Flip-flop pharmacokinetics – delivering a reversal of disposition: challenges and opportunities during drug development

Flip-flop pharmacokinetics is a phenomenon often encountered with extravascularly administered drugs. Occurrence of flip-flop spans preclinical to human studies. The purpose of this article is to analyze both the pharmacokinetic interpretation errors and opportunities underlying the presence of flip-flop pharmacokinetics during drug development. Flip-flop occurs when the rate of absorption is slower than the rate of elimination. If it is not recognized, it can create difficulties in the acquisition and interpretation of pharmacokinetic parameters. When flip-flop is expected or discovered, a longer duration of sampling may be necessary in order to avoid overestimation of fraction of dose absorbed. Common culprits of flip-flop disposition are modified dosage formulations; however, formulation characteristics such as the drug chemical entities themselves or the incorporated excipients can also cause the phenomenon. Yet another contributing factor is the physiological makeup of the extravascular site of administration. In this article, these causes of flip-flop pharmacokinetics are discussed with incorporation of relevant examples and the implications for drug development outlined.

Drug absorption is a complex process involving various physicochemical and physiological variables [1]. In the case of extravascularly administered drugs, it could be misleading to define the absorption by plotting drug concentration–time data only. This may be particularly important for drugs with prolonged absorption periods, or apparent sustained absorption profiles that exhibit bi-exponential elimination. These factors can often be accompanied by the lack of intravenous (iv.) drug concentration–time data available to the pharmacokineticist, making it impossible to determine whether **absorption rate constant** (k_a) > **elimination rate constant** (k_{el}) or $k_a > k_{el}$. This could result in not realizing the presence of *in vivo* **flip-flop pharmacokinetics**.

A review of the biomedical literature clearly demonstrates that the occurrence of the flip-flop phenomenon in the **disposition** of a variety of xenobiotics during drug development (preclinical phase), in subsequent clinical studies and during use postapproval **(Supplementary Table 1)**. Overall, for drugs exhibiting flip-flop, there is a switch in the k_a for k_{el}. Therefore, the elimination phase of the drug profile reflects the input k_a, rather than the output k_{el}. This causes the k_a to be the rate-limiting step ($k_{el} > k_a$), making it slower and causing an increase in half-life.

In linear pharmacokinetics, the elimination half-life of a drug from the body is constant, regardless of the route of administration and, thus, becomes a defining parameter for the drug. However, if a much longer apparent elimination half-life following extravascular dosing is observed compared with the iv. route, it suggests flip-flop pharmacokinetics is occurring. The decline of the terminal slope during flip-flop pharmacokinetics will depend greatly on how fast absorption is taking place. In this case, the terminal slope is not controlled by the usual clearance and volume of distribution, but instead by bioavailability and the k_a. Since the drug cannot be eliminated until it is absorbed, the decline of the terminal slope can depend greatly on how fast absorption is taking place. Thus, when modeling or examining data of drugs exhibiting flip-flop pharmacokinetics, the k_a may not parallel the iv. k_{el}, as is normally the case. Recognizing the occurrence of flip-flop pharmacokinetics will avoid the incorrect calculation of terminal elimination half-life, volume of distribution, clearance, time to steady-state and mean residence time (MRT) [2–4].

In order to manage flip-flop pharmacokinetics, a longer duration of sampling may be necessary [5–7] in order to avoid high estimates of extrapolated area under the curve (AUC) leading to overestimation of fraction of dose

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Key Terms

Absorption rate constant: Value used in pharmacokinetics to calculate the rate at which a drug is absorbed from its site of administration in the body.

Elimination rate constant: Value used in pharmacokinetics to calculate the rate at which a drug is removed from the body.

Flip-flop pharmacokinetics: Phenomenon in which the absorption rate constant (k_a) is much slower than the elimination rate constant (k_{el}). This reversal or flip-flop of the drug concentration–time profile is the so-called flip-flop pharmacokinetics phenomenon.

Disposition: Another term used concomitantly with pharmacokinetics to represent the fate of a drug once it enters the body.

absorbed [8]. In flip-flop pharmacokinetics, the terminal k_{el} is controlled by the k_a. Therefore, without an iv. reference, extrapolation of AUC and area under the first-moment curve from time of the last quantifiable concentration to infinity will require the use of k_a instead of k_{el} [9]. Furthermore, it is not possible to apply compartmental analysis or statistical moments methods to extravascular data so that disposition parameters can be determined accurately [10–13]. Nevertheless, for all drugs without an iv. reference, the extravascular profile cannot provide the bioavailability of the administered drug but only its rate of appearance and disappearance.

The physicochemical and physiological mechanisms underlying the occurrence of the flip-flop phenomenon are multifactorial and include, but are not limited to, solubility-limited absorption, modified-release formulations and alterations in permeability of membranes. Anatomical and physiological differences between species also need to be considered [14]. Quantitative and qualitative assessment of absorption data is often part of regulatory submission requirements for characterization of new drugs and is critical to the establishment of *in vitro–in vivo* correlations (IVIVCs). The aim of this article is to present, discuss and provide a comprehensive, rather than exhaustive, appraisal of the flip-flop pharmacokinetics phenomenon. Illustrative pertinent examples from the literature in which flip-flop pharmacokinetics have been reported or presumed are presented to explain the disconnect between intravascular and extravascular routes of administration. A better understanding of this phenomenon may greatly aid drug development at an early stage, enabling the necessary corrections to be made during pharmacokinetic parameter analysis.

The flip-flop mathematical behavior

Flip-flop pharmacokinetics can be described as a mathematical behavior that is related to the structural identifiability of parameter values in a model described by differential equations [15]. Structural identifiability can be defined as a computational approach to aid in obtaining information about the internal structure of a system that contains input–output measurements. It also determines which experiments are necessary to uniquely characterize the internal couplings. This concept can be applied to a pharmacokinetic model since compartmental structures are employed with input and output measurements (rate constants), which have a certain degree of coupling for the determination of various pharmacokinetic parameters. Furthermore, the structural identifiability concept also dictates that it is possible to determine all the rate constants of a compartmental model by taking measurements of the n^{th} compartment [15]. In the case of extravascular administration, the n^{th} compartment will be the central compartment where, ultimately, the drug will reside. The identifiable measurement will be the drug concentrations measured in blood or excreta, and the rate of change of drug in the body equals k_a minus k_{el} [16,17].

The rather simple but widely utilized compartmental modeling is not uniquely identifiable because there is no unique set of parameter values that can be calculated. Therefore, there are actually two solutions when concentration–time data is fit, both giving valid solutions to the parameter values. The typical model used for extravascular modeling has an absorptive compartment X1, k_a, a volume of distribution of the central compartment (V), the central compartment X2 (from which blood is collected), and a k_{el} from the body **(Equation 1 & 2)**. The two equal solutions to fitting the data are the reason for the problem of identifiability in the model.

$$dX1/dt = -k_a X1$$

Equation 1

$$dX2/dt = k_a X1 - k_{el} X2$$

Equation 2

The model output equation, called Y1, for the blood concentration is represented by:

$$Y1 = X2/V$$

Equation 3

From the model, three invariants can be uniquely calculated:

$$A1 = k_a + k_{el}$$

Equation 4

$$A2 = k_a k_{el}$$

Equation 5

$$V1 = k_a/V$$

Equation 6

A fourth invariant – oral clearance (CL/F) of the drug from the body – can also be derived from **EQUATION 5 & 6**:

$$CL/F = A2/V1 = k_{el}V$$

EQUATION 7

As stated, the majority of xenobiotics present an k_a that is more rapid than the k_{el}. For example, if we set the $k_a = 2$, $V = 3$, $k_{el} = 1$, the invariants can be calculated as:

$$A1 = k_a + k_{el} = 2 + 1 = 3$$

$$A2 = k_a k_{el} = 2 \times 1 = 2$$

$$V1 = k_a/V = 2/3$$

$$CL/F = A2/V1 = k_{el}V = 1 \times 3 = 3$$

If we were to reverse the values of the rate constants to represent the flip-flop phenomenon, k_a becomes slower than k_{el}. For example, if we set $k_a = 1$ and $k_{el} = 2$, the invariants V1 and A2/V1 remain 2/3 and 3, respectively. Therefore, the other invariants can be calculated as:

$$A1 = k_a + k_{el} = 1 + 2 = 3$$

$$A2 = k_a k_{el} = 1 \times 2 = 2$$

$$V1 = 2/3 = k_a/V = 1/V$$

$$\text{so } V = 3/2$$

In this example, it can be observed that V changed from 3 (nonflip-flop pharmacokinetics) to 3/2 (flip-flop pharmacokinetics). This change in V is the real source of the flip-flop phenomenon. However, it needs to be considered that following extravascular administration the volume of distribution is masked by bioavailability (F) and, depending on the route of administration (i.e., oral vs intraperitoneal), first-pass metabolism. It also needs to be considered that different dosage forms will affect both the dissolution rate of a drug and its k_a, ultimately affecting the bioavailability and distribution of a drug.

Methods to manage flip-flop pharmacokinetics

The different methods available to manage flip-flop pharmacokinetics, their pros, cons and necessary data to be performed are summarized in **TABLE 1**.

■ Parameterization

Because the flip-flop behavior is dependent on the magnitude of k_a and k_{el}, these rates can be parameterized by adding some other constant, C, to always make them faster than the other depending on the desired solution [18]. If the desired solution involves k_a being faster than k_{el} ($k_a > k_{el}$), which is the case for the majority of xenobiotics, k_a can be parameterized to make it equal to $k_{el} + C$. This guarantees that k_a is always faster than k_{el}, which would lead to the following corrections in the model equations and model invariants:

$$dX1/dt = -(k_{el} + C)X1$$

EQUATION 8

$$dX2/dt = (k_{el} + C)X1 - k_{el}X2$$

EQUATION 9

$$A1 = (k_{el} + C) + k_{el} = 2k_{el} + C$$

EQUATION 10

$$A2 = (k_{el} + C)k_{el} = k_{el}^2 + Ck_{el}$$

EQUATION 11

$$V1 = (k_{el} + C)/V$$

EQUATION 12

$$CL = A2/V1 = k_{el}V$$

(this equation remains the same)

EQUATION 7

On the other hand, if flip-flop pharmacokinetics is present ($k_a < k_{el}$), k_{el} can be parameterized to make it equal to $k_a + C$, which guarantees that k_{el} is always faster than k_a, this would lead to the following corrections in the model equations and model invariants:

$$dX1/dt = -k_a X1$$

(this equation remains the same)

EQUATION 1

$$dX2/dt = k_a X1 - (k_a + C)X2$$

EQUATION 13

$$A1 = k_a + (k_a + C) = 2k_a + C$$

EQUATION 14

$$A2 = k_a(k_a + C) = k_a^2 + C\,k_a$$

EQUATION 15

$$V1 = {}^{k_a}\!/_{V}$$

(this equation remains the same)

EQUATION 6

$$CL/F = {}^{A2}\!/_{V1} = (k_a + C)V = Vk_a + VC$$

EQUATION 16

This method can be useful when the presence of flip-flop is known and can prevent the estimated pharmacokinetic parameters from becoming miscalculated and misinterpreted by using an otherwise faster k_{el}. However, it needs to be recognized that this is a manual fix to the problem and will require re-graphing the plasma–concentration profiles and re-calculating the pharmacokinetic parameters with the parameterized k_a. Furthermore, this is a theoretical correction to flip-flop pharmacokinetics; to the best of our knowledge, it has not been published in any actual preclinical or clinical study.

■ Feathering, stripping, or the method of the residuals

Drug absorption can exhibit two limiting cases, the first one is the usual case in which absorption is more rapid ($k_a > k_{el}$). In this case, the terminal concentration–time phase is pure elimination (yields k_{el}); while the absorption phase and rate is obtained by feathering or the method of residuals (yields k_a). At some time point, which is greater than the half-life ($t > t_{1/2}$), as absorption is completed, the e^{-kat} term in **EQUATION 17** approximates zero, leading to **EQUATION 18 & 19**.

$$C_p = k_a F\left({}^{A_0}\!/_{V}[k_a - k_{el}]\right)(e^{-k_{el}t} - e^{-k_a t})$$

EQUATION 17

Table 1. Necessary data, pros and cons of the different methods available to manage flip-flop pharmacokinetics.

