permeation[11] barrier being the major rate determinants for transport. Passive diffusion depends to a large extent on three interdependent physicochemical properties[12], namely lipophilicity, polarity (charge, hydrogen bonding) and molecular size.

In some studies, single physicochemical properties of the drug molecule, such as octanol/water partition coefficients, hydrogen bonding capacity or desolvation energy have been correlated to intestinal absorption rate or cell membrane permeability. The octanol/water partition coefficient (P_{oct}) is the most wide spread predictor of drug absorption and P_{oct} is routinely determined for new chemical entities. The disadvantage of using single physicochemical factors is that they are only roughly correlated to passive drug absorption.

Carrier-mediated transport in Caco-2 cells

Intestinal absorptive cells express a number of carrier systems that are responsible for the absorption of amino acids[13], bile acids, nucleosides, vitamins and peptides. Efforts to determine the potential exploitability of these transporters in drug delivery has led to a greater understanding of carrier-mediated intestinal drug transport. Various properties of Caco-2 cells like the presence of tight junctions, brush border enzymes, drug metabolizing enzymes etc, makes this system useful for structure transport relationships for both passive and carrier-mediated transport.

Intestinal carrier systems

Amino acid transport:

Several amino acid transport systems have been identified in the brush border of the small intestine. Some of these transport systems exhibit overlapping substrate specificities. They also vary in their Na^+-dependence and on the involvement of other ions, such as Cl^- and K^+. In Caco-2 cells, the apical uptake of the large neutral amino acid, phenylalanine, showed Na^+-dependence and inhibition by the amino acids, His and Lys. Although most amino acid transport systems are Na^+-dependent and H^+-independent. Despite the increasing knowledge on amino acid transport, there is little evidence supporting the role of amino acid carriers in drug transport.

Nucleoside transport:

Nucleosides and nucleotides act as precursors in the synthesis of nucleic acids and play a role in the synthesis of lipids, carbohydrates, and proteins. Most cells do not

require purines and pyrimidines because they can synthesize these compounds. The mechanisms responsible for the intestinal absorption of purines and pyrimidines include facilitated diffusion and Na^+-dependent active transport. The uptake of a natural nucleoside, thymidine and a deoxy nucleoside analogue, zidovudine (AZT), in Caco-2 cells were studied. The uptake of thymidine was saturable. Moreover, the uptake of thymidine was inhibited by the structural analogues, uridine and cytidine by the specific nucleoside transporter inhibitors. In contrast, the uptake of zidovudine was neither saturable nor inhibited by any of the compounds that inhibited the uptake of thymidine. Based on observations, it was concluded that the uptake of zidovudine in Caco-2 cells was mainly passive.

Bile acid transport:

Bile acids are secreted into the duodenum after the ingestion of meals. Bile acids emulsify lipids thus facilitating their digestion. Approximately 90% of the secreted bile acids are recycled back to the liver. In the jejunum, bile acids are reabsorbed by passive diffusion; however in the ileum, bile acid absorption involves active carrier systems. In small intestine, bile acid transport in Caco-2 cells is Na^+-dependent. The bile acid carriers expressed by Caco-2 cells is similar to that in human intestine, thus validating the utility of Caco-2 cell model for studying intestinal bile acid transport.

Vitamin transport:

Vitamin B_{12} is absorbed[14] from the intestinal lumen by a process that involves the intrinsic factor. After being secreted by gastric parietal cells intrinsic factor binds vitamin B_{12} to form a complex, which interacts with receptors in the apical membrane of

ileal enterocytes. The transport of vitamin B_{12} was studied in Caco-2 cells. Thus, the potential utility of this pathway to achieve carrier-mediated enhancement of intestinal drug absorption appears to be very low.

Oligopeptide transport:

The intestinal oligopeptide transporter has received much attention as a potential vehicle for rational drug delivery of β-lactam antibiotics[15], renin inhibitors and angiotensin converting enzyme inhibitors. The intestinal oligopeptide transport system is distinct from the Na^+-dependent transport systems used by glucose and amino acids. The carriers are located on the intestinal brush border membrane and mediate the cotransport of di-/tripeptides and H^+ across the enterocyte membrane. Thus, peptide transport requires a transmembrane H^+ gradient. This gradient is produced by the action of the Na^+-K^+ ATPase system located on the basolateral membrane and the Na^+-H^+ exchanger on the apical membrane.

