Comparison of In Vitro and In Vivo Dissolution for the Study of Colonic Drug Absorption

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I. INTRODUCTION

Drug therapy is predominated by oral formulations, since the oral route is the most convenient and popular means of applying a drug. The systemic availability of orally administered drugs is determined routinely as part of drug development during phase I, and later as part of in vivo quality control. The reduced systemic availability of some oral formulations may be explained, or even predicted, by their pharmacokinetic fate in the body after intravenous administration. However, the reduction in availability of many drugs or formulations is not fully understood. The reasons for this have not been further explored, but were often attributed to lacking absorption or incomplete dissolution.

Since oral formulations play such an important role in drug therapy, expressing availability as percentage alone is unsatisfactory. Interest should also focus on the reasons for the reduced availability. Particularly with sustained-release formulations or drugs with

slow dissolution rates, it is important to know whether the drug is absorbed throughout the gastrointestinal tract at the same rate and extent or whether the rate and extent differs markedly from site to site. Since the residence time of a formulation within a certain part of the gastrointestinal tract is limited, a small or limited rate of absorption may affect the extent of absorption. In the same way, the rate of dissolution from a solid oral dosage form may change the extent of absorption, depending on the total residence time of the formulation in the gastrointestinal tract or on the residence time in the absorbing part.

Kübler (1) has transformed these considerations into a pharmacokinetic model and applied this model to findings with sulfhathiocarbamid, propicilline, xylose, and small amounts of ascorbic acid (1, 2). The complex "window" model of Kübler was later modified in several ways (3–5) and used to explain the concentration–time profiles of griseofulvin, buformin, and sulfisoxazole (4), piretanide, and furosemide (5, 6).

The authors developing various "window" models were not referring to certain segments of the gastrointestinal tract. However, the dominant site of absorption for some drugs was localized (2) and certain sites like the large bowel were shown to be nearly incapable of absorbing relevant amounts of drug (7, 8).

Experimental results with theoretical considerations on the physiology of the gastrointestinal tract led to the assumption that drug absorption occurs primarily in the upper part of the gastrointestinal tract. In the anatomical reserve length concept for intestinal absorption this presumption became part of the consideration, but is not necessarily absolutely linked to it (9).

Hirtz questioned the "window" concept because of the "absence of convincing experimental proof for its existence" (10) and his own results with metoprolol (11). Since then, equipment and experimental procedures have been developed or adjusted to allow for study of the absorption properties of different sites of the gastrointestinal tract generally, and of the colon particularly. They consist of intubation or endoscopy techniques, the high-frequency (HF) capsule, or the gamma-scintigraphic visualization technique.

Recently, a new approach was elaborated to study the absorption properties of the gastrointestinal tract, and particularly of the colon by comparing the in vitro results of drug dissolution with the in vivo profile of dissolution or absorption (12–15). It is a mathematically based approach that was deduced from moment analysis for both in vitro dissolution testing and in vivo studies of the same formulation. The results obtained by this method encouraged the hypothesis that controlled-release formulations can be used as probes to explore the rate and extent of absorption along the entire gastrointestinal tract.

Since the transit times of formulations through the different sections of the gastrointestinal tract have been studied extensively and are known, the result obtained by the method described here can also be used to specify which fraction of the dose is absorbed from the small intestine and which from the colon. Furthermore, it can be judged whether the rate constant of absorption is changing along the intestinal tract in general and with the passage of the formulation into the colon in particular. However, the controlled-release formulation can only function as a probe if a relevant amount of the dose is still delivered when it has reached the colon.

II. METHODOLOGIC BACKGROUND

The new approach focuses on continuous (point by point) comparison of the in vivo with an in vitro dissolution profile. However, it must be stressed that the in vivo dissolution profile can only be determined indirectly (e.g., by deconvolution). Therefore, it seems more appropriate to use the term *hypothetical in vivo dissolution profiles*.

Candidates for such a comparison are only formulations that show a sustained- or controlled-release profile in vitro that fulfill certain prerequisites (12). For example, the in vitro dissolution profile must characterize the formulation, but not the dissolution apparatus used. These prerequisites will be discussed later.

In general, the in vitro and in vivo dissolution profiles are not immediately superimposable. Comparison of in vitro and in vivo dissolution profiles starts with the assumption, which must be confirmed or rejected, that the in vivo dissolution curve has in principle the same profile as the in vitro curve. They differ only in the absolute units of their time axes, that is, both dissolution profiles can be superimposed by linear transformation of the time axes of either (13). This concept is illustrated in Figure 1.

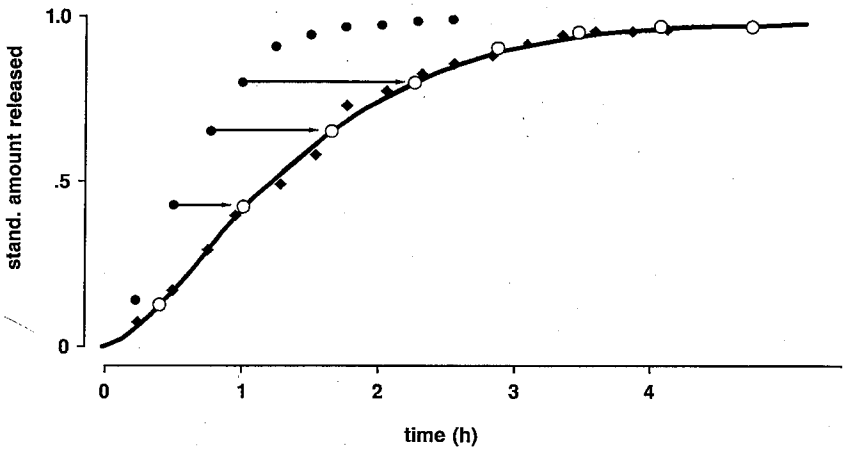

Figure 1 Homomorphism of two dissolution profiles and equivalence of the experimental conditions. Results shown are from dissolution testing of film-coated tablets of molsidomine in the Sartorius (●) and the USP paddle (◆) dissolution model. Least-square adjustment of a cumulative gamma function from data with the paddle model (solid line). Release profiles are superimposable by uniform linear transformation of the time base (arrows). The transformed data (○) are randomly distributed around the gamma function, showing that the two dissolution profiles provide the same information.

