Commentary

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Scientific Perspectives on Extending the Provision for Waivers of *In vivo* Bioavailability and Bioequivalence Studies for Drug Products Containing High Solubility-Low Permeability Drugs (BCS-Class 3)

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Abstract. Recently, there has been increased interest in extending the provision for waivers of *in vivo* bioavailability and bioequivalence (BA–BE) studies that appeared in the guidance published by the Food and Drug Administration (FDA) (1) to pharmaceutical products containing Class 3 drugs (High solubility–Low Permeability). The extension of the Biopharmaceutics Classification System (BCS) to Class 3 drugs is meritorious because of its impact on public health policy considerations. The rate limiting step in the absorption of Class 3 drugs is the permeability through the intestinal membrane. This commentary will focus its attention on the scientific considerations which need to be examined to assess the risk and the benefit prior to granting a waiver of *in vivo* bioavailability and/or bioequivalence studies for Class 3 drugs. It will examine the forces affecting the interconnectivity of the neuronal, immunological and hormonal systems in the gastrointestinal tract that may affect its permeability and functionality. It will also challenge the assumption that *in vitro* dissolution and *in vitro* permeability studies in tissue cultures in the presence and absence of excipients are good predictors for *in vivo* dissolution and *in vivo* permeability which are at the heart of the BCS.

KEY WORDS: BCS Class 3 drugs; biopharmaceutics classification system; biowaivers; excipients.

INTRODUCTION

Health-care spending in the USA will hit \$4.3 trillion by 2017, nearly double the 2007 amount. That would equate to nearly 20% of gross domestic product. In 2007, health-care spending accounted for 16.3% of GDP (2). In 2007, pharmaceutical companies increased wholesale prices for the 50 top-selling branded drugs by an average of 7.82%, after increases of 6.73% and 6.22% in the previous two years. The most recent increase is almost double the overall US economy's 4.1% annual inflation rate last year (3). The Congressional Budget Office estimates that generic drug use results in savings of \$10 billion per year. During the next few years, \$60 to \$70 billion in brand name drugs will lose their patent protection, and FDA must be poised to respond to the growing number of generic drug applications to help ensure that consumers enjoy a wide selection of lower-cost generic drugs (4). The increased use of generic products has resulted in an increased number of filings of Abbreviated New Drug Applications (ANDAs) with the Office of Generic Drug

Products (OGD) of the FDA. In 2007, the total number of ANDAs reached 683 consisting of 188 tentative approvals and 495 full approvals (5). This number of ANDAs will continue to increase because of the increased number of brand name drug products that are expected to lose their patent protection. The FDA has estimated that the OGD will likely receive 857 applications in FY 2008. Extension of BCS biowaivers to Class 3 drugs may result in the acceleration of generic drug approvals. The impact would be greater if the provision of waivers in the FDA guidance could be expanded to Class 2 drugs. In addition, developing countries that do not have the infrastructure to perform BA–BE *in vivo* studies will also benefit by the extension of the biowaivers provision of BCS to drug products that contain BCS Class 3 drugs. The exuberance elicited by the proposal to extend the biowaivers provision to Class 3 drugs needs to be tempered by a careful scientific evaluation of the risks and benefits that may result from this extension.

A provision for waivers of *in vivo* BA–BE studies (biowaivers) under certain conditions is provided in the Federal Register 21 CFR 320.22. In addition, the Food and Drug Administration published a guidance based on the work of Amidon and co-workers (1,6) that explains when biowaivers can be requested for immediate release (IR) solid oral dosage forms based on the BCS. The BCS takes into account three major factors that govern the rate and extent of drug absorption from IR solid oral dosage forms: *in vivo*

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dissolution, solubility, and intestinal permeability. According to the BCS, drug substances are classified as follows:

- Case 1: High Solubility–High Permeability
- Case 2: Low Solubility–High Permeability
- Case 3: High Solubility–Low Permeability
- Case 4: Low Solubility–Low Permeability