Permeability modulation of Caco-2 cell monolayers by interferons

Drug transporters play a vital role in the disposition and elimination of xenobiotics. In particular, P-glycoprotein[16] is an important ATP-dependent membrane transporter which is expressed in many normal tissues, including liver, brain, kidney, adrenal gland and intestinal epithelial cells. P-glycoprotein is regulated by a number of substances, including xenobiotics, hormones and cytokines[17]. Interferons, which consist

of type I interferons and type II interferons, are endogenous cytokines induced in a variety of inflammatory diseases and also used clinically as anticancer or antiviral agents. P-glycoprotein-mediated drug transport activity in Caco-2 cells is itself not influenced by interferons-β or interferons-γ treatment. However, the expression and function of intestinal P-glycoprotein *in vivo* may be modulated indirectly by interferons because of a large variety of biological activities of cytokines. These results suggest that interferons modulate the permeability[18] of Caco-2 monolayer through effect on paracellular transport rather than effect on P-glycoprotein activity.

Rotavirus induces apoptosis in Caco-2 cells

Rotavirus[19], which belong to the *Reoviridae* family, exhibit a marked tropism for the enterocytes of the intestinal epithelium and are recognized as the major cause of viral gastroenteritis in young children. The infection of Caco-2 cells by the rotavirus[20] strain induces structural and functional alterations such as cytoskeleton disorganization and increase in intracellular Ca^{+} concentration. Calcium plays a major role in the events leading to cell injury and cell death, especially by apoptosis. Apoptosis plays a major role in many physiological processes such as development, morphogenesis, tissue modeling and immune regulation. The apoptosis is directly induced by rotavirus infection. The rotavirus infection of Caco-2 cells leads to an intracellular increase in Ca^{2+}. Ca^{2+} is recognized as an important regulator of apoptosis and both early and late increase in Ca^{2+} can be apoptogenic. Apoptosis may also be associated with functional changes in the course of rota virus infection, such as modifications of cell permeability.

Rotavirus infection in Caco-2 cells is followed by a dilatation and/or opening of the tight junctions resulting from the rearrangements in structural and functional tight junction proteins. Thus, it led to the hypothesis that apoptosis plays a role in the increase of epithelial permeability during infection.

Endogenous lipase activity in Caco-2 cells

Triglycerides, the major component of dietary fat, must first undergo hydrolysis and micellar dispersion before their translocation into the enterocyte. Caco-2 cells have frequently been used for study of fatty acid uptake and metabolism, an endogenous lipolytic activity allowing the hydrolysis of triglycerides into monoglycerides and free fatty acids. The majority of the lipase activity[21] was found in the cytosolic cell fraction and to a lesser extent in the apical brush border membrane and other organelles. Caco-2 cells remain a widely utilized cell model for the study of human intestinal fat absorption and lipid metabolism. The activity of this lipase resulted in an increase of free fatty acids uptake in a time and dose-dependent manner. Caco-2 cells have been extensively investigated regarding their ability to synthesize and secrete triglyceride-rich lipoproteins after the uptake of free fatty acids.

Toxic and biochemical effects of zinc in Caco-2 cells

Zinc[22-23] is an essential micronutrient that is involved in vital functions such as wound healing, immune response, growth and cell division. Zinc also induces the

formation of metallothioneins, which play an important role in scavenging toxic nonessential metals such as cadmium and in protection of cells against reactive oxygen species which cause oxidative stress. Zinc also acts as an antioxidant due to protection of protein sulfhydryl groups. Apoptosis is induced with increasing amounts of zinc in both cell types of Caco-2, nevertheless the induction is higher and significant in preconfluent cells only. Zinc in relatively high concentrations can be toxic to intestinal cells. The mechanisms of zinc-induced damage might be mediated both intra- and extracellularly. In particularly high levels of zinc sulphate might be quite harmful to non-differentiated cells as well as to absorptive enterocytes.

