International Journal of Pharmaceutics 419 (2011) 43–51

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Evaluation of gastrointestinal motility directly from human pharmacokinetic data

John A. Roush*

GlaxoSmithKline, Five Moore Drive, Research Triangle Park, NC 27709, United States

ARTICLE INFO

Article history:
Received 25 March 2011
Received in revised form 20 June 2011
Accepted 8 July 2011
Available online 18 July 2011

Keywords:
Compartmental absorption
Absorption rate analysis

ABSTRACT

Since the 1980s, a considerable body of research has been dedicated to the development of in silico models for the prediction of human pharmacokinetic data based on absorption in a series of discreet intestinal compartments. While some of these models have been successfully used to predict future pharmacokinetic results or to explain previous results, evidence for compartmental absorption in individual pharmacokinetic data has not been published. This article presents in vivo evidence for compartmental drug absorption along with an empirical method for determining gastrointestinal (GI) tract location during absorption, using individual time–absorption rate profiles. Comparisons are shown between the absorption rate profiles and corresponding gamma scintigraphy images, to demonstrate the reliability of the GI position assignments and a hypothesis is proposed to explain the appearance of peaks and troughs in absorption rate profiles. Absorption rate analysis is shown to be a reliable and low cost tool for interpretation of unexpected pharmacokinetic data. Pharmaceutical scientists should find it useful for understanding the in vivo performance of drug products and it is hoped this will result in fewer delays and lower costs during drug development programs.

1. Introduction

Unexpected problems related to oral absorption have been a major cause of attrition during the drug development process. Clearly, it is desirable to predict the absorption characteristics of a drug product prior to dosing in humans and numerous in silico predictive models have been developed for this purpose. Dokoumetzidis et al. recently published a review describing the various types of predictive models as well as their relative merits [1]. Predictive models have been developed based on the physical and chemical properties of the drug, release characteristics of formulations and physiological factors affecting drug absorption. Physiological models can be divided into two broad categories: dispersion models and compartmental absorption models.

The compartmental absorption model views the intestine as being divided into a series of absorbing segments [2]. These models are based on the assumption that absorption is continuous throughout a specific segment of the gastrointestinal (GI) tract but that the rate of absorption varies as the dose traverses the gut. Suttle et al. developed a model based on discontinuous absorption throughout the GI tract to explain the frequent appearance of double peaks in plasma time–concentration profiles [3]. The Compartmental Absorption Transit (CAT) model, as developed by Yu et al. [4] became the forerunner of the widely used commercial software, GastroPlus. The various compartments were ascribed to specific regions of the GI tract and relied on a calculated term, effective permeability P_{eff}, which is a function of the physical conditions within a specific absorption compartment. Sawamoto et al. [5] proposed a similar model called the GI Transit Absorption (GITA) model which permitted the additional flexibility of variable drug transit rates. Kimura and Higaki also employed in vivo techniques, such as gamma scintigraphy, to validate their model [6].

The number of intestinal compartments has remained a topic of uncertainty since the first compartmental model was described. As few as one and as many as seven have resulted in satisfactory predictions of drug absorption [7–11]. However, no single value has proved to be suitable for every circumstance. Furthermore, while compartmental absorption models are believed to simulate in vivo absorption processes, in vivo evidence for compartmental absorption has not been published.

The goal of predictive modeling is to estimate the mean bioavailability and time course of the fraction of dose that reaches systemic circulation in a population. While there are numerous instances where the various compartmental models have made satisfactory predictions, the problem of unexpected or undesired pharmacokinetic results continues to persist. In such instances, there is a need to interrogate the individual subject time–concentration profiles and if possible, attribute a likely cause for the unexpected results. For example, when similar formulations are shown to be non-bioequivalent, it is desirable to conduct an absorption analysis of individual pharmacokinetic data in hopes that it may yield clues to a specific cause for the failure, prior to embarking on expen-

* Corresponding author. Tel.: +1 919 483 8205; fax: +1 919 483 0443.
E-mail address: john.a.roush@gsk.com

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.07.011

sive and time consuming investigations in the laboratory. The goal of absorption analysis is to interpret individual subject absorption characteristics with respect to chemical and physical properties of the drug product, as well as to individual physiological conditions, such as rapid GI transit or prolonged gastric residence. As such, absorption analysis of existing pharmacokinetic data can be viewed as being dissimilar but complimentary to predictive absorption modeling.

Interpretation of individual subject absorption characteristics can be made with far greater certainty when details of GI transit are available. Consequently, gamma scintigraphy and other non-invasive techniques have been widely used to supplement pharmacokinetic data. However, all such supplementary techniques require advanced planning and incur additional costs and delays, so more often than not, they are not used. Recently, comparisons have been made between time–drug absorption rate profiles and corresponding gamma scintigraphy data for the corresponding subject/dose combinations. These comparisons appear to support the concept of compartmental drug absorption, as proposed in the various compartmental models referred to above, in that there are successive periods of high and low rate of absorption. The periods of high drug absorption rate appear to be consistently associated with identifiable regions of the GI tract. The following paragraphs will describe a simple empirical method for determining details of drug transit through the GI tract using individual subject absorption rate data. Locations of regions of high absorption rate will be verified by comparison with corresponding gamma scintigraphy data. Also, explanations for periods of low drug absorption rate and the apparently variable number of absorbing GI compartments will be proposed.

The new method, called absorption rate analysis, is used specifically to obtain a detailed view of how a drug product performs in vivo or why it has not performed as anticipated. A systematic method of inquiry has been offered, so that investigators can begin to extract high value information from pharmacokinetic data quickly after becoming familiar with the method. Finally, some examples are presented to illustrate how absorption rate analysis may be used for the interpretation of unexpected pharmacokinetic data. These examples are drawn from a number of drugs no longer in development and have been chosen based on the fact that PK and gamma scintigraphy data exist for each subject and the data illustrates common problems which pharmaceutical scientists have struggled repeatedly to understand and deal with successfully. Specific therapeutic indications for the drugs will be identified as each drug is introduced in the text, although these are not necessary to gain an understanding of the method being presented. The object of this article is to introduce a new technique for interpretation of PK results as clearly as possible. Specific subjects presented in the text and figures have been chosen on the basis that these individual's absorption rate profiles clearly illustrate the points being discussed for readers who are unfamiliar with the topic presented by this article. It should be noted that the method being described is applicable to any oral drug product and has no limitation with respect to BCS classification. If plasma concentration can be measured for 12–16 time well chosen time points, location of absorption sites can be determined with good precision.

2. Methods

Pharmacokinetic and gamma scintigraphy data were drawn from a series of regional absorption studies conducted in the previous decade for drugs that are no longer in development. Dosage forms used include tablets, capsules and suspensions. Specific formulations may sometimes affect the appearance of absorption rate profiles. For example, enteric coated tablets may sometimes begin to absorb a few hours after gastric emptying. However, the examples shown were designed to be rapidly disintegrating immediate release formulations and in vivo absorption characteristics are indistinguishable from each other, unless specifically noted.