Method	Necessary data	Requires iv. data?	Pros	Cons	Ref.
Parameterization	k_a and k_{el}	No	Simple manual correction	Theoretical manual correction that has not been fully applied	[18]
Feathering, striping or the method of the residuals	k_a and k_{el}, and iv. profile	Yes	Widely used and accepted	It can be used only when $k_{el} > 3\,k_a$	[16,17]
Flip-flop pharmacokinetics conundrum	Extravascular profile	No	Simple method, allows one to obtain the k_a profile and the fraction absorbed at time t Prevents the use of deconvolution	It has not been fully applied	[19,21]
Wagner–Nelson	Extravascular profile	Yes	Implemented in various software for pharmacokinetic modeling The absorption process doesn't have to be first order	Requires k_{el} after iv. administration	[20,22]
Loo–Riegleman	Extravascular and iv. profile	Yes	Implemented in various software for pharmacokinetic modeling	It only applies to drugs that can be administered iv.	[25–27]
Deconvolution	Extravascular and iv. profile	Yes	Widely used and implemented in various software for pharmacokinetic modeling Model-independent approach that requires no utilization of a compartmental structure Various applications on different fields	Limited to a linear system Might utilize nonphysiological conditions such as negative input functions Mechanistic interpretation of derived pharmacokinetic parameters is usually not possible	[24,32–37]

k_a: Rate of absorption; k_{el}: Rate of elimination; iv.: Intravenous.

$$C_p = k_a F\left(A_0/V[k_a - k_{el}]\right)e^{-k_{el}t}$$

EQUATION 18

$$\log C_p = \log k_a F\left(A_0/V[k_a - k_{el}]\right) - k_{el}\left(t/2.3\right)$$

EQUATION 19

where, F = bioavailability; A_0 = administered dose; and C_p = plasma concentration at time t.

The second case is when flip-flop pharmacokinetics occurs. The terminal phase is pure absorption (yields k_a), while the pure elimination phase is obtained by feathering or the method of residuals (yields k_{el}). In this case, at times much greater than half-life ($t > t_{1/2}$), the e^{-kelt} term approaches zero and leads to **EQUATION 20**.

$$\log C_p = \log k_a F\left(A_0/V[k_a - k_{el}]\right) - k_a\left(t/2.3\right)$$

EQUATION 20

In order to identify the true elimination phase and absorption phase, the slope of the terminal phase following iv. administration will be used to calculate k_{el}. k_a will be obtained from the slope of the terminal phase of the extravascular administration for non-flip-flop pharmacokinetics. The process of 'stripping' and deducing the k_{el} is by the method of the residuals.

Feathering, stripping or the method of residuals entails an initial extrapolation of the post-absorptive concentration to time zero of the iv. administration, the obtained slope is the k_{el}. This is followed by a subtraction of the actual concentrations from the extravascular administration during the absorptive phase from the extrapolated concentrations. Then, the residual concentrations versus time are plotted and the slope (k_a) is calculated from a logarithmic-linear plot [17]. This method is only possible if k_a and k_{el} are significantly different, and in the case of flip-flop pharmacokinetics is only a valid procedure when $k_{el} > 3\ k_a$ [16]. This is illustrated in **FIGURE 1**, where the nonlogarithmic formulation of the original data C can be subtracted at any time (t) from the nonlogarithmic formulation of C′ to obtain an extrapolated terminal phase so that a semi-logarithmic plot of the natural logarithm of the difference (C′–C) against time has a slope, (k), that is equivalent to k_a or k_{el}, depending on the situation [17]. Feathering is a manual data correction widely used to correct for flip-flop pharmacokinetics, but, as stated, is limited to cases with significantly different rate constants.

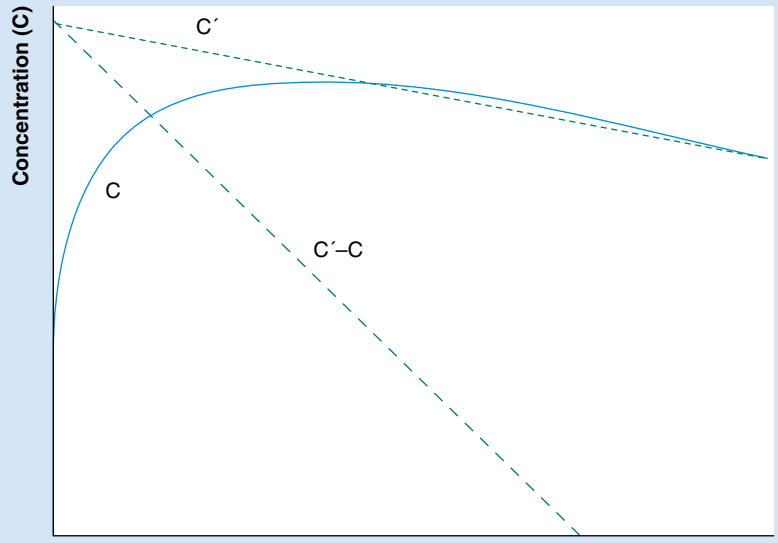

Figure 1. Feathering (the method of residuals) applied to a semi-logarithmic plot of plasma concentration. If the antilogarithmic values of C (solid line) are subtracted from the antilogarithmic values of C′ (dotted line) the negative slope of the natural logarithm of the difference (C′–C, dashed line) is k_a; however if $k_{el} > k_a$, flip-flop occurs and the respective slopes of the logarithms of C′ and C′–C are reversed and are k_a and k_{el}, respectively.
C: Concentration; k_a: Rate of absorption; k_{el}: Rate of elimination.

■ Flip-flop pharmacokinetics conundrum

Disentangling the tricks and the traps of flip-flop pharmacokinetics has been succinctly reviewed [19]. It has been reported that the Wagner–Nelson equation [20], which characterized drug absorption in a one-compartmental model can be rearranged:

$$k_a = V\left(k_{el} + dCp/dt\right)$$

EQUATION 21

where, C_p = plasma concentration at time t; k_{el} represents the true k_{el} calculated from iv. administration.

EQUATION 21 can be further simplified to represent a flip-flop system where k_a approximates k_{el}:

$$k_a \approx V k_{el} C_p \approx CL C_p \approx k_{el}$$

EQUATION 22

Theoretically, k_a can approximate k_{el} during flip-flop pharmacokinetics when k_a is the rate-limiting step in the sequential/parallel processes

of drug absorption, distribution and elimination. This can occur at different segments or moments of the plasma concentration–time profile. As previously stated, the main trap in flip-flop pharmacokinetics where an analyst can fail in correct interpretation is to assume that the terminal half-life following extravascular administration represents the terminal phase, when it truly represents the absorption phase. However, as described by Boxenbaum [19] and Katakam *et al.* [21], a useful trick in flip-flop pharmacokinetics is to realize that the plasma concentration–time profile tends to closely parallel k_a **(FIGURE 2)**. The use of this simple method provides an easy way to assess the shape of a k_a profile in data where flip-flop pharmacokinetics occurs as well as preventing the use of deconvolution [22–24] techniques to obtain the k_a profile.

Furthermore, since the plasma concentration–time profile tends to parallel k_a when the flip-flop phenomenon is present, the fraction absorbed at time t can be calculated:

$$f = \frac{(C_p + k_{el} AUC_{0\text{-}t})}{(k_{el} AUC_{0\text{-}\infty})}$$

EQUATION 23

where, f = fraction absorbed.

■ The Wagner–Nelson procedure & the Loo–Riegelman method

In general, the fraction absorbed or percent of drug absorbed can be calculated by model-dependent techniques, such as the Wagner–Nelson procedure [20,22] and the Loo–Riegelman method [25–27] or by model-independent numerical deconvolution (see the section titled 'Deconvolution'). The Wagner–Nelson procedure uses a one-compartment model, while the Loo–Riegelman method uses a multicompartment system. However, both require iv. data to be able to adequately determine k_a by using the true k_{el} [28].

According to the Wagner–Nelson procedure, the cumulative fraction of drug absorbed (f) can be calculated from:

$$f = \frac{C_p + k_{el} \int C dt}{k_{el} \int C dt}$$

EQUATION 24

Furthermore, k_a can be obtained from the least-square-fitted log–linear plot of the percent unabsorbed versus time, while the absorption half-life can be calculated by dividing $0.693/k_a$ [29].

Conversely, the Loo–Riegelman method allows the estimation of the fraction of drug absorbed based on:

$$f = \frac{C_p + k_{el} \int C dt + \left(X_p / V_c\right)}{k_{el} \int C dt}$$

EQUATION 25

where, X_p = amount of drug in the peripheral compartment following oral administration; and V_c = apparent volume of the central compartment.

More details about the derivation of equations for both procedures have been widely discussed elsewhere [20,22,25–28]. However, it needs to be mentioned that both of these methods have been widely employed and included in a variety of software for pharmacokinetic modeling.

■ Deconvolution

Extravascular administration is dependent on a rate of input (k_a) and a rate of output (k_{el}). Since, k_a can often not be measured directly, pharmacokinetic analysis must frequently be performed with k_{el}. However, as we have seen, this becomes problematic when there is reversal of these two rates, as in flip-flop pharmacokinetics. In a typical extravascular analysis, one follows the input–output order of events; however, when flip-flop pharmacokinetics is present the order is reversed resulting in the order of events following the output–input design. This reversal of order of events has been resolved by

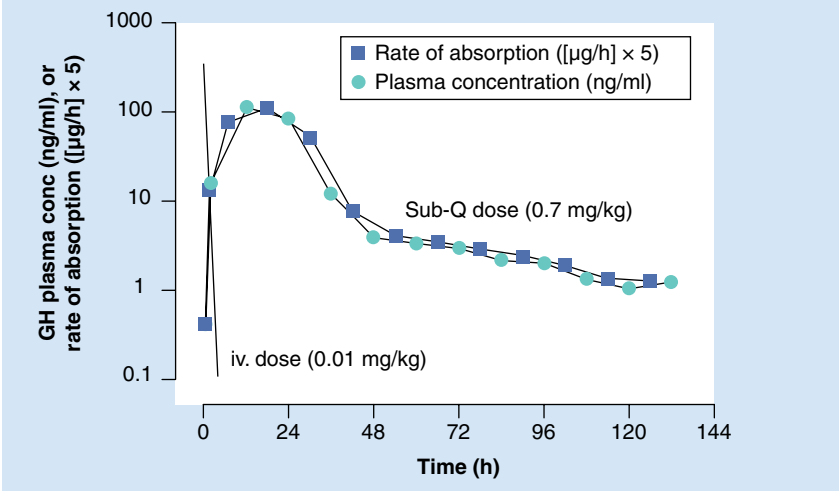

Figure 2. Recombinant HGH mean semilogarithmic plasma concentration–time profiles from dogs. The rate of absorption was calculated by Wagner's modification of the Loo–Riegelman equation [22], while the rate of absorption was multiplied by five to closely approach the plasma concentration profile for easier comparison.
Reproduced with permission from [19].

using deconvolution. This method has been successfully applied to flip-flop pharmacokinetics as well as other disciplines including magnetic resonance imaging and differentiation of composite peaks in chromatography [24,30,31].

Deconvolution in pharmacokinetics has been widely used for almost 40 years [24,32–37]. It is an algorithm-based process employing the reverse of the effects of convolution by trying to solve a convolution equation:

$$f \times g = h$$

EQUATION 26

where, f = variable we want to quantify; g = variable that convolved f; and h = measured variable.

It can be observed from **EQUATION 26**, that the recorded variable h is equal to f, which has been convolved by g as part or in the totality of the dynamic system when h was being measured. These variables can be applied to typical pharmacokinetics as h being the plasma concentration measure at different time points while f equals the elimination process and g the absorption process or convoluting variable. In this case, deconvolution is not typically applied since the pharmacokinetic parameters can be calculated from the terminal elimination phase. However, in flip-flop pharmacokinetics, f equals the absorption process and g the elimination process or convoluting variable. Therefore, deconvolution is necessary to solve for g in order to adequately estimate the pharmacokinetic parameters [7,24]. It needs to be kept in mind that pharmacokinetics is a dynamic system in which we encounter instances of simultaneous absorption and distribution, absorption and elimination, distribution and elimination, or solitary elimination.

Even though deconvolution is an algorithm-based process, its application in pharmacokinetic fitting has also been performed by algebraic expressions such as curve fitting, splines, or polynomials [24,34,38]. Interestingly, these algebraic expressions have been found to be more robust than numerical algorithmic routines [36–40]. It also needs to be noted that deconvolution algorithms are available as part of various commercial pharmacokinetic analysis software such as GastroPlus™ (Simulations Plus, Inc., Lancaster, CA, USA), Kinetica® (InnaPhase Corp., Philadelphia, PA, USA) and WinNonlin® (Pharsight Corporation, Mountain View, CA, USA).

Deconvolution is widely utilized in practice for IVIVC. For example, deconvolution can be used to determine *in vivo* absorption characteristics based on the *in vitro* dissolution profile of a drug. Specifically, it has been applied to controlled release dosage forms by using the immediate release (IR) formulation dissolution profile as the weighing function in an analytical deconvolution method [41]. Furthermore, deconvolution application for flip-flop pharmacokinetics involves its capacity to estimate the rate and extent of systemic availability following extravascular routes of administration such as oral, intranasal, rectal and transdermal, among others [42,43]. In addition to being used to characterize the absorption of different dosage forms, deconvolution is used to assess drug–drug interactions [44] by taking the iv. data as the weighing function and the test formulation data, following extravascular administration, as the response function. Good examples of the application of deconvolution in the determination of k_a have been reported and reviewed elsewhere [24,45,46]. Numerical deconvolution has also been applied to estimate the sufficient sampling time to characterize the totality of the absorption phase and, in that way, adequately calculate bioavailability for amiodarone [47,48] and hydroxychloroquine [49].