Iron uptake and toxicity in Caco-2 cells

Iron[24-25] is an essential micronutrient that is involved in oxygen transport and energy metabolism. However, free iron can be detrimental to cells, as it is involved in forming reactive oxygen species which cause oxidative stress to cells. In addition to dietary sources, iron salts are also ingested for therapeutic supplementation, primarily in cases of iron deficiency anemia. Efficiency of iron uptake is dependent on solubility, linkage and valence. Caco-2 cells might be used as a rapid screening model to predict potential iron availability for absorption. Many *in vitro* studies describing toxicity and uptake of iron are carried out with iron bound to nitrilotriacetic acid, which is a form of iron that is not normally present in the human gut. Nitrilotriacetic acid seems to enhance the toxicity of iron, while citric acid inhibits iron uptake and toxicity. Therefore the choice of iron salts and complexes is critical in studies dealing with uptake and toxicity.

The adverse effects of iron in Caco-2 cells were tested with cell viability and proliferation as well as membrane stability.

Applications of Caco-2 cells

1. Rapidly assess the cellular permeability[26] of potential drug candidates
2. Elucidate pathways of drug transport (e.g., passive versus carrier-mediated)
3. Assess formulation strategies designed to enhance membrane permeability
4. Determine the optimal physicochemical characteristics for passive diffusion of drugs
5. Assess the potential toxic effects of drug candidates or formulation components on this biological barrier
6. Screening of drug absorption and intestinal permeability
7. Reference model for theoretical predictions[27] of drug absorption
8. In classifying a compounds absorption characteristics in the biopharmaceutics classification system
9. Study of pre-systemic drug metabolism[28]
10. Determine the efflux mechanisms of phase II conjugates of drugs and natural products
11. Study the functionality of intestinal transporters[29] in the Caco-2 cell model
12. *In vitro* assays to predict the absorption rate of candidate drug compounds[30] across the intestinal epithelial cell barrier

CONCLUSION

Comparision of drug transport in Caco-2 monolayers with intestinal drug transport *in vivo* indicates that the monolayers can be used to predict drug transport by different ways across the intestinal epithelium. The best correlation to the *in vivo* situation is obtained for drugs that are transported by the passive transcellular type. The absorption of drugs transported via carrier-mediated mechanisms can also be predicted in some of the cases. Theoretical prediction of drug absorption usually relies on a single physicochemical property of the drug molecule such as lipophilicity or hydrogen bonding capacity. Computational methods built optimally from high quality data, taking into account all the different processes of drug transport, will probably replace a large part of the conventional Caco-2 screening. However, until these computational methods are more accurate prediction models, artificial membranes for reconstructing the passive component of drug permeability and biological *in vivo* assays for obtaining of a net flux value in combination with knowledge of transport processes, should be used in parallel to gain the best information for new drug design.

REFERENCES

1. Hilgers A R, Conradi R A, Burton P S, Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa, Pharm. Res. 1990, 7(9): 902.
2. Anna-Lena B U, Caco-2 replace or refine, Drug Discovery Today: Technologies, 2004, 1(4): 423.
3. Artursson P, Palm K, Luthman K, Caco-2 monolayers in experimental and theoretical predictions of drug transport, Adv. Drug Deliv. Rev., 2001, 46(1-3): 27.
4. Shah P, Jogani V, Bagchi T, Misra A, Role of Caco-2 cell monolayers in prediction of intestinal drug absorption, Biotechnol. Prog., 2006, 22(1): 186-98.
5. Per A, Katrin P, Kristina L, Caco-2 monolayers in experimental and theoretical predictions of drug transport, Adv. Drug Deliv. Rev., 1996, 22: 67.
6. Nellans H N, Paracellular intestinal transport: Modulation of absorption, Adv. Drug Del. Rev., 1991, 7: 339.
7. Hilgendorf C, Spahn L H, Regardh C G, Lipka E, Caco-2 versus Caco-2/HT29-MTX co-cultured cell lines: Permeabilities via diffusion, inside- and outside-directed carrier-mediated transport, J. Pharm. Sci., 2000, 89(1): 63.
8. Ano R, Relationships between structure and high-throughput screening permeability of peptide derivatives and related compounds with artificial membranes: Application to prediction of Caco-2 cell permeability, Bioorg. Med. Chem., 2004, 12: 257.

