

SESSION II:

**In Vitro and In Vivo Correlations: Applications in Animal Drug
Development and Regulation**

Chair: Marilyn N. Martinez

***In Vitro* and *In Vivo* Correlations: Applications in Animal Drug Development and Regulation**

Marilyn N. Martinez, Ph.D.
Center for Veterinary Medicine
Food and Drug Administration

This session focused on recent progress in our understanding of the physiologic and physico-chemical factors affecting oral drug absorption. Numerous physiologic and formulation variables impact the rate and extent of oral drug absorption. These can be summarized as follows:

1. Dissolution:

A. Physico-Chemical Properties of the Drug

- Aqueous solubility
- Drug pKa
- Particle size, density and effective surface area
- Chemical stability
- Boundary layer thickness and drug diffusivity

B. Physiological Variables

- GI motility
- Volume of contents
- Composition of GI contents
- Flow rate of contents
- pH
- Blood flow
- GI surfactants

2. Absorption of Dissolved Drug:

- Enzymes (e.g., cytochrome P450 system, hydrolases, etc.)
- Efflux proteins (e.g., the p-glycoproteins)
- Local pH
- Drug pKa
- Effective permeability
- Hydrogen bonding with membrane constituents
- Lipid/aqueous solubility
- Conformational changes of drug in membrane (e.g., cyclosporin)
- Drug interaction with mucous barrier layer
- Membrane carriers and receptors (carrier mediated transport)
- Integrity of tight junctions (paracellular transport)

Biopharmaceutical scientists are becoming increasingly cognizant of the differences in mucosal properties that exist across the length of the GI tract of both human and veterinary species (e.g., absorptive surface, lumen composition, carriers, efflux proteins, and metabolic enzymes). Since

this heterogeneity can lead to poor oral bioavailability, it must be considered when developing oral dosage forms. Possible sources of incomplete drug absorption include:

1. Failure of the formulation to deliver solubilized drug to the site(s) of absorption.
2. Decomposition of drug in the GI tract
3. Formation of a nonabsorbable complexes
4. Poor permeability from the apical to basal direction.
5. Drug metabolism and/or elimination en route to the systemic circulation
 - A. Gut metabolism
 - Bacterial
 - Mucosal enzymes
 - Luminal enzymes
 - B. Liver metabolism
 - C. Biliary excretion

These potential sources of incomplete drug absorption reflect the complex inter-relationships that exist between animal physiology, drug physico-chemical properties and product formulation. By understanding these inter-relationships, we enhance our ability to predict potential problems in oral drug delivery. Pharmaceutical scientists can use this information to facilitate the development of alternative formulations or modified methods of drug delivery. These formulation changes may be sought as a method for improving user compliance and/or increasing oral bioavailability. Regulatory scientists can apply this information toward the development of *in vitro* methods for evaluating *in vivo* drug bioavailability. Furthermore, in identifying the critical variables associated with the absorption of specific compounds, we strengthen our ability to predict differences in product bioavailability that may exist across a population of animals differing with respect to age, gender, breed, disease condition, prandial state, and concomitant drug use. Ultimately, this will lead to better dosing instructions on approved product labels.

Recent advances in oral drug absorption were the subjects of presentations by the world-renowned pharmacokinetics experts, Leslie Z. Benet, Ph.D. and Gordon L. Amidon, Ph.D. Dr. Benet's presentation focused on intestinal multi-drug resistance proteins and the cytochrome P450 enzymes as barriers to oral drug delivery. Dr. Amidon spoke about permeability and solubility barriers to oral drug absorption. The significance of these two topics is well recognized within the human pharmaceutical community, and many aspects of these presentations have already been incorporated into human drug regulatory guidances.

Individuals interested in these topics may wish to explore the following guidances published by the Center for Drug Evaluation and Research (CDER):

1. GUIDANCE FOR INDUSTRY: Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies *In Vitro* (April 1997).
2. GUIDANCE: Dissolution Testing of Immediate Release Solid Oral Products. (August 1997)
3. GUIDANCE: Extended Release Dosage Forms: Development, Evaluation, and Application of *In Vitro/In Vivo* Correlations (September 1997).

4. CDER's GUIDANCE: Waiver of *In Vivo* Bioavailability And Bioequivalence Studies For Immediate Release Solid Oral Dosage Forms Containing Certain Active Moieties/Active Ingredients Based on a Biopharmaceutics Classification System (January 1999)
5. SUPAC GUIDANCE DOCUMENTS (Scale-Up and Postapproval Changes)
 - Guidance for Industry: Immediate Release Solid Oral Dosage Forms. Scale-up and Postapproval Changes: Chemistry, Manufacturing, and Controls, *In Vitro* Dissolution Testing, and *In Vivo* Bioequivalence Documentation (November, 1995)
 - Guidance for Industry: SUPAC-MR: Modified Release Solid Oral Dosage Forms (October 1997).

CDER guidances can be accessed through the web at <http://www.fda.gov/CDER/guidance>.