As noted, deconvolution is a widely used method and offers significant advantages compared with other methods in the management of flip-flop pharmacokinetics. Of particular note, deconvolution is a model-independent approach that requires no utilization of a compartmental structure. This characteristic allows for the determination of the absorption process without the need for first-order input or other pharmacokinetic processes. Furthermore, it allows greater insight into the input profile since it is more detailed than the sole calculation of k_a. It also allows the calculation of systemic availability of various dosage forms and routes of administration; enabling the calculation of absolute bioavailability without the need to extrapolate to infinity for the estimation of AUC. However, deconvolution also has its limitations. It is limited to a linear system that in some instances might utilize non-physiological conditions, such as negative input functions. Moreover, the mechanistic interpretation of deconvolution-derived pharmacokinetic parameters is usually not possible [24].

Factors that affect rate constants

■ Formulation

It is often important to maintain therapeutic plasma concentrations for drugs exhibiting a short terminal half-life. To address this,

dosage forms with a slower input rate have been developed. Flip-flop pharmacokinetics is important in the pharmaceutical manufacturing of controlled-, extended- and sustained-release formulations, as it can often occur with them. The downward part of the plasma concentration–time curve becomes a reflection of the actual k_a while the upward part of the curve reflects k_{el} **(FIGURE 3)**. The following are several examples of flip-flop pharmacokinetics caused by modified-release formulations.

Introduced clinically in the 1960s, injectable depot antipsychotics were synthesized by esterification of the active drug followed by dissolution in an oil formulation. Examples include: fluphenazine enanthate and decanoate, haloperidol decanoate, clopenthixol decanoate and flupenthixol decanoate. Following intramuscular (im.) administration, these formulations demonstrate a slower k_a than the k_{el} and, thus, exhibit flip-flop pharmacokinetics; with the diffusion and release from the oily depot site as the rate-limiting step [45,47].

Population pharmacokinetic parameters of sustained-release and enteric-coated oral formulations and suppository formulations of diclofenac sodium were fit using mixed-effect modeling [50]. The sustained-release formulation demonstrated slow first-order kinetics and follows flip-flop pharmacokinetics since k_{el} is greater than k_a. This indicates that the slow diffusion process of diclofenac through the ethylcellulose derivative into the gastrointestinal (GI) fluid controls the k_a [50].

A theophylline once-daily administered preparation (Uniphyl®) made from hydrated cellulose with aliphatic alcohol allows for a controlled *in vivo* release rate with peak concentrations of theophylline 12 h postdose. A meta-analysis was made from data collected in a premarketing clinical trial in Japan compared with iv. infusion data previously reported. The first-order k_a for a 200 mg tablet under fasting conditions was 0.0773 h^{-1}, which was slower than the k_{el} of 0.168 h^{-1}, indicating flip-flop pharmacokinetics for this formulation. The decline following the peak is thus dependent on the k_a [51].

Using mixed-effect modeling, the absorption of a conventional tablet and a controlled-release form of carbamazepine were compared from plasma samples of epileptic patients. The k_a and k_{el} appeared to be transposed in a flip-flop manner as expected for the controlled-release product. The difference in k_a between the two products was evident (k_a of tablet = 0.312 h^{-1} versus k_a of controlled release = 0.149 h^{-1}) [52].

Polymeric nanoparticles are used for oral delivery of drug molecules as they can potentially shield entrapped drug from the adverse conditions of the GI tract, allowing avoidance of first-pass effects. These formulations can also be taken up by M-cells in Peyer's patches and be released over long periods. A pharmacokinetic study comparing estradiol solution administered iv. with estradiol administered as a suspension or as estradiol administered in poly(lactic-co-glycolic) acid nanoparticles demonstrated that polymeric nanoparticles exhibited flip-flop pharmacokinetics with slow and sustained release from the polymeric matrix and an increase in AUC and t_{max} compared with suspension [53]. Compared with an iv. formulation, the k_{el} decreased with the nanoparticulate formulations indicating slower elimination from the body, which translates into an increased apparent half-life and consequently prolonged release. The half-life at 100 µg/kg dose was approximately 4.3 h following iv. administration, 7.98 h following a drug suspension, and 12.32 h following polyglycolic-lactic acid (PGLA) nanoparticles [51].

The pharmacokinetics in mice of alternative formulations for delivery of paclitaxel including, gel, film prodrug, liposomes and micelles have been assessed. The apparent half-lives were

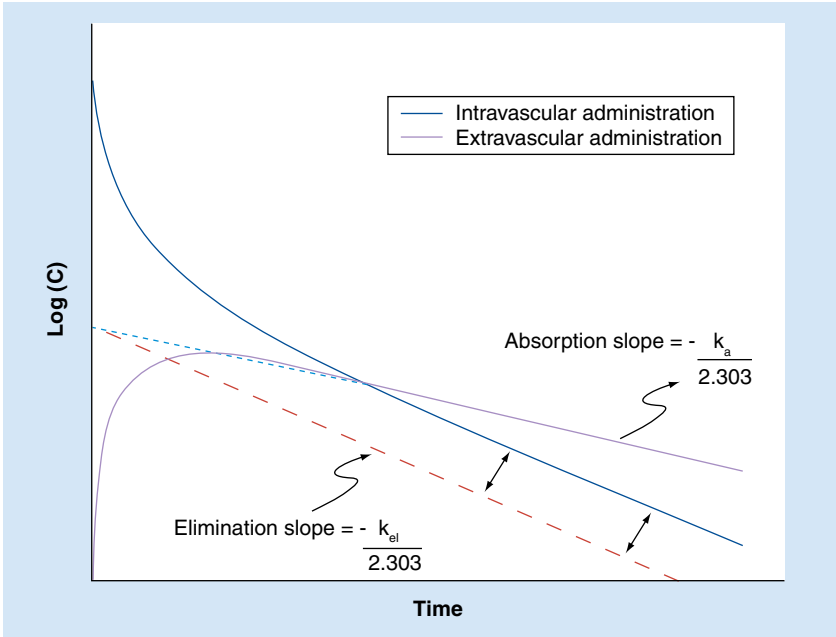

Figure 3. Plasma concentration–time profile following an extravascular dose (oral administration) and an intravascular dose exhibiting flip-flop kinetics exhibiting absorption rate-limited elimination. It can be observed that the terminal portion of the extravascular curve is not parallel with the iv. curve. The latter reflects the true elimination of the drug.

6.2, 5.1, 3.4, 2.4 and 2.1 h for micelles, prodrug liposome, paclitaxel-liposome, Cremophor EL-paclitaxel and prodrug, respectively. Following subcutaneous (sc.) administration of liposomes and gel-liposomes, initial plasma concentrations of paclitaxel were lower than the concentrations obtained following iv. administration. The half-lives of paclitaxel liposome and liposome-gel were 18.0 and 15.0 h, respectively, which is indicative of sustained release and were significantly longer than the gold standard, Cremophor EL iv. formulation, which has a half-life of 2.4 h. This result is indicative of flip-flop pharmacokinetics [54]. A thermoreversible poloxamer gel incorporating paclitaxel in liposomes was injected subcutaneously in rats. Polymers can retard the release due to rigidity of gels restricting the rate of water diffusion. Absorption of paclitaxel occurred slowly with a prominence of the absorption phase suggesting flip-flop pharmacokinetics. A half-life of approximately 40 h following administration of pure paclitaxel in ethanol was achieved, while a half-life of 3 h for paclitaxel alone and 4 h in Cremophor EL were obtained [54].

Recently, the pharmacokinetics of isoxsuprine hydrochloride was determined from orally and im.-administered doses to healthy female volunteers [55]. The isoxsuprine was an extended-release formulation administered orally, and as expected the oral absorption of the drug release from the core of the extended-release formulation followed zero-order kinetics with a half-life of 2.2 h following im. administration and approximately 10 h following oral dosing with a sustained behavior and profile [55].

A long-acting naltrexone extended-release formulation was developed to have continuous exposure for 1 month for the treatment of alcohol dependence following im. injection. The product was based on microspheres incorporated into a biodegradable polymer matrix of polylactide-co-glycolide. Long apparent half-lives (5–8 days) for both naltrexone and 6β-naltrexol were attributed to the slow release of naltrexone and k_a-limited elimination or flip-flop pharmacokinetics [56].

Ranolazine has an elimination half-life of 1.4–1.9 h following iv. or oral administration of an IR capsule. However, administration and utility of the IR formulation was limited by its short elimination half-life; which can be prolonged on average to 7 h for the extended-release formulation as a consequence of extended absorption and flip-flop pharmacokinetics with C_{max} at 4–6 h postdose [57].

■ Drug physicochemical factors

The Biopharmaceutics Classification System (BCS) classifies oral drug absorption characteristics according to their solubility and permeability characteristics. According to the BCS, drug substances are classified into four groups as follows [58]:

- Class I – high permeability, high solubility: these compounds are rapidly dissolved and gastric emptying tends to be critical;
- Class II – high permeability, low solubility: for these compounds, *in vivo* dissolution is critical and formulation changes can affect absorption;
- Class III – low permeability, high solubility: for these compounds, absorption is permeability-rate limited;
- Class IV – low permeability, low solubility: these compounds tend to have very poor oral bioavailability.

This classification system identifies the fundamental parameters governing the rate and extent of drug absorption, which are solubility and permeability. For instance, class I compounds, such as an aqueous solution, are generally well absorbed; however, gastric emptying can be the rate-limiting absorption step. Class II compounds exhibit dissolution rate-limited absorption and their bioavailability is very difficult to predict because of the large variability in the absorption and/or dissolution kinetics. Class III compounds exhibit permeability rate-limited absorption, while class IV compounds tend to have very poor oral bioavailability [58]. This system applies to oral absorption only, while extravascular administration must traverse different membranes than the GI tract with very different permeabilities. In addition, solubility may differ as the volume of fluid associated with extravascular injection or other dosage forms becomes far less than the GI tract volume. The flip-flop phenomenon may be found with drugs that are slowly absorbed as a result of: low intrinsic first-order k_a; drugs with poor water solubility; or drugs administered in a modified-release dosage form.

The Biopharmaceutics Drug Disposition Classification System (BDDCS) [58,59] gives scientists and clinicians a tool for predicting drug disposition early on with little additional expense. It is generally believed that GI absorption is faster than elimination for most IR orally dosed drugs. It has been described that drugs exhibiting poor intestinal membrane

permeability would be those most likely to exhibit flip-flop pharmacokinetics [60]. In general, these drugs are characterized by low oil-to-water partition coefficients and low metabolization. They often belong to classes III and IV in the BDDCS. These descriptors indicate the importance of absorptive transporters in GI absorption. The absorption:elimination half-life ratio has been calculated with numerous drugs that exhibit flip-flop pharmacokinetics and are characterized as either class III or IV drugs based on the BDDCS. These drugs include acamprosate, amoxicillin, carbovir, cephalexin, cefuroxime, furosemide, metformin, pravastatin, rebamipide and zidovudine. Furthermore, based on the absorption:elimination half-life ratio it was determined that absorptive transporters can play an important role and should be considered for poorly metabolized drugs that exhibit flip-flop pharmacokinetics [60].

■ Physiological factors

Following extravascular administration, the concentrations of drug reaching the systemic circulation are dependent on the absorption across barriers. Once a drug enters the oral cavity it passes through the GI tract and it may be absorbed in different segments of the GI tract depending on:

- The surface area of the particular segments (i.e., small intestine has the largest surface area);
- Residence time of the drug in a particular GI segment (i.e., gastric emptying and intestinal motility), which can be influenced by the input rate dosage form of the drug (i.e., enteric coating and sustained release);
- The physicochemical properties of the drug (i.e., pK_a and solubility).

Depending on the formulation of the drug (i.e., controlled release), significant changes in C_{max} and t_{max} can be observed (compared with an IR formulation) and the appearance of a lag time in plasma concentrations (t_{lag}) can also be observed. Therefore, it is important to note that different oral dosage forms (i.e., solution, capsule, sustained-released tablet and so forth), disease states (i.e., intestinal atrophy and Crohn's disease), and GI contents (i.e., fasting, administration before, during or after a meal and so forth) may alter the k_a of a drug. k_{el} may also be altered due to a variety of disease states in particular renal or hepatic impairment. If the dose remains constant, parameters t_{max} and C_{max} are dependent on the k_a and k_{el}, while $AUC_{0-\infty}$ is dependent on the k_{el} only [61].

Regulatory guidance recommendations

Flip-flop pharmacokinetics has become a recognized phenomenon in different stages of drug development. However, no specific guidance has been drafted to propose specific recommended methods to assess k_a when flip-flop pharmacokinetics is identified. But various guidelines recommend certain studies to better assess pharmacokinetics and toxicokinetics during drug development. These recommendations are described below based on the regulatory agency.

