Pharmaceutical Research, Vol. 14, No. 12, 1997

Commentary

Intestinal Drug Absorption and Metabolism in Cell Cultures: Caco-2 and Beyond

Per Artursson[1,3] and Ronald T. Borchardt[2]

Received September 6, 1997; accepted September 11, 1997

KEY WORDS: intestinal drug transport; intestinal drug metabolism; *in vitro* epithelial models; Caco-2 cells.

At the 4th International Conference on Drug Absorption, Edinburgh, 13–15 June 1997, more than 50 % of the posters dealing with *in vitro* and *in vitro–in vivo* aspects of drug absorption were solely or partly based on studies using intestinal epithelial cell cultures (1). The popularity of cell cultures, predominantly Caco-2 cells, in studies of intestinal drug transport and metabolism can probably be explained by the ease with which new information can be obtained from these simple *in vitro* models. Drug transport experiments using cell cultures are easy to perform in large numbers and under controlled conditions, resulting in the generation of a wealth of information. This information can often be easily translated into fundamental principles since the cell culture models lack many of the obscuring variables found in more complex (and complete) drug absorption models. For instance, much of our more recent knowledge on intestinal active drug transport mechanisms has been derived from cell culture studies as has new information on passive transcellular and paracellular drug transport mechanisms. The good correlation between passive drug transport through the cell monolayers and that seen *in vivo* has made it possible to use the cell cultures for studies on structure-absorption relationships.

More recently, interest in the application of Caco-2 monolayers in high throughput screening of intestinal drug absorption has increased, and many drug companies are now using Caco-2 monolayers to screen for potential absorption problems in drug discovery programs. As a result, the FDA is currently evaluating the role of Caco-2 monolayers in the biopharmaceutical classification of drugs. The intestinal cell culture models have also been used in preformulation studies of the cellular effects of various pharmaceutical excipients such as co-solvents and absorption enhancers.

Thus, intestinal epithelial cell cultures have proven their value in various types of drug transport studies and have provided a major conceptual advance in our understanding of intestinal drug transport at the cellular level. The recent advances in this research area have been reviewed in several textbooks (2,3) and theme issues of review journals (4,5).

Although the rapid development of research using intestinal epithelial cell cultures is encouraging, as in all mature research fields, there are also signs of stagnation. Repetitive work on the characterization of the permeability of Caco-2 monolayers continues to be published (there are always some differences to be found between Caco-2 cells from different laboratories and there will always be a neverending supply of drug analogues available for permeability studies in Caco-2 monolayers). To our mind, some of these studies are a waste of energy and scientific resources and do not fulfill the demand for originality that most scientific journals require for publication. Are these signs of stagnation simply an indication that this research field is saturated and that there is not much innovative research left to be done? We do not think so. We are convinced that the application of cell culture models to the study of drug transport and metabolism will continue to be one of the most rapidly moving areas in pharmaceutical research for some time. We present here some observations that appear to us to offer interesting opportunities for original and much needed research involving intestinal cell culture models and their applications. We emphasise that the current intestinal cell culture models are not perfect and refinements are needed to better predict human intestinal permeability. Modifications of the cell culture models will also be needed to accommodate the screening of combinatorial libraries.

Further Refinements of the Cell Culture Models Are Needed to Better Predict Human Intestinal Permeability

After the first encouraging reports a few years ago on the correlations between Caco-2 permeability and drug absorption in humans after oral administration, permeability studies in cell culture models were sometimes over-interpreted by enthusiastic researchers. For instance, claims that Caco-2 permeability predicted the absolute bioavailability of drugs were made, while other contributing factors such as drug solubility and liver metabolism were neglected. Similarly, since Caco-2 cells were

[1] Department of Pharmaceutics, Uppsala University, P.O. Box 580, S-751 23 Uppsala, Sweden.

[2] School of Pharmacy, The University of Kansas, 2095 Constant Avenue, Lawrence, Kansas 66047-2504.