Figure 1 shows the cumulative amount released under two different in vitro dissolution conditions, A and B, and how both are correlated. The same considerations are valid if one of the two profiles represents a hypothetical in vivo dissolution. A cumulative gamma function, depicted as a solid line, has been adjusted to the data for condition B. After one rescales the time values of all observations related to condition A by uniform linear transformation, the data represented by open circles are obtained. This linear transformation is illustrated for three data pairs by arrows. The transformed data for condition A are randomly distributed around the function adjusted to the data for condition B.

The profiles are perfectly superimposable after linear transformation of the time bases of either. They are, therefore, homomorphic and the dissolution conditions are termed equivalent (13). Dissolution profiles superimposable without transformation of the time axes are termed isomorphic (16).

This method of comparing two dissolution profiles is the essential feature of continuous in vitro–in vivo correlation and any conclusions based on it. It simply means that the elapse times under conditions A and B are different. There is an easy way to show homomorphism of two dissolution profiles without describing the mathematics. The procedure is illustrated in Figure 2.

The aim of the technique is to determine the time at which the same amounts have been dissolved under both conditions (14). For example, one starts with a certain amount dissolved under condition A at the t_A and determines the same amount dissolved under condition B at time t_B, if necessary by interpolation. Then follows a check of whether t_A and t_B are related linearly for the entire dissolution profiles by plotting t_B against t_A.

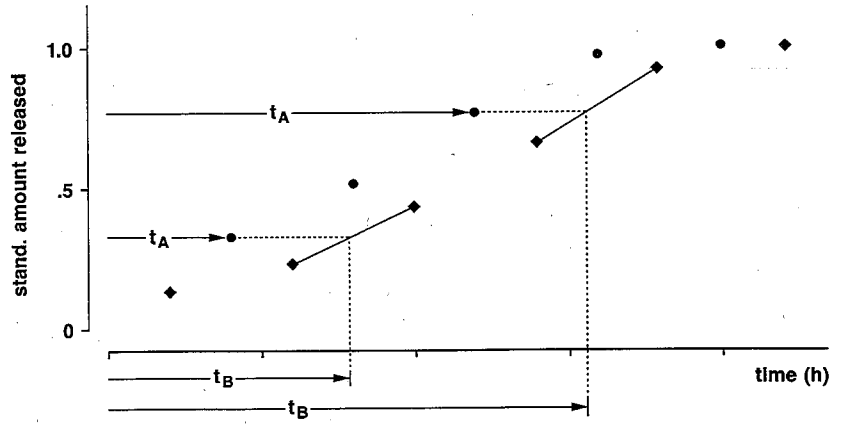

Figure 2 Technique for testing homomorphism of dissolution profiles. Cumulative fractions of drug dissolution as functions of time (●, ◆) originate from experiments under different dissolution conditions or from different apparatuses. Homomorphism of both dissolution profiles (i.e., equivalence of apparatuses or conditions) was tested by correlating dissolution times t_B with t_A related to identical amounts dissolved under condition A (●) and B (◆), respectively. Related time pairs were determined by suitable interpolation of the dissolution data; linear interpolation is shown (adapted from ref. 14).

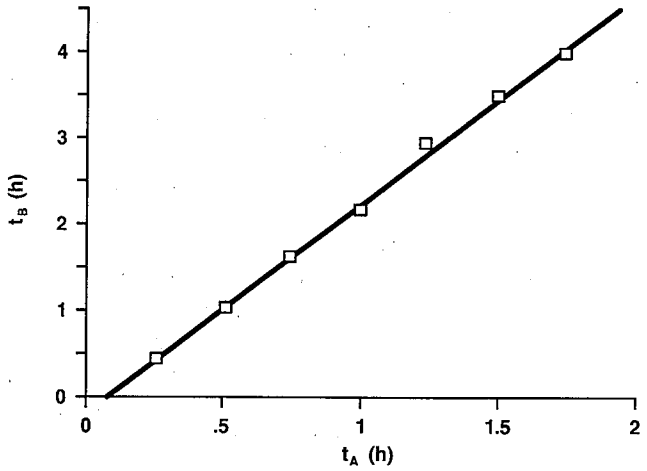

Figure 3 Correlation of dissolution times. Correlation of dissolution time, t_A and t_B, related to the same amounts dissolved under condition A and B for the data depicted in Figure 1. Intercept and slope provide the parameters to scale the time axis related to condition A to the time axis related to condition B (adapted from ref. 13).

This analysis was carried out for the two dissolution curves in Figure 1; the result is shown in Figure 3. Since both profiles are homomorphic, the time related to the same amounts dissolved under both conditions is clearly linearly related. As rule, if two dissolution profiles are homomorphic, the time at which the same amounts are dissolved is linearly related and vice versa.

This type of plot has been called the Levy-plot by our working group (14) since Gerhard Levy suggested this technique. He stated that

> Another approach to interpretation of the data is to plot the time required for the absorption of a given fraction of the dose versus the time required for the same fraction to dissolve in vitro. Such a plot yields a straight line nearly intercepting the origin, which suggests that the lag time referred to above is very short (17).

Levy compared in vivo absorption and in vitro release intuitively after the adjustment of in vitro dissolution to in vivo dissolution by varying the in vitro conditions. If the absorption of an aqueous solution is rapid, this correlation is correct.

Based on a more rigorous, mathematical definition of homomorphism of dissolution profiles, alternative algorithms to the Levy plot analysis can be used (13, 14).

III. APPLICATION

The two methods of continuous in vitro–in vivo correlation are illustrated in Figure 4.

