Evaluation of Excipient Risk in BCS Class I and III Biowaivers

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Abstract. The objective of this review article is to summarize literature data pertinent to potential excipient effects on intestinal drug permeability and transit. Despite the use of excipients in drug products for decades, considerable research efforts have been directed towards evaluating their potential effects on drug bioavailability. Potential excipient concerns stem from drug formulation changes (e.g., scale-up and post-approval changes, development of a new generic product). Regulatory agencies have established in vivo bioequivalence standards and, as a result, may waive the in vivo requirement, known as a biowaiver, for some oral products. Biowaiver acceptance criteria are based on the in vitro characterization of the drug substance and drug product using the Biopharmaceutics Classification System (BCS). Various regulatory guidance documents have been issued regarding BCS-based biowaivers, such that the current FDA guidance is more restrictive than prior guidance, specifically about excipient risk. In particular, sugar alcohols have been identified as potential absorption-modifying excipients. These biowaivers and excipient risks are discussed here.

KEY WORDS: bioavailability; bioequivalence; Biopharmaceutics Classification System (BCS); biowaiver; excipient.

INTRODUCTION

Following oral administration, solid dosage formulations must first disintegrate in the gastrointestinal (GI) tract and dissolve in solution for drug absorption to occur. Based on the drug’s physiochemical properties, intestinal permeation occurs by passive diffusion or active/facilitative transport (1). Of the fraction of the oral dose that is absorbed from the intestinal lumen, the fraction that becomes available in systemic circulation (i.e., is bioavailable) is further reduced by metabolism through the gut wall into the hepatic portal circulation, metabolism by first pass elimination through the liver, and biliary excretion (2).

Oral drug absorption is a process that is influenced by key biopharmaceutical and physiological factors. Important physiochemical properties of the drug include its solubility, intrinsic dissolution rate, ionization (pKₐ), lipophilicity (log P), stability, surface area, crystallinity, polymorphism, salt form, and molecular size. Physiological factors such as gastrointestinal pH, gastric emptying, small intestinal transit time, bile salts, and mechanisms of membrane permeability also influence oral drug absorption (3). Non-drug components of the dosage formulation, i.e., excipients, may also impact absorption of the drug. Excipients are typically used in dosage formulations to ensure manufacturability and content uniformity but are also used to modulate drug substance or active drug ingredient (API) stability and bioavailability.
Generally, excipients can potentially have an impact on drug absorption by altering the dosage formulation’s disintegration, dissolution, or stability, or by directly impacting GI physiological processes. It is well appreciated that excipients can alter drug release rate and/or extent of release from dosage formulations. However, there are several anticipated mechanisms through which excipients in the GI tract could impact drug absorption. For example, excipients may potentially modify GI transit time and luminal volumes, alter permeability, or modify metabolism within the GI tract (4). Osmotically active excipients such as sugar-alcohols (e.g., mannitol, sorbitol) and polyethylene glycol (PEG) 400 are known to potentially reduce drug absorption by increasing GI fluid volume, which in turn dilutes intraluminal drug concentration and reduces small intestinal transit time (5). However, other potential concerns such as excipient impact on drug membrane permeability have much less evidence of an effect in vivo (5–9).

Given the potential excipient risks to drug absorption, changes to drug formulations should consider excipient amount, mechanism(s) in which excipient may impact absorption, and the drug’s absorption properties (10,11). In vivo bioequivalence (BE) studies are generally needed to demonstrate a lack of impact of significant formulation changes on a drug’s bioavailability during its development, for post-approval manufacturing changes, and when developing generic products. A regulatory framework to provide regulatory relief based on the in vitro characterization of the drug substance and drug product, termed Biopharmaceutics Classification System (BCS), allows the waiving of clinical BE studies for some immediate-release (IR) solid oral dosage formulations. Not needing human BE trials provides a great benefit in that it reduces drug development costs and eliminates unnecessary clinical trials (12). For various reasons, in vitro studies are sometimes better than conventional human pharmacokinetic in vivo studies in assessing BE of IR solid oral dosage formulations (13).

**BCS CLASSIFICATION AND BCS-BASED BIOWAIVERS**

The BCS classifies orally administered immediate release drug products based on the fundamental principles that control the rate and extent of drug absorption, i.e., solubility, dissolution rate, and intestinal permeability. The categories are high solubility-high permeability (Class I), low solubility-high permeability (Class II), high solubility-low permeability (Class III), and low solubility-low permeability (Class IV) (14).

The first finalized US Food and Drug Administration (FDA) BCS guidance for industry was issued in August 2000 and indicated that evidence of BE via in vitro dissolution studies in lieu of in vivo pharmacokinetic profiles may be sufficient for BCS Class I drugs. Such biowaivers were also supported by the European Medicines Agency (EMA) in their guidance issued in 2001. EMA and FDA expanded BCS-based biowaivers to include Class III drugs in 2010 and 2017, respectively (15,16). Although the World Health Organization (WHO) considered granting BCS biowaivers for Class II weak acids, the organization published a guideline in 2015 for only Class I and Class III generic drugs (17).