Observed *in vivo* differences in the rate and extent of absorption of a drug from two pharmaceutically equivalent solid oral products may be due to differences in drug dissolution *in vivo*. However, when the *in vivo* dissolution of an IR solid oral dosage form is rapid in relation to gastric emptying and the drug has high permeability, the rate and extent of drug absorption is unlikely to be dependent on drug dissolution and/or gastrointestinal transit time. Under such circumstances, demonstration of *in vivo* BA or BE may not be necessary for drug products containing Class 1 drug substances, as long as the inactive ingredients used in the dosage form do not significantly affect absorption of the active ingredients. The BCS approach outlined in the FDA guidance can be used to justify biowaivers for highly soluble and highly permeable drug substances (i.e., Class 1) in IR solid oral dosage forms that exhibit rapid *in vitro* dissolution. The American Association of Pharmaceutical Scientists (AAPS) held a Workshop in May of 2007 in which the author was invited to discuss if Class 3 drugs should be considered for biowaivers (7). This commentary summarizes some of the information presented by the author at the workshop. The BCS framework can be very useful if the assumptions on which it was founded are truly enforced. The scientific community is in agreement that BCS is a useful tool for regulatory agencies to grant waivers from *in vivo* BA–BE studies and that granting waivers for drug products containing Class 1 drug substances does not appear to pose a safety or efficacy problem. However, regulatory agencies should be careful in evaluating products containing new excipients, and highly variable drugs. It should be noted that even if the drug is in solution does not ensure fast absorption.

Extension of biowaivers to drug products containing Class 3 drugs needs to be based on scientific evidence to prevent potential safety and efficacy problems. This commentary raises scientific issues that regulatory agencies should examine when considering granting a biowaiver of *in vivo* BA–BE studies for drug products containing a Class 3 drug. The real question is: What scientific evidence exists to justify this extension.

Class 3 contains high solubility/low permeability drug substances. Thus, it is safe to assume that the intestinal permeability is considered to be the rate-controlling step in oral drug absorption. This implies that the absorption kinetics of BCS Class 3 drugs from the gastrointestinal (GI) tract could be controlled by the biopharmaceutic and physiologic properties of the drug substance, rather than formulation factors, provided excipients do not affect drug permeability or drug intestinal residence time. The assumptions that excipients do not affect drug permeability and intestinal transit time are significant assumptions that deserve further investigation. Human studies to determine excipient effects in a dose-response fashion could be considered unethical, not practical, and cost prohibitive. *In vitro* culture studies using Caco-2 cells have been conducted to determine the effect of

some common excipients (8). Briefly, the objective of their work was to evaluate the effect of nine individual excipients on the Caco-2 permeability of seven low-permeable compounds that differ in their physiochemical properties. Although these studies were well conducted, the authors concluded that further work is needed to interpret the *in vivo* consequences of the observations made from the cell culture experiments. Additionally, the authors concluded that generally, most excipients had no influence on drug permeability. This statement suggests that some excipients did influence drug permeability. In fact, the authors reported that sodium lauryl sulfate did influence the permeability of almost all drugs tested. Tween 80 significantly increased the apical-to-basolateral direction permeability of furosemide, cimetidine, and hydrochlorothiazide, presumably by inhibiting their active efflux, without affecting mannitol permeability. Additionally, docusate sodium moderately increased cimetidine permeability. Cell culture methods are easily impacted by solvents used to facilitate the solubility of drugs and or excipients due to volume limitations. In addition, studies clearly show that the behavior of the gastrointestinal tract is controlled by immunological, neuronal and hormonal controlling mechanisms (9). It can not be ignored that these physiological systems within the wall in the gut are functionally interconnected and that cell culture methods can not yield any information on these mechanisms. Excipients can have a profound effect on the functionality and interconnectivity of these mechanisms. Dose excipients-response relationships need to be established to better understand their impact in the translocation of a solute from one side of the membrane to the other side. This will help prevent the risk that granting biowaivers for class 3 drugs may pose on the safety and efficacy of drugs products. This risk is minimized if the same excipients used in the reference product are used in the test product. However, the risk increases as newer and more efficient excipients are used in the manufacturing process. Thus, careful attention should be given to the excipients used. The most conservative approach is to use the same excipients.