Gamma scintigraphy studies were performed at Scintipharma, Inc., Lexington, Kentucky, USA. Studies were conducted in accordance with "good clinical practice" (GCP) and all applicable regulatory requirements, including, where applicable, the 1996 version of the Declaration of Helsinki. Written informed consent was obtained from each subject prior to the performance of any study-specific procedures. Case report forms were provided for each subject's data to be recorded. Radio labeled tablets or capsules were prepared at Scintipharma and pharmacokinetic analysis was performed either by Scintipharma or at GlaxoSmithKline. Subjects in the Naproxen Sodium example were migraine headache sufferers up to 60 years of age and the examples shown were obtained in the absence of migraine. All remaining subjects in the other regional absorption studies were young, male healthy volunteers. Unless specifically noted, drug was dosed in the fasted state.

Absorption rate profiles were obtained by deconvolution of individual plasma time–concentration data, using WinNonLin version 5.2. Deconvolution is a mathematical process for separating time–concentration data into its respective absorption and elimination phases, mainly for the purpose of determining drug absorption characteristics. WinNonLin uses an algorithm called numerical deconvolution by least squares, which is a difference minimization technique. The mathematics have been explained in detail by D.J. Cutler, who first developed the algorithm [12,13].

From a theoretical perspective, the observed plasma time–concentration profile is divided into uniform time intervals. The unit impulse response function over the interval $(t_1 - t_0)$ is the rate of change in plasma concentration required C_δ,or(t_1) to achieve the change in cumulative amount absorbed over that interval. The overall unit impulse response is the sum of the areas under the unit impulse response function: UIR = ΣC_δ,or(t_1), C_δ,or(t_2), C_δ,or$(t_3) \ldots C_\delta$,or(t_n). A general limitation for deconvolution is that the sampling interval just described must be set so that the area under the unit impulse function, $g(t)$, satisfies the requirement $g(1) > g(2) > g(3)$, etc. The theoretical requirements and limitations of the deconvolution method have been described by Li and Cutler [14].

Usually for oral drugs, no corresponding IV data is available and absolute bioavailability is unknown. To address this shortcoming, the unit impulse response is calculated for an oral dose which is likely to have the highest bioavailability among the study regimens. For deconvolution of pharmacokinetic data, the unit impulse response (UIR) for each individual in a study was determined using the WinNonLin v.5.2 IVIVC wizard. The individual UIR terms were then averaged. Deconvolution was performed using the WinNonLin deconvolution tool. The first derivative or drug time–input rate profile is relevant to the assessment of GI motility and will be the only WinNonLin output referred to throughout this article. For the purpose of comparison to gamma scintigraphy data, most time–drug input rate profiles have been normalized with respect to the individual's highest observed input rate.

No differences in compartmental absorption characteristics or gastrointestinal motility have been noted with respect to age, gender or ethnic heritage. However, certain disease states have been noted to alter absorption rate profiles significantly. For example, type II diabetic patients have been observed to have very prolonged gastric emptying times. Recently, a small population ($n = 8$) of hepatitis C patients exhibited very rapid gastric emptying compared to a group of healthy volunteers. It is not known if this observation is typical for hepatitis patients. No examples of these effects have been intentionally shown in the following discussion but it is important to be aware that disease state may affect GI motility.

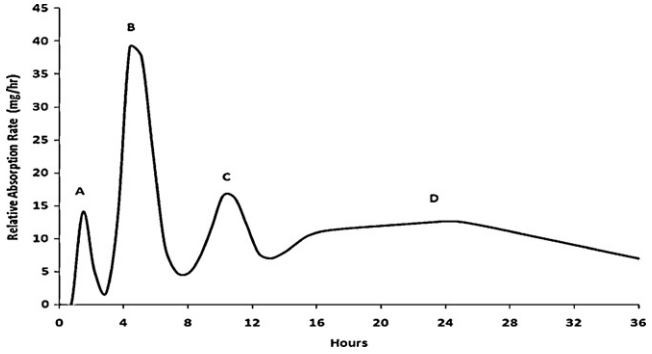

Fig. 1. Time versus drug absorption rate for one individual after dosing with a drug known to absorb throughout the entire GI tract.

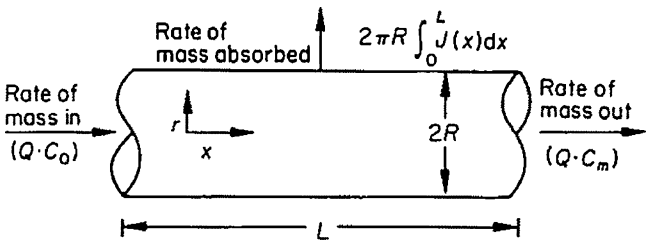

Fig. 2. The macroscopic mass balance for the intestinal perfusion experiment described by Johnson and Amidon. The difference between the mass flowing into and out of the intestine is equal to the rate of mass absorbed.

3. Results

The evaluation of GI transit from pharmacokinetic data evolved empirically, during an investigation involving a drug known to absorb in both the small intestine and throughout the colon. Fig. 1 shows one individual's time–drug absorption rate profile. It can be seen that drug absorbs in a series of rate peaks and troughs, consistent with the predictive compartmental GI transit models previously referred to. Individual differences in GI transit can be observed within a study population. However, observations from several pharmacokinetic studies indicate there is no inter-subject variation in the number of absorbing compartments. Four absorption compartments have been identified, although that number is not always observed. Reasons for the appearance of more or fewer compartments will be given in succeeding paragraphs, as well as a method for consistent identification.

Tentative assignment of GI tract locations can be made a process of rational deduction and the validity of the location assignments can later be verified by comparison with gamma scintigraphy. Obviously, absorption does not take place prior to the onset of gastric emptying. Consequently, the absorption rate peak labeled "A" is most likely to be associated with absorption in the proximal (early) region of the small intestine. The drug is known to absorb throughout the colon and considering the time elapsed since dosing, the absorption rate peak labeled "D" can reasonably be attributed to the late (transverse or descending) colon. The distal (late, including the ileum) small intestine succeeds the proximal region and residence time in the distal region is typically longer than in the proximal region. Therefore, it is reasonable to assign the absorbing region labeled "B" to the distal small intestine. Upon leaving the small intestine, whatever drug remaining in the distal small intestine collects for a time in the cecum before being passed to the ascending colon. Since the absorption rate peak labeled "C" lies between compartments previously attributed to the distal small intestine and the transverse colon, it is reasonable to attribute "C" to absorption in the ascending colon. It will be seen later that passage through the cecum results in a leveling effect, so that variability in early GI transit has no discernable effect on absorption in the ascending colon.