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), which involves experts from both regulatory and industry agencies, finalized a guideline intended to be recognized worldwide, entitled “M9 guideline on biopharmaceutics classification system-based biowaivers (Step 5)” in 2020 (10). With the support of ICH, FDA recently finalized a guidance for industry in May 2021 entitled “M9 Biopharmaceutics Classification System-Based Biowaivers,” which replaced the 2017 FDA guidance (11). Relative to the 2017 FDA guidance, the 2021 FDA guidance (i.e., M9 document) has some biowaiver acceptance criteria changes, as summarized in Table I. Both documents indicate that BCS Class I and III drug products may be eligible for a biowaiver for IR oral dosage formulations with the same strength as the reference product. Acceptance criteria consist of the composition (i.e., excipients) and in vitro dissolution performance of the drug product depending on its BCS classification (11). It should be noted that M9 is a notable step forward, as it is the first harmonized allowance of BCS-based regulatory relief, including for example, in Japan.

However, M9 guidance is more restrictive than the prior 2017 FDA guidance, specifically about excipient risk. The 2017 FDA guidance indicated, in the context of BCS-based biowaivers, “In general, using excipients that are currently in FDA-approved IR solid oral dosage forms will not affect the rate or extent of absorption of a highly soluble and highly permeable drug substance that is formulated in a rapidly dissolving IR product.” On the other hand, M9 lacks such a statement. The prior 2017 FDA guidance also indicates, “Unlike for BCS class 1 products, for a biowaiver to be scientifically justified, BCS class 3 test drug product must contain the same excipients as the reference product,” and further describes evaluation of “the same excipients” (e.g., qualitatively the same and quantitatively similar). While the prior 2017 FDA guidance anticipates common excipients to not be a concern for BCS Class I, this expectation is less evident from the M9 guidance. M9 does indicate “[BCS Class I drugs] generally represent a low risk group of compounds in terms of the potential for excipients to affect absorption, compared to other BCS classes. Consideration of excipient effects for BCS Class I drug products should focus on potential changes in the rate or extent of absorption.” However, this statement is only relative to other BCS classes and apparently does not convey the anticipation that common excipients are not a concern for BCS Class I drugs. An additional restriction is observed where Caco-2 is indicated as the only in vitro permeability assessment in M9, while the prior guidance states in vitro permeability methods using excised intestinal tissues, or monolayers of suitable epithelial cells, may be used. This difference is a notable narrowing.

EMA and FDA have published product-specific guidelines, and the International Pharmaceutical Federation (FIP) has published over 50 drug monographs that assess potential usage of BCS biowaivers (17). BCS-based biowaiver monographs are a series of literature reviews on IR solid oral dosage formulations published in the Journal of Pharmaceutical Sciences. Evaluation of the API’s physiochemical properties, pharmacokinetics, and interactions with excipients are
Table I: Comparison of 2017 FDA Versus 2021 M9 BCS-Based Biowaiver Criteria

| Criteria                  | 2017 FDA Guidance                                      | 2021 M9 Guidance                                      |
|---------------------------|-------------------------------------------------------|-------------------------------------------------------|
| Dosage form               | Solid oral dosage forms                               | Solid oral dosage forms or suspensions                |
| Drug substance            | Must be the same                                       | Different salt form may be applicable (BCS Class I);  |
|                           |                                                       | ester, ether, isomer, mixture of isomers, complex or   |
|                           |                                                       | derivative are not applicable                         |
| Solubility class boundary  | Highest strength                                       | Highest single therapeutic dose                       |
| or drug amount            | Ionization determines number of pH conditions          | At least three pHs within 1.2–6.8, including buffers  |
| Solubility assessment     | within 1–6.8, including pH=pKa; pH=pKa+1; pH=pKa−1,  | at pH 1.2, 4.5, and 6.8                                |
|                           | and at pH = 1 and 6.8                                  |                                                       |
| Permeability assessment   | Preference for human PK studies                        | Preference for human PK studies (e.g., absolute       |
|                           | (e.g., absolute bioavailability or mass balance); in  | bioavailability or mass balance); Caco-2 permeability |
|                           | vivo human intestinal perfusion; animal in vivo or in  | is considered for passively absorbed drugs           |
|                           | situ intestinal perfusion, excised animal/human       |                                                       |
|                           | intestinal tissues, or epithelial cell monolayers      |                                                       |
|                           | possible for passively absorbed drugs, although human  |                                                       |
|                           | data supersedes                                        |                                                       |
| Excipients                | BCS Class I: Generally, excipients will not affect rate| BCS Class I: Excipients that may affect absorption     |
|                           | or extent of absorption; consider excessive quantities | of the particular API are qualitatively the same and |
|                           | of surfactants (e.g., polysorbate 80) and sugar        | quantitatively similar (i.e., within ±10% of the    |
|                           | alcohols (e.g., mannitol or sorbitol)                  | weight of excipient in the reference product and a   |
|                           | BCS Class III: Excipients must be qualitatively       | cumulative difference within ±10%; any qualitative    |
|                           | the same and quantitatively similar, except for        | and quantitative differences are acceptable for all   |
|                           | excipients used in limited amounts such as the        | other excipients                                       |
|                           | coating/shell                                         |                                                       |
| In vitro dissolution      | Demonstrate f₁ similarity factor of ≥ 50; not         | BCS Class I: test and reference should both have,    |
| performance               | necessary if test and reference both have very        | - very rapid properties (≥85% for the average percent |
|                           | have rapid properties (≥85% for the average percent   | dissolved in ≤ 15 min), or                            |
|                           | dissolved in ≤ 15 min)                                 | - rapid properties (≥85% for the average percent      |
|                           | To allow the use of mean data for f₁, the             | dissolved in ≤ 30 min) and f₂ similarity factor of ≥ 50|
|                           | coefficient of variation should not be more than 20%  | If one product has rapid and the other has very      |
|                           | at the earlier time points (e.g., 15 min), and should  | rapid characteristics, demonstrate f₂ similarity     |
|                           | not be more than 10% at other time points              | factor of ≥ 50                                        |
|                           |                                                       | When the coefficient of variation is too high, f₂     |
|                           |                                                       | calculation is considered inaccurate and a conclusion |
|                           |                                                       | on similarity in dissolution cannot be made          |
|                           |                                                       | BCS Class III: test and reference should both produce |
|                           |                                                       | very rapid properties (≥85% for the average percent   |
|                           |                                                       | dissolved in ≤ 15 min)                                |