The risks associated with biowaivers of Class 3 drugs or those of any other class may also be related to the overconfidence that we have placed on *in vitro* dissolution. *In vitro* dissolution has served well during formulation development, and to help control the quality of drug products, but it has not been efficient in predicting bioavailability of drug products. There are occasions when the *in vitro* dissolution rate or profile appears to be the same in the statistical sense, but the products are found to be bioinequivalent. Thus, the risk of using *in vitro* dissolution as a surrogate for *in vivo* dissolution which is one of the basic assumptions of the BCS should not be underestimated. The failure of *in vitro* dissolution to predict bioavailability may be related to the principle of solute-solvent interactions and its influence on solubility and solubilization which has been studied extensively (10–17). The Hildebrand–Scatchard model (10–11) has been extended to handle the solubility prediction of drugs in polar solvents. Adjei used this model to study the membrane solubility parameter and *in situ* release of theophylline (18). Figure 1 shows that the closer the solubility parameter of theophylline and the solubility parameter of a particular solvent mixture, the greater the

Fig. 1. Disappearance [of ^{14}C salicylic acid from the gut lumen with 20% glycerol (filled triangle), 20 % PEG 4000 (filled square), 10% ethanol (open circle), and control (filled circle). Vertical bars correspond to one standard deviation (20)

attraction of solvent vehicle for the drug. A solvent whose solubility parameter is identical to that of theophylline would, according to regular solution theory, lead to the greatest solubility of the drug, barring solute-solvent interactions. The authors concluded that the interplay of multiple physico-chemical attractive forces in the drug release process is due to differences between the membrane parameter and that of the solvent and solute. Thus, the membrane solubility parameter would be important in evaluating the effects of solvent parameters on drug absorption. Figure 1 shows that as the solubility parameter of the solvent gets closer to that of the solute, there is a rapid fall in the *in situ* partition parameter and a significant increase in half-life for absorption (disappearance from the intestine) of theophylline. This was considered to be because of stronger solute-solvent interactions. Thus, the fact that the drug is in solution does not guarantee faster absorption. Inspection of the lines in Fig. 1 and the parameters of Eq. 1 used by the authors reveals a dependence of K_p (drug partitioning parameter) on δ_2 and δ_0 , the membrane solubility parameter.

$$\log K_p = \frac{V_2 \Phi_1}{2.303RT} [(\delta_1 - \delta_2) - (\delta_0 - \delta_2)^2] \quad (1)$$

Where R is the gas constant, T is the absolute temperature, and V_2 is the liquid molar volume of the solute, the terms Φ_1 and δ_1 are, respectively, the volume fraction and the solubility parameter, and δ_2 and δ_0 are, respectively, the solubility parameter of the solute and the solubility parameter of the membrane.

The authors concluded that a solvent mixture for optimum drug release and bioabsorption would have a solubility parameter that operates in conjunction with δ_2 and δ_0 so that the solubility and partitioning effects were simultaneously changed. The solubility parameter of the drug

and the solvent vehicle must be sufficiently different to allow ready release of theophylline from the solvent, yet must be similar enough to provide a reasonable concentration of the drug. Also δ_2 and δ_0 must be sufficiently alike to favor drug absorption into the membrane.

Loper and Stavchansky (19) studied the effect of solvent composition on drug absorption, and concluded that even when drugs dissolved in the gastrointestinal fluid without incident and were readily transported to the small intestine, the properties of the solvent would affect absorption (Fig. 2). Ethanol has been shown to affect glucose and water transport (20). Houston and Levy reported that various alcohols increased net water flux with concomitant increase in theophylline absorption from solution in rats (21). The results of all of these investigations although conducted in several solvents that are not often used in solid dosage forms suggest that excipients in pharmaceutical preparations can not only affect permeability and solubility, but also membrane partitioning. It is clear from these investigations that even if the drug is in solution, which is the ideal environment for drug absorption, differences in drug permeability are observed. Thus, in extending the provision of biowaivers for Class 3 drugs it is suggested that proper studies of excipients be conducted. Again, the most conservative approach would be to use the same excipients.

Another scientific consideration that should not be underestimated is the effect of the "unstirred layer" (UL). The UL is a water-layer adjacent to the membrane that is not subject to the same gross mixing that take place in the rest of the medium. The UL can be thought as a region of slow