[3] To whom correspondence should be addressed. (e-mail: per.artursson@galenik.uu.se)

0724-8741/97/1200-1655$12.50/0 © 1997 Plenum Publishing Corporation

found to express most of the known drug transporters of the small intestine, claims were made that Caco-2 cells could predict the transport of any drug, independent of the transport mechanism. We now know that the expression level and substrate specificity of the carriers and efflux systems found in Caco-2 monolayers tend to be variable in comparison to the *in vivo* situation (6,7). Despite this, there are still researchers advocating the use of Caco-2 cells to predict the absorption of any drug, regardless of transport mechanisms (8). Generalizations such as these could be dangerous, especially when they are based on very limited data sets. Similar extrapolations of limited data cannot be made about intestinal drug metabolizing enzymes, since the expression of these enzymes is so clearly different in intestinal cell cultures and the *in vivo* situation. Many researchers are therefore now attempting to express relevant intestinal carriers and drug metabolising enzymes in Caco-2 cells and other cell lines by molecular cloning strategies (9,10). The advantage of this approach is that it allows the insertion of a human intestinal transporter or enzyme of known (primary) structure that is not easily found in more complex systems. Once established, these new cell culture models will offer the opportunity to study human drug transport at a more refined level.

Although these models will soon provide new and important information on, for example, structure-transport relationships, one drawback of the molecular cloning strategy is that the cloned genes are usually under the regulation of viral promoters. Thus, the natural up- and down-regulation of gene expression cannot be controlled in these systems. Studies of the relative contribution of potentially co-regulated transporters and enzymes (e.g. MDR-1 P-glycoprotein and CYP 3A4) to the observed drug permeation are likely to give incorrect information in these models. It is therefore encouraging to see that conventional cell biological methods can be used in Caco-2 cells as an alternative approach to induce the expression of under-expressed drug metabolizing enzymes such as CYP 3A4 to levels comparable to those seen in the small intestine *in vivo* (11). It is becoming apparent that such targeted optimization of cell culture models for specific purposes has not been fully exploited. It is therefore possible that similar approaches can be used to induce the expression of other relevant enzymes and transporters that display low expression levels in Caco-2 cells. Optimization of the microenvironment through co-culture with other cell types may also be used to dramatically alter the phenotype of the cell culture, as recently shown for Caco-2 cells in co-culture with Peyer's patch lymphocytes (12). In addition, the optimization of the culture conditions may reduce the time-consuming 3-week differentiation period required to obtain reproducible Caco-2 cell cultures. Preliminary results have suggested that, using an optimized differentiation environment, it is possible to obtain at least partly differentiated Caco-2 cells after only 3 days in culture (13).

Other imperfections of the Caco-2 cell cultures in need of improvement include a lower paracellular permeability (which makes it difficult to study poorly permeating drugs, such as peptides and peptidomimetics) and the absence of other intestinal absorption barriers, such as the mucus layer. The low level of paracellular permeability is often explained by anatomical differences between intestinal segments and by noting the colonic origin of this cell line. Caco-2 cell populations with increased permeability, more similar to that of the small intestine, are available, but these appear to contain a sub-population of dead cells, which would explain the high permeability (14). With regard to the absence of a mucus layer, the key constituent of this layer, mucin, is produced by a different intestinal cell population from that represented by Caco-2 cells, the goblet cells. Thus, the sources of these imperfections are less related to abnormal or unexpected properties of the Caco-2 cells themselves than to properties not inherent in this cell culture model. It is therefore likely that the best solutions to these deviations from the normal human intestine can be found in models other than Caco-2 cells, as exemplified by studies using new cell culture models with a permeability more closely resembling that of the small intestine (15) or mucus-producing goblet cell cultures (16). These are only isolated examples of attempts to obtain alternative cell culture models, and much more research is needed in this area. The recent establishment of normal human duodenal epithelial cell lines offers new research opportunities in this respect (17). Whether similar cell lines from other human intestinal segments can be established remains to be seen. If such cell lines are established, it may become possible to study segment-specific intestinal drug transport and metabolism in cell culture.

The ideal cell culture model for drug transport and metabolism studies would have passive transcellular and paracellular permeabilities comparable to those of the human small intestine. In addition, the levels of carriers and enzymes expressed by this ideal model would be similar to those expressed *in vivo* in the small intestine. Finally, the model would comprise not only enterocytes but also mucin-producing goblet cells and other relevant cell populations. Attempts have been made to establish co-cultures of Caco-2 cells and goblet cell lines (derived from the HT29 cell lineage), but with limited success, since different cell lines seem to prefer to grow in mono- rather than co-cultures, probably because of differences in their repertoires of cell adhesion molecules (18). It could be speculated that more fruitful ways of transferring the heterogeneity of the small intestinal epithelium into cell culture might involve the use of human intestinal epithelial stem cells or transgenic animals expressing inducible genes that promote the selective growth of the intestinal epithelium *in vitro*. Again, a traditional cell biological approach, involving the optimization of microenvironmental factors such as the extracellular matrix, growth medium and differentiation inducing factors will be a requirement for success (19).