A. First Alternative: From in Vitro Dissolution to in Vivo

Let us consider that a formulation is available and shows homomorphic dissolution profiles under various in vitro dissolution conditions. In general, each of these dissolution profiles has to be re-

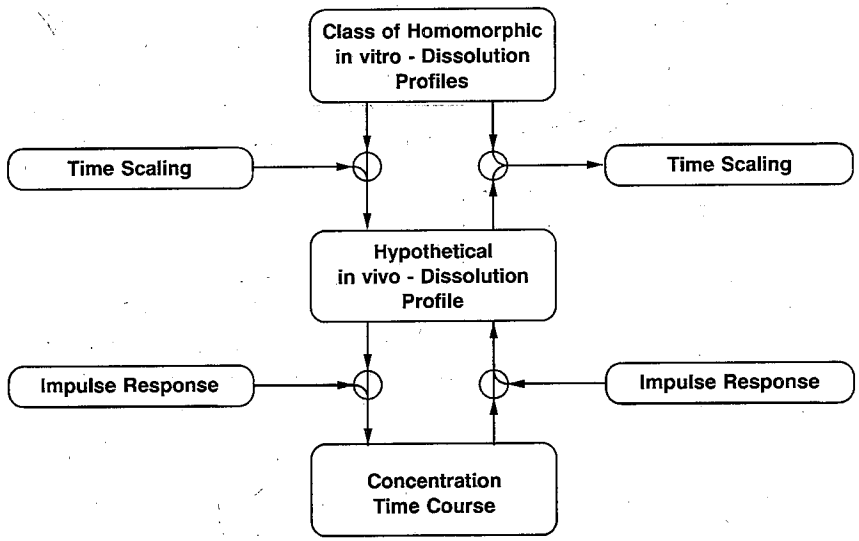

Figure 4 Two alternatives for the continuous comparison of in vitro and in vivo results. Left side of the scheme shows sequence of computational steps with the aim of comparing a predicted concentration profile with the actual readings. Right side shows procedure to obtain the hypothetical in vivo dissolution profile, which is then compared with the in vitro dissolution profile. See text for further details (adapted from ref. 15).

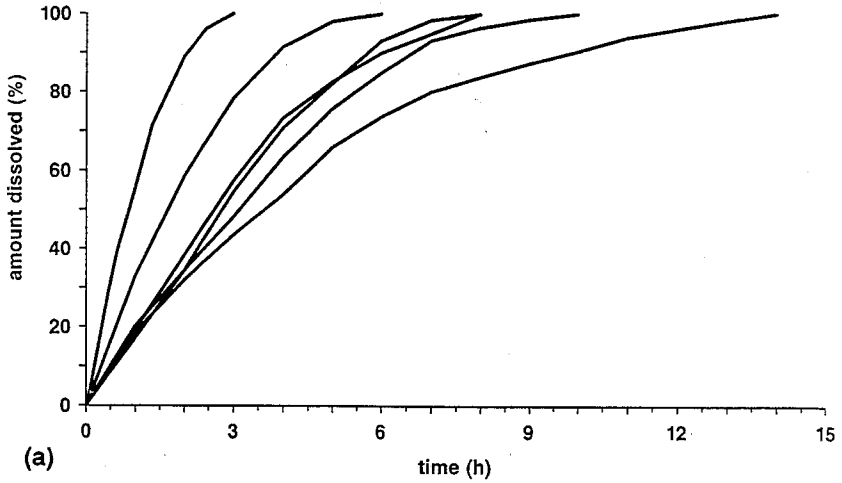

Figure 5 In vitro dissolution profiles for a sustained-release theophylline formulation. (a) Cumulative dissolution profiles of the sustained-release theophylline formulation. Depending on the dissolution condition, duration of dissolution ranged from 3 to 14 hr. Dissolution was tested under various in vitro conditions including different apparatuses (Sartorius, paddle, rotating flask), different revolutions per minute or agitation modes, and different dissolution media. Profiles represent means of five samples originating from the same batch.

scaled individually to be superimposable with the hypothetical in vivo dissolution profile (first step on left of Fig. 4). The correct transformation of the time axis is essential. After transformation, either of these profiles can be used to predict the concentration–time course by convolution. Convolution is a mathematical technique that allows one to predict the concentration–time profile (18, 19). For this it is necessary to know the input of drug to the body system (hypothetical in vivo dissolution, Fig. 4) and the response of the body system to an instantaneous input. In the context described here, the instantaneous input would be an oral solution and the concentration–time profile following its administration (standardized by dose) would be the response (impulse response, Fig. 4). Under certain conditions the concentration–time profile following intravenous application can substitute for that after an oral solution to deduce the impulse response.

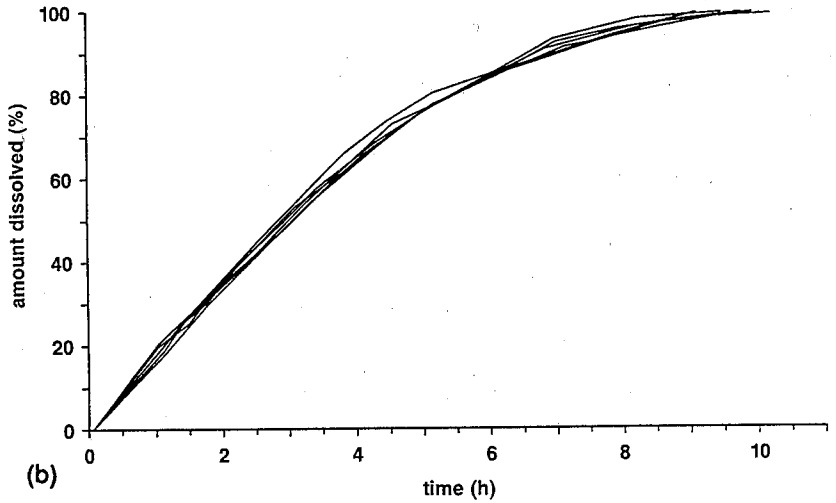

Figure 5 (b) Five dissolution profiles were rescaled to the time axis of the sixth (arbitrarily chosen as the reference) according to the methods for testing homomorphism of profiles (see ref. 16). Transformed profiles are practically superimposable (i.e., differences at each sampling point are less than the difference regularly observed between samples originating from the same batch) (adapted from ref. 22).

This first alternative is illustrated by data taken from a study with a sustained-release formulation of theophylline tested in eight healthy volunteers (20, 21). The sustained-release formulation and an oral solution was administered in a randomized crossover manner. The in vitro release of theophylline from this sustained-release formulation was tested under different conditions with different equipment (22). The resulting dissolution profiles are shown in Figure 5a. The release profiles are markedly different depending on buffer and agitation.

From this result it becomes apparent that one cannot specify which of the in vitro dissolution profiles, if any, is like the in vivo dissolution. However, for this formulation the dissolution profiles were homomorphic since they were adequately superimposable by linear transformation of the time axes (Fig. 5b). Since the in vitro dissolution profiles were homomorphic, it can be concluded that they reflect mainly a formulation property and not the dissolution

condition. This is one of the more stringent requirements that must be fulfilled by any formulation used in the continuous in vitro–in vivo correlation. None of the untransformed in vitro dissolution profiles was like the in vivo dissolution profile. This can be easily shown by their convolution with the impulse response function, followed by comparison with the actual concentration–time data (15). The result of convolution is shown in Figure 6.