Considered for biowaiver risk-based analysis. Many of the monographs support biowaivers for specific drugs and their corresponding IR dosage formulations, such as metformin, sitagliptin, and moxifloxacin (18–20). Meanwhile, a smaller number of monographs suggest against biowaivers for specific drugs and their corresponding IR dosage formulations, such as carbamazepine (i.e., due to its narrow therapeutic index) (21).

It should be noted that when comparing BE in vitro test results to in vivo test results, in vivo BE studies can have type I (i.e., consumer risk/false positive) and type II (i.e., producer risk/false negative) errors. Hence, a reason for discordance between a BCS biowaiver conclusion and an in vivo BE conclusion is type II error in \( C_{\text{max}} \) from in vivo pharmacokinetic (PK) BE studies. Of the in vivo BE metrics for rate and extent, \( C_{\text{max}} \) is the more common reason for BE failure (22–25). Limitations of \( C_{\text{max}} \) as a BE metric are well described (26–29). In a retrospective study performed in Brazil, 12 of 115 studies of Class III drug products provided non-bioequivalent (i.e., non-BE, where confidence interval exceeds 80–125% range) result, with 5 of those being bioinequivalent (i.e., point estimate is outside the range of 80–125%) (30). Specifically, among the 12 non-BE studies, 7 were due to only \( C_{\text{max}} \), 4 were due to both \( C_{\text{max}} \) and \( \text{AUC}_{0\text{--}t} \), and 1 was due to only \( \text{AUC}_{0\text{--}t} \). Of the 5 bioequivalent studies, 4 were caused by only \( C_{\text{max}} \) and 1 was due to \( \text{AUC}_{0\text{--}t} \). Similarly, for Class I drug products, 22 out of 140 studies provided a non-BE result, with 8 of those being bioequivalent. Of the 22 non-BE studies, 18 were due to only \( C_{\text{max}} \) while 4 were due to both \( C_{\text{max}} \) and \( \text{AUC}_{0\text{--}t} \). Of the 8 bioequivalent results, 7 were due to only \( C_{\text{max}} \) while 1 was due to both \( C_{\text{max}} \) and \( \text{AUC}_{0\text{--}t} \). Thus, it is important to consider that in vivo bioequivalence can be due to limitations of \( C_{\text{max}} \).
CLASSIFICATION OF BCS CLASS I AND III AND ELIGIBILITY FOR BIOWAIVER

According to the current FDA guidance, to be considered highly soluble, the highest single therapeutic dose must be soluble in 250 mL or less of aqueous media in at least three pHs within the range of 1.2-6.8 at 37±1°C. To be considered highly permeable, the drug product must have human pharmacokinetic results with an absolute bioavailability of ≥85% or a urine recovery of ≥85% via mass balance with demonstrated GI stability. Other in vivo data such as drug recovery in feces or data obtained from the literature (e.g., product knowledge and bioavailability studies) may be acceptable. In vitro methods include Caco-2 cell permeability assays, but they should be used alongside available in vivo data to estimate intestinal drug absorption. If Caco-2 permeability assays are used alone to classify a drug as highly permeable, classification is limited to passively absorbed drugs due to the lack of certain intestinal transporters in the Caco-2 cell model (11).

Using comparative in vitro dissolution tests, the test and reference products of BCS Class I drugs must dissolve very rapidly (≥85% for the mean percent dissolved in ≤15 min), or rapidly (≥85% for the mean percent dissolved in ≤30 min) with similar 2f2 (≥50) comparison. BCS Class III drugs must demonstrate very rapid comparative in vitro dissolution (11). Interestingly, the 2021 FDA Guidance does not allow for other methods to compare in vitro dissolution profiles other than 2f2.

POTENTIAL ABSORPTION-MODIFYING EXCIPIENTS

FDA’s Inactive Ingredient Database (IID) catalogs all excipients used in approved New Drug Application (NDA) and Abbreviated New Drug Application (ANDA) products, regardless of current market availability of the drug product (31). Information on each excipient includes ingredient name, route of administration, dosage formulation, chemical abstracts service (CAS) number, unique ingredient identifier (UNII), maximum potency per unit dose, and maximum daily exposure (MDE).

