Commentary

Theme: Landmark Publications in the Pharmaceutical Sciences: The Backstory

Editor: Ho-Leung Fung

Hidalgo, I. J., Raub, T. J., and Borchardt, R. T.: Characterization of the Human Colon Carcinoma Cell Line (Caco-2) as a Model System for Intestinal Epithelial Permeability, *Gastroenterology*, 96, 736–749, 1989—The Backstory

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In 1983, I was offered the opportunity to become Chairman and Solon E. Summerfield Distinguished Professor in the Department of Pharmaceutical Chemistry in the School of Pharmacy at The University of Kansas (KU), Lawrence, KS. Prior to that time, I had been a faculty member in the Departments of Biochemistry and Medicinal Chemistry at KU for 12 years and my research interests were focused on the design and synthesis of enzyme inhibitors having potential medical applications in the treatment of viral and parasite infections and neurological diseases.

My decision to accept these new academic positions in KU's Department of Pharmaceutical Chemistry was based in part on the opportunities that I saw to expand the scientific breadth of this department to include pharmaceutical biotechnology and cellular and molecular biopharmaceutics. Through the leadership provided by the late Professor Takeru Higuchi, who was the founder and the first Chairman of KU's Department of Pharmaceutical Chemistry, this department had already earned a national and international reputation for excellence in the application of physical, physical organic, and analytical chemistry to the formulation and delivery of organic chemistry-based drug candidates. In addition, I saw in

this move to the Department of Pharmaceutical Chemistry the opportunity to expand my personal research interests into pharmaceutical biotechnology with a focus on elucidating chemical pathways of peptide and protein degradation and the development of chemical and formulation strategies for stabilization of biotechnology-based drug candidates to chemical degradation. I also saw an opportunity to expand my personal research interests into cellular and molecular biopharmaceutics, an area that I felt would become very important to drug discovery scientists as they attempted to “design drugs with ADME (absorption, distribution, metabolism, excretion) in mind.” This new drug discovery paradigm, which started in the late 1980s in some companies, focused on designing drugs with optimal affinity and specificity for their pharmacological targets as well as optimal ADME properties, thus enhancing the delivery of the drugs to their pharmacological targets in animals and humans.

Shortly after assuming my new responsibilities as Chairman and Professor in KU's Department of Pharmaceutical Chemistry, I began to realize the full impact of this career change, particularly the impact it would have on my research interests. For example, this career change required me to “phase out” most of the drug discovery-oriented research projects ongoing in my laboratory in 1983 because they had been designed for graduate students and postdoctoral fellows with research interests in biochemistry and medicinal chemistry. While I was phasing out productive research

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projects that had contributed significantly to my early successes in academia, I had to “phase in” new research projects in the areas of cellular and molecular biopharmaceutics and pharmaceutical biotechnology, which I felt were more suited to the interests of graduate students and postdoctoral fellows with research interests in pharmaceutical chemistry.

One aspect of cellular and molecular biopharmaceutics that immediately attracted my interest was the idea of developing cell culture models of the blood–brain barrier (BBB) and the intestinal mucosa. I envisioned these *in vitro* cell culture models as having significant potential for estimating the oral absorption and the brain permeation of drug candidates, thus reducing the need to conduct expensive and sometime controversial animal experiments.

I was fortunate early in this career to change from a “biochemist/medicinal chemist” to a “pharmaceutical chemist” to be able to establish research collaborations with some excellent scientists at The Upjohn Company (Kalamazoo, MI) and INTERx Research Corporation-Merck Sharp and Dohme Research Laboratories (Lawrence, KS). Both of these companies had established by the early 1980s drug delivery groups that had “physiology/cell biology orientations” to their research and development activities. I was also fortunate that senior managers in both companies saw significant merit in the new research programs that we were setting up at KU; thus, they were willing to provide financial support to establish these new university–industry collaborations.

Our efforts to develop cell culture models of the major biological barriers (e.g., intestinal mucosa, BBB) that limit drug delivery started in 1984 when Dr. Ken Audus joined my research group as a postdoctoral fellow. For the next 2 years, Ken focused his research in my laboratory on the development and characterization a cell culture model of the BBB using primary cultures of bovine brain microvessel endothelial cells (BMECs) grown onto nylon mesh (1). After Ken completed his postdoctoral training, he accepted an appointment in KU’s Department of Pharmaceutical Chemistry as an Assistant Professor and focused his independent research programs on refining this BMEC cell culture model and using it to conduct mechanistic studies of drug transport. Ken is now a Professor of Pharmaceutical Chemistry and Dean of KU’s School of Pharmacy.