The appearance of absorption rate peaks and troughs may be explained by a slight modification of the mass transport model proposed by Johnson and Amidon, illustrated in Fig. 2 [15]. Johnson and Amidon defined the term P_{eff} or effective intestinal permeability rate constant (Eq. (1)) as a function of the physical parameters illustrated in Fig. 2. Q is the intestinal fluid flow rate, which is

$$P_{\text{eff}} = \frac{Q(1 \sim C_m/C_0)}{2\pi RL} \quad (1)$$

assumed to be constant. C_0 and C_m are the drug concentrations passing into and out of the intestine, respectively. Finally, R and L are the radius and length of the intestine. However, if the GI tract is thought of as being composed of a series of absorbing compartments, rather than a single, continuous compartment, then P_{eff} can be defined for each individual compartment and bulk fluid flow may proceed at a variable rate. Intestinal fluid would initially flow into a compartment, then briefly collect and pause in a specific, narrow region of the compartment before passing on to the next compartment. In such a scenario, the bulk drug concentration in any intestinal segment, C_0, becomes a function of the intestinal flow rate, Q. When the flow rate is high, drug is widely dispersed in a compartment, so bulk drug concentration C_0 is low. At a point of collection within an absorption compartment, flow rate is low and C_0 is high. This view of discontinuous compartmental flow is supported by gamma scintigraphy images, such as those seen in Fig. 3. For clarity, specific regions of interest in the GI tract are indicated in Fig. 3. The stomach, as well as the ascending colon (C) and transverse colon (D) are easily distinguished in gamma scintigraphy and these areas are manually outlined by the scintigraphy analyst in each successive image. The numerous bends and folds in the small intestine do not allow for resolution of this organ in gamma scintigraphy, so regions of interest are outlined by the analyst. These regions of interest are identified as the proximal (A) and distal (B) regions of the small intestine.

For the individual shown in Fig. 3, gastric emptying occurs from 0.15 to 0.47 h. By 0.47 h, the drug and radiation are seen to be concentrated primarily in the proximal region of the small intestine and this time point corresponds with the first absorption rate peak. From 0.73 to 1.46 h, radiation and drug can be seen migrating from the proximal to distal region of the small intestine. This time period corresponds to a period of reduced drug absorption rate or trough. From 1.95 to 2.60 h, the radiation and drug are concentrated in a specific region of the distal small intestine, with presumably little or no flow during this interval. During this time, another absorption rate peak occurred. From 3.00 to 4.47 h, intestinal fluids were seen to migrate from the distal small intestine to the ascending colon. This migration corresponded to a new absorption rate trough. During the interval 4.47–7.96 h, radiation and drug remained confined mostly in the ascending colon, with a resulting absorption rate peak centered at 6 h. In the final absorption phase form 12 to 24 h, the remaining radiation and drug can be seen to be confined mostly in the transverse region of the colon.

Comparison of time–absorption rate profiles for Figs. 4–7 shows that this view of compartmentalized intestinal flow applies consistently across a number of individuals, regardless of the drug being tested. For all of the following figures, the absorption compartments are identified by the same convention used initially in Fig. 1:

A = absorption in the proximal small intestine
B = absorption in the distal small intestine
C = absorption in the ascending colon
D = absorption in the transverse colon

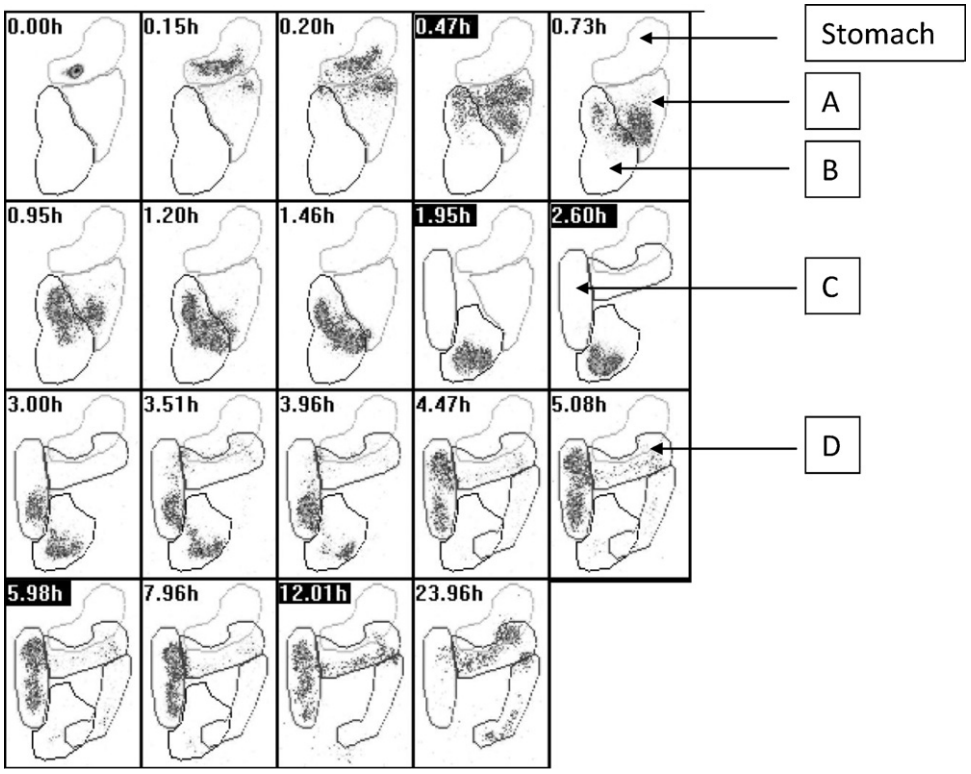

Fig. 3. Gamma scintigraphy images for an individual who received 363 mg GW634695, radio labeled with indium (1 1 1) chloride.

Fig. 4 shows the absorption rate profile for an individual after dosing with 500 mg Naproxen Sodium. The absorption rate peaks at 2.00, 4.53 and 8.01–10 h correspond to periods of high concentration and low flow through the proximal small intestine, distal small intestine and ascending colon, respectively. Periods of migration from one compartment of the GI tract to the next correspond to intervals of low absorption rate. During these periods of migration from one compartment to the next, radiation is widely dispersed and drug concentration in any specific segment of the GI tract is presumably low, resulting in a low P_{eff} value.

Fig. 5 shows the absorption rate profile for the same individual whose gamma scintigraphy images were presented in Fig. 3. This subject was dosed with 363 mg of GW695634, a pro-drug of GW678248 which is a non-nucleoside reverse transcriptase inhibitor (NNRTI) for treatment of HIV infection. GW695634 is rapidly converted to the parent drug GW678248, in the liver. Absorption rate peaks were assigned empirically, as described for Fig. 1, and then compared to the individual's gamma images for confirmation. As stated previously, periods of high local concentration and low flow rate correspond with absorption rate peaks. Periods of migration from one compartment to the next coincide with wide dispersion of radiation and drug, resulting in absorption rate troughs.