None of the predicted profiles fits the true observations. In each case the in vitro release is faster than the in vivo. The time axis of the in vitro dissolution profiles must therefore be transformed to become identical with the in vivo, that is, the in vitro clock and the in vivo do not run at the same speed. Transformation of the time axis of the in vitro dissolution profiles is necessary. Since all in vitro profiles are homomorphic, one can choose any of them as

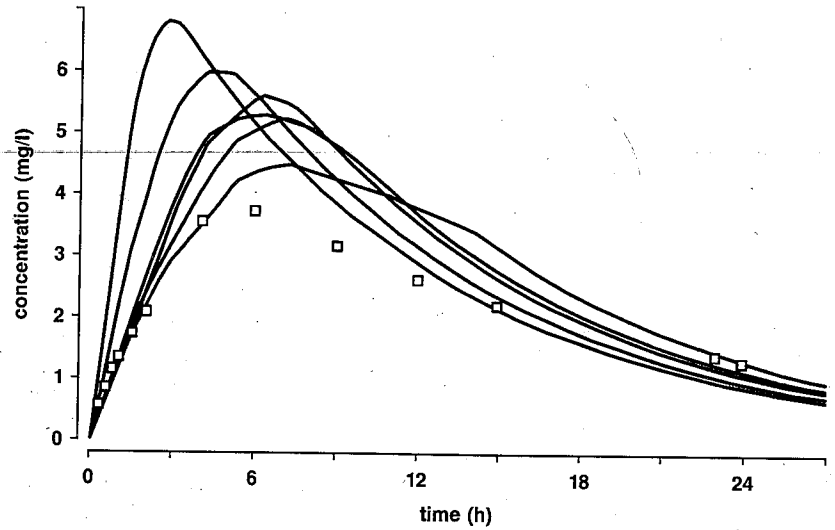

Figure 6 Predicted concentration–time profile for a sustained-release theophylline formulation without scaling the in vitro time towards the in vivo. Open squares (□) represent the mean serum concentrations of theophylline after administration of a sustained-release formulation. Solid lines are the results of prediction by convolution, with the various untransformed in vitro dissolution profiles as input functions. Body system was characterized by a weighting function, which is the dose-standardized and lag-time-corrected response to an oral solution (adapted from ref. 15).

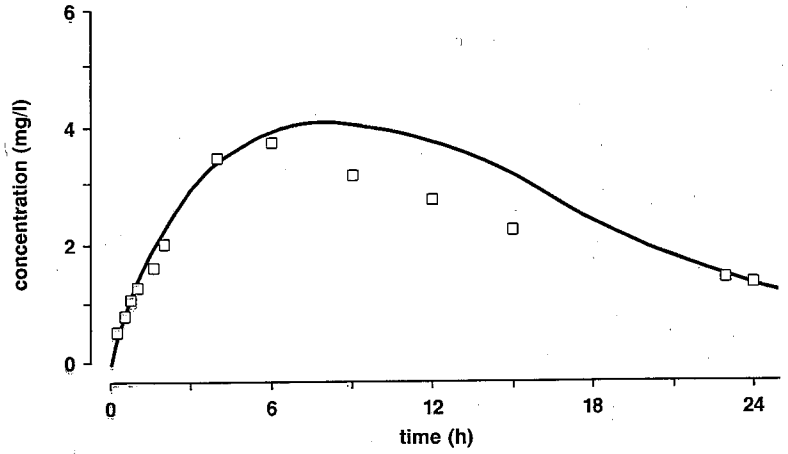

Figure 7 Predicted concentration–time profile for a sustained-release theophylline formulation with scaling of the in vitro time towards the in vivo. Open squares (□) represent the mean serum concentrations of theophylline. The solid line represents the result of prediction by convolution taking the time-scaled in vitro dissolution profiles as input function into the body system (adapted from ref. 15).

in vitro reference. The parameters for the transformation can be computed from the statistical moments of dissolution and transit times (15). After the time axis was rescaled, the prediction of the concentration–time course by convolution was reasonable, but not perfect (Fig. 7). Between 6 and 24 hr, the prediction differed clearly from the actual observations. This mode of analysis does not indicate any reason for the difference. Therefore, the alternative approach was applied to compare in vitro with in vivo results (right part of Fig. 4) (15).

B. Second Alternative: From in Vivo Concentration–Time Course to in Vitro

The hypothetical in vivo dissolution profile was estimated by deconvolution (first step on right of Fig. 4). The inversion of the convolution, that is, the determination of the input of drug to the body system, is termed deconvolution (19, 23–25). The result of deconvolution (the hypothetical in vivo dissolution profile) was then com-

pared with one of the in vitro dissolution profiles by the Levy plot technique (second step on right of Fig. 4).

Levy plots were produced for each individual. They did not show a single linear relation between the time at which the same amount was dissolved under in vitro and in vivo conditions. Therefore, the in vitro and the hypothetical in vivo dissolution profiles are not homomorphic. However, the plots showed two sections, each of which could be described by a straight line. The same type of Levy plot was determined for each of the individuals. They differed in the slopes for the first and second section of the Levy plot and in the ratios between the two slopes (15).

The results for the two volunteers with the smallest and largest ratios between slopes are shown in Figure 8.

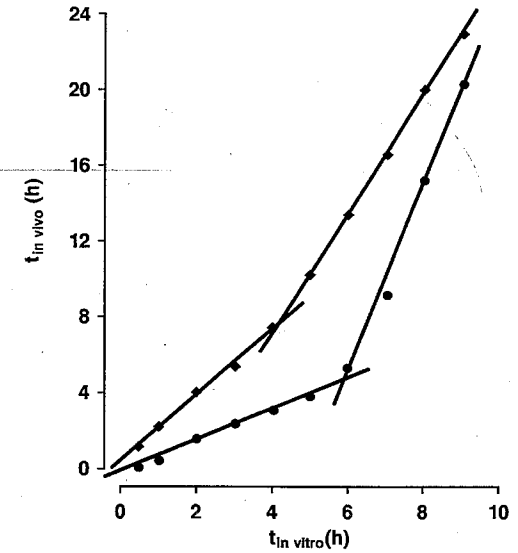

Figure 8 Correlation of in vitro and in vivo dissolution times for a sustained-release theophylline formulation. Correlation of dissolution times, $t_{in\ vitro}$ and $t_{in\ vivo}$, related to identical amounts dissolved under both conditions for the two subjects (◆ and ●), with smallest and largest ratio between the slopes for the two sections of the biphasic linear correlation. Same pattern for all subjects. The in vitro and the hypothetical in vivo dissolution profiles are not homomorphic and the dissolution conditions are apparently not equivalent (from ref. 15).