In addition to the refinements in the cell culture systems that are described above, we are also of the opinion that other improvements in technology are needed before these systems can become an integral part of the drug discovery process. These needed improvements in technology include: (I) miniaturization of the cell culture apparatus; (ii) automation of the actual transport/metabolism experiments; (iii) adaptation of these cell culture systems to handle insoluble compounds and/or complex formulations; (iv) coupling of these systems to sophisticated and sensitive analytical methodologies (e.g., LC-MS/MS); (v) development of standardized methods for quantitation of transport/metabolism data; and (vi) development of sophisticated data systems for the analysis, storage and retrieval of transport and metabolism data.

These advances in technology are necessary because of the changes that have occurred in recent years in drug discovery. In the past, medicinal chemists focused on optimization of the

pharmacological properties of lead compounds by altering their molecular structures so as to have the highest possible affinity for macromolecular targets (e.g., receptor and enzyme). To achieve this goal more rapidly, drug discovery scientists have developed high throughput biological screens and combinatorial chemistry (20). The introduction of these technologies into the drug discovery process has permitted the rapid optimization of the pharmacological properties of lead molecules through an iterative process. This iterative process has frequently led to the identification of high affinity ligands that did not always make good drugs. Often, these molecules lacked optimal pharmaceutical properties (e.g., cell membrane permeability). Only recently have drug discovery scientists realized that they must implement similar iterative processes for optimizing the pharmaceutical properties of lead compounds (5). In addition, they have come to realize that the processes of optimizing the pharmacological and pharmaceutical properties of lead molecules need to be done in parallel rather than sequentially.

This increased demand for information about the pharmaceutical properties of lead compounds has led pharmaceutical scientists to modify existing technologies (e.g., Caco-2 cells grown in the Transwell® system) in an attempt to develop high throughput screens. Alternatively, pharmaceutical scientists have tried to expedite the screening process by conducting experiments with libraries of compounds (21). In reality, however, what is really needed is the development of new technologies not simply modification of existing technologies. For example, transport systems need to be miniaturized so as to accommodate the small amounts of compounds forthcoming from combinatorial chemistry. This will require the engineering of new cell growth and transport apparati. The transport/metabolism experiments themselves, including the analytical chemistry and data analysis and storage steps, need to be automated to reduce the time and costs associated with these types of experiments. In addition, these improvements are needed to permit the generation of data about the pharmaceutical properties of a large number of compounds in a time frame consistent with the high throughput screens used to generate data about the pharmacological properties of these molecules. If information about the pharmaceutical properties of a molecule does not reach the medicinal chemists at the same time as the data about the pharmacological properties, they will not factor this information into their drug design strategies. In addition, since many lead compounds forthcoming from discovery research are insoluble, methodologies need to be developed to routinely handle these types of molecules in a typical transport/metabolism experiment. Finally, standardized methods for quantitation of transport and metabolism data and for the storage and retrieval of these data are needed so that data bases describing how chemical structure affects the transport and metabolism of molecules can be developed. Establishment of these structure-transport and structure-metabolism data bases will ultimately facilitate the overall process of optimizing the pharmaceutical properties of drug candidates and lead to safer and more efficacious drugs.

In conclusion, the development of these cell culture systems represents one of the most exciting advances in the pharmaceutical sciences in the past 10 years. These systems, if properly used, could not only lead to an improved understanding of the biological barriers that often limit the efficacy of a drug molecule; but they also have the potential to expedite the process of drug discovery and development and, thus, improve the efficiency of the pharmaceutical industry. Hopefully, by optimizing both the pharmacological and pharmaceutical properties of drug candidates by structural manipulation, the pharmaceutical industry will ultimately realize an improvement in the number of drug candidates that succeed in clinical trials. This would reduce the costs involved in developing a drug as well as the time necessary to introduce a new therapeutic agent into clinical practice. However, in order to get maximum benefit from this technology, we cannot let the field stagnate. Instead, we must to continue to make meaningful refinements in this technology and to develop new technologies that will allow pharmaceutical scientists to make significant contributions to drug discovery and development in the future.

ACKNOWLEDGMENTS

PA's research in this area was supported by The Swedish Medical Research Council (9478), Centrala försöksdjursnämnden (97-46). ASTRA AB and SmithKline Beecham Corp. RBS's research in this area was supported by research grants from the United States Public Health Service (DA-09315. GM-51633, GM-088359), Glaxo-Wellcome, Inc., Pharmacia & Upjohn Inc., Costar Corporation, and SmithKline Beecham. Corp.