The uncertainty surrounding the actual number of GI absorption compartments is understandable in light of the time–absorption rate profile shown in Fig. 6. In this individual, gastric emptying is observed to occur in two distinct phases, separated by a brief

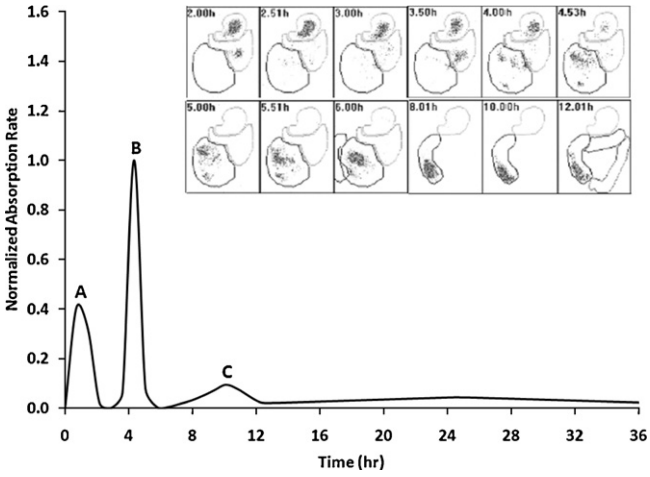

Fig. 4. The time–absorption rate profile is shown for subject 1, after being dosed with 500 mg Naproxen Sodium. Gamma scintigraphy images for the same individual are shown for comparison.

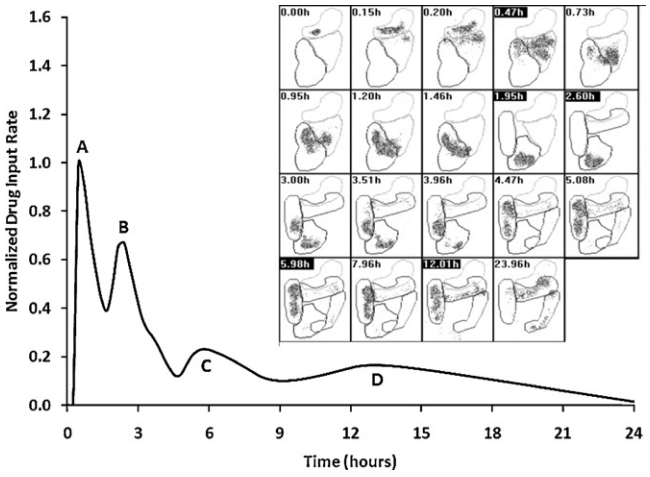

Fig. 5. Absorption rate profile for GW678248, subject 9. The gamma scintigraphy images for this subject/dose are displayed to the right and image times corresponding to absorption rate peaks are highlighted.

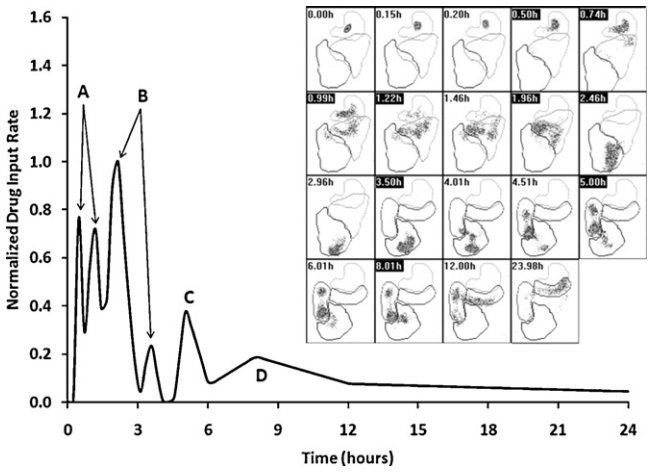

Fig. 6. Absorption rate profile for GW678248, subject 11. The gamma scintigraphy images for this subject/dose are displayed to the right and image times corresponding to absorption rate peaks are highlighted.

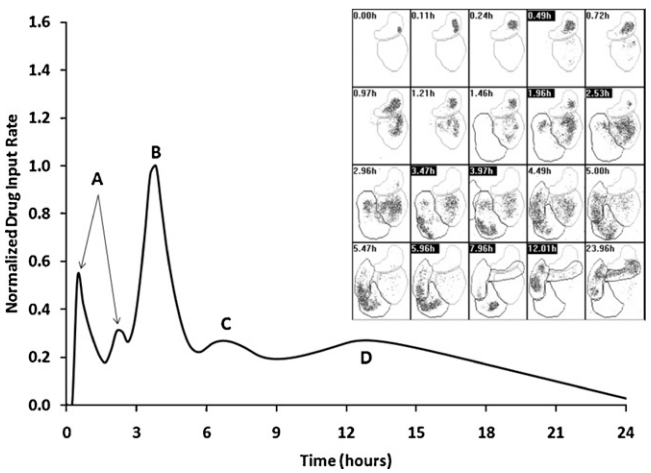

Fig. 7. Absorption rate profile for GW678248, subject 7. The gamma scintigraphy images for this subject/dose are displayed to the right and image times corresponding to absorption rate peaks are highlighted.

pause. The pause was long enough to divide absorption in both the proximal and distal regions of the small intestine into two phases each. However, passage through the cecum appears to have recombined the separate fractions, so that drug absorption from the ascending and later colon were unaffected by dual phase gastric emptying. The result is that four absorption compartments have the superficial appearance of six. However, the fact that absorption in the ascending colon has been unaffected by earlier events makes it easy to identify in the time–absorption profile. Therefore, the ascending colon compartment can serve as a landmark, to assist in identifying the earlier compartments. By interpreting the absorption rate profile in this way, it is possible to attribute the earlier absorption rate peaks to biphasic gastric emptying even in the absence of gamma scintigraphy data. It should be noted that drugs subject to enterohepatic recirculation may also seem to exhibit additional absorption compartments. To date, the author has had no opportunity to study absorption rate profiles for such a drug and it is presently unknown how the additional peaks would alter the appearance of the absorption rate profile.

In Fig. 7 gastric emptying was also biphasic. However, for this individual there was no bi-phasic absorption beyond the proximal small intestine. The reason for this can be understood by close inspection of the gamma scintigraphy images. After the first phase of gastric emptying, radiation and drug have remained in the proximal small intestine in a locally high concentration for a brief period of time. However, the 2nd phase of gastric emptying coincides with a period of rapid migration from the proximal to distal small intestine. The two initially separated fractions of radiation and drug