Fig. 2. Influence of solubility parameter $(\delta_1 - \delta_2)$ on theophylline *in situ* partitioning (K_p) and the biological half-life ($t_{1/2}$) for absorption across the rat gut membrane. δ_1 is the solubility parameter of the mixed solvent; δ_2 is the solubility parameter of the drug, theophylline. Key (filled circle) half-life for theophylline absorption; (open circle) partition coefficient for theophylline absorption from solvent mixtures of varying solubility parameters (18)

laminar flow parallel to the membrane. The effective thickness of the UL may be as much as 500 μm . What is particularly significant is that the UL cannot be reduced below approximately 20 μm , even by vigorous mechanical stirring. The question that needs to be answered is when the UL is significant when studying the transport of drug molecules through a biological membrane like the epithelium of the small intestine. For example, assume that the typical diffusion coefficient for small solute particles in water is about $10^{-5} \text{ cm}^2/\text{sec}$ and that the effective thickness of a membrane is 100 μm then the permeability coefficient can be expressed as $10^{-5} \text{ cm}^2/\text{sec} / 10^{-2} \text{ cm} = 10^{-3} \text{ cm/sec}$. In this particular case the unstirred layer would become a significant factor in determining the flux through the membrane. The BCS for Class 3 drugs needs to take this into consideration because the rate limiting step in the transport across the intestinal membrane is the permeability coefficient of the drug. Thus, under these conditions, a correction of the permeability is advisable. If the flux is J and the concentration gradient is ΔC , the observed permeability is given by Eq. 2.

$$P_{\text{observed}} = J / \Delta C \quad (2)$$

However the intrinsic or true permeability would be given by Eq. 3.

$$P_{\text{true}} = J / [\Delta C - (\Delta C)_1 - (\Delta C)_2] \quad (3)$$

Each of the two concentration differences developed across the UL is related to the layer's thickness "h" and the approximate diffusion coefficients D_1 and D_2 given in Eqs. 4 and 5.

$$(\Delta C)_1 = \frac{Jh_1}{D_1} \quad (4)$$

and

$$(\Delta C)_2 = \frac{Jh_2}{D_2} \quad (5)$$

Thus,

$$P_{\text{true}} = \frac{1}{\frac{1}{P_{\text{observed}}} - \frac{h_1}{D_1} - \frac{h_2}{D_2}} \quad (6)$$

It should be noted that when the movement of lipophilic compounds through a membrane is fairly rapid, the effect of the UL becomes more significant and may become the rate limiting step in the permeability process. Thus, excipients that may impact the UL may change the translocation of a highly lipophilic drug (22–24).

Most biological membranes possess fixed charges on their surfaces (i.e., ionic channels, charged proteins). When a membrane having a uniform or discrete charge density is exposed to ionic excipients, a diffuse ionic layer may form near the surface, thus modifying the electrical potential it generates, probably impacting the translocation of ionic species. Also, extrinsic proteins (on the surface of the membrane) may be affected by excipients, resulting in an overall effect on drug permeability. Further studies in humans are needed to answer these intriguing scientific observations.

Another scientific consideration is the effect of excipients on the modulation of tight junctions. Tight junctions are cell-to-cell connections found in the epithelial and endothelial layers that regulate transport across the natural boundaries that comprise the intestinal mucosa or other mucosa such as the nasal mucosa, blood vessels and the blood brain barrier. It is possible that excipients may influence the modulation of the tight junctions and thus the paracellular transport of drug molecules. In fact, modulating tight junctions has been used to modulate the transport of drugs through the nasal mucosa (25).

Blume and Schug (26) have suggested that biowaivers for Class 3 drugs may be considered on a case-by-case basis. The assessments for a biowaiver must include a proper explanation for the low permeability of the drug throughout the GI tract, including the proximal colon. If there is an absorption window or a gradient in the permeability of the gut wall to the drug (with decreasing permeability in distal regions), excipients that accelerate gut motility could significantly reduce the residence time with the sites at which the permeability is favorable. This may result in lower bioavailability of the drug. Excipients that accelerate transit time in the upper gastrointestinal tract such as sodium acid pyrophosphate and sorbitol have been clearly shown to reduce the extent of absorption of ranitidine and cimetidine, respectively (26,27,28). Recently, PEG-400 was shown to significantly change intestinal transit time (29). It appears that "excipient effects" continue to be a potential area for further research. For example, "dose-response" effects of excipients could be systematically studied. Excipients that significantly affect the permeability *in vitro* include surfactants, fatty acids, medium chain glycerides, steroids, detergents, acylcarnitine and alkoyl cholines. Also, the effect of excipients on the solubility of the drug needs to be considered.