■ US FDA

The Bioequivalence Guidance [201] indicates that a multiple dose bioequivalence study may be appropriate when there are concerns where prolonged or delayed absorption exist (flip-flop pharmacokinetics). It is also stated that the assessment of prolonged or delayed absorption may be made from pilot data, literature, information contained with the Freedom of Information summaries of the specific drug or the family of drugs. Furthermore, it is stated that a one-period parallel design may be preferable for drugs exhibiting prolonged or delayed absorption [201].

Similarly, the Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations [202] recommends that there needs to be a change in focus for both the direct (rate constant and rate profile) and indirect (C_{max}, t_{max}, mean absorption time, MRT and C_{max} normalized to AUC) pharmacokinetic measures. It is recommended that C_{max} and AUC can continue to be used as measures for product quality bioavailability and bioequivalence, but in terms of their capacity to assess exposure rather than rate and extent of absorption [202].

The Guidance for Industry and Other Stakeholders Toxicological Principles for the Safety Assessment of Food Ingredients [203] recognizes the caveats of data analysis when flip-flop pharmacokinetics is identified. It states that k_a and k_{el} are often complicated when the absorption and elimination phases reverse (flip-flop pharmacokinetics) and becomes even more complex when the k_a and k_{el} differ by less than a factor of three. Furthermore, it recommends

that when flip-flop pharmacokinetics occurs, the calculation of k_a and k_{el} should not be attempted on the basis of oral data alone, but iv. data is also necessary [203].

■ European Medicines Agency

The Guidance on Quality of Modified Release Products: A: Oral Dosage Forms B: Transdermal Dosage Forms [204] recommends and sets specifications for IVIVC studies. The EMA recommends a 1:1 correlation between the dissolution profile *in vivo* and *in vitro*. Furthermore, it specifies that the *in vivo* profile can be derived from the plasma concentration–time profile using deconvolution. It also details that a point-to-point relationship can be generated between the *in vitro* dissolution curve of the product and the *in vivo* dissolution curves that were generated by numeric deconvolution, Wagner–Nelson or Loo–Riegelman methodology of the plasma level data [204]. Similarly, the Guideline on the Investigation of Bioequivalence [205] indicates that in bioequivalence studies, the plasma concentration–time profile is used to determine various pharmacokinetic parameters. For instance, the AUC reflects the extent of exposure, while the C_{max} and t_{max} are parameters that are influenced by the k_a

Drug examples

A variety of drugs in different classes, in different formulations and after various routes of administration in both preclinical and clinical situations have demonstrated flip-flop pharmacokinetic phenomena. For simplicity of reading, the drug examples have been sub-divided by route of administration and by drug class. A summarized version of the drugs that have been reported to exhibit flip-flop pharmacokinetics is presented in **SUPPLEMENTARY TABLE 1.**

■ Oral

The absorption of a solid chemical entity from the GI tract occurs via four major steps:

- Gastric emptying and small intestinal transit to deliver the xenobiotic to an absorptive site
- Disintegration and dissolution of the solid into solution available for absorption
- Permeation of the dissolved xenobiotic through the GI membrane
- Movement of the xenobiotic away from the site of absorption into the systemic circulation

A classical example of flip-flop pharmacokinetics was demonstrated with bioavailability studies of calcium dobesilate. Following iv. injection, a relatively short half-life of 1.9 ± 0.6 h was evident whereas with an oral ampule or tablet formulation, t_{max} occurred at approximately 4 h with a half-life between 5.6 to 6.3 h. It appears that absorption is the velocity-determining step in the pharmacokinetics of calcium dobesilate [62]. Acamprosate (calcium *bis*-acetyl-homotaurine) is a hydrophilic drug used to prevent relapse in patients with alcohol dependence. It is hydrophilic and soluble in water with poor permeation across intestinal mucosa. It can be observed in **FIGURE 4** that acamprosate, following iv. bolus and oral administration of a 9.3 mg/kg solution, follows flip-flop pharmacokinetics since the terminal slope in the oral curve is significantly lower than the slope observed in the iv. curve [63]. This observation suggests that the terminal slope in the oral plasma concentration–time curve is not representative of the elimination process. This indicates that the real rate-limiting step is related to acamprosate absorption. Specifically, k_a is considerably slower than k_{el} due to a low intrinsic first-order k_a or k_{el} that is sufficiently rapid to make the absorption step rate controlling. Consequently, the terminal half-lives of orally administered acamprosate are six- to sevenfold higher than following iv. administration. There are a number

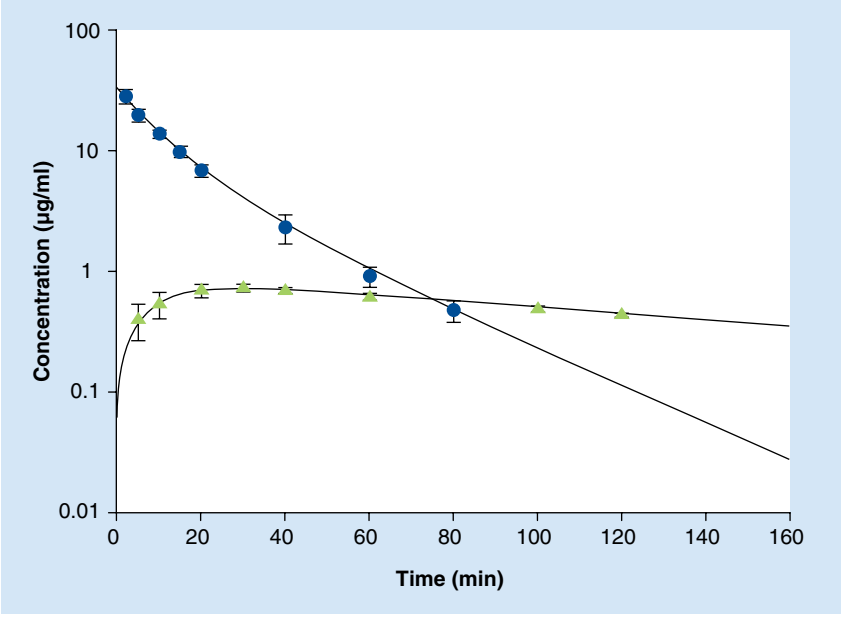

Figure 4. Mean plasma concentrations (± standard deviation, n = 6) of acamprosate after intravenous bolus and oral administration of 9.3 mg/kg.
Reproduced with permission from [63].

of unpublished internal company clinical studies that also demonstrate flip-flop pharmacokinetics in humans [64].

Antifungal & antiviral

Amphotericin B is a polyene antibiotic used for treating systemic fungal infections. It is intravenously administered in the clinic, and is characterized as a BCS class IV drug. A novel oral formulation of amphotericin B in Peceol/1,2-distearoyl-sn-glycero-3-phosphoethanolamine *N*-poly(ethylene glycol) 2000 (DSPE-PEG2000) was developed and the pharmacokinetics compared with commercially available iv. formulations in rats [65]. The half-life of this oral formulation of amphotericin B at 10 mg/kg was approximately 25 h compared with a half-life of approximately 13–15 h for a lower amphotericin B 4.5 mg/kg dose and a 5 mg/kg dose of AmBisome iv. It is suggested that amphotericin B in Peceol/distearoylphosphatidyl-ethanolamine *N*-polyethylene glycol 2000 promotes the absorption of amphotericin B by enhancing solubility and affecting the fluidity of the enterocyte membrane. This improves the permeability of this amphiphilic drug rather than relying on absorption of particles by endocytosis via Peyer's patches (M-cells). At higher dosages, a slower k_a was evident that may be a consequence of a higher volume of the coadministered lipids that may delay gastric emptying and slow peristaltic movements. This is an example of flip-flop pharmacokinetics due to a prolonged absorption phase at higher dosages. Furthermore, as rats do not have a gallbladder, it was suggested that rats are unable to digest relatively large volumes of lipids. In consideration of these differences, the flip-flop pharmacokinetics might not be seen in other species [65].

Carbovir demonstrates activity against HIV. Its terminal elimination half-life was 21.4 ± 4.37 min following iv. administration; however, after an oral dose the terminal half-life was 81.0 ± 67.6 min. This suggests that oral carbovir follows flip-flop pharmacokinetics with absorption being much slower than elimination of the drug from the body [66]. Another example includes the antiretroviral drug zidovudine in which the effects of food and propantheline on the pharmacokinetics of orally administered zidovudine were assessed in rats [67]. Urinary excretion rate plots were created for fasting, nonfasting, propantheline bromide-treated rats to inhibit GI motility. Urine excretion plots are useful tools as they can reflect differences in rate and extent of drug absorption. There was no difference in the extent of absorption of zidovudine as measured by the cumulative amount excreted unchanged in urine. However, the terminal phase of the urinary excretion rate versus time plots yielded half-lives of 1.58 ± 0.63 h, 4.35 ± 2.1 h and 3.42 ± 0.86 h for fasting, nonfasting and nonfasting propantheline bromide-treated rats, which are different to the reported zidovudine half-life of 0.76 ± 0.35 h following an iv. dose [67]. It was suggested that the terminal half-lives reflect drug absorption rather than elimination and that the presence of food in the GI tract of the rats or pretreatment with propantheline bromide increased the mean terminal phase half-life consistent with their abilities to inhibit gastric emptying and, thereby, delay drug absorption. This has been suggested to occur in rats and does not appear to occur in other species.

Anti-inflammatory

The pharmacokinetics of meclofenamic acid was studied in thoroughbred horses [68]. Following iv. administration of 4 mg/kg an elimination half-life of 0.88 ± 0.1 h was determined. However, following oral administration of either meclofenamic acid in granules or sodium meclofenamate (4 mg/kg) solution, a longer time to peak concentration (t_{max} of 2–5 h) occurred and a longer terminal elimination half-life was calculated (2.62 ± 0.22 h and 1.58 ± 0.48 h, respectively). This suggests that flip-flop pharmacokinetics was occurring and that k_a was represented by the decline of plasma levels following oral administration and there is a marked difference in the slopes of the terminal linear portions of the oral and iv. plots of meclofenamic acid [68].

In poultry, the pharmacokinetics of indomethacin following iv. and oral administration demonstrated flip-flop pharmacokinetics. The half-life of indomethacin administered iv. was 0.97 ± 0.39 h, while following oral administration of an aqueous solution, an increase in plasma concentrations and a terminal elimination of 3.3 ± 0.8 h were observed [69]. However, it needs to be acknowledged that the digestive tract of chickens is distinct from mammals caused by a higher pH and an unpredictable gastric emptying time, which may affect absorption [69].

A nonsteroidal anti-inflammatory prodrug, AU-8001, which is metabolized to tolmetin and acetaminophen was administered by both oral and iv. routes to rats. It was observed that the

rapid hydrolysis of the ester moiety causes rapid elimination following iv. and oral administration and that the prodrug exhibited flip-flop behavior and slow bioconversion of the prodrug possibly due to aqueous insolubility of the metabolites. The half-lives of the parent compound were 3.19 min following iv. and 126.48 min following oral administration [70].

Thalidomide appears to exhibit flip-flop pharmacokinetics although iv. studies of the racemate have not been performed [71,72]. For two 100 mg tablets of thalidomide, the absorption and elimination half-lives were 1.7 ± 1.05 h and 8.70 ± 4.11 h [72]. For a 50 mg capsule formulation and an undisclosed clinical trial formulation, elimination half-lives of 6.17 ± 2.56 h and 5.42 ± 1.33 h were seen, respectively [73]. This contrasts with a 100 mg capsule formulation that exhibited a terminal elimination half-life of 15.3 ± 5.99 h and a lower C_{max} suggesting a slower k_a. If the true k_{el} was used, a calculation of approximately 17 l was obtained for V_d/F rather than the 84–94 l when the terminal k_a was used [73]. Furthermore, the effects of a high-fat meal on thalidomide pharmacokinetics resulted in a 0.5–1.5 h absorption lag time and a subsequent delay in t_{max}. The tablet formulation had a mean terminal half-life of 13.5 ± 6.77 h compared with a terminal half-life of 5.80 ± 1.72 h and 5.09 ± 1.03 h for capsule formulations in fasted and fed subjects, suggesting flip-flop pharmacokinetics [74]. Thalidomide dose proportionality was assessed following single doses to healthy subjects [75]. Interestingly, V_d/F was found to increase with dose using the terminal rate constant to calculate V_d/F, which represents absorption and not elimination due to flip-flop. For the highest dose (400 mg capsule), the terminal rate constant was 50% less than the other two doses (50 and 200 mg). This is potentially due to the low aqueous solubility of thalidomide of 40–65 mg/l. This poor solubility causes the rate of drug absorption to depend on the dissolution of thalidomide in the GI tract. This is also consistent with an increase in t_{max} from 2.9 to 4.55 h with an increasing dose from 50 to 400 mg, with a less than proportional increase in C_{max}, and a longer terminal half-life from 5.5 h (50 mg) to 7.3 h (400 mg) [75]. Increase in half-life of thalidomide with doses >800 mg was observed by Figg *et al.* [76] and Fine *et al.* [77]; however, flip-flop was not recognized and the calculation of V_d/F is possibly incorrect in these cases.