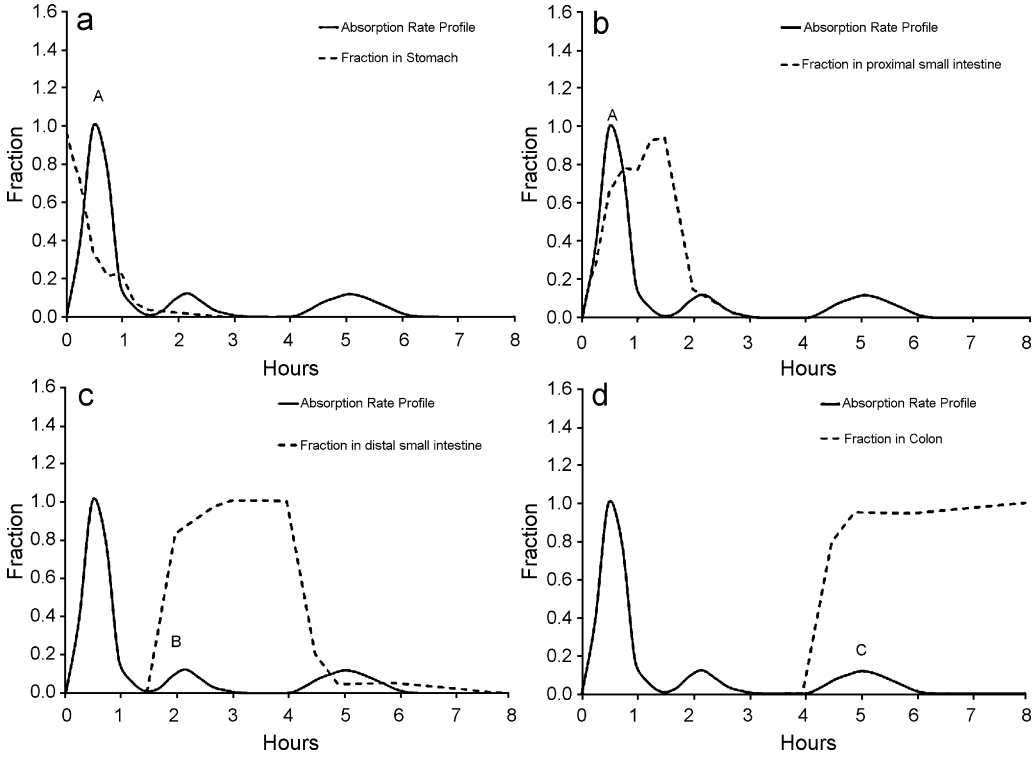

Fig. 8. Absorption rate profile for 20 mg GW353162, subject 112, with gamma count fractions from various regions of the GI tract overlaid. (a) Fraction remaining in the stomach, (b) fraction in the proximal small intestine, (c) fraction in the distal small intestine and (d) fraction in the colon, respectively.

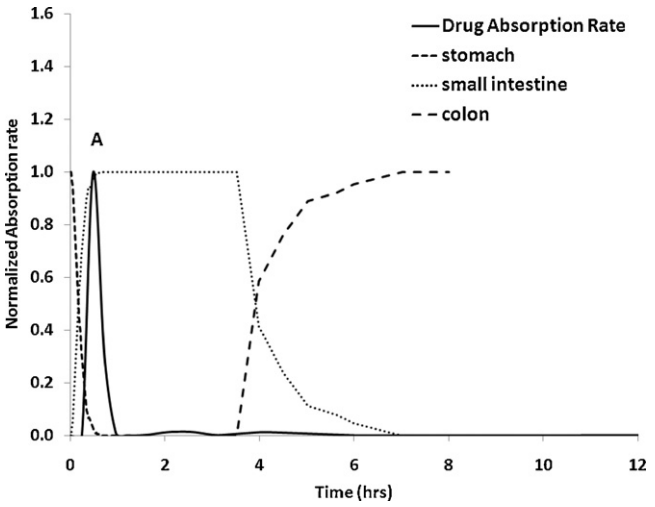

Fig. 9. Absorption rate profile for 100 mg GSK561679, subject 5. The fraction remaining in stomach, combined proximal and distal small intestine and colon are shown overlaid on the normalized absorption rate profile.

have arrived almost simultaneously in the distal small intestine at 3.47–3.97 h, resulting in a single absorption rate peak from this compartment. The final two absorption phases can be seen to correspond to the ascending and transverse colon, as described earlier.

Inspection of gamma scintigraphy images provides a useful qualitative demonstration of the drug migration through the GI tract. However, it is difficult to estimate the proportion of drug in each region of interest, directly from these images. Alternatively, gamma scintigraphy can be viewed as a plot of fraction remaining in a region of interest versus time. In Figs. 8–10, the fraction of radiation remaining in the gastric phase, small intestine and colon are overlaid onto the time–absorption rate profiles for the corresponding individual. Absorption in the proximal small intestine corresponds with the interval of gastric emptying. Absorption in the distal small intestine occurs after gastric emptying is complete but before migration to the colon. When conditions are favorable for absorption in the ascending colon, this will be seen at or near the time when there is overlap between the small intestine and colonic phases of radiation migration. Generally, the transit time through the ascending colon appears to be similar to distal small intestinal transit, so this absorption rate peak will have a similar width at the baseline. Finally, transit through the transverse or later colon is

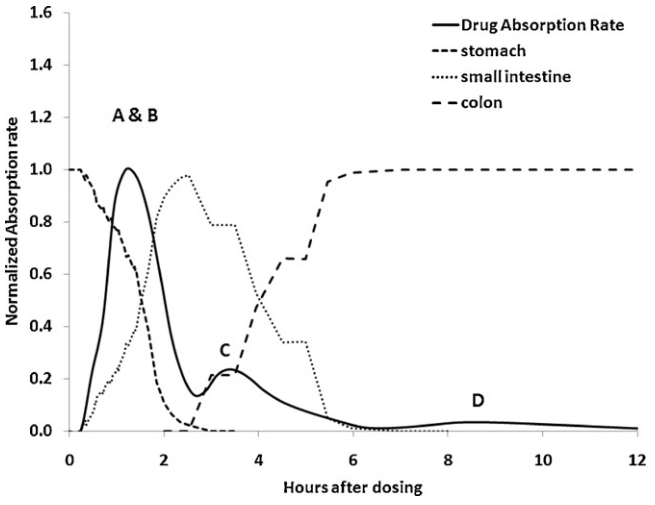

Fig. 10. Absorption rate profile for 100 mg GSK561679, subject 2, 30 min after a moderate fat meal.

longer than for the three previous compartments. If it is observed at all, the peak absorption rate is rarely as high as that of the earlier absorption compartments.

Although all individuals seem to have four separate absorption compartments along the GI tract, there are cases where fewer compartments are actually observed. For example, if a drug is soluble in gastric fluid but precipitates soon after entering the small intestine, only one absorption compartment may be observed. In the case of enteric coated products, it is conceivable that absorption in the proximal small intestine will not be observed, while absorption in the distal small intestine and later compartments is evident. The time–absorption rate profile shown in Fig. 9 shows that absorption is essentially complete in the proximal small intestine. The subject received a 100 mg tablet of GSK561679, a CRF1 receptor antagonist. For this drug, solubility and permeability are favorable for absorption in the distal small intestine and ascending colon and absorption in these compartments was observed for other individuals. However, the dose was relatively low and proximal intestine absorption rate was high, so it is likely that available drug was absorbed before passing to the distal small intestine.

Finally, there are instances where the absorption rate peaks for proximal and distal small intestine are unresolved. This condition is characteristic of prolonged gastric emptying. It is frequently seen when drug is dosed in the fed state but is also observed in some individuals in the fasted state. With prolonged gastric emptying, drug is expected to be migrating to the proximal and distal regions of the small intestine simultaneously, so what is normally observed as two distinct absorption rate peaks will be merged into one. The time–absorption rate profile shown in Fig. 10 illustrates the effect of prolonged gastric emptying, after the subject received a 100 mg tablet of GSK561679 in the fed state. The absorption peak attributable to the ascending colon would be easily discerned even without gamma scintigraphy data, so it is clear that the first two absorption phases have merged.