The transition from the first linear section to the second occurs, on average, after 5.3 hr (15), which is essential for the following. Possible theoretical reasons for such biphasic continuous in vitro–in vivo correlation include increasing clearance while absorption is still in progress, decreasing agitation in the gastrointestinal tract before complete in vivo dissolution, pH-dependent change in solubility, site-dependent degradation in the gastrointestinal tract, decreasing diffusion within the chymous mass, or decreasing absorption rate constant. Each of these has been discussed in detail (15) and most of them can be ruled out for theophylline. Therefore, it was concluded that most probably the rate constant of absorption for theophylline changes along the gastrointestinal tract and that this change occurs, on average, after 5.3 hr.

This interpretation was later confirmed by Staib and co-workers (26, 27). They used the HF capsule to release a theophylline solution at different sites of the gastrointestinal tract and determined the rate and extent of absorption. The extent of absorption (as measured by the area under the curve) was nearly the same for all sites of administration. However, the rate of absorption (as measured by the absorption half-life) differed to sixfold at the different sites (Table 1).

In the present example the time of transition from the first to the second linear section of the continuous in vitro–in vivo correlation apparently reflects the passage of the formulation from the small intestine to the ascending colon. The time of transition for this study was, on average, 5.3 hr. The average orocecal transit time reported for different formulations using the scintigraphic technique is about 5 hr (28–37).

IV. HYPOTHESES

The continuous in vitro–in vivo correlation was applied to data from a study with a sustained-release formulation of theophylline that had homomorphic in vitro dissolution profiles. In contrast to the expectations based on in vitro studies, the results showed that the hypothetical in vivo dissolution profile was not homomorphic with the in vitro dissolution profiles. The difference was explained by a possible change in the rate constant of absorption during transit of the formulation along the gastrointestinal tract (15). This

Table 1 Pharmacokinetic Characteristics of Theophylline

	N	AUC (mg h/l)	$t_{1/2.abs}$ (min)	$t_{1/2.elim}$ (hr)	MT (hr)
Oral solution	8	16.1 ± 3.6	9.5 ± 6.4	5.7 ± 1.4	8.5 ± 2.0
Stomach	3	16.3 ± 6.5	13.8 ± 8.8	6.3 ± 2.5	9.3 ± 3.6
Ileum	10	14.0 ± 4.5	25.6 ± 17.1	5.8 ± 1.3	9.0 ± 1.8
Ascending colon	6	15.0 ± 6.7	39.6 ± 27.6	7.4 ± 1.9	11.6 ± 3.1
Descending colon	4	14.4 ± 5.7	39.6 ± 36.4	6.9 ± 2.2	11.2 ± 3.2
Sigmoid colon	7	14.4 ± 4.8	64.0 ± 30.6	6.5 ± 1.7	10.8 ± 2.6

Source: Compiled from ref. 27.
Data were deduced from the concentration–time profile after administration of 100 mg theophylline at different sites of the gastrointestinal tract using the HF capsule and after administration of an oral solution.
AUC, Area under the concentration–time data curve; $t_{1/2.abs}$, absorption half-life; $t_{1/2.elim}$, elimination half-life; MT, total mean residence time.

interpretation was confirmed by Staib and co-workers using the HF capsule (26, 27). Therefore, the following hypotheses were formulated: Provided that a sustained-release formulation shows at least homomorphic in vitro dissolution profiles under different dissolution conditions. If a biphasic or polyphasic correlation is obtained when applying the continuous in vitro–in vivo correlation to this formulation, it suggests that the rate constant of absorption is different for the different parts of the gastrointestinal tract.

The larger the ratio of the slopes for the separate linear sections of the continuous in vitro–in vivo correlation, the larger the difference in the rate constant of absorption between the different sites of the gastrointestinal tract for the drug studied.

The region of the gastrointestinal tract in which the change of the rate constant of absorption takes place can be deducted from the continuous in vitro–in vivo correlation, that is, from the in vivo time related to the transition from the first linear section to the second.

V. SCRUTINY OF THE HYPOTHESES

A. Further Results for Theophylline

In a study with eight healthy volunteers, an oral solution of theophylline was compared with a theophylline formulation, which by content and release rate should have been suitable for once daily administration. The study followed a randomized crossover design. The in vitro dissolution of the formulation was determined by different apparatuses and under different conditions comparable to those described in Figure 5. Homomorphism was proven for the resulting dissolution profiles (38).

The concentration–time curve after administration of the 24 hr formulation was deconvoluted using the dose-standardized response after administration of the solution. The resulting hypothetical in vivo dissolution profile was subjected to Levy plot analysis; the in vitro dissolution profile obtained with a Sartorious apparatus was chosen as the in vitro reference. However, any of the in vitro dissolution profiles could have been used as reference since they were homomorphic with each alternative.

The correlation of time related to the same amounts dissolved under in vitro and in vivo conditions was biphasic, as already shown in Figure 8. A biphasic correlation was obtained for all subjects but one. The correlation for the mean curve is depicted in Figure 9, together with the mean for the study described first.