Vitamins & antioxidants

The pharmacokinetics of folic acid following im., iv., and oral administration to pigs have been studied [78]. Following oral administration, folic acid demonstrated limited bioavailability and a k_a-limited elimination, while the elimination profiles were similar following iv. and im. The slow absorption following oral administration produced flip-flop pharmacokinetics. Folate may be absorbed via saturable mechanisms through Michaelis–Menten kinetics or nonsaturable mechanisms such as passive diffusion [78].

The oral absorption of three tea catechins, (-)-epicatechin, (-)-epicatechin gallate and (-)-epigallocatechin, following iv. and oral administration to rats has been examined [71]. The terminal elimination half-lives after oral dosing ranged between 451 and 479 min, representing 1.4- to tenfold longer than the half-life following iv. dosing. Additionally, oral dosing produced a longer t_{max} and lower C_{max} coupled to an increased terminal elimination following oral administration suggesting flip-flop pharmacokinetics [79].

Antibiotics

The pharmacokinetics of the combination product amoxicillin and clavulanic acid following iv. and oral administration has demonstrated a flip-flop phenomenon for both drugs in goats [80]. The elimination half-lives were twice as long following oral (2.1 ± 0.20 h and 1.94 ± 0.16 h for amoxicillin and clavulanic acid, respectively) than following iv. administration (1.2 ± 0.16 h and 0.86 ± 0.09 h, respectively) [80].

The low solubility of cilostazol has precluded the ability to develop an iv. formulation; thus, the poor solubility suggests that dissolution may be rate-limiting to absorption. The disposition of an oral solution compared with a suspension and a tablet formulation, and the effect of a high-fat meal have been studied [81]. Absorption from a suspension was more rapid than the tablet formulation. Additionally, the apparent half-lives of cilostazol, as well as its major metabolite, were shorter following administration of a suspension compared with the half-lives of a tablet. Furthermore, the half-life of cilostazol decreased from 15.5 h following a tablet administration to 2.5 h following administration of an ethanolic solution; which correlated to an increase of approximately threefold in the k_a. The co-administration with a high-fat meal

increased the C_{max} and AUC; however, the half-life decreased from 15.1 h in the fasted state to 5.4 h in the fed state. Thus, the observed differences in half-life between the tablet, suspension and solution as well as the effect of food suggest flip-flop pharmacokinetics [81].

The disposition of amprolium in fasting and nonfasting chickens demonstrated an oral elimination half-life of 0.292–0.654 h, which is 1.5–3.2-times longer than the elimination half-life following iv. administration, representing flip-flop pharmacokinetics [82]. The t_{max} and terminal half-life were shorter during the fasting group than the nonfasting (t_{max} of 0.958 and 1.667 h, and half-life of 0.326 and 0.654 h, respectively). The iv. terminal half-life was 0.212 ± 0.079 h, whereas for nonfasting chickens the terminal half-life was 4.4–5.38 h and for fasting chickens a terminal half-life of 3.8 h was observed. It was observed that the absorption increased during fasting perhaps due to accelerated gastric emptying time in the absence of food [82].

Ungulates such as the camel have also demonstrated flip-flop pharmacokinetics. For instance, the disposition of sulfadimethoxine, a lipophilic antibiotic, was assessed following both iv. and oral doses. For the orally administered drug, an elimination half-life of 11.7 ± 3 h and a t_{max} of 11.407 ± 2 h were observed, while following iv. administration the half-life was 4.5 ± 1.2 h indicating flip-flop pharmacokinetics [83]. It was proposed that the sulfadimethoxine may concentrate in resident bicarbonate stores in the small intestine of the camel, and that this may slow the k_a sufficiently to reduce its C_{max} [83].

Antidiabetics

Metformin is a strong base (pK_a = 11.5) that is protonated at physiologic pH. Diabetic rats received an iv. bolus, oral solution, intraduodenal bolus and intra-colonic bolus doses of metformin [84]. In addition, two controlled-release gastroretentive dosage forms based on a polymeric slow-release matrix were designed to produce a constant input of the drug to the absorption sites at the upper part of the GI tract. These formulations were retained in the stomach for 8–10 h and it was observed that metformin was absorbed slowly with prolonged absorption even following oral solution administration. An analysis of iv. versus oral administration demonstrated that the k_{el} are distinctly different (0.31 ± 0.06 h^{-1} and 0.57 ± 0.08 h^{-1}) for oral and iv. bolus, respectively, suggesting flip-flop pharmacokinetics. High affinity of metformin to the negatively charged intestinal wall is reported to occur resulting in an absorption depot situated in the GI tract [84].

Vildagliptin is a hydrophilic compound that was administered orally and via an iv. infusion to healthy volunteers. Population modeling identified two absorption sites with lag-times of 0.23 and 2.46 h and both of the k_a were significantly slower than the k_{el} suggesting flip-flop pharmacokinetics following oral administration. The mean half-life following oral administration was 2.13 h while it was only 1.67 h following iv. administration [85].

Anticoagulants

Although no iv. data is available, population pharmacokinetic analysis of the new oral thrombin inhibitor, dabigatran etexilate, in patients that had undergone primary elective total hip replacement surgery was performed. It was observed that this orally bioavailable double prodrug demonstrated a flip-flop phenomenon after days 0 and 1 postsurgery but not after days 2 to 10. The k_a (during the first 24 h postsurgery) was 0.022 h^{-1} resulting in an absorption half-life of 31.5 h; however, the k_a (after 24 h postsurgery) was 0.265 h^{-1} resulting in an absorption half-life of 2.62 h. The differences found in the disposition during and after the first day postsurgery are likely to be the consequence of alterations in gastric motility and pH and or comedication with opioids following surgery, which reduced the k_a [86].

Antihistamines

Fexofenadine is a substrate to active transporters such as P-glycoprotein, and a highly soluble, low-permeability compound (BCS Class II). Its transport across the intestinal membrane may be a rate-limiting step. It has been suggested to exhibit flip-flop pharmacokinetics although iv. data are lacking [87]. Its apparent elimination half-life ranges from 3 to 17 h and it is suggested that this is dictated by the slow absorption of the zwitterionic molecule, the duration of study and the sensitivity of the assay method [87].

Disease states

Patients with compensated congestive heart failure (CHF) have considerably prolonged absorption compared with normal subjects with resultant lower peak concentrations of drug. For both bumetanide and furosemide, the elimination half-life was twice as long as compared

with healthy subjects. This delayed absorption also caused delayed urinary excretion rates in comparison to healthy subjects. For bumetanide, the elimination half-life in normal subjects was 44–45 min while CHF patients dosed with bumetanide the half-life was 103–110 min. For furosemide, the healthy subjects reported an elimination half-life of 64–84 min, while for CHF patients dosed with furosemide had an elimination half-life of 143–199 min [88]. This study was followed up by a subsequent study of furosemide absorption in decompensated CHF [89]. Furosemide absorption in decompensated CHF was compared with the absorption after attaining normal weight. There were qualitative alterations in furosemide absorption in decompensated CHF when compared with compensated CHF patients. It was observed that eight out of 11 patients had a decrease in lag time and in time to peak plasma concentrations when compensated (t_{max} = 2.95 h) as compared with decompensated (t_{max} = 4.04 h) with nine patients achieving higher peak concentrations **(FIGURE 5)**. As patients' clinical status improved, the absorption also improved suggesting that the disease process alters absorption; however, even in clinically stable CHF patients, absorption was altered compared with healthy patients.

Patients with cirrhosis caused by alcohol received furosemide through iv. and oral administration and its pharmacokinetic disposition was assessed [90]. In nine out of 12 patients, the mean absorption time was longer than the MRT determined following iv. administration suggesting flip-flop pharmacokinetics in these patients. The mean absorption time in cirrhosis was prolonged regardless of edema relative to healthy subjects. A slower absorption was evident in patients with cirrhosis suggesting that this may be the consequence of edema in the gut wall and perhaps disease-associated changes in GI motility. The half-life following iv. administration was 166 ± 149 min while following oral administration it was 296 ± 119 min **(FIGURE 6)** [90]. Interestingly, this flip-flop pattern occurred in patients with cirrhosis and adequate renal function but not in those patients with both cirrhosis and concomitant renal insufficiency. In patients with poor

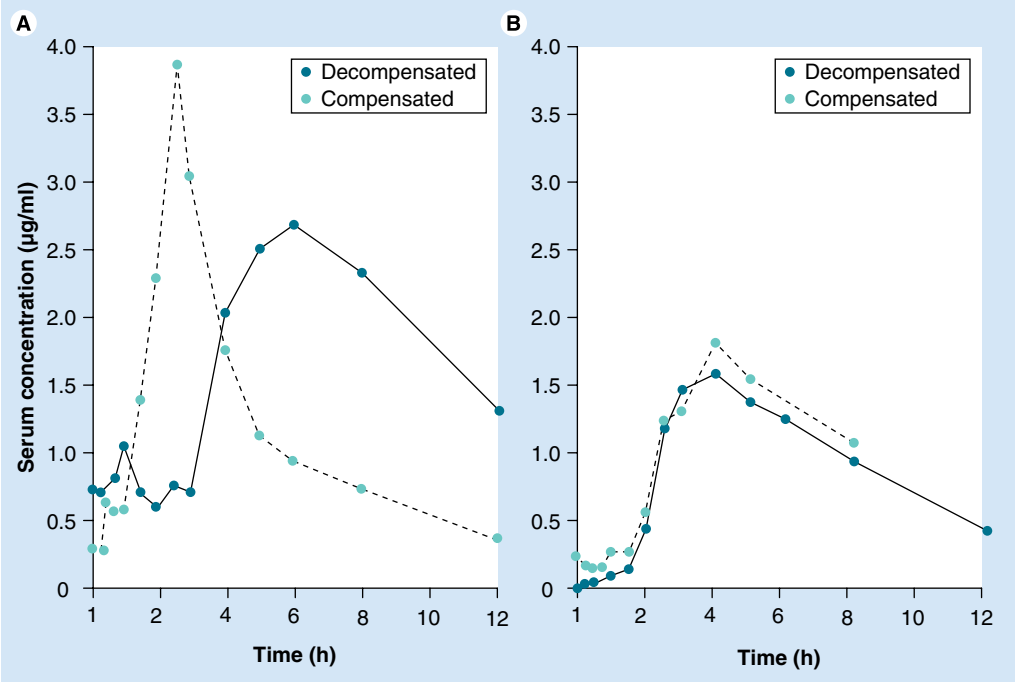

Figure 5. Serum concentration compared with time profiles for two representative patients after oral administration of 160 mg of furosemide. Each patient was administered furosemide while in the decompensated phase of congestive heart failure (solid lines) and again after attaining dry weight (dashed lines). **(A)** Patient ten is representative of eight out of 11 patients studied, with a considerable decrease in lag time and time to peak concentration, and a higher peak concentration when the patients achieved dry weight. **(B)** Patient six is representative of three patients with no changes in pharmacokinetic values between the decompensated and compensated states.
Reproduced with permission from [89].