Excipients are usually in much greater amounts than the API and can typically make up to ~90% of the entire drug product. They are used in dosage forms to facilitate manufacturability, stability of the API, dose uniformity, and delivery of the API to the systemic circulation. Commonly used excipients can be grouped into several categories based on their functions such as binders (e.g., hypromellose, starch, povidone), fillers (e.g., lactose, mannitol), lubricants (e.g., magnesium stearate, stearic acid), and surfactants (e.g., sodium lauryl sulfate, polysorbates). Excipients are generally considered as “inactive ingredients.” Some excipients (e.g., polymers) are utilized as enhancers of solubility or dissolution rate for poorly soluble drugs.

It can be conceived that absorption-modifying excipients (AMEs) that do not affect in vitro dissolution testing can be considered “critical” (i.e., concerning) for biowaivers since their effect on GI absorption (e.g., transit, intestinal permeability) would be overlooked. In the literature, only a subset of mechanisms exist by which excipients could function as AMEs and potentially affect the absorption of BCS Class III drugs. In contrast, BCS Class I drug absorption is not likely to be impacted by such excipients. For many potential AMEs, there is no in vivo evidence of such an effect. For example, we know of no common excipient that decreased drug absorption of a BCS Class I drug by drug-excipient binding where in vitro testing did not anticipate such binding. Many of the excipients with such reported effects, such as surfactants, would not normally be used in IR solid oral dosage forms for highly soluble drugs, i.e., BCS Class I and III. Only a few potential AMEs have been identified, such as excipients that can impact intestinal transit (e.g., sorbitol, mannitol). Evidence of an effect in humans for several of these AMEs has largely been observed with only high quantities of excipient (e.g., 1.6 g of sorbitol) (9). Although surfactants have also been associated as potentially critical AMEs, there is no clear evidence in humans. Rather, evidence has been observed in the preclinical and in vitro domains.

Sorbitol and Mannitol

The sugar alcohols sorbitol and mannitol are known to generate, in sufficient doses, significant osmotic effect after oral administration (i.e., decreases transit time that reduces drug absorption, particularly for low permeability drugs). The molecular weights of sorbitol and mannitol are the same (i.e., 182 Da), as they are stereoisomers, and they exhibit a dose-dependent proportional decrease in small intestinal transit time (32,33). It is important to consider the amount of excipient used, the degree at which that amount affects BCS Class I or III drug absorption, and the absorption properties of the drug substance such as the location, rate, and mechanism (4,11).

Chen et al. showed BCS Class III drugs were more sensitive than BCS Class I drugs to sorbitol, with respect to the ability of 5 g sorbitol to reduce drug absorption. Chen et al. employed metoprolol and ranitidine as Class I and Class III drugs, respectively. Test product containing 5 g of sorbitol had no impact on metoprolol extent of absorption compared to reference product containing sucrose in healthy volunteers. Meanwhile, ranitidine absorption was reduced by 7%, 25%, and 45% by 1.25 g, 2.5 g, and 5 g sorbitol, respectively (6). In general agreement with Chen et al.’s observation regarding metoprolol, Fassihi et al. observed that 10 g of sorbitol had no impact on the extent of theophylline, a BCS Class I drug (7,8).

Vaithianathan et al. assessed commercial solutions containing sorbitol of each cimetidine and acyclovir, BCS Class III drugs, in bioequivalence studies (9). The cimetidine and acyclovir solutions contained about 1.6 g and 1.5 g of sorbitol, respectively. The dose containing 1.6 g sorbitol reduced cimetidine absorption by 19%. Conversely, 1.5 g sorbitol did not impact acyclovir absorption. It is not clear why these similar doses of sorbitol showed apparently different impacts, although cimetidine and acyclovir are of course different drugs, including with different formulation compositions. Vaithianathan et al. also compared the cimetidine commercial solution (with about 1.6 g of sorbitol) against a sorbitol-free solution of cimetidine (9). While Cmax was reduced by about 13%, cimetidine absorption was not impacted. Overall results support Chen et al.’s observation that 1.25 g sorbitol can impact Class III drug absorption. An in silico model
conducted by Yamane et al. estimated that a threshold of 400 mg of sugar alcohols will not impact drug absorption (34).

Other studies examined quantities more than 2 g of sugar alcohols. Adkin et al. evaluated the impact of 2.264 g of mannitol on cimetidine absorption, as well as small intestinal transit times. Mannitol reduced cimetidine absorption by about 31%, as well as small intestinal transit time by 23% (35). Also, Adkison et al. observed that 3.2, 10.2, and 13.4 g of sorbitol decreased absorption of lamivudine, a BCS Class III drug, by 20%, 39%, and 44%, respectively (36,37).

**Surfactants**

Surfactant effect on permeability has been studied in vivo and in vitro, in regard to reducing small intestinal transit time or modifying passive or active permeation (4). However, in vivo human data has shown no effect.

The surfactant sodium lauryl sulfate (SLS) has been shown to increase permeability of mannitol and other drugs in Caco-2 monolayers via opening of tight junctions (38). SLS has also been classified as a modulator of paracellular transport from ex vivo data (39). Parr et al. showed SLS at ≥ 0.1 mg/mL increased permeability across Caco-2 monolayers of four BCS Class III drugs due to damaging membrane integrity, but not the BCS Class I compound antipyrene. Concentrations of 0.01–0.04 mg/mL did not have any effect on the permeability of all five drugs (40). Although García-Arieta considered the in vivo impact of SLS where two studies showed bioinequivalence (with 3.64 mg or 1.5 mg SLS), other studies with very high amounts (9 g) have demonstrated bioequivalence (5). Vaithianathan et al. found that sodium lauryl sulfate (25 mg) had no significant impact on the in vivo bioavailability of cimetidine nor acyclovir (9).