With respect to developing a cell culture model of the intestinal mucosa, this proved to be very

challenging. Based on the scientific literature in the mid-1980s, it was apparent that earlier attempts by cell biologists to either culture intestinal epithelial cells or to establish cell lines from enterocytes had been unsuccessful. Therefore, in 1984, my laboratory entered into collaboration with scientists at INTERx Research Corporation-Merck Sharp and Dohme Research Laboratories to assess the utility of brush border membrane vesicles, isolated mucosal cells, and everted intestinal rings to estimate oral absorption and/or to elucidate pathways of oral absorption of drugs. While the experiments conducted as part of this KU–INTERx research collaboration generated some valuable insights into the utility of these tissue, cell, and subcellular models of the intestinal mucosa to estimate oral absorption and/or to elucidate pathways of oral absorption of drugs (2–4), the experiments also highlighted some of the weaknesses of these models. Basically, these tissue, cell, and subcellular models of the intestinal mucosa were technically challenging to set up, the materials (i.e., cells, tissues) were derived from animals and not humans, the preparations lacked robustness and were not readily amenable to automation, and for all practical purposes, the experiments could provide some valuable information about a drug’s cellular uptake but very little information about a drug’s transcellular transport.

Therefore in 1986, when Dr. Ismael J. Hidalgo joined my research group as a Postdoctoral Fellow, we revisited the idea of developing a cell culture model of the intestinal mucosa. From our perspective, the “ideal cell culture model” would consist of human intestinal mucosal cells grown onto microporous membranes that would form monolayers with tight junctions and polarized expression of key transporters and metabolic enzymes. This “ideal cell culture model” might then be used to study transcellular transport of drugs. Alternatively, experiments might be designed using this “ideal cell culture model” to determine polarized cellular uptake and/or efflux of drugs across either the apical membrane or basolateral cell membrane. In addition, this “ideal cell culture model” had to be robust and amenable to automation, which would then allow it to be used in a drug discovery setting.

Upon joining my research group in 1986, Ismael conducted a thorough literature search focusing on finding examples of human cell lines that exhibited some or all of the morphological and biochemical properties of intestinal mucosa cells *in vivo*. Ismael’s literature-searching efforts identified several papers published in the 1970s and

early 1980s, which described human colon adenocarcinoma cell lines (i.e., Caco-2, HT-29, SW1116, LS174T) that underwent enterocytic differentiation in culture (5–7). Ismael immediately focused his laboratory work on Caco-2 and HT-29 cells, which were both readily available from ATCC (Rockville, MD). Ismael's initial experiments with HT-29 cells suggested that it was very difficult to reproducibly stimulate these cells to differentiate by altering the culture media as suggested in the literature. In addition, the HT-29 cells tended not to consistently form confluent cell monolayers. Therefore, Ismael quickly refocused his laboratory work toward Caco-2 cells, which were reported to spontaneously differentiate into polarized, confluent monolayers with tight cellular junctions in approx. 15–20 days. Once Ismael was able to optimize the conditions for growing Caco-2 cells on polycarbonate membranes (i.e., Transwell®), experiments could then be designed and undertaken to characterize and validate this Caco-2 cell monolayer as a model of the intestinal mucosa.

While Ismael and several other Postdoctoral Fellows and graduate students in my research group at KU had the technical skills to characterize the transport properties of the Caco-2 cell monolayers grown on Transwell® polycarbonate membranes, we realized that a collaboration with Dr. Thomas J. Raub, a recently hired cell biologist at The Upjohn Company (Kalamazoo, MI), would enhance our ability to characterize these Caco-2 cell monolayers. Through my consulting activities with The Upjohn Company, I had met Tom and identified him as a very skilled cell biologist, who had the expertise to characterize the Caco-2 cell monolayers grown on Transwell® polycarbonate membranes from the morphological and the biochemical perspectives. Subsequently, Ismael and Tom began to collaborate on this project in early 1988. This research collaboration required Ismael to visit The Upjohn Company several times in order to transfer the technology for growing Caco-2 cells in a Transwell® configuration to Tom's laboratory and for them to conduct experiments designed to characterize the morphological and the biochemical properties of the Caco-2 cell monolayers. This KU–Upjohn collaboration ultimately led to poster presentations at the American Cell Biology Society meetings in 1988 (8) and 1989 (9) and the publication of a seminal paper in *Gastroenterology* in 1989 (10). In addition, Ismael and other scientists from my research group at KU made several poster presentations at the American Association of Pharmaceutical Scientists meetings