REFERENCES

1. Supplement: Abstracts, 4th International Conference on Drug Absorption, Edinburgh. *Eur. J. Pharm. Sci.*, **5/Suppl. 2**:(1997).
2. A. J. Shaw. Epithelial cell culture—a practical approach, Oxford University Press, Oxford, 1996.
3. R. Borchardt, P. Smith, and G. Wilson. Models for assessing drug absorption and metabolism, Plenum Press, New York, 1996.
4. J. Hochman and P. Artursson. Cell cultures to assess drug transport and metabolism. *Adv. Drug. Deliv. Rev.*, **22**:1–265 (1996).
5. P. Smith. *In vitro* models for selection of development candidates. *Adv. Drug. Deliv. Rev.*, **23**:1–235 (1997).
6. R. Borchardt. The application of cell culture systems in drug discovery and development. *J. Drug Targeting*, **3**:179–182 (1995).
7. D. Brayden. Human intestinal epithelial cell monolayers as prescreens for oral drug delivery. *Pharm. News*, **4**:11–15 (1997).
8. S. Yee. *In vitro* permeability across Caco-2 cells (colonic) can predict *in vivo* (small intestinal) absorption in man- fact or myth. *Pharm. Res.*, **14**:763–766 (1997).
9. K. Covitz, G. Amidon, and W. Sadee. Human dipeptide transporter, hPEPT1, stably transfected into Chinese hamster ovary cells. *Pharm. Res.*, **13**:1631–1634 (1996).
10. C. Crespi, B. Penman, and M. Hu. Development of Caco-2 cells expressing high levels of cDNA-derived cytochrome P450 3A4. *Pharm. Res.*, **13**:1635–1641 (1996).
11. P. Schmiedlin-Ren, K. Thummel, J. Fischer, M. Paine, K. Lown, and P. Watkins. Expression of enzymatically active CYP3A4 by Caco-2 cells grown on extracellular matrix-coated permeable supports in the presence of 1-alpha, 25-dihydroxyvitamin D3. *Mol. Pharmacol.*, **51**:741–754 (1997).
12. S. Kerneis, A. Bogdanova, J. Kraehenbuhl, and E. Pringault. Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. *Science*, **277**:949–952 (1997).
13. D. Asa and P. LaRocca. Comparison of the Biocoat® intestinal epithelial environment with the conventional 21-day Caco-2 system. Poster presentation at the 4th International Conference on Drug Absorption, Edinburgh, 1997.
14. E. Walter and T. Kissel. Heterogeneity in the human intestinal cell line Caco-2 leads to differences in transepithelial transport. *Eur. J. Pharm. Sci.*, **3**:215–230 (1995).
15. V. Milovic, S. Olsson, J. Hochman, E. Paul, and P. Artursson. Conditionally immortalised rat fetal intestinal epithelial cell line

(2/4/A1) for studying epithelial differentiation, apoptosis and permeability. *Gastroenterology,* **110**:A822 (1996).

16. A. Wikman, J. Karlsson, I. Carlstedt, and P. Artursson. A drug absorption model based on the mucus layer producing human intestinal epithelial goblet cell line HT29-H. *Pharm. Res.,* **10**:843–852 (1993).
17. G. Pang, A. Buret, E. O'Loughlin, A. Smith, R. Batey, and R. Clancy. Immunologic, functional and morphological characterization of three new human small intestinal epithelial cell lines. *Gastroenterology,* **111**:8–18 (1996).
18. A. Wikman and P. Artursson. Co-cultures of human intestinal goblet (HT29-H) and absorptive (Caco-2) cell for studies of epithelial transport and integrity. *Eur. J. Pharm. Sci.,* **3**:171–183 (1995).
19. A. Quaroni and J. Hochman. Development of intestinal cell culture models for drug transport and metabolism studies. *Adv. Drug. Deliv. Rev.,* **22**:3–52 (1996).
20. W. H. Moos, G. D. Green, and M. R. Pavia. Recent advances in the generation of molecular diversity. *Annu. Rep. Med. Chem.,* **28**:315–324 (1993).
21. E. W. Taylor, J. A. Gibbons, and R. A. Braeckman. Intestinal absorption screening of mixtures from combinatorial libraries in the Caco-2 model. *Pharm. Res.,* **14**:572–577 (1997).