The surfactants Vit-E-PEG, AOT, polysorbate 80, CTAB, polysorbate 20, Cremophor® EL, Solutol® HS 15, and Brij® 58 and the polymer NaCMC have been shown to be inhibitors of P-glycoprotein (P-gp) in MDCK-MDR1 cell culture, as determined by significant intracellular increase in digoxin (41). In an in vivo rat experiment, surfactants modified the pharmacokinetic profile of orally administered digoxin and celiprolol (BCS Class III), although the overall AUC was not increased. An early peak of absorption was observed consistently across surfactants, most likely due to the higher concentration of excipient in the proximal intestine where P-gp expression is lower (42).

**REGULATORY FRAMEWORK IN FORMULATION**

Biowaivers are allowed for scale-up and post-approval changes (SUPAC) to drug formulations. The FDA guidance on “Immediate Release Solid Oral Dosage Forms Scale-Up and Post-Approval Changes” (SUPAC-IR) published in November 1995 outlines post-approval changes in the composition of formulation, manufacturing location, batch size, and manufacturing equipment and process. SUPAC-IR provides regulatory relief in the context of BCS. Specifically, excipient changes are divided into three impact levels on formulation quality and performance that are accepted by dissolution and in vivo BE requirements. The categories include level 1 (negligible impact), level 2 (could have a significant impact), and level 3 (likely to have a significant impact) (43).

Changes in excipients at level 1 are unlikely to affect the quality or performance of the formulation such as in the color or flavor of the drug product or excipient amounts less than or equal to a percentage (w/w) of the total formulation. Specifically, ±5% for fillers, ±3% for the disintegrant starch and ±1% for other disintegrants, ± 0.5% for binders, ±0.25% for the lubricants calcium or magnesium stearate or ±1% for other lubricants, ±1% for talc and ±0.1% for other glidants, and ±1% for film coating. Additionally, the total additive excipient changes must not be >5%. BE is demonstrated in level 1 via in vitro dissolution testing (i.e., biowaiver) (43).

Changes in excipients at level 2 could significantly alter the quality or performance of the formulation. Examples include a change in the technical grade of an excipient or in the percent (w/w) of the total formulation greater than level 1 but less than or equal to a two-fold increase over level 1 changes. Additionally, the total additive excipient changes must not be >10%. BE for level 2 is demonstrated via dissolution profile similarity factor f2 (i.e., biowaiver) for BCS Class I, II, and III, with an exemption for BCS Class I drugs that show ≥85% dissolution in 900 mL 0.1N HCl in 15 min.

Changes in excipients at level 3 significantly alter the quality or performance of the formulation due to additive excipient changes of >10% and require in vivo BE testing for qualification (43,44).

FDA expanded its SUPAC-IR requirements in the guidance for ANDAs, in which excipient changes must be “Q1/Q2,” i.e., the test formulation must be the same excipients (qualitatively the same; Q1) and in the same concentration (quantitively the same; Q2) to the reference formulation (45). Allowable qualitative excipient differences to be Q1 include those that affect the color or flavor of the drug product, printing ink, technical grade and/or specification, and particle size. Allowable quantitative excipient differences to be Q2 include excipient amounts less than or equal to a percentage (w/w) of the total formulation. Specifically, the guidance for ANDAs states ≤10% for fillers, ≤6% for starch and ≤2% for other disintegrants, ≤3% for binders, ≤0.5% for the lubricants calcium or magnesium stearate or ≤2% for other lubricants, ≤2% for the glidant talc or ≤0.2% for other glidants, and ≤2% for film coating. Additionally, the total additive excipient changes must be ≤10% (45).

**EXCIPIENT RISK FOR BCS CLASS I**

BCS Class I drug products have minimal risk regarding excipient changes since they are very well absorbed given their high solubility and high permeability characteristics. Since the rate-determining steps are dissolution, permeation, or gastric emptying, excipients that could alter the rate or extent of the drug’s absorption should still be evaluated. Such cases include excipients that modulate uptake transporters that the drug relies on for its high permeability, or excipients that increase the absorption rate of drugs that are absorbed slowly. A biowaiver is acceptable for BCS Class I drugs if the excipients that may affect absorption are qualitatively the same (i.e., identical chemistry, grade, and characterization) and quantitatively similar (i.e., within ±10% of the weight of excipient in the reference product and a cumulative difference within ±10%). For all other excipients, any qualitative and quantitative differences in excipients are acceptable when granting a biowaiver (10,11).
Cephalexin, a BCS Class I drug, has high intestinal permeability due to active uptake across the apical membrane of enterocytes via the proton-coupled oligopeptide transporter PEPT1. Variations in the expression of PEPT1 in vitro and in vivo (i.e., Caco-2 cells, human duodenum, and rat jejunum) are correlated with differences in the permeability of cephalexin (46). Hypothetically, excipients that have the potential to modulate PEPT1 activity or expression could affect the extent of absorption of cephalexin and would be important to consider during formulation development. In general, a 10–15% change in extent of absorption can be expected to cause bioinequivalence (47).