As can be seen in Figure 9, the slopes for the first and second section of the correlation plot differ for the two studies, although in both cases the same dissolution conditions from the Sartorious dissolution model were used. However, on average the time for the transition from the first to the second section is very similar for the two studies: 5.3 and 5.9 hr. Also, a large interindividual variation of the transition time was seen in both studies: 2.6–8.2 hr for the first study and 4.9–7.7 hr for the second study. The ratio of the slopes for the first and the second linear section of the correlation for both studies was broadly the same: 4.4 and 4.7 hr. In the first study 64% of the dose was dissolved and absorbed during the time period represented by the first section of the Levy plot, that is, 0–5.3 hr. Twenty-five percent of the dose was absorbed during the second section, which reflects absorption from the colon. Due to the slower release from the aimed 24 hr formulation, 41% of the

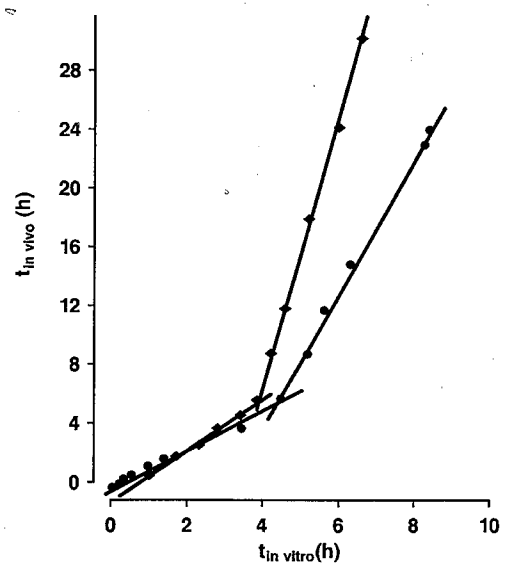

Figure 9 Correlation of in vitro and in vivo dissolution times for two different sustained-release theophylline formulations. Continuous in vitro–in vivo correlation for two studies with different sustained-release theophylline formulations. Mean values for both studies (◆ and ●). For both formulations, correlation of dissolution times $t_{in\ vitro}$ and $t_{in\ vivo}$, related to identical amounts dissolved under in vitro and in vivo conditions showed the biphasic linear relation. Slopes differed slightly from one formulation to the other. Times for the transition from the first linear phase to the second were nearly identical.

dose was dissolved and absorbed during the first time period of the Levy plot (0–5.9 hr), and 20% during the colonic phase. The formulations tested in the two studies differed clearly in content and rate of release. However, the same pattern of continuous in vitro–in vivo correlation was obtained and the parameters describing these correlations were very similar.

These results show that reasonable amounts of theophylline have already been absorbed before the formulations reached the colon. However, the absorption of theophylline is not restricted to the upper intestine; it continues when the formulation reaches the colon. Therefore, the passage of a sustained-release formulation into the colon is not the sole cause for reduced bioavailability in the case of the 24 hr-formulation. For this formulation the release was too slow and probably the entire dose was not released during passage through the gastrointestinal tract.

B. Testing the Hypotheses for Piretanide

The best way to confirm our hypotheses (section IV) seemed to be the use of a drug for which a change in absorption along the gastrointestinal tract had been verified and for which also a sustained-release formulation had been studied under in vitro and in vivo conditions. Piretanide, a loop diuretic, is a drug that meets both requirements. Figure 10 shows the concentration–time curve of piretanide after endoscopic administration of a 3 mg solution into the stomach, duodenum, and ascending colon (8).

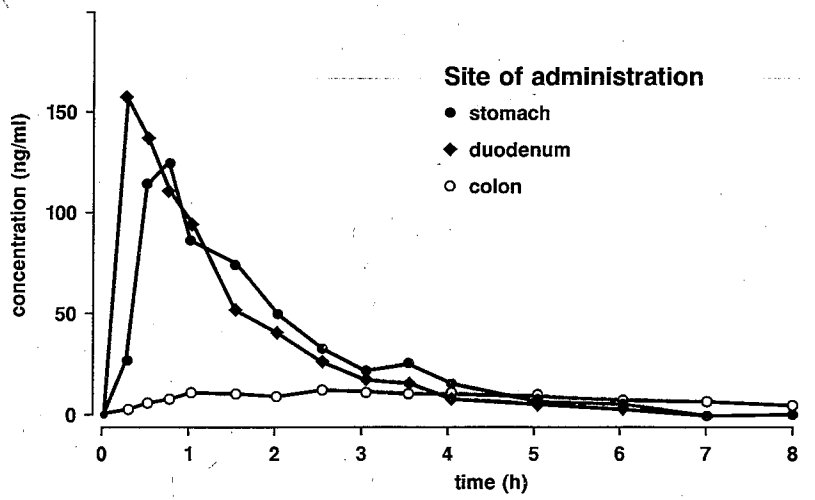

Figure 10 Absorption of piretanide from different sites of the gastrointestinal tract. Median serum concentration–time profiles of piretanide after endoscopic administration at three different sites of the gastrointestinal tract. About the same amount is absorbed when piretanide is instilled into the stomach and into the duodenum. A striking difference is observed (amount and rate) when piretanide is instilled into the ascending colon (from ref. 8).

The absorption of piretanide was delayed after gastric administration compared to intraduodenal. After administration of this agent into the duodenum, the concentration–time curve was practically like that after intravenous administration, indicating extremely rapid absorption. However, a remarkable difference in the concentration–time profile was observed when the drug was administered into the colon. Only 25% compared with intraduodenal instillation is very slowly absorbed. In one subject, the concentration never exceeded the detection limit. The concentration–time profiles for the remaining subjects resembled very much those seen after continuous infusion.

According to these absorption properties of the gastrointestinal tract, piretanide must show a biphasic Levy plot in the continuous in vitro–in vivo correlation to support our hypotheses. If not, the new approach would be invalid.

In a study with 27 volunteers, piretanide was administered intravenously and orally as conventional tablet and as an experimental sustained-release formulation following a randomized crossover design (39, 40). The dose for all routes was 6 mg. Data after administration of an oral solution were not available. Therefore, the hypothetical in vivo dissolution profile for the sustained-release formulation was estimated by deconvolution using the dose-standardized profile for intravenous application as impulse response. This is not a serious drawback. It only implies that the result of deconvolution cannot be regarded as the hypothetical in vivo dissolution profile, but encompasses the absorption profile. The in vivo dissolution profile obtained by deconvolution is always hypothetical and represents the actual in vivo dissolution profile only in cases in which the absorption rate constant does not change along the gastrointestinal tract. For deconvolution, the impulse-response function used is assumed to be valid for the entire gastrointestinal tract. Since the concentration–time response after administration into the duodenum is almost the same as that after intravenous bolus application, the use of the response after intravenous administration in the continuous in vitro–in vivo correlation equates to the situation in which the impulse response has been deduced from administration of a solution into the duodenum.