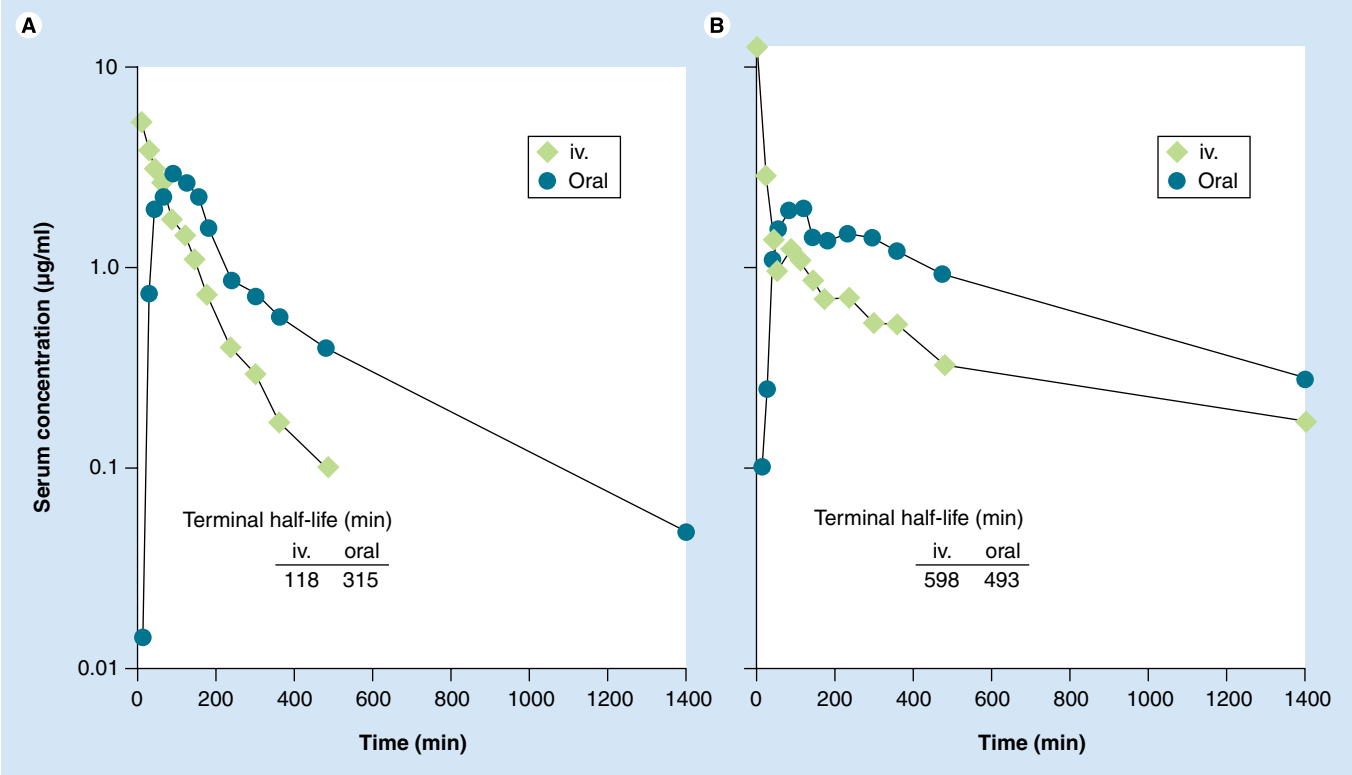

Figure 6. Representative patients with absorption-limited kinetics (A) and decreased renal function (B) in whom terminal phase represents elimination.
iv.: Intravenous.
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kidney function, the terminal portion of the serum concentration–time curve represented the terminal elimination phase whereas in patients with adequate renal function the terminal phase represented absorption. It is until moderate renal insufficiency intervenes where k_a is faster than k_{el}. In eight healthy volunteers, absorption-limited kinetics was also demonstrated [91]. Furosemide was administered as an iv. bolus, as a tablet in the fasting state and as a tablet and a solution after food intake. The iv. data demonstrated a half-life of 3.39 h, and food delayed the t_{max} to values down to 45 and 225 min for solution and table formulation, respectively. The MRT following iv. administration was 51 ± 1.5 min and following all oral doses the MRT was longer than iv., which is further evidence of flip-flop pharmacokinetics [91]. A meta-analysis of the various studies examining furosemide pharmacokinetics outlines the variability in the studies to date [92].

Miscellaneous

COL-3 is a chemically modified matrix metalloproteinase inhibitor that demonstrates an irregular absorption profile. COL-3 was administered via various routes of administration such as iv., orally and intraduodenally in fine and coarse suspensions, and in bile-duct cannulated animals with and without food. Flip-flop pharmacokinetics of the absorption and k_{el} was noted but only following intraduodenal administration of the coarse suspension to bile-duct cannulated rats [93]. COL-3 is almost water insoluble but solubility increases and stability decreases with increasing pH. The terminal half-life following iv. administration was 5.81 ± 0.76 h; however, intraduodenal administration of a coarse suspension to bile-duct cannulated fed rats reported a terminal half-life of 15.91 ± 1.43 h. This pattern is consistent with dissolution rate-limited absorption, formulation effects, endogenous bile effects and food effects on COL-3 [93].

Chitooligosaccharides are being scrutinized as biomaterials. Chitobiose and chitotriose were eliminated rapidly following iv. administration to rats. However, following oral administration the first-order k_a were less than 1.0 h^{-1} and smaller than the k_{el} (2.2 ± 0.3 and 2.7 ± 0.1 h^{-1}, respectively), indicating flip-flop pharmacokinetics.

Furthermore, only low-molecular weight chitooligosaccharides administered orally were absorbed [94].

■ Subcutaneous & intramuscular

The absorption of a solid chemical entity from the injection site following sc. or im. administration is affected by several processes:

- The surface area of the absorptive surface that is in contact with the injected volume
- Diffusion of drug into surrounding tissues and the local pH
- The rate of removal of the solvent and rate of diffusion through the tissues
- Blood and lymphatic flow and modifications resulting from exercise or disease
- Anatomical differences in vascularity
- Permeability of blood and lymphatic vessels
- Binding of drug or metabolites to tissue

Movement of the drug away from the site of absorption into the systemic circulation is critical. It has been demonstrated that following im. or sc. administration of drugs, the rate and extent of a drug's absorption depends on its physicochemical properties, such as lipid solubility and pK_a but also on factors such as the vascularity of the injection site, the volume of the formulation deposited and the concentration of the active moiety in the dosage form. Administration at diverse injection sites may result in differences in blood flow and absorptive surfaces and hence disposition kinetics may differ [14].

Many peptides and proteins demonstrate short half-lives following iv. administration. Delayed and prolonged absorption can often be observed with large macromolecules (>4 kDa) following im. or sc. administration since lymphatic uptake from injection sites leads to increased exposure and k_a-limited disposition. Following extravascular drug administration, the terminal half-life can be more prolonged than following an iv. administration. There are many long-acting formulations obtained using slow sustained-release dosage forms and subdermal implants that are designed to provide prolonged duration of action through maintenance of plasma concentration above a minimal therapeutic concentration. They are created to be more clinically practical in extending dosing intervals.

Antibiotics

Intramuscular administration of five different products of ampicillin to ruminant calves demonstrated influence of formulation on plasma-concentration profiles [95]. im. administration of ampicillin trihydrate (Polyflex®) and Ampikel 20 in the lateral neck demonstrated apparent half-lives of 3.8 ± 1.7 h and 2.1 ± 0.5 h, respectively. The formulations of Albipen® and Duphacillin resulted in lower plasma concentrations; however, they also had a more sustained profile with half-lives of 22.2 ± 7.6 h and 11.9 ± 3.7 h, respectively. While Penbritin® administration concentrations were slightly higher with a half-life of 5.9 ± 2.0 h [95]. The pharmacokinetics of injectable ampicillin formulations: Polyflex (a water-based suspension) and Ampi-kel 10 (an oil-based suspension) were administered to calves, sheep and swine via im. injection. The terminal elimination half-lives were estimated to be at least 2.4 h, which was greater than the literature values for sheep and calves (literature value for swine is not available) and it was concluded that this is likely to be associated with flip-flop pharmacokinetics [96].

Not only the formulation but the location of the site of injection may affect the disposition profile and pattern of a xenobiotic. For instance, penicillin G was administered as procaine penicillin G to horses in a variety of muscle groups. Bioavailability was highest following im. injection in the neck and bicep musculature while im. gluteal and sc. sites resulted in lower concentrations that were more sustained [97]. Overall, the terminal elimination half-life following iv. administration was 3.72 ± 0.60 h, while following sc. administration the half-life was 21.8 ± 8.8 h and following im. administration ranged from 8.0 ± 2.7 h to 14.9 ± 3.4 h. All the routes exhibited flip-flop pharmacokinetics [97]. Therefore, it can be observed that the route and site of injection affects the disposition of drugs.

The pharmacokinetics of recombinant human IFN-β and fibroblast-derived human IFN-β were compared following iv. and im. administration to rabbits. The serum concentrations in the iv. group demonstrated terminal half-life of 1.55 ± 0.05 min and 2.05 ± 0.30 min, while the terminal half-lives following im. administration were 39.6 ± 6.3 min and 21.3 ± 1.1 min, representing flip-flop pharmacokinetics [98]. This parallels to the findings in humans with IFN-β1a following iv. and sc. administration, where it was observed that slow and incomplete absorption

of the sc. dose resulted in prolonged concentrations of IFN-β1a reflecting flip-flop pharmacokinetics [99]. Similarly, the pharmacokinetics of T-2 toxin following im. and iv. administration demonstrated a half-life of 21 ± 5 min that was four-times longer than following iv. administration (5.6 ± 2.3 min). A slow absorption following im. administration was apparent since the k_a was slower than k_{el}, suggesting flip-flop pharmacokinetics [100].

Ruminants, such as cattle, during the first few weeks of life are functionally monogastric and absorption is more comparable to those of nonruminants. Following oral and sc. administration to calves, the disposition of sulfadiazine and trimethoprim was examined [101]. The effect of age and diet was assessed. One group was fed with milk-replacer for 13 weeks while the second group was fed with a chopped-grain fiber mix when 5 weeks old. The sulfadiazine absorption following oral administration was very slow in all the calves between 8.2 and 12.67 h and slightly faster in grain-fed ruminants. After 1 week of diet (<42 days of age) the terminal half-life was 11.5 ± 0.6 h, while the true elimination half-life was determined to be 5.0 ± 0.6 h with t_{max} at approximately 15 h. However, calves older than 42 days did not exhibit flip-flop pharmacokinetics. It was observed that the k_a increases with age and the development of the rumen since the half-life in grain-fed cattle for 6 and 12 weeks was 8.5 ± 1.3 h and 8.2 ± 0.4 h, respectively [100]. Another example in ruminants can be observed after enrofloxacin was administered via iv., im. and sc. routes. It was observed that the mean serum half-lives were 1.7, 5.9 and 5.6 h, respectively demonstrating flip-flop pharmacokinetics [102]. Furthermore, delayed absorption was evident following im. and sc., which limited the elimination and demonstrated sustained release from the site of injection allowing for a more prolonged dosing interval [102].

Epitiostanol absorption and disposition was determined following im., intrabursal and oral administration to rats. The concentration–time curve following im. administration demonstrated flip-flop pharmacokinetics as the epitiostanol remaining at the injection site exhibited a k_a smaller than the k_{el} obtained from iv. data. It was observed that the half-life was approximately 21 min following iv. administration and 756 min following im. administration [103]. The semi-simultaneous im. administration of a water-based suspension was compared with the iv. solution formulation of flumequine and a mean elimination half-life of 3.9 ± 1.95 h and an absorption half-life of 8.3 h were observed. A predominantly slow absorption of the drug from the im. depot at the injection site was rate-limiting while the distribution and the elimination are much faster. Flumequine is a weak acid and may dissolve slowly at the pH in the muscle following im. administration [104].

Rabbits were administered with the antibiotic florfenicol via iv., oral and im. routes. Results demonstrated that following iv. administration a terminal half-life of 1.54 h was observed; however, following both im. and oral dosing the terminal elimination half-life was 3.01 and 2.57 h, respectively. This suggested that flip-flop pharmacokinetics occurred as k_a was slow and achieved a t_{max} at 0.5 h postdose [105].

Horses were administered with difloxacin via iv., im. and oral routes with mean serum half-lives of 2.66, 5.72 and 10.75 h, respectively, demonstrating flip-flop pharmacokinetics [106]. Furthermore, delayed absorption was evident following im. and oral administration, which limited the elimination and demonstrated sustained release from the site of injection and the absorption site. Cefazolin sodium was also administered to horses via iv. and im. injections with an apparent half-life that ranged from 49 to 99 min compared with a true half-life that ranged from 35 to 46 min. It was observed that the im. administration influenced the rate of absorption and cefazolin was eliminated more slowly [107].

Healthy lactating Israeli-Holstein cows were administered with a single dose of danofloxacin, a fluoroquinolone antibiotic, via iv. and im. routes at a 1.25 mg/kg dose. It was observed that mean elimination half-lives in serum were 54.9 and 135.7 min following iv. and im. administration, respectively. The drug was eliminated from serum following im. administration at a significantly lower rate than following iv. administration [108]. The pharmacokinetics of oxytetracycline following iv. and im. administration to sheep also demonstrated flip-flop pharmacokinetics. A conventional iv. formulation demonstrated a half-life of 3.29 h while a conventional formulation (T-100) and an aqueous solution with 1% lidocaine (OTC_L)-administered im. reported longer half-lives of 14.1 and 58.2 h, respectively. In these formulations, the initial phase of the curve represented the apparent k_{el} while the later phase represented the k_a [109].

Different formulations of amoxicillin including a conventional and a long-acting amoxicillin trihydrate suspension were studied in sheep following iv. and im. injection. im. administration of the conventional suspension resulted in a flip-flop phenomenon. This suggests that the absorption of amoxicillin is the rate-limiting step. The injection of the long-acting formulation also created a depot and resulted in lower but remarkably prolonged serum concentrations and an increase in half-life [110]. The half-life following iv. and im. administration of a conventional suspension was approximately 1.62 h and 10 h, respectively, while approximately 30 h following a prolonged action formulation **(FIGURE 7)** [110]. Amoxicillin is also formulated as a sodium salt in aqueous solution or as amoxicillin trihydrate suspensions for im. or sc. administration. The influence of the injection site on the pharmacokinetics of amoxicillin following im. administration of a conventional and long-acting amoxicillin trihydrate suspension was demonstrated in sheep. im. administration in the neck and the hind limb demonstrated that absorption is the rate-limiting step in the overall disposition. The long-acting formulation formed a depot, which caused prolonged serum concentrations and an increase in terminal half-lives [110]. It was also observed that the iv. administration half-life was 1.62 ± 0.30 h, while the im. administration of Amoxykel in the neck and thigh was 10.21 ± 1.69 and 9.77 ± 3.2 h, respectively, and Clamoxyl LA reported a half-life of 29.88 ± 8.09 h and 35.44 ± 9.35 h, respectively **(FIGURE 7)** [110]. This is consistent with the results of Fernandez *et al.* [111] where flip-flop pharmacokinetics was observed following im. injection of amoxicillin trihydrate suspension in sheep, horses [112,113] and pigs [114].