In vitro data has shown the non-ionic surfactants Solutol® HS15 (poly-oxyethylene esters of 12-hydroxystearic acid), polysorbate 20, and polysorbate 80 inhibit PEPT1 in transfected MDCKII cells (48). Notably, surfactants are also known to enhance the penetration of drugs through the intestinal membrane by disrupting its integrity and function. Therefore, it is important to consider an overall net effect since multiple mechanisms may (or may not) be at play when surfactants are present in the GI tract (49). Similarly, in vitro and in situ experiments have shown the excipient caprylocapril macroglucosylceides to enhance cephalexin transport. However, these experiments fail to emulate the in vivo human environment that includes active transport and fail to consider an already high permeability of cephalexin (46).

EXCIPIENT RISK FOR BCS CLASS III

BCS Class III drug products are thought to be at risk of excipient changes since they have low permeability and may only be locally absorbed at specific sites along the gastrointestinal tract (e.g., only small intestine as colonic permeability is too low). Therefore, according to the current FDA guidance, a biowaiver is acceptable for BCS Class III drugs if all excipients are qualitatively the same (i.e., identical chemistry, grade, and characterization) and quantitatively similar (i.e., within ±10% of the weight of excipient in the reference product and a cumulative difference within ±10%), except for excipients that are used in limited amounts such as the film coating or capsule shell. This criterion assumes that all excipients have the potential to affect absorption of the drug, regardless of known or suspected capability (10,11).

Osmotically active excipients at amounts used in formulations have been shown to alter the bioavailability of BCS Class III drugs (50). Sorbitol decreased ranitidine absorption by increasing intestinal fluid volume, and thus enhancing GI motility and decreasing ranitidine transit time. Mannitol decreased the bioavailability of cimetidine. PEG 400 accelerated transit time and altered the absorption of ranitidine (50).

Valacyclovir, a BCS Class III drug and prodrug of acyclovir, is more permeable than administration as the parent drug acyclovir due to active uptake of valacyclovir (but not acyclovir) via PEPT1 such that bioavailability is improved to >50% compared to 15% (9,51,52). Non-ionic surfactants have been shown in vitro to inhibit intestinal transporters, including via modulation of membrane fluidity (53–55). For example, polysorbate 80 has been shown to inhibit the intestinal transporter PEPT1 (48). Hypothetically, the PEPT1 substrate valacyclovir could be impacted by polysorbate 80, which may potentially decrease valacyclovir absorption, although FDA M9 regulatory guidelines do not describe methods to assess transporter-mediated excipient-drug interactions.

If inhibition of intestinal efflux transporters affects permeability, there could be a potential increase in bioavailability, although not concerning for passive permeability drugs. Cimetidine and famotidine, which are both BCS Class III drugs, are substrates for intestinal efflux mediated by P-gp. Concentration-dependent decrease of the secretion of both drugs in situ via single-pass intestinal perfusion studies in rats was obtained by P-gp inhibitors. Notably, the in vivo permeability of both drugs along the small intestine correlated with P-gp expression levels, thereby exhibiting segmental dependent intestinal absorption. Site-specific P-gp inhibition along the intestine, as observed by verapamil in the literature, may impact overall drug absorption (56). Many surfactants and one polymer have also been shown in vitro to inhibit P-gp in MDCK-MDR1 cells while five dyes and one suspending agent showed minimal inhibition in HEK293 cells (41,57).

The surfactant vitamin E TPGS (d-α-tocopheryl polyethylene glycol 1000 succinate) has been classified as an inhibitor of P-gp-mediated drug transport in Caco-2 monolayers and other cell lines (58,59). It has also been shown to enhance the oral bioavailability of the BCS Class III drug colchicine in rats (60). Notably, in vitro findings involving intestinal absorption of P-gp substrates have been performed using Caco-2 monolayers, although these cells have variable P-gp expression based on the culture conditions (61) and it is indicated that they overexpress P-gp (62).

Rege et al. assessed the influence of nine excipients (lactose, SLS, polysorbate 80, HPMC, docusate sodium, EDTA, propylene glycol, PEG 400, and anhydrous cherry flavor) on the Caco-2 permeability of seven low permeability compounds. Polysorbate 80 significantly increased apical-tobasolateral permeability of low permeability compounds via inhibition of active efflux as assessed by the lack of effect on mannitol permeability. SLS moderately increased drug permeability and affected Caco-2 monolayer integrity. The rest of the excipients showed minimal impact on the overall permeability of these compounds (54).

The surfactants salcaprozate sodium (SNAC) and sodium caprate (C10) are two of the most advanced AMEs that have gone through clinical testing for the oral delivery of macromolecules. Oral semaglutide, which has gone through multiple phase 3 clinical trials, is the first oral peptide therapeutic for type 2 diabetes in the form of a daily capsule. It is thought that SNAC promotes semaglutide absorption in the stomach by raising local pH to protect drug from degradation by gastric enzymes, as well as by inducing transcellular flux of semaglutide across the gastric epithelium of the stomach. C10 was assessed for use with oral insulin although dosage formulation development was discontinued. Low concentrations of C10 act via openings of tight junctions, while high concentrations via membrane perturbation (63).