The hypothetical in vivo dissolution profile obtained differed clearly from the in vitro dissolution profile. Application of the continuous in vitro–in vivo correlation resulted in a biphasic Levy plot. Figure 11 shows the two subjects with the largest and smallest ratio between the slopes for the first and second section of the correlation.

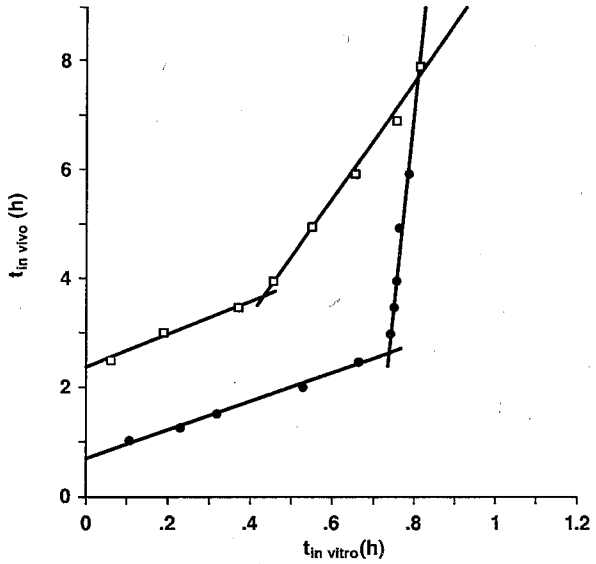

Figure 11 Correlation of in vitro and in vivo dissolution times for a sustained-release piretanide formulation. Correlation is biphasic, as expected from the absorption characteristics of the gastrointestinal tract for piretanide (see Fig. 10). Results for the two subjects with the smallest (□) and the largest (●) ratio between slopes for the two sections of the biphasic correlation are shown. Same pattern of biphasic linear correlation for all but one subjects. It is concluded that the rate constant of absorption changes abruptly after about 3 hr, with only small amounts of piretanide being absorbed thereafter.

This pattern was observed in 26 of the 27 subjects. The Levy plots differed only in the slopes for the first and second linear section and in the transition time, from the first to the second section. This time varied from 2.1 h to 5 hr, with an average of 3.6 hr (41).

These results clearly support the first hypotheses stated above. At least a biphasic Levy plot is obtained if the absorption rate constant changes along the gastrointestinal tract. Furthermore, the ratio between the first and second slope was 12 on average, in con-

trast to 4 for theophylline. The larger the difference in the rate constant of absorption for the different parts of the gastrointestinal tract becomes, the larger becomes the ratio between the slopes. Based on the information on gastrointestinal transit times compiled from other studies, it can be concluded that the time of change (3.6 hr) is too small to reflect the passage of the formulation from the small intestine to the colon.

Piretanide is absorbed mainly in the upper small intestine and only to a very small extent in the lower small intestine and colon. Therefore, its systemic availability is defined by the time the formulation resides in the well-absorbing part of the gastrointestinal tract (window). Figure 12 confirms this conclusion; it shows a positive correlation between systemic availability of piretanide and the time period in this window. The time for formulation resides in this window can be read from the continuous in vitro–in vivo correlation. However, the absorption capability is not entirely present or absent in the sense of an all-or-nothing characteristic, but the gastrointestinal tract shows a part with a rapid and almost complete absorption of piretanide and a part with very slow and, therefore, incomplete absorption.

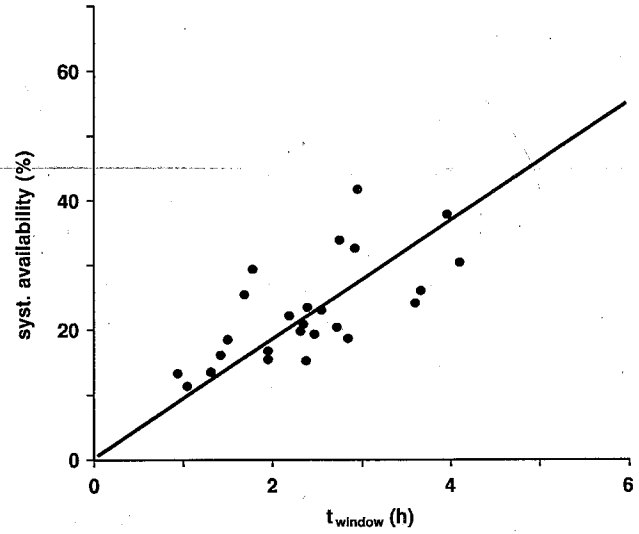

Figure 12 Correlation of the amount of piretanide absorbed with the time the formulation resides in the absorption window. Amount of piretanide absorbed from the lower part of the gastrointestinal tract is very small. Bioavailability of piretanide depends on the time the sustained-release formulation resides in the part with rapid and complete absorption (window). Bioavailability plotted over window time correlates well. The time the sustained-release formulation resides in the well-absorbing part of the gastrointestinal tract (window time) was read from the individual continuous in vitro–in vivo correlation.

The absorption of piretanide in the gastrointestinal tract can be assessed in a less invasive manner using the continuous in vitro–in vivo correlation. Furthermore, the average residence time of the formulation in the absorption window can be determined more precisely. Based on the knowledge of transit times of different formulations through the gastrointestinal tract, it can be concluded that piretanide is absorbed only to a small extent from the distal part of the small intestine and from the colon. This has a direct impact on the development of therapeutic formulations: the in vivo dissolution must be adjusted to the time the formulation resides in the well-absorbing upper part of the intestinal tract.