While the method of determining sites of drug absorption is interesting it must be conceded that this information is of little practical value unless it can be used to shed additional light on the in vivo fate of drug products as they pass along the gastrointestinal tract. Pharmacokinetic studies often turn out differently than expected, resulting in long delays in product development and expensive investigations which may or may not actually contribute to understanding of the problem. Among problems frequently encountered by pharmaceutical scientists are: failure to show bioequivalence, significant loss of exposure on repeat dosing and excessive inter-subject variability, among many others.

Determination of drug sites of absorption is only the first phase of an investigational process that has been called absorption rate analysis. It is the author's contention that each subject in every well designed pharmacokinetic study reveals the in vivo fate of a drug product in precise detail. However, the ability to obtain this information and take advantage of it requires highly developed observation skills. The investigator must be able to see the details within the absorption rate profile and recognize the significance of what has been seen. For example, the investigator may observe two subjects who have received the same regimen. For one of the subjects, it is observed that gastric emptying occurred soon after dosing, that the majority of absorption has occurred in the distal small intestine and that the maximum rate of absorption is about 30 mg/h. For the second subject, it is observed that gastric emptying is prolonged over 2 h, that most absorption has occurred in the proximal intestine and that the maximum rate of absorption is 3 g/h. Anyone will recognize that these are significant details. With due consideration, the investigator will conclude that if the dose has an opportunity to dissolve in the stomach, the proximal small intestine represents a more favorable absorption site than the distal small intestine. Furthermore, for individuals whose absorption

occurred in a less favorable site, it would be reasonable to conclude that a physical or physiological process prevented absorption in the proximal small intestine. A tablet may have ejected from the stomach prior to disintegration, for example. Assuming that similar observations are made for the entire study population, it would seem the best way to optimize drug exposure and reduce variability would be through the use of a very rapidly disintegrating formulation.

A systematic approach to observation of in vivo absorption involves answering a few basic questions:

Where was the drug located at the start of absorption?

(1) Where was the drug located when absorption was last observed?
(2) How much time elapsed between the onset and end of absorption?
(3) Does GI motility change with multi-day dosing?
(4) Does one compartment seem to be a more favorable site of absorption than the others?
(5) If so, does drug often seem to bypass the most favorable site and absorb largely in other sites?
(6) Does an increase or decrease in dose alter the observed absorption characteristic?
(7) How does food, antacid or dose frequency alter absorption characteristic?
(8) Do one or more individuals have absorption characteristics that seem atypical?
(9) If so, describe the atypical phenomena.

This is not meant to be an exhaustive list of appropriate observations but is should cover most circumstances that investigators are likely to encounter. Once the questions have been answered for each subject in one or a series of clinical studies, it is necessary for the investigator to develop a hypothesis that can account for the circumstances that have been observed. For example, drug absorption never begins in the ascending colon unless some physical cause has prevented absorption in the small intestine. With knowledge of the composition and the results of in vitro tests for the formulation that has been dosed, the investigator will easily form a rational and testable hypothesis to account for the observed clinical results.

With that brief explanation of how absorption rate analysis should be conducted, it is appropriate to offer a few examples of its use in actual practice. The object such an investigation is first of all, to explain why the specific results were obtained. Once the factors contributing to absorption characteristics have been identified, options for formula optimization may become apparent. The following examples have been encountered many times by pharmaceutical scientists but the underlying causes have often defied explanation. Through the application of absorption rate analysis, the cause for each of the following circumstances can be readily understood.

Example 1. Drug exposure drops during repeat dosing: A common observation during drug development is that during repeat dosing, AUC drops substantially, relative to the first dose. While this phenomenon may be common, the cause is rarely understood satisfactorily. However, inspection of individual absorption rate profiles may reveal an obvious cause. Fig. 11 shows the absorption rate profile for an individual after dosing with 500 mg Naproxin Sodium. Naproxin is a known GI tract irritant. After repeated dosing of the irritating compound, gastric residence time prior to onset of absorption is observed to be much longer than after the first dose. Furthermore, after repeated dosing, transit through the small intestine is observed to be more rapid than after the first dose. Gamma scintigraphy for the individual represented in Fig. 11 shows that the bulk of the dose transited from stomach to ascending colon

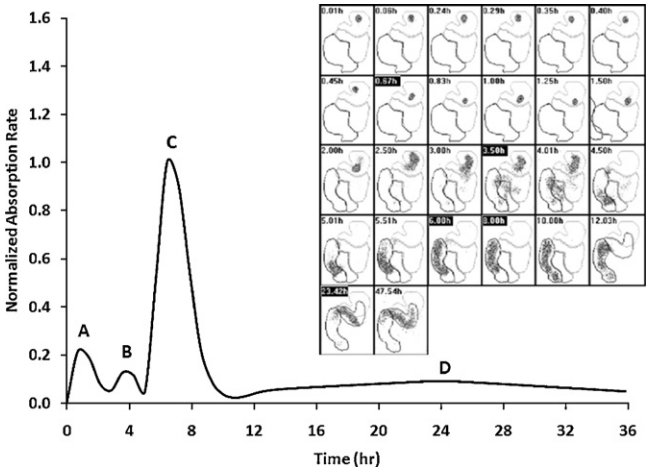

Fig. 11. Absorption rate profile and corresponding gamma scintigraphy images for an individual after dosing with 500 mg Naproxin Sodium.

in just a few minutes, between 4 and 4.5 h after dosing. The same information can be readily deduced from the absorption rate profile. The ascending colon presents less favorable conditions for drug absorption than the proximal and distal regions of the small intestine but the majority of drug absorption is in the ascending colon. Consequently, it is reasonable to conclude that most of the drug transited the small intestine too rapidly for significant absorption to occur. This mechanism for rapidly bypassing two important sites of absorption would certainly account for reduced drug exposure.

Example 2. Investigation of a failed bioequivalence study: One factor that contributes to the utility of absorption rate analysis is the reproducibility of an individual's GI function. Some variation is to be expected but the similarity from day to day is sufficient that within a small population of subjects, individuals can be easily identified and distinguished from each other on the basis of the patterns observed in the absorption rate profiles.

In the case of a bioequivalence study comparing capsules and tablets, the individual reproducibility of absorption rate profiles was used to attribute the likely cause of the failure. Fig. 12 has been taken from a study in which a tablet formulation failed to show bioequivalence to a previously developed capsule formulation. The figure shows a comparison of one individual's absorption rate profiles for capsule and tablet. The absorption rate profiles are similar through the regions attributable to the proximal and distal small

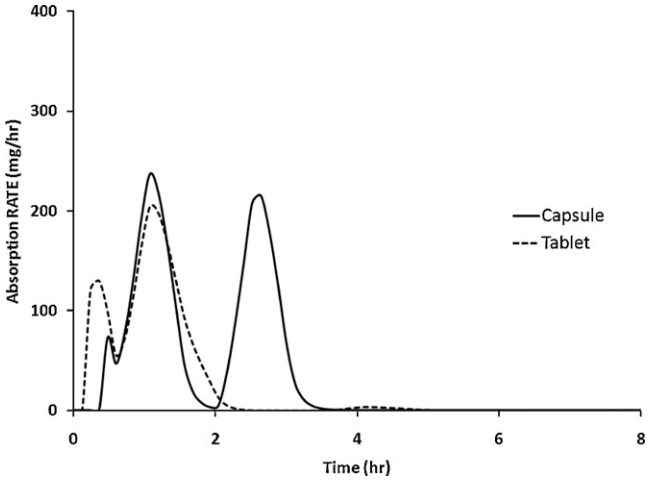

Fig. 12. Comparison of the absorption rate profiles for one individual participating in a failed bioequivalence study.