9. Gian C, Jochem A, Han V W, Gerd F, Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs lipophilicity and molecular weight, Eur. J. of Pharm. Sci., 1998, 6: 313.
10. Gian C, Gerd F, Han V W, Comparision of passive drug transport through Caco-2 cells and artificial membranes, Int. J. Pharm., 1997, 147: 61.
11. Sibylle N, Anna-Lena Y, Ismeal Z, Per A, P^H-Dependent passive and active transport of acidic drugs across Caco-2 cell monolayers, Eur. J. Pharm. Sci., 2005, 25 (2-3): 211-220.
12. Camenisch G, Comparision of passive drug transport through Caco-2 cells and artificial membranes, Int. J. Pharm., 1997, 147: 61.
13. Ismael J H, Jibin L, Carrier-mediated transport and efflux mechanisms in Caco-2 cells, Adv. Drug Del. Rev., 1996, 22: 53.
14. Dix C J, Hassan I F, Obray H Y, Shah R, The transport of vitamin B_{12} through polarized monolayers of Caco-2 cells, Gastroenterology, 1990, 98: 1272.
15. Bergan T, Pharmacokinetics of beta-lactam antibiotics, Scand J. Infect. Dis., 1984, 42: 83.
16. Hunter J, Jepson M A, Tsuruo T, Simmons N L, Functional expression of P-glycoprotein in apical membranes of human intestinal Caco-2 cells, J. Biol. Chem., 1993, 268: 14991.
17. Walsh S V, Hopkins A M, Nusrat A, Modulation of tight junction structure and function by cytokines, Adv. Drug Del. Rev., 2000, 41: 303.

18. Hiroko K, Yukiko A, Yoshihiko W, Yoshinobu T, Permeability modulation of human intestinal Caco-2 cell monolayers by interferons, Eur. J. Pharm. and Biopharm., 2005, 59: 45.
19. Charlotte C, Jacqueline C L, Catherine S, Audrey E, Rotavirus induces apoptosis in fully differentiated human intestinal Caco-2 cells, Virology, 2005, 332: 480.
20. Greenberg H B, Clark H F, Offit P A, Rotavirus pathology and pathophysiology, Curr. Top. Microbiol. Immunol., 1994, 185: 255.
21. Johannes H S, Ernest G S, Daniel M, Emile L, Biochemica. Biophysica. Acta, 1998, 1393: 119.
22. Bettina Z, Michaela Z, Mansour S, Norman B R, Toxic and biochemical effects of zinc in Caco-2 cells, J. Inorg. Biochem., 2003, 97: 324.
23. Vidal J E, Kaori O, Tohru S, Bruce A, Contact with enterocyte-like Caco-2 cells induces rapid upregulation of toxin production by *Clostridium perfringens* type C isolates, Cellular Microbiology, 2009, 11(9): 1306.
24. Bettina Z, Michaela Z, Patricia P, Ilse S, Iron uptake and toxicity in Caco-2 cells, Microchem., 2005, 79: 393.
25. Aubry M, Monnier A, Chicault C, Galibert MD, Burgun A, Mosser J, Iron-related transcriptomic variations in Caco-2 cells: in silico perspectives, Biochimie., 2008, 90(4): 669.
26. Meunier V, Bourrie M, berger Y, Fabre G, The human intestinal epithelial cell line Caco-2: Pharmacological and pharmacokinetic applications, Cell Biol. Toxicol., 1995, 11(3-4): 187.

27. Stenberg P, Norinder U, Luthman K, Artursson P, Experimental and computational screening models for the prediction of intestinal mucosa, Crit. Rev. Ther. Drug Carrier Syst., 1991, 8: 305.
28. Artursson P, Borchardt R T, Intestinal drug absorption and metabolism in cell cultures: Caco-2 and beyond, Pharm. Res., 1997, 14: 1655.
29. Artursson P, Cell cultures as models for drug absorption across the intestinal mucosa, Crit. Rev. Ther. Drug Carrier Syst., 1991, 8: 305.
30. Engle M J, Goetz G S, Alpers D H, Caco-2 cells express a combination of colonocyte and enterocyte phenotypes, Journal of Cellular Physiology, 1998, 174(3): 362.

CURRENT AUTHOR ADDRESSES:

[*]**Associate Professor,**

Sri Vishnu College of Pharmacy,

Vishnupur, Bhimavaram- 534 202, A.P.

Phone No. 08816-250863

Mobile No. 098660 99916

E-mail: dralrao@gmail.com

[**]**Associate Professor,**

A.U. College of Pharmaceutical Sciences,

Andhra University,

Visakhapatnam- 530 003, A.P.