Antineoplastics

Polyestradiol phosphate used in prostate cancer is a long-acting, parenteral depot estrogen. It is a mixture of 13 estradiol polymers that are hydrolyzed to free estradiol at the injection site but will also diffuse at different rates into the systemic circulation. The terminal half-life of depot estradiol was 70 ± 21 days compared with a half-life of 2–4 h following administration of pure estradiol via the iv. route [115]. The flip-flop pharmacokinetics are further supported by secondary estradiol metabolites with similar elimination, which is governed by the rate of metabolite formation. The serum concentrations of estradiol are governed by flip-flop pharmacokinetics and a limited rate of hydrolysis following im. injection, and the median rate of input was reported to be 0.21 days while the elimination output was 0.001 $days^{-1}$[116].

Degarelix is a novel gonadotropin-releasing hormone blocker that exhibited flip-flop pharmacokinetics in humans following sc. dosing and was modeled with both a slow and fast first-order process of absorption. The sc. administration demonstrated flip-flop pharmacokinetics with a prolonged terminal phase with concentrations detectable until 60 days following single-dose

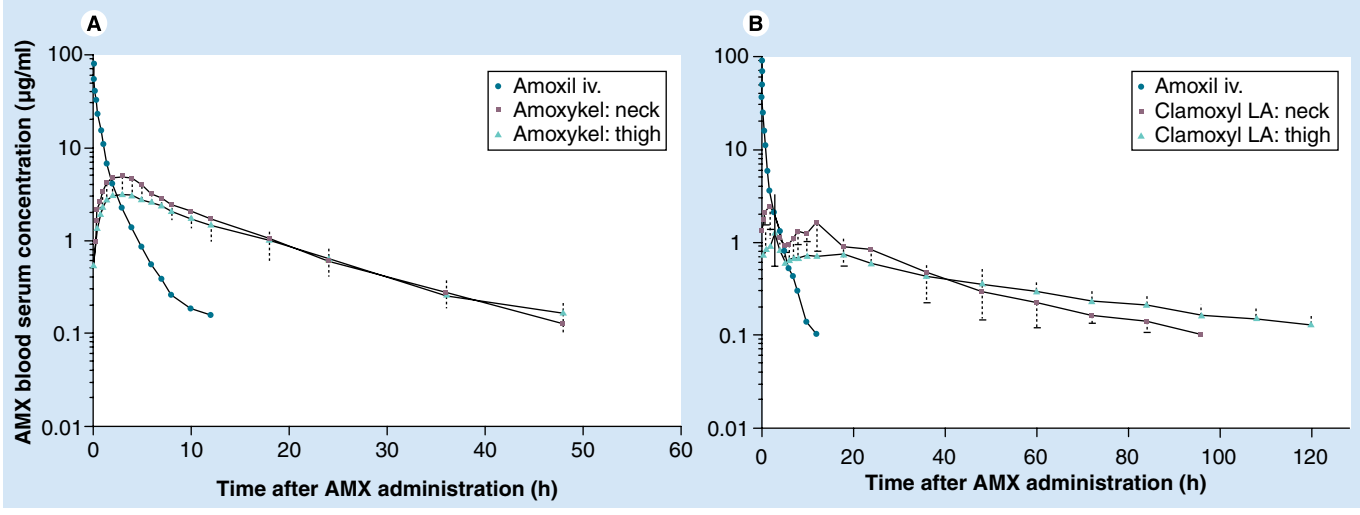

Figure 7. Amoxicillin blood serum concentration versus (A) time after intravenous and intramuscular administration in sheep at 15 mg/kg dose and (B) intravenous and long-acting intramuscular administration in sheep at 15 mg/kg dose.
AMX: Amoxicillin; iv.: Intravenous.
Reproduced with permission from [110].

administration. An *in situ* depot formation is responsible for the prolonged degarelix release and it has been suggested that interaction with the tissue proteins following sc. injection forms a gel-like structure [117]. In humans, cetrorelix demonstrates a similar disposition pattern with a terminal half-life of 56.9 h following sc. administration [118].

Antidiabetics

Following sc. injection of insulin, absorption from the injection site is often delayed as regular insulin forms complexes that have to dissociate before absorption can occur. Insulin lispro is structurally different from human insulin at the amino acids proline and lysine positions 28 and 29; this prevents association and may lead to faster absorption. Pharmacokinetic studies of insulin lispro in Type II diabetic patients with normal renal function and with hemodialysis demonstrated that insulin lispro following sc. injection in patients with normal renal function is absorbed faster compared with regular insulin. While in patients undergoing hemodialysis, plasma insulin concentration increased more rapidly (t_{max} 20 vs 40 min) and was higher after insulin lispro compared with regular insulin. Differences in elimination half-life (52 vs 192 min) suggest flip-flop pharmacokinetics with regular insulin [119].

Anti-inflammatory

The pharmacokinetics of methylprednisolone succinate following iv. and im. administration in normal and adrenolectomized (ADX) rats demonstrated that following im. administration, it was absorbed slowly with two first-order k_a indicating flip-flop pharmacokinetics. The disposition of methylprednisolone succinate at the injection site exhibited slow dual k_a. The terminal half-lives for ADX rats and normal rats following im. dosing were longer (normal and ADX rats = 1.1 h) compared with iv. dosing (normal rats = 0.5 h, and ADX rats = 0.33 h) [120].

Miscellaneous

The skeletal muscle-derived creatine kinase (CK) was investigated in dogs following im. and iv. administration to dog-muscle homogenates in order to use the disposition of plasma CK activity to quantify muscle damage. iv. injection demonstrated a terminal half-life of 2.5 h while following im. administration the terminal half-life of CK was 6.5 h, demonstrating flip-flop pharmacokinetics. There was a rate-limiting absorption process for CK from the site of injection into the plasma [121]. This has also been demonstrated in horses where CK deposition parameters were evaluated following iv. and im. administration and a plasma terminal half-life of 112 ± 18 min and 11.8 ± 5.3 h, respectively [122].

Pharmacokinetics of recombinant human IL-10 in humans following sc. and iv. dosing demonstrated an iv. terminal half-life of 1.94 ± 1.16 h while sc. dosing demonstrated a depot at the injection site with absolute bioavailability of 42% and a lower but sustained concentration–time profile. A small amount was rapidly absorbed directly into the circulation followed by a more gradual release of a second fraction that might be controlled by lymphatic flow with a terminal elimination half-life of 4.3 h [123].

The pharmacokinetics of human growth hormone administered subcutaneously with two different injection systems (a transcutaneous jet injection device and a sc. cannula) demonstrated a t_{max} of approximately 4 h and a terminal half-life of 2–4 h [124]. Erythropoietin is a performance-enhancing hormone that stimulates production of red blood cells. sc. injection of recombinant human erythropoietin to athletes demonstrated a mean half-life of 35.5 h, which was four- to five-times longer (~42 h) compared with iv. administration where half-life was 4–7 h, suggesting flip–flop pharmacokinetics [125]. Modeling of iv. and sc. dosing in cynomolgus monkeys demonstrated that following sc. dosing of erythropoietin, flip-flop pharmacokinetics with dual absorption pathways may result from entry via blood vessels and via the lymphatics [126]. This was followed up in a clinical study in healthy volunteers with a similar disposition pattern observed with slow absorption (t_{max} ~24 h) [127]. Modeling of sc. pharmacokinetic data of erythropoetin demonstrated flip-flop pharmacokinetics (k_a = 0.7 day^{-1} and the k_{el} = 2.2 day^{-1}) [128].

■ Epidural

Epidural route of administration is typically employed to inject anesthetics (to reduce sensation) and analgesics (to reduce pain) through a catheter placed into the epidural space in order to block transmission of signals through nerves near or in the spinal cord [129]. It has been observed that some local anesthetics exhibit flip-flop pharmacokinetics following epidural administration, such as ropivacaine [130–132]. For instance, ropivacaine 0.2% was continuously infused (5 ml/h)

epidurally for 48 h postoperation in patients scheduled for major abdominal or urologic surgery [130]. The pharmacokinetic data was fitted to a one-compartment model with sequential bolus and infusion inputs, which was selected based on the Akaike information criterion [133]. However, because of difficulties in estimating the time for ropivacaine to reach steady-state after epidural administration, a mixed hybridized absorption/distribution/elimination half-life estimate was selected rather than the iv. half-life or the half-life at the end of infusion [130]. The mixed hybridized absorption/distribution/elimination half-life was calculated by the ratio between the estimated clearance (CL/F) and volume of distribution (V_d/F). Clearance was calculated using the model-independent parameter approach (CL = dose/AUC_{tot}) [130]. It was observed that the mixed hybridized absorption/distribution/elimination half-life was 10.8 h, which was higher than the terminal half-life at the end of infusion (7.4 h) and the iv. half-life (1.8 h) [130].

Other investigations of formulation approaches to prolong the duration of action of local anesthetics administered via an epidural have been attempted [8]. A hyaluronate-based formulation of lidocaine demonstrated a decreased k_a from the epidural space compared with a solution formulation. A viscous lidocaine–hyaluronate formulation and a 2% lidocaine solution following iv. administration and epidural administration were compared. The terminal slope of the plasma lidocaine concentration–time profile following iv. administration (half-life of 48.1 ± 19.1 min) was significantly greater than following epidural administration of either the lidocaine solution or the lidocaine–hyaluronate complex [8]. The slow release of drug from formulation was evident by the increase in the AUC. The absorption of the epidural-administered solution appeared to occur biphasically with both slow and fast absorption phases. Furthermore, the k_a half-life was greater than the terminal elimination rate [8]. The slower absorption of drug from the lidocaine-hyaluronate formulation appeared to be a single absorption pathway and was attributed to both diffusional and ionic mechanisms of drug release. Both formulations administered epidurally demonstrated flip-flop pharmacokinetics as the rate-limiting release of drug from the formulation and the terminal elimination phase represents the slow absorption phase [8].

There appears to be different absorption characteristics following epidural administration to rabbits and dogs owing to anatomical differences in the dimension of the epidural spaces [8]. A smaller body weight in the rabbit may render a larger surface area of absorptive blood vessels:volume (of injectate) ratio resulting in rapid appearance of drug in the systemic circulation. On the other hand, a larger body weight in the dog would have a smaller absorptive surface:volume ratio and subsequently the residence time in the epidural space would increase, which could enhance fat and neural tissue. Therefore, in the dog's epidural space a drug could be sequestered, facilitating a built-in slow-release process that represents a rate-limiting role on the appearance of drug in the systemic circulation [8].

■ Intra-articular

Intra-articular route of administration consists of a direct injection of anti-inflammatory agents and anesthetics directly into the facet joints in order to achieve therapeutic benefit in patients with joint-related ailments [129]. Local injections of anti-inflammatory drugs into inflamed joints have been used clinically to maintain high concentrations in the synovial joint while minimizing systemic concentrations. There is a paucity of data available on the pharmacokinetic profile of drugs following intra-articular administration. The intra-articular administration of methylprednisolone acetate in cattle demonstrated slow release of methylprednisolone from methylprednisolone acetate at the site [134]. Methylprednisolone acetate was not detected in plasma; however, methylprednisolone concentrations increased slowly and decreased slowly from the synovial joint area with an elimination half-life of approximately 21.7 ± 4.49 h. Following iv. administration, elimination half-life was 1.43 ± 0.315 h, suggesting flip-flop pharmacokinetics [134]. This appears to mirror the clinical disposition in humans of triamcinolone acetonide, triamcinolone hexacetonide and a combination of β-methasone phosphate and acetate when administered intra-articularly [135]. Owing to the lower solubility, triamcinolone hexacetonide appeared to be absorbed to a slower extent than triamcinolone acetonide. The terminal half-life of triamcinolone acetonide varied between 3.2 and 6.4 days compared with 1.5 h following iv. administration, suggesting flip-flop pharmacokinetics. The triamcinolone hexacetonide absorption took place over a

long period of time with a terminal half-life of 4.6 days; and a terminal half-life of β-methasone in plasma of 6.3 days compared with 7 h following iv. injection [135].

The pharmacokinetics of rimexolone suspension following intra-articular injection into knee joints of patients with rheumatoid arthritis dissolved slowly and provided sustained release of the steroid locally. Absorption was rate limiting with a k_a of 0.035 $days^{-1}$, which is equivalent to a half-life of 20 days, while the elimination half-life was approximately 30 min [136]. The absorption of ulinastatin following intra-articular administration to rabbits demonstrated a slow increase in plasma concentrations with a t_{max} of 4.3 h followed by a slow decline with a half-life of 10.8 h [136]. Ulinastatin distributed throughout the body but was retained for a long period of time in the joint tissues. Following iv. administration to rabbits, ulinastatin declined biphasically with a half-life of 6.1 h while following intra-articular administration a plasma half-life of 10.8 h was evident [137]. The transport of ulinastatin from synovium to the plasma occurred predominantly via pores in the capillary wall and very little was transported via the lymphatic system. The apparent elimination half-life of the ulinastatin from plasma reflects its rate of transfer to the plasma from the injected site and that the apparent k_a of ulinastatin into plasma reflects the rate of elimination of ulinastatin from the plasma [137].