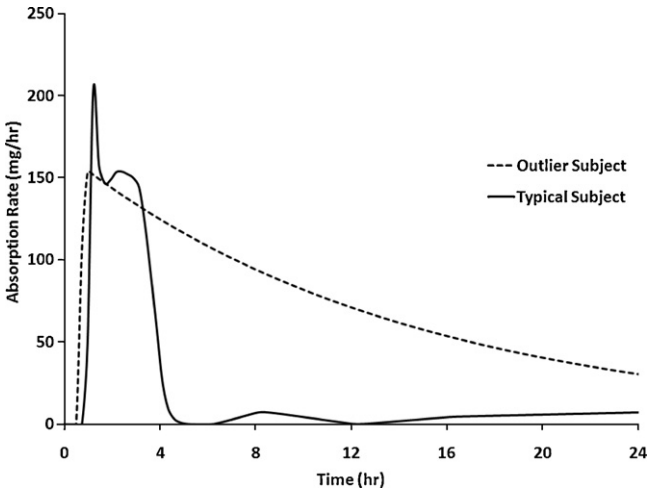

Fig. 13. A comparison of absorption rate profiles from two individuals who have been received a high dose of a drug with low oral bioavailability 30 min after a high fat meal.

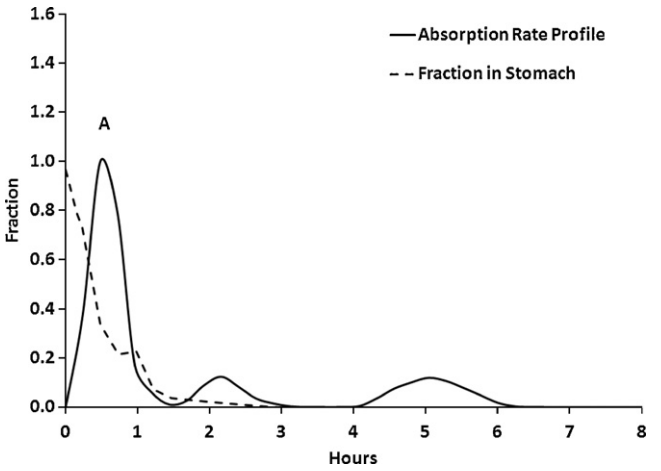

Fig. 14. The absorption rate profile and stomach gamma scintigraphy reproduced from Fig. 8a, above.

intestine, but the tablet dose exhibited no absorption in the ascending colon despite the observation of substantial absorption from the capsule. For this individual, the difference in exposure from capsule and tablet was proportional to the fraction of drug absorption that seemed to be "missing" from the ascending colon absorption phase. The same relationship appeared to apply to the study population, as a whole. When the absorption rate profiles were similar throughout, the two formulations were bioequivalent for that individual. However, when dissimilarity was observed between the absorption rate profiles, a significant part of the tablet absorption rate profile was observed to be reduced or absent and drug exposure from the tablet was observed to be reduced by a proportional amount.

The observation that differences in individual absorption rate profiles corresponded to proportional differences in drug exposure was suggestive of excessive in vivo disintegration time from the tablet. A portion of the tablet has not been absorbed in some individuals because it passed through the relevant absorption compartments in its original solid state. Further in vitro tests supported this interpretation and once the problem was identified, it was easily corrected.

Example 3. Identification of outlier individuals in a PK study: There are occasions when the interpretation of pharmacokinetic data may be strongly biased by the inclusion of data from a single individual. The individual's drug exposure may be several times greater or less than the study population for a particular regimen. Every instinct identifies the individual as an outlier but without adequate justification, the individual's data cannot be treated separately from the rest. However, individual absorption rate profiles may provide sufficient justification for identifying and individual as an outlier.

For a drug with low oral bioavailability and/or large dose required, the amount of drug absorbed in a specific GI compartment is a function of both the effective permeability (P_{eff}) and the dwell time in that compartment. Fig. 13 compares the absorption rate profiles for two individuals after dosing such a drug in the fed state. As expected, the proximal small intestine represents a favorable site of absorption for both individuals. However, for the individual identified as a "typical" subject, gastric emptying and proximal intestine transit appear to be complete by 2 h after dosing. Therefore, most of the drug absorption is observed to occur from the distal small intestine phase for this individual. In the case of the "outlier" subject, gastric emptying is prolonged over a period of at least 24 h after dosing, so the proximal small intestine is exposed to a high concentration of drug for the entire time. As might be expected, high drug concentration for a prolonged period of time in an absorbing compartment leads to high exposure. In this instance, the subject identified as an outlier had drug exposure almost three times higher than the population mean. The observation that the gastric emptying period exceeded 24 h would seem to justify his identification as an outlier, since it is not typical in a population of healthy volunteers.

Example 4. Attributing a cause for high PK variability: In many drug development programs, high inter- and intra-subject variability is identified as a factor that needs to be addressed to ensure that the drug is both safe and efficacious. Unfortunately, such efforts are bound for disappointment unless the excessive variability can be reasonably attributed to a controllable variable. Once again, however, a thorough interrogation of absorption rate characteristics may indicate the cause of variability without incurring large expense or long delays in the development program.

In one instance, a basic drug with high gastric solubility exhibited such high inter- and intra-subject variability in exposure that the viability of the development program was threatened. Since gastric solubility was high, absorption in the proximal small intestine (subsequent to gastric emptying) was thought to play a significant role in PK variability. Using absorption rate analysis, the absorption period associated with gastric emptying can be readily identified, as seen previously in Fig. 8a. This figure is reproduced in larger size as Fig. 14. For absorption in the proximal small intestine, the peak in absorption rate corresponds in time with the point of inflection in the time-fraction remaining gastric emptying profile. Consequently, the gastric emptying rate (1/h) can be easily calculated for each individual.

Pharmacokinetic data from three similar relative bioavailability PK studies was combined into a single data set. AUC and gastric emptying rate were determined for each individual and are presented in Fig. 15. It can be seen that drug exposure is a function of gastric emptying rate and that variable gastric emptying rate is the largest contributor to variable exposure. As with the "outlier" subject identified in Fig. 13, a slow gastric emptying rate resulted in high drug exposure. It may be noted that the distribution of data in Fig. 15 is not particularly random. Although none of the formulations was designed with the intent of altering the gastric emptying rate, two of the formulations seemed to have done so. In the crossover study, those formulations prolonged the gastric emptying time by several hours, compared to the original reference dose.