in 1988 (11, 12) and 1989 (13, 14). These poster presentations started the process of characterizing the transport properties of Caco-2 cell monolayers grown on Transwell® polycarbonate membranes. In September 1989, we had the first opportunity to present our cell culture work at a NATO Advanced Research Workshop entitled “Drug Transport in Cell Culture,” which was held in Bandol, France. This international meeting provided pharmaceutical scientists worldwide the opportunity to present their most recent experimental data, to discuss the pros and cons of using cell culture systems (e.g., Caco-2 cells) as *in vitro* surrogates of biological barriers (e.g., intestinal mucosa), and to meet and interact with Professor Alain Zweibaum, whose pioneering work on Caco-2 cells stimulated and guided pharmaceutical scientists to examine the drug transport properties of this human colon carcinoma cell line (6, 7).

Over the next 2 years (1990–1991), our laboratory started to publish experimental data on the transport properties of Caco-2 cell monolayers grown on Transwell® polycarbonate membranes (15–21). During this same period, other academic and industrial laboratories, including Professor Per Artursson's laboratory at Uppsala University in Uppsala, Sweden (22–25) and Dr. Philip S. Burton's laboratory at The Upjohn Company in Kalamazoo, MI (26, 27) began publishing their experimental data describing the transport properties of this cell culture model of the intestinal mucosa.

The flurry of poster and podium presentations at national and international meetings and the publication of numerous peer-reviewed papers by academic and industrial laboratories from 1988 to 1991 stimulated significant interest among pharmaceutical scientists worldwide about the potential use of this cell line as a surrogate for the intestinal mucosa. For example, starting in 1988, many scientists from North America, Europe, and Asia visited our laboratory at KU to be trained on growing Caco-2 cell monolayers on Transwell® polycarbonate membranes and on using these cell monolayers to conduct drug transport experiments. To accommodate the numerous requests to visit our laboratory for training, we decided in 1988 to offer a short course (4 days) entitled “Fundamental Aspects and Applications of Cell Culture Models in Drug Transport and Metabolism Studies.” This short course included background lectures on the various biological barriers to drug delivery and cell culture techniques as well as practical laboratory training on culturing cells and con-

ducting drug transport experiments using these cell culture models. The presentation of this short course at KU and the frequent visits by academic and industrial scientists for technical training in our laboratory continued well into the early 1990s. By this time, experiments using Caco-2 cell monolayers grown on Transwell® polycarbonate membranes were being conducted in academic and industrial laboratories worldwide and the numbers of poster presentations at national and international meetings and published papers dealing with Caco-2 cell monolayers as a model of the intestinal mucosa were growing significantly.

Through the mid-1990s, my laboratory at KU continued to contribute to the "Caco-2 field" by publishing numerous papers describing the results of our studies, which at that time were largely focused on elucidating the functional transport and metabolic properties of Caco-2 cells. In the late-1990s, my research group became a "user" of this cell culture model of the intestinal mucosa in our experiments designed to characterize the transport properties of peptides, peptido-mimetics, and cyclic prodrugs of peptides.

After Ismael completed his postdoctoral training in 1990, he entered the pharmaceutical industry, first working at Glaxo-SmithKline and then at Rhone-Poulenc Rorer. In 1996, Ismael co-founded Absorption Systems, Inc where he currently serves as the company's Chief Scientific Officer. After our collaboration ended, Tom continued to excel as a scientist at The Upjohn Company until 2003 when he moved to Eli Lilly and Company where he currently serves as a Research Fellow and Director of Pre-Lead ADME and Computational ADME/Toxicology.

Like other scientists who have made important scientific discoveries, the authors of the 1989 Gastroenterology paper, which describes for the first time the use of Caco-2 cell monolayers as a model of the intestinal mucosa (10), did not foresee the significance and the impact of their research findings on the pharmaceutical sciences. While the specific details about Caco-2 cell monolayers as a potential model of the intestinal mucosa turned out to be important to pharmaceutical scientists, I firmly believe that this research also lead to a radical change in the methodology that pharmaceutical scientists were willing to use to study drug transport across biological barriers. This radical change involved moving from animal, tissue, and isolated cell models to cell culture models. The eventual acceptance by many pharmaceutical scientists of Caco-2 cells as a model of the intestinal mucosa

in the mid-1990s emboldened other pharmaceutical scientists to develop cell culture models for other biological barriers to drug delivery.

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