DISCUSSION

Suitability of Experimental Models to Assess Drug Absorption

Intestinal absorption is often determined using in situ rat perfusion models or in vitro epithelial cell culture models. It is
important to consider the utility of these alternative methods to drug absorption in humans. In situ rat perfusion models exhibit physiological differences from humans such as dilution, gastric emptying, degradation, and intestinal transit. However, this model favorably assesses drug transport in small intestinal tissue, the main in vivo absorption site. Notably, although rat and human tissue show similar drug absorption profiles, they exhibit distinct transporter and metabolic enzyme expression in the intestinal wall. Therefore, a rat model can be used to predict oral drug absorption in the small intestine of human, but not to predict oral bioavailability (64).

Caco-2 cell monolayers are a sensitive tool capable of distinguishing between high and low permeability values. However, they present practical limitations as an in vitro model to assess excipient effects on drug permeability. For example, a theoretical increase in drug intestinal permeability of a Class III drug by a theoretical absorption-modifying excipient from 60% absorption to 65% may or may not be detectable by Caco-2 monolayers, or by in vivo human bioequivalence testing. Since the Caco-2 permeability assay has an intraday variability of ~10% that is comparable to the bioequivalence similarity assessment (e.g., 10–15%), it would be difficult to reliably detect small excipient effects for low permeability drugs by Caco-2 monolayers. That is, a true enhancement of drug permeability of 10% across Caco-2 monolayers may not reliably be detected, even though a 10% increase in in vivo drug absorption may be important. In general, a 10–15% change in extent of absorption can be expected to cause bioinequivalence (47).

However, in spite of a lower limit of sensitivity of Caco-2 monolayers to drug permeability enhancement, in the literature, Caco-2 cells have been highly sensitive to excipient effects on drug permeability compared to in vivo (54). Consistent with the sensitivity of Caco-2 monolayers, excipients such as surfactants, disintegrants, and chitosans have shown an effect on drug permeability across Caco-2 monolayers but not in vivo. Caco-2 monolayers can be expected to frequently over-predict in vivo effect of excipients, such as SLS. This over-prediction is in part because Caco-2 monolayers do not secrete mucus, such that Caco-2 is much more sensitive to membrane disruptors (i.e., surfactants) than in vivo. Mucus creates a steric and interactive barrier against intestinal permeation such that its presence (or absence) may impact drug permeation (65). Therefore, the in vivo implication of an enhancement in in vitro Caco-2 permeability by an excipient is not clear.

A practically challenging topic is the comparison of permeability values (e.g., with and without excipient). There is currently no known universal method to assess permeability similarity when employing an in vitro model such as Caco-2 in assessing potential excipient effect. Although intraday variability in Caco-2 permeability is low, there is appreciable variability between studies (even conducted on the same day) that is often unexplained. This may lead statistical analysis via t-tests to false-positives, even before considering the high sensitivity of Caco-2 to predict in vivo human permeability. For example, Rege at al. reported a false-positive outcome in about 10% of all studies (54). These apparent effects were about 1.3-fold in magnitude, were not systematic, and attributed to variability effects than true excipients effects. Two studies were repeated and showed no subsequent excipient effect. In another two studies, “tighter” monolayers, as assessed by mannitol permeability, explained the decreased drug permeability. Parr et al. examined excipient effects on BCS Class III permeability using Caco-2 monolayers and rat perfusion (40). It was concluded that the four BCS Class III compounds would not be greatly impacted by the excipients. Permeability values were examined in the presence and absence of excipient, but no statistical tests were conducted. It would appear that permeability comparisons should not be limited to straight-forward t-tests (either with or without multiple comparisons correction).

Madin-Darby canine kidney (MDCK) cells have also been used in permeability assays since they favorably grow more rapidly than Caco-2 cells. In drug discovery/development interface programs that examine drug biopharmaceutic properties, MDCK monolayers are perhaps even more commonly used than Caco-2 monolayers. MDCK and Caco-2 cells both form into polarized epithelial monolayers. Although Caco-2 and MDCK cells differ biological in their source (human colon and canine kidney, respectively), their monolayers have comparable apparent permeability coefficient values. Notably, they each differ and vary in their own transporter expression levels (66–68).

Other cell culture models may be a more practical permeability model worth further evaluation, such as human colon carcinoma cell lines HT29-H and HT29-MTX, which form a monolayer with a mucosal barrier (69,70). Co-culture models of enterocyte-like Caco-2 cells with mucus-producing HT29-MTX cells have also been assessed for correlation with human in vivo studies, although relevant intestinal transporters are still not expressed (71–73). Bioengineering approaches that involve 3D co-cultures have also been reported in the literature as biomimetic models (74). Nonetheless, there is a need for novel in vitro models alongside the use of human and animal in vivo techniques to assess permeability (75).

Considerations of Excipient-Transporter Interaction

M9 guidance requires a BCS-based biowaiver proposal to include a mechanistic and risk-based approach in assessing if differences between test and reference product (e.g. pre- and post-change SUPAC products, brand versus proposed generic) will not affect drug absorption. One such potential mechanism is transporter-mediated drug absorption. Although M9 guidance references important intestinal transporters, i.e., P-gp and BCRP, Caco-2 cell monolayers are limited in M9 to only assessing high permeability of passively transported drugs due to the potential lack of transporter expression. Transporter-mediated risk assessment of excipients is discussed. A critical question is “Are there excipients in the formulation with known or suspected effects on drug absorption?”