C. Isosorbide-5-Mononitrate

Another continuous in vitro–in vivo correlation was directly compared with the results obtained by gamma scintigraphy. Wildfeuer and co-workers have shown that the rate of absorption of isosorbide-5-mononitrate (ISN) from the colon was only slightly slower than from the upper intestine (42). The extent of absorption was, however, about the same for all sites of administration. They used the HF capsule that was opened in the stomach, duodenum, jejenum and ascending colon. Since the difference between the rate constants of absorption was small, ISN was a crucial candidate for testing the hypotheses stated, especially the sensitivity of the method. Applying continuous in vitro–in vivo correlation to ISN should therefore lead to a biphasic correlation, but only a small difference between the slopes of the two sections of the correlation is expected. Fischer and co-workers studied the absorption of ISN after administration of a slow-release pellet formulation (43). They characterized it as controlled-release because the in vitro release profile was independent of the dissolution conditions used (i.e., resulting in isomorphic dissolution profiles). They monitored the

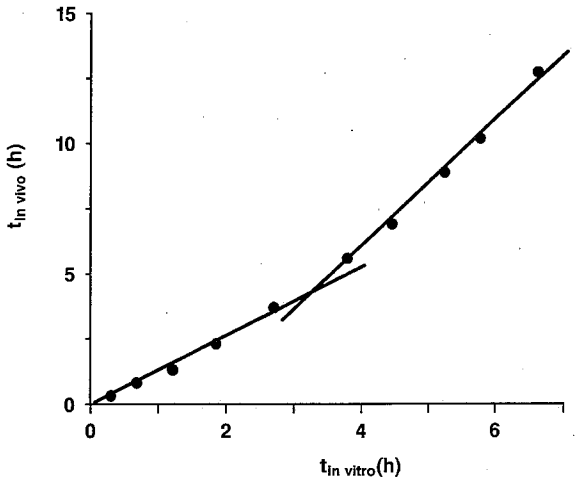

Figure 13 Correlation of in vitro and in vivo dissolution times for a controlled-release isosorbide-5-mononitrate (ISN) formulation. Correlation of dissolution times, $t_{in\ vitro}$ and $t_{in\ vivo}$, related to identical amounts dissolved under both conditions (mean values). According to expectation, a biphasic linear relation results for the controlled-release ISN formulation. The change of the rate constant of absorption for the upper and lower part of the gastrointestinal tract is less pronounced than with piretanide and theophylline. The ratio between the slopes for the two sections of the biphasic correlation is therefore smaller than for those substances.

intestinal transport of the ^{111}In-DTPA-labeled pellets by gamma scintigraphy after having proved that the release from labeled and unlabeled pellets was the same.

The results of the continuous in vitro–in vivo correlation were deduced from their figures on the in vitro and in vivo drug release and are shown in Figure 13.

Even the small difference between the rate constant of absorption from the upper intestine and from the colon was revealed by the continuous in vitro–in vivo correlation. The difference in the rate constant of absorption was not very pronounced. This also became obvious in the slopes for the first and second section of the Levy plot. They did not differ as clearly as for piretanide or theophylline. The ratio between both slopes was only 1.8. The time at which the first line passes to the second is 4.6 hr, which is about the same as the orocecal transit time. The data show that ISN is absorbed from the colon with only a small difference in the rate constant of absorption when compared to that for the upper intestine.

The arrival in the colon determined by Fischer et al. started at 4 hr. After 6.8 hr, an average of 50% of the pellets were found in the colon. They reported that the pellets accumulated at the ileocecal junction and entered the colon in one or a few boluses. The propulsion as bolus can explain the clear break in the transition from the first linear section in the Levy plot to the second.

VI. DISCUSSION

The theoretical and methodologic basis for the continuous in vitro–in vivo correlation (12–15) has been developed by elaborating the concept of in vitro–in vivo correlation using the mean in vitro dissolution time and the mean residence time in the body (44, 45). The results of a study with a sustained-release theophylline formulation have been analyzed according to this technique. In contrast to our expectation, this approach resulted in a biphasic linear in vitro–in vivo relationship (15). It was thought most probable that this is caused by a change in the absorption rate constant for theophylline along the gastrointestinal tract. This interpretation was later confirmed by Staib and co-workers (26, 27), who showed that the rate constant of absorption was slower, particularly when the drug was administered into the colon.

Studies on continuous in vitro–in vivo correlation call for sustained-release formulations with isomorphic or at least homomorphic dissolution profiles under different in vitro dissolution conditions. First, suitable formulations can be developed in vitro. In vivo studies designed to apply the methods of continuous in vitro–in vivo correlation would follow. Such a formulation must be studied in volunteers with an oral solution or an intravenous application. The findings of Staib et al. with theophylline encouraged the hypothesis that the continuous in vitro–in vivo correlation can be used to screen the absorption properties of the gastrointestinal tract for site-dependent (shown as time-dependent) differences in rate and amount. This hypothesis was confirmed for different sustained- and controlled-release formulations of theophylline (40, 41).

The hypothesis was further scrutinized by inverting it. Continuous in vitro–in vivo correlation should lead to at least biphasic, linear correlations for drugs with known changes of the absorption rate constant along the gastrointestinal tract. Two drugs were selected: piretanide, for which it has been shown that the rate constant of absorption changes extremely, and ISN, for which the change is only small. Our hypothesis was confirmed (40, 41) for both drugs. Further studies with sustained-release piretanide formulations showed the same biphasic in vitro–in vivo correlation, with remarkable consistency of the transition time from the first to the second section of the biphasic linear relation.

The time of transition from the first to the second section reflects generally the passage of the formulation from the site with a higher rate constant of absorption to the site with a lower rate constant. Knowledge of the gastrointestinal transit time of various formulations allows us to predict the anatomical site where the change in rate constant of absorption takes place.

Therefore, it is usually possible to judge whether the drug is absorbed from the large bowel or not. It requires a formulation that shows a sustained in vivo release exceeding the orocecal transit time. If the result shows that the drug is not or only barely absorbed from the colon, the release of drug has to be adjusted. The same is valid for drugs that are minimally soluble in the gastrointestinal tract. The continuous in vitro–in vivo correlation would indicate whether the residence time in the well-absorbing part of the gastrointestinal tract is sufficient to dissolve the dose administered.

With the method of continuous in vitro–in vivo correlation, patterns of biphasic linear correlation have been found for other drugs not reported here. Most of them showed a transition time that correlated with the orocecal transit time reported in the literature. The results also showed that in most cases the colon is capable of absorbing the drug well. In some cases the continuous in vitro–in vivo correlation resulted in Levy plots with three linear sections. Whether this indicates that the gastrointestinal tract consist of three parts with different rate constant of absorption must be confirmed by direct measurement using the intubation technique or the HF capsule.

In summary, it has been shown that the absorption features of the gastrointestinal tract, particularly of the colon, can be studied by using sustained-release formulations as pharmaceutical probes with application of the continuous in vitro–in vivo correlation. A two-step strategy should be adopted in the future. First, a formulation is developed to serve as pharmaceutical probe only. Second, the oral dosage form of that drug is optimized with consideration given to both the therapeutic goal and the site-dependent rate of drug absorption.

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