■ Percutaneous

Skin is an effective defense barrier of the body against external xenobiotics due to its stratum corneum filled with keratin and a dense intracellular space. The transdermal delivery is a means of sustaining action for drugs following systemic or local administration. The percutaneous route avoids hepatic first-pass metabolism; thus, transdermal delivery of drugs represents an approach to delivery of a variety of xenobiotics.

The feasibility of topical delivery of naproxen for both local and systemic anti-inflammatory effects was assessed following application to the stifle joints of dogs [137]. Following intrabursal administration the half-life in serum was 39.4 ± 9.70 h. Interestingly the administration of a topical gel resulted in long sustained concentration–time profiles in serum with a t_{max} of approximately 20 h postdose and lasting for the next 30 h with an estimated half-life of 61.2 ± 12.8 h [137]. The longer half-life and low bioavailability were the consequence of large drug accumulation in the skin.

The pharmacokinetics of ^{14}C-thymoxamine was studied in hairless rats following different routes of administration including oral, iv., and percutaneous. Following percutaneous topical administration, the elimination half-lives of ^{14}C-thymoxamine and its metabolites were approximately 15 h, which are longer than the half-lives following oral or iv. administration (~9 h). As a result of the observed penetration into the stratum corneum, the absorption by the cutaneous microcirculation and the overall absorption phenomena of thymoxamine, it was determined that flip-flop pharmacokinetics through the skin was observed [138]. The pharmacokinetics of ^{14}C/^{13}C-labeled orthophenylphenol following dermal application to human volunteers following a single 8 h dermal dose demonstrated that 43% of the dose was absorbed with a slow rate of dermal absorption with t_{max} within 4 h postexposure. The average half-life for dermal absorption was 9.7 ± 2 h with absorption through the skin (rate-limiting step) and a mean elimination half-life of 0.8 ± 0.1 h [139].

■ Rectal

A rectal suppository is a drug-delivery system that is inserted into the rectum where it dissolves and can deliver drug either systemically or locally. Suppositories containing ketoprofen were administered to patients following anal surgery. Compared with healthy subjects, patients who had anal surgery demonstrated reduced plasma concentrations, increased t_{max} and a longer terminal half-life of both fatty suppositories and gelatin-capsulated suppositories [140]. The half-life increased two- to four-times longer in patients with anal surgery than healthy subjects. The k_a in patients was significantly smaller than in healthy subjects, and a flip-flop phenomenon could be seen in the disposition profiles in plasma. The half-life in healthy subjects was approximately 0.78 ± 0.18 h and 0.77 ± 0.12 h versus 3.09 ± 1.30 h and 2.01 ± 0.92 h for fatty suppositories and gelatin capsulated suppositories, respectively. These results suggest that patients operated under spinal anesthesia demonstrated a decrease in k_a owing to a decrease in blood flow rate in the rectum due to spinal anesthesia, and an impairment of tissue at the absorption site by surgical injury due to the change in absorption site by bed rest [140].

A follow-up study on the clinical pharmacokinetics of two different preparations of ketoprofen suppositories following spinal or local anesthesia for anal surgery also demonstrated

significant differences in the peak concentrations, t_{max} and terminal half-lives. In patients operated under spinal anesthesia, the C_{max} decreased by one-half while the t_{max} and half-life increased two- to four-times compared with patients operated under local anesthesia. k_a was significantly lower following spinal anesthesia compared with either local anesthesia or healthy controls [141]. The pharmacokinetics of the anti-inflammatory drug ximoprofen in healthy subjects has been evaluated following iv., oral and rectal dosing. The terminal half-life of 1.9 h in healthy subjects was prolonged leading to flip-flop pharmacokinetics in rectal dosing with a half-life of 3.4 h [142].

■ Ocular

The absorption of ocularly applied tritiated [D-ala2]-metenkephalinamide was studied in rabbits with peak plasma concentrations within 20 min. The apparent k_a was slower than k_{el} with approximately 36% absorbed with both conjunctival and nasal mucosa playing an important role (k_a = 0.0017 min^{-1} < k_{el} = 0.097 min^{-1}) [143]. The slope of the terminal phase following iv. administration was rapid and much steeper than following topical administration, suggesting flip-flop pharmacokinetics. Ocular instillation was undertaken in rabbits in open nasolacrimal ducts, conjunctival instillation with closed nasolacrimal ducts, and instillation directly in the nasolacrimal duct. Each mode of delivery produced k_a < k_{el}. In the eye, the contact time of the instilled dose, the intrinsic permeability and dilution are critical factors [143].

■ Respiratory

Inhalational

The aim of inhalation therapy is often utilized to deliver drugs to sites of infection or affliction in order to achieve effective tissue concentrations while minimizing systemic concentrations.

Antibiotics

Inhaled antibiotics are a part of current cystic fibrosis therapy and can improve pulmonary function. Six patients with cystic fibrosis inhaled tobramycin 600 mg using a jet nebulizer, and compared with iv. administration, a longer terminal elimination half-life of 9.47 ± 3.28 h was evident compared with a half-life of 2.32 ± 0.28 h (following iv. administration) suggesting a prolonged absorption [144].

Anti-inflammatory

The pharmacokinetic disposition following iv. administration of sodium cromoglycate, a water-soluble acid drug, was compared with inhalation administration via nebulizer solutions and capsule inhaled as dry powder via Spinhaler. iv. administration demonstrated rapid elimination from the body with a mean k_{el} of 11.5 h^{-1}. After inhalation, there was a terminal k_{el} of 29 h^{-1} from capsules, 18 h^{-1} from a 10 mg/ml solution and 34 h^{-1} from a 30 mg/ml solution [145]. On a separate study, following iv. administration, sodium cromoglycate demonstrated rapid elimination from the body with a half-life of 13.58 min and after inhalation there was a terminal half-life of approximately 91 min [146].

A model for absorption from the lungs has been described and involves absorption at two different rates, which may reflect absorption from different sites in the lungs. The material deposited deeper into the alveoli may be absorbed rapidly while the material deposited higher would be absorbed slower, and the terminal half-life represents the k_a. The pharmacokinetic disposition following iv. administration of nedocromil was compared with inhalation via a metered-dose inhaler and an oral solution. iv. administration demonstrated rapid elimination from the body with a plasma half-life of 53 min [147]. The inhalation dose rapidly plateaued and then decreased monoexponentially with a half-life of 2.3 ± 0.3 h in volunteers and 1.5 ± 0.2 h in asthmatic patients with absorption of 6% of the dose. The terminal elimination half-lives following oral, iv., and inhalation administration routes are similar at approximately 13.8 h. It was observed that the terminal elimination half-life represents the absorption half-life from the lungs and becomes the rate-limiting step indicating flip-flop pharmacokinetics. Therefore, it appears that more than one k_a may be occurring in the lungs [147].

The neutrophil elastase inhibitor, FK706, was administered via inhalation to smokers and nonsmokers, and significantly different plasma concentrations were observed between the two groups. Interestingly, the smokers demonstrated a shorter t_{max} and a shorter elimination half-life (1.23 ± 0.40 h vs 2.73 ± 0.57 h, respectively) [148]. k_a was reported to be ten-times greater in smokers than nonsmokers. The C_{max} values of the smokers were two- to four-times higher than nonsmokers. Although IV data were not available, it is evident that flip-flop pharmacokinetics was occurring. These findings may suggest that

smoking causes increasing pulmonary epithelial permeability. FK706 is a small hydrophilic molecule and this phenomenon could apply to other xenobiotics that are delivered to the lungs with similar physicochemical properties [148].

Antidiabetics

Oral inhalation of insulin may have utility in glycemic control. A physiologically realistic insulin–glucose model was applied to a meta-analysis of insulin–glucose profiles from clinical studies of inhaled and iv. insulin to derive pharmacokinetic disposition of insulin in the lung following inhalation [149]. The model assumed first-order absorption and parallel nonabsorptive loss (k_{mm}) from metabolism and mucociliary clearance. The k_a for the inhaled formulation was estimated to be from 0.020 to 0.032 h^{-1} across doses, formulations and subjects with an estimated half-life ranging between 22 and 33 h, while the half-life following iv. administration of insulin was <0.2 h. Passive diffusion and absorption were reported to follow flip-flop pharmacokinetics as both k_a and k_{mm} were smaller than the systemic k_{el} [149]. Another study employed a two-compartment pharmacokinetic model with one (inhaled: technosphere insulin) or two sequential first-order absorption processes and first-order elimination. The model was employed using data from two studies with a total of 651 concentrations from 16 healthy volunteers. The estimated first-order k_a for technosphere insulin was 2.35 h^{-1} while the first-order k_a associated with subcutaneously administered insulin were 0.63 and 1.04 h^{-1}, respectively [150].

Antineoplastics

A nanoparticle formulation of the chemotherapeutic cisplatin was developed using the polysaccharide hyaluronan. The nanoparticles were instilled to rats as a 3.5 mg/kg total platinum solution and compared with cisplatin at 3.5 mg/kg administered iv. The instilled nanoparticles had an increased half-life of 82 h compared with 38 h for the nanoparticles administered iv. and a half-life of 41 h for cisplatin (not in nanoparticles) administered iv. [149]. The instilled nanoparticles also increased the $AUC_{0\text{-}96\ h}$ by 61% compared with cisplatin administered iv. The flip-flop phenomenon was likely caused by a depot effect of the nanoparticles in the lung, which prevented the rapid release of the cisplatin and limited the rate of absorption into the plasma [149].

Intrapleural

In patients with malignant pleural effusion, the antibiotic neothramycin was intrapleurally administered and the half-life ranged from 3.45 to 6.48 h with a t_{max} at 1–2 h [151]. The intrapleural concentrations declined with half-life ranging from 6.54 to 17.80 h. The elimination half-life was longer than that following iv. administration indicating flip-flop pharmacokinetics. In this case, the rate of transfer from the pleural space to the plasma was much slower than that of the elimination from the plasma [151].

Intranasal

Nasal administration is an alternative for the delivery of proteins and polypeptides. For instance, enkephalins are absorbed through the nasal mucosa into the systemic circulation. After nasal administration, the serum concentrations of these proteins are not significantly different from iv. administration. The elimination of metkephamid following nasal administration had a terminal elimination half-life of 34.9 min and following iv. administration of 18.7 min. No metkephamid was detectable after oral administration [152].

Conclusion

A review and analysis of the biomedical literature suggests that flip-flop in the blood fluid concentration–time curve profiles occurs in a variety of different situations and can complicate the determination and the interpretation of pharmacokinetic parameters. With the advent of new delivery systems, formulations, biomaterials, technologies and a plethora of exciting therapeutic approaches, the occurrence of flip-flop phenomenon will undoubtedly continue to be observed and reported. Pharmacokinetic software is widely used to estimate pharmacokinetic parameters. Use of any modeling program without a clear understanding of the drug formulation, routes of administration and physiology affecting the parameters may result in erroneous interpretation of data when flip-flop pharmacokinetics is encountered.

Future perspective

Therapeutic delivery of macromolecules is evolving with the aim of controlling absorption, distribution, metabolism and cellular uptake through programmed drug delivery. Specifically, drug targeting by carriers utilizing prodrugs, synthetic polymers and various nanocarrier approaches is becoming more frequently incorporated into drug design. This alteration

and manipulation of input and output rates of xenobiotics is becoming increasingly complex. An understanding of the conceptual limitations of pharmacokinetic software packages is essential for the detection of pharmacokinetic phenomena such as flip-flop pharmacokinetics. Classically trained pharmacokineticists, although in decline, remain critically important for pharmacokinetic parameter interpretation in both preclinical and clinical pharmaceutical science research. A comprehensive understanding of the mathematical and physiological attributes of flip-flop pharmacokinetics can aid in the avoidance of pharmacokinetic parameter interpretation errors.

Supplementary data

Supplementary data accompanies this paper and can be found at **www.future-science.com/doi/suppl/10.4155/TDE.11.19**

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Executive summary

- Flip-flop pharmacokinetics can occur for extravascularly administered drugs (i.e., rate of absorption < rate of elimination).
- Pharmacokinetic parameters may be miscalculated and misinterpreted when this phenomenon is not recognized.
- Flip-flop disposition can occur as a consequence of modified-release formulations.
- Intravenous pharmacokinetic data is necessary for an unequivocal estimate of rate of elimination.
- Flip-flop pharmacokinetics can be exhibited by, but is not limited to, many class III or IV biopharmaceutics classification system drugs that are slowly absorbed and have poor water solubility.
- Flip-flop pharmacokinetics has been demonstrated across a wide variety of species and extravascular routes of administration.

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