The excipients' antigenic effects can not be underestimated. It has been reported that alterations in microvillus surface area is a common response to a variety of insults and may represent an important adaptive response to limit absorption or attachment and invasion of injurious agents such as infectious organisms or allergenic food proteins. The accumulated evidence suggests that regulation of microvillus length and thus brush border surface area may constitute an additional physiological mechanism whereby the gut can regulate absorptive function (30). Recent experiment in our laboratories suggests that excipients such as olive oil may have an impact on the microvillus length obtained from the intestinal jejunum of rats. Transient shortening of the microvillus has been reported after cycloheximide treatment (30).

Intestinal anaphylaxis was examined in Hooded-Lister rats sensitized to egg albumin (31). The results suggested that the morphology of the small intestine assessed by light microscopy showed no abnormalities. There was no evidence of increased cellular infiltration in the lamina propria, and villous and crypt architecture appeared unaltered. However, differences were observed when quantitative measurements were made. Villous height was reduced and crypt depth increased in the proximal intestine of sensitized animals exposed to antigen. In addition, the authors reported significant decrease in brush border disaccharidase and maltase activity, suggesting injury to the microvillus mem-

brane as reflected by changes in the height of the microvilli. Further studies are needed with diverse excipients to fully understand their impact on the translocation of drugs from the intestinal membrane when the immune system is activated. Thus, luminal antigens, possibly including excipients, may affect the barrier function of the gut and the ability of the gut epithelium to prevent uptake of molecules, including immunologic molecules, and may also affect the movement of ions, water, and solutes such as drugs. In conclusion, immediate hypersensitivity reactions to luminal antigens occur in the intestine, resulting in changes in intestinal permeability and ion secretion.

Recently, Benet *et al.* (32,33) suggested the Biopharmaceutics Drug Disposition Classification System (BDDCS) for classifying the permeability of marketed drugs. This approach is interesting in that the classification is based on drug metabolism as a surrogate for drug permeability. They suggested that if the major route of elimination for the drug was metabolism, then the drug exhibited high permeability. However, if the major route of elimination was renal and biliary excretion of unchanged drug, then that drug should be classified as low permeability. The authors recommended to regulatory agencies that the extent of drug metabolism (i.e., $\geq 90\%$ metabolized) be used as an alternate method for the extent of drug absorption (i.e., $\geq 90\%$ absorbed) in defining class I drugs suitable for biowaivers of *in vivo* BA–BE studies. BDDCS may be extended to Class 3 drugs, and the excipients concerns may be minimized and/or eliminated if the extent of metabolism of the drug released from the drug product, not the drug substance administered alone, is reported to regulatory agencies. A thorough knowledge of the effect of excipients on drug metabolism in the intestinal wall and on the intestinal, hepatic, and biliary transporters is necessary.

Considerable progress has been made in the development of tools and techniques to study drug transport and the forces that affect the translocation of a solute from one side of the membrane to the other side. These advances have helped us understand the impact of solubility, partitioning, permeability, surface area, viscosity, transporters, transit time, and other factors on drug release and intestinal absorption. These efforts have resulted in the BCS and the BDDCS. However, further investigation is needed at the molecular level to understand the functional interconnectivity of the hormonal, neuronal, and immunological function of the gastrointestinal tract. Thus, the question of whether Class 3 drugs should be granted a waiver from *in vivo* bioequivalence studies is not a black and white decision. The decision and assessment of risk and benefit has to take into consideration the scientific issues that have been briefly raised in this commentary. Thus, waiver of *in vivo* bioequivalence studies for drug products containing Class 3 drugs should be done on a case-by-case basis. Furthermore, if the excipients in the test product are different from the excipients in the reference product, biowaivers of *in vivo* BA–BE studies should not be automatically granted, even if the products show similar *in vitro* dissolution profiles. The impact of the excipients on the physiological functionality of the gastrointestinal tract and the membrane architecture and function should be clearly understood. However, if the excipients are the same, one should expect similar *in vivo* performance. We need to move

away from generalities such as, “commonly used excipients will not affect the rate of absorption of highly soluble and highly permeable drugs”, and move in the direction of creating a national formulary of monographs for generic products, including excipients and the quality grade used in the reference product. This will reduce the uncertainties associated with drug permeability and its impact on bioequivalence assessment. We have the moral responsibility to reduce the number of studies in humans, increase access to and decrease cost of medicines, and to reduce the regulatory burden. However, the risk-benefit assessment should be done based on the best available science.

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