For additional reading, the following citations may also be of interest:

1. Kararli, T. (1995). Comparison of the gastrointestinal anatomy, physiology and biochemistry of humans and commonly used laboratory animals. Biopharm. Drug Disp., 16: 351-380.
2. Gali, E., Nicolaidis, E., Horter, D., Lobenberg, R., Reppas, C. and Dressman, J.B. (1998). Evaluation of various dissolution media for predicting *in vivo* performance of Class I and II drugs. Pharm. Res., 15: 698-705.
3. Piquette-Miller, M., Pak, A., Kim, H., Anari, R. and Shahzamani, A. (1998). Decreased expression and activity of p-glycoprotein in rat liver during acute inflammation. Pharm. Res., 15: 706-711.
4. Arimori, K. and Nakano, M. (1998). Drug exsorption from blood into the gastrointestinal tract. Pharm. Res., 15: 371-376.
5. Ito, K., Kusuhara, H. and Sugiyama, Y. (1999). Effects of intestinal CYP3A4 and p-glycoprotein on oral drug absorption – theoretical approach. Pharm. Res., 16: 225-230.
6. Kaus, L.C., Gillespie, W.R., Hussain, A.S. and Amidon, G.L. (1999). The effect of *in vivo* dissolution, gastric emptying rate and intestinal transit time on the peak concentration and area-under-the-curve of drugs with different gastrointestinal permeabilities. Pharm. Res., 16: 272-280.
7. Shastri, S., Mrosczak, E., Prichard, R.K., Parekh, P., Nguyen, T.H., Hennessey, D.R. and Schiltz, R. (1980). Relationship among particle size distribution, dissolution profile, plasma values and anthelmintic efficacy of oxfendazole. Am. J. Vet. Res., 41: 2095-2101.
8. Michalets, E.L. (1998). Update: clinically significant cytochrome p-450 drug interactions. Pharmacotherapy, 18: 84-112.

Reference recommended by speaker:



Advanced Drug Delivery Reviews 20 (1996) 99-112

advanced
drug delivery
reviews

Active secretion and enterocytic drug metabolism barriers to drug absorption

Vincent J. Wacher^a, Laurent Salphati^b, Leslie Z. Benet^{b,*}

^aAvMax Incorporated, 890 Heinz Avenue, Berkeley, California 94710, USA

^bDepartment of Pharmacy, University of California, San Francisco 94143-0446, USA

Abstract

Intestinal phase I metabolism and active extrusion of absorbed drug have only recently been recognized as major determinants of oral drug bioavailability. Both CYP3A4, the major phase I drug metabolizing enzyme in humans, and the multidrug efflux pump, P-glycoprotein (P-gp), are present at high levels in the villus enterocytes of the small intestine, the primary site of absorption for orally administered drugs. Moreover, these proteins are induced by many of the same compounds and demonstrate a broad overlap in substrate and inhibitor specificities, suggesting that they act as a concerted barrier to drug absorption. Clinical studies have demonstrated that inhibition of CYP3A4-mediated intestinal metabolism can significantly improve the oral bioavailability of a wide range of drugs. Intestinal P-gp is a major route of elimination for both orally and intravenously administered anticancer drugs in animal models, and experiments with the Caco-2 cell line have provided strong evidence that inhibition of intestinal P-gp is another means by which oral drug bioavailability could be enhanced.

Keywords: P-glycoprotein; Cytochrome P450 3A; Bioavailability; Intestine; Metabolism; Active transport

Reference recommended by speaker:

Pharmaceutical Research, Vol. 12, No. 3, 1995

A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of *in Vitro* Drug Product Dissolution and *in Vivo* Bioavailability

Gordon L. Amidon,^{1,2} Hans Lennernäs,³
Vinod P. Shah,^{4*} and John R. Crison⁵

Received March 10, 1994; accepted October 3, 1994

A biopharmaceutics drug classification scheme for correlating *in vitro* drug product dissolution and *in vivo* bioavailability is proposed based on recognizing that drug dissolution and gastrointestinal permeability are the fundamental parameters controlling rate and extent of drug absorption. This analysis uses a transport model and human permeability results for estimating *in vivo* drug absorption to illustrate the primary importance of solubility and permeability on drug absorption. The fundamental parameters which define oral drug absorption in humans resulting from this analysis are discussed and used as a basis for this classification scheme. These Biopharmaceutic Drug Classes are defined as: Case 1. High solubility-high permeability drugs, Case 2. Low solubility-high permeability drugs, Case 3. High solubility-low permeability drugs, and Case 4. Low solubility-low permeability drugs. Based on this classification scheme, suggestions are made for setting standards for *in vitro* drug dissolution testing methodology which will correlate with the *in vivo* process. This methodology must be based on the physiological and physical chemical properties controlling drug absorption. This analysis points out conditions under which no *in vitro-in vivo* correlation may be expected e.g. rapidly dissolving low permeability drugs. Furthermore, it is suggested for example that for *very rapidly* dissolving high solubility drugs, e.g. 85% dissolution in less than 15 minutes, a simple one point dissolution test, is all that may be needed to insure bioavailability. For slowly dissolving drugs a dissolution profile is required with multiple time points in systems which would include low pH, physiological pH, and surfactants and the *in vitro* conditions should mimic the *in vivo* processes. This classification scheme provides a basis for establishing *in vitro-in vivo* correlations and for estimating the absorption of drugs based on the fundamental dissolution and permeability properties of physiologic importance.

KEY WORDS: bioavailability; drug absorption; mathematical modeling; *in vitro-in vivo* correlation; intestinal permeability.