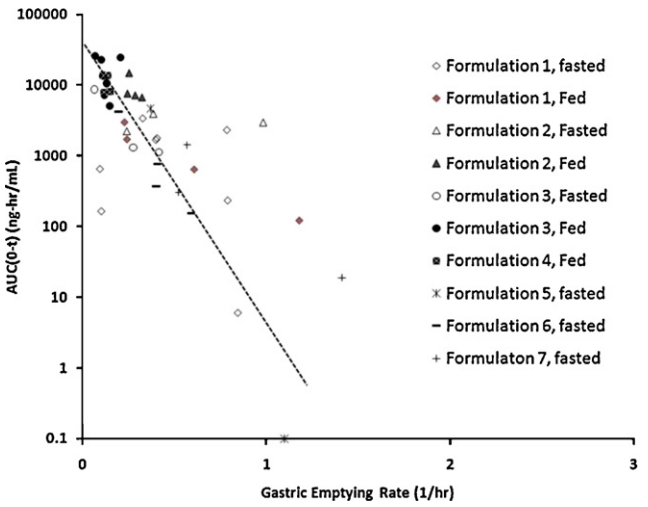

Fig. 15. Exposure versus gastric emptying rate for individuals combined from three small PK studies.

4. Conclusions

Deconvolution of individual plasma time–concentration data shows that drug absorption occurs in discreet compartments of the GI tract. Inspection of corresponding gamma scintigraphy images makes it clear that the compartmental absorption rate phenomena is the result of discontinuous flow through the GI tract. During periods of migration from one absorbing compartment to another, radiation and drug are widely dispersed, resulting in low local bulk drug concentrations and coinciding low absorption rates. Once fluid enters a new compartment, it is seen to collect or concentrate in a narrow region of the compartment, coinciding with a new absorption rate peak.

It has also been shown that the four absorption compartments are consistently identifiable in time–absorption rate profiles, regardless of the individual or drug being studied. Though the system of absorption rate peak identification was derived empirically, it has been verified by comparison with gamma scintigraphy in a number of individuals from four different pharmacokinetic studies. Of 70 absorption rate profiles calculated from the four studies, agreement with gamma scintigraphy was observed for 66 (>90%). Therefore, absorption rate analysis can be used as a reliable way of tracking a drugs' progress through the GI tract. It is expected that other investigators will observe the same absorption rate phenomena in other clinical PK data and will easily learn to identify absorption rate peaks without the aid of gamma scintigraphy data.

Absorption rate analysis is a new technique for interpretation of PK data, and is still in development at the time of this writing. However, in the first few months of use, it has been found to be a powerful and flexible tool for extracting valuable information from a wide range of pharmacokinetic data. Among the variety of information that has been extracted are:

- Location of the most favorable site for dissolution and whether or not the dose can be kept there.
- Location of the most favorable site for absorption.
- Whether or not the characteristics of the formulation take advantage of the most favorable sites of dissolution and absorption.
- Whether the drug alters GI motility.
- An estimate of a drug's solubility limited dose.
- Whether exposure can be increased by particle size reduction.
- Whether therapeutic drug concentration can be prolonged with an MR formulation.
- Whether a drug product is a potential candidate for an IVIVC. That is, whether the rate and extent of in vivo dissolution depends on the formulation, is a property of the unformulated drug substance or the physiological characteristics of the patient.

The value derived from absorption rate analysis depends largely on disciplined and methodical observation skills. A systematic routine for applying the method to PK interpretation has been described and it is hoped that interested readers will find the recommendations valuable in developing their own observation skills.

Finally, it should be recognized that absorption rate analysis offers the most value to pharmaceutical scientists when applied early in a drug development program. There is certainly a benefit in being able to explain what went wrong but there may be more satisfaction in avoiding problems. Gaining a clear understanding of how a drug behaves in the GI tract during the first time in humans study will be of great assistance in designing the most appropriate drug formulation. It is believed that the routine practice of absorption rate analysis at the beginning of product development programs will lead to a reduction in the number of phase I clinical studies required for product development, as well as fewer program delays, investigations and reformulation efforts.

References

Dokoumetzidis, A., Kalantzi, L., Fotaki, N., 2007. Predictive models for oral drug absorption: from in silico methods to integrated dynamic models. Expert Opin. Metab. Toxicol. 3, 491–505.
Goodacre, B.C., Murray, R.J., 1981. A mathematical model of drug absorption. J. Clin. Hosp. Pharm. 6, 117L 133.
Suttle, B.A., Pollack, G.M., Brouwer, K.L.R., 1992. Use of a pharmacokinetic model incorporating discontinuous gastrointestinal absorption to examine the occurrence of double peaks in oral concentration–time profiles. Pharm. Res. 9, 350–356.
Yu, L.X., Lipka, E., Crison, J.R., Amidon, G.L., 1996. Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. Adv. Drug Deliv. Rev. 19, 359–376.
Sawamoto, T., Haruta, S., Kurosaki, Y., 1997. Prediction of plasma concentration profiles of orally administered drugs in rats on the basis of gastrointestinal transit kinetics and absorbability. J. Pharm. Pharmacol. 49, 450–457.
Kimura, T., Higaki, K., 2002. Gastrointestinal transit and drug absorption. Biol. Pharm. Bull. 25, 149–164.
Dressman, J.B., Fleisher, D., Amidon, G.L., 1984. Physicochemical model for dose-dependent drug absorption. J. Pharm. Sci. 73, 1274–1279.
Dressman, J.B., Fleisher, D., 1986. Mixing-tank model for predicting dissolution rate control of oral absorption. J. Pharm. Sci. 75, 109–116.
Hintz, R.J., Johnson, K.C., 1989. The effect of particle size distribution on dissolution rate and oral absorption. Int. J. Pharm. 51, 9–17.
Oberle, R.L., Amidon, G.L., 1987. The influence of variable gastric emptying and intestinal transit rates on plasma level curve of cimetidine: an explanation for the double peak phenomenon. J. Pharmacokinet. Biopharm. 15, 529–544.
Luner, P., Amidon, G.L., 1993. Description and simulation of a multiple mixing tank model to predict the effect of bile sequestrants on bile salt excretion. J. Pharm. Sci. 82, 311L 318.
Cutler, D.J., 1978. Numerical deconvolution by least squares: use of prescribed input functions. J. Pharmacokinet. Biopharm. 6, 227–241.
Cutler, D.J., 1978. Numerical deconvolution by least squares: use of polynomials to represent the input function. J. Pharmacokinet. Biopharm. 6, 243–263.
Johnson, D.A., Amidon, G.L., 1988. Determination of intrinsic membrane transport parameters from perfused intestine experiments: a boundary layer approach to estimating the aqueous and unbiased membrane permeabilities. J. Theor. Biol. 131, 93–106.
Li, J., Cutler, D.J., 1998. Stability of finite difference deconvolution. I: Theoretical analysis. Biopharm Drug Dispos. 19, 547–554.