Regarding the potential for transporter-mediated excipient-drug interactions, two other FDA guidances are more comprehensive in assessing such risks than M9: “In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions” and “Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions.” These companion guidances are largely aimed at drug development in anticipating or assessing drug-drug interactions. The guidances are additionally supported with a website concerning tables that list substrates, inhibitors, and inducers of P450.
enzymes and transporters (78). Nine transporters are discussed, including two apically localized efflux transporters that have significant expression in the intestine: P-gp and breast cancer resistance protein (BCRP). Given their location and directionality of transport, they have the potential to translocate drug back into the gut lumen and reduce drug absorption. Correspondingly, for a drug that is incompletely absorbed due to such efflux, an excipient that inhibits P-gp and/or BCRP has the potential to increase drug absorption. In vitro dissolution would presumably not detect such an excipient effect.

The quality of experimental tools to evaluate transporter-mediated drug interactions varies and depends upon the question to be addressed. There are in vitro assays that are viewed as reliable to demonstrate that a drug, or presumably an excipient, is not an inhibitor (76). For example, vitamin E TPGS was shown to not inhibit human PEPT1 (55).

Meanwhile, in vitro assays showed the BCS Class III drug cimetidine to be a P-gp substrate and inhibitor (9,79,80). This situation exemplifies the general challenge in addressing the question about whether or not excipients in a formulation have potential effects on drug absorption. It is well appreciated that, even for perpetrator drug substances much less perpetrator excipients, that in vitro tools to predict in vivo transporter impact or transporter-mediated drug interactions have limitations. These limitations include relevance of in vitro studies to in vivo impact, as well as specificity to one transporter over another transporter, as transporters such as P-gp and BCRP can have overlapping activities. Of note, M9 guidance lists four model drugs for permeability assay method validation: digoxin, paclitaxel, quinidine, and vinblastine. Of these, only digoxin and quinidine are listed as example probes in the FDA clinical drug-drug interaction guidance (77). This lack of convergence reflects that in vitro and in vivo tools, as well as overall understanding about transporter-mediated interactions at the level of the gut, are still often only modestly developed.

Such limitations are highlighted in examining the current, state-of-the-art recommendations for conducting in vivo clinical studies to assess transporter-mediated drug interactions, even for perpetrator excipients (77). This guidance notes clinical substrates of P-gp to include dabigatran etexilate, digoxin, and fexofenadine. The guidance further notes that criteria for selecting P-gp clinical substrates are (a) AUC fold-increase ≥2 with verapamil or quinidine co-administration and (b) in vitro transport by P-gp expression systems, but not extensively metabolized. More importantly for one with an interest in assessing excipient risk to modulate P-gp (e.g., risk of excipient to increase drug absorption via P-gp inhibition), the guidance notes that criteria for selecting P-gp clinical inhibitor are (a) AUC fold-increase of digoxin ≥2 with co-administration and (b) in vitro inhibitor. That is, it is clear that digoxin is the state-of-the-art in vivo victim drug to assess a P-gp-mediated drug interaction by a potential perpetrator drug (or excipient). Trueck et al. employed digoxin as the P-gp probe in a five-drug cocktail that aims to serve as a clinical tool to screen for transporter-based interactions (81).

However, there are specificity and sensitivity limitations in using digoxin as the P-gp probe for in vivo clinical studies (82). Oral absorption of digoxin from tablets is 60–80% (83), reflecting its intestinal permeability is less than high (84). However, with only 20–40% incomplete permeation due to perhaps P-gp, there is a modest amount that P-gp inhibition can increase digoxin absorption. Fexofenadine and dabigatran etexilate have been suggested to be more appropriate P-gp probes than digoxin to assess intestinal P-gp inhibition, although they also have significant limitations (85). Limitations in the availability of a suitable P-gp probe for in vivo clinical studies generally reflect the experimental challenges in assessing whether or not excipients in a formulation have potential in vivo effects on drug absorption. In other words, transporter effects are difficult to demonstrate or characterize, particularly in vivo.

Future research should be aimed to help better answer the critical question — “Are there excipients in the formulation with known or suspected effects on drug absorption?” Currently, in vitro and in vivo tools as well our overall understanding about transporter-mediated interactions at the level of the gut are often only modestly developed, making this critical question difficult to fully address. This difficulty is further challenged if there is the presumption of an excipient effect, which M9 appears to assume for even BCS Class I drugs.

CONCLUSION

Excipients affecting GI drug absorption limit the granting of BCS-based biowaivers. Excipients may impact small intestinal transit, passive permeability, or active transport for BCS Class III drugs. BCS Class I drugs are not likely to be impacted by common excipients. However, experience to date supports that common excipients in solid oral IR dosage formulations generally do not modify in vivo drug permeability or transit. A few potentially critical absorption-modifying excipients have been identified at high quantities in vitro and preclinically, including excipients that can impact intestinal transit (e.g., sorbitol, mannitol). Nonetheless, the current FDA M9 guideline has conservative limits for excipient changes. These restrictions, especially for that of BCS Class III drugs, merit regulatory relief. A database of failed BE clinical trials because of excipient changes could help identify disallowable excipient changes to dosage formulations due to impacting performance.

AUTHOR CONTRIBUTION

Both authors contributed to the writing and approval of the version to be published.

DECLARATIONS

Conflict of Interest The authors declare no competing interests.

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