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Full Length Research Paper

Development of *in vitro-in vivo* correlation for pharmacokinetic simulation

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This article presents a comprehensive overview of systematic procedure for establishing and validating an *in vitro* - *in vivo* correlation (IVIVC) level A, B, and C. It encompasses all experimental, statistical and mathematical concepts of IVIVC development and its validation. A Level A IVIVC is an important mathematical tool that can help to save time and cost during and after the development of a formulation.

Key words: Bioavailability, correlation, dissolution, predictability, Wagner-Nelson equation.

INTRODUCTION

In recent years, a number of drugs are being developed. Thus there is a growing need of pharmacokinetic studies; subsequently it has become a very tedious, expensive and time consuming task to collect and handle huge pharmacokinetic data. It is therefore, useful to develop pharmacokinetic simulation models for the prediction of pharmacokinetic parameters. Pharmacokinetic simulation model is defined as a computational and/or mathematical tool that interprets drug kinetics in living environment under specific conditions (Ahmad et al., 2009).

In addition to routine quality control tests, comparative dissolution test have been utilized to waive off bioequivalence requirements called biowaiver studies, for lower strength of a formulation. For a biowaiver study, a dissolution profile should be established and characterized using model dependent and independent approaches. Biowaiver study is generally conducted for multiple strengths with different release rates, after the approval of a bioequivalence study performed on one strength (Murtaza et al., 2009). For the prediction of *in vivo* performance of a formulation, the use of dissolution test as a quality control tool is significantly increased if an *in vitro- in vivo* correlation (IVIVC) is developed. The application of the IVIVC involves the selection of the bio-relevant *in vitro* dissolution test method in bioequivalence studies (Rasool et al., 2010). Another important use of the validated IVIVC is to provide an explanation for a biowaiver during scale-up or post approval changes. Certainly, a biowaiver will only be awarded if the prophecy of the *in vivo* act of the formulation with the modified *in vitro* release rate remains bioequivalent with that of the originally tested formulation (Rasool et al., 2010).

The IVIVC for a formulation is a mathematical relationship between an *in vitro* property of the formulation and its *in vivo* act. The *in vitro* drug release profiles commonly act as distinguished *in vitro* characteristic. Whereas, the *in vivo* act is elaborated by plasma drug profiles, these profiles are then treated mathematically to assess whether a correlation exists; a

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Table 1. Biopharmaceutics drug clarification system.

Class	Solubility	Permeability	Absorption rate control	IVIVC expectation
BCS Class I	High	High	Gastric emptying	IVIVC expected if dissolution rate is slow than gastric emptying rate otherwise limited or no correlation
BCS Class II	Low	High	Dissolution	IVIVC expected if *in vitro* dissolution rate is similar to *in vivo* dissolution, unless very high dose
BCS Class III	High	Low	Permeability	Absorption (permeability) is rate determining, limited or no IVIVC with dissolution
BCS Class IV	Low	Low	Not defined (case by case)	Limited or no IVIVC is expected

correlation can generally be expected when drug release from the formulation is the step controlling the subsequent absorption kinetics (Rasool et al., 2010).

The IVIVC assessment depends on mean *in vitro* and *in vivo* data. Based on regulatory guidelines, the *in vitro* profiles are regarded acceptable for an IVIVC: (i) When the dissolution rate is a mean of twelve individual determinations and (ii) the coefficient of variation at each sampling point is less than 10%; however the initial release data may exhibit a larger variability (Rasool et al., 2010).

The *in vitro* dissolution testing is used to accept or reject formulation. The acceptable formulations are considered bioequivalent in terms of *in vivo* performance and vice versa. For the establishment of IVIVC, a minimum of three formulations with different release rate are required. In addition, a reliable and discriminating *in vitro* dissolution test is also required which can be established changing its different variables so that it may perform as *in vivo* environments. Before *in vivo* performance of a formulation, the discriminating dissolution test can be utilized as a quality assurance tool (Murtaza et al., 2009). Briefly based as IVIVC, dissolution test acts as surrogate of the bioavailability study (U.S. Department of Health and Human Services, 1997).

A theoretical basis has been developed for developing correlation between *in vitro* dissolution and *in vivo* bioavailability depending on the solubility and permeability of drug. Using this approach, solubility and permeability of different representative drugs have been studied and is presented in a biopharmaceutics drug classification system (Table 1) (Khan et al., 2010)

The Biopharmaceutics Classification System (BCS) is a predictive approach for developing correlation between physicochemical characteristics of a drug formulation and *in vivo* bioavailability. The BCS is not a direct IVIVC.

The IVIVC is calculated from *in vitro* and *in vivo* data of the same formulation using some mathematical functions based on the type of parameters employed. The IVIVC is divided into five categories. The following five levels of IVIVC correlations are narrated in FDA guidelines (U.S. Department of Health and Human Services, 1997; Khan et al., 2010).

1. Level A correlation
2. Level B correlation
3. Level C correlation
4. Level D correlation
5. Multiple level C correlation

Level A correlation, a higher level of correlation, elaborates point to point relationship between *in vitro* dissolution and *in vivo* absorption rate of drug from the formulation. The mean *in vitro* dissolution time ($MDT_{in\ vitro}$) and mean *in vivo* dissolution time ($MDT_{in\ vivo}$) can be evaluated using statistical moment theory for developing level B correlation which compares $MDT_{in\ vitro}$ to $MDT_{in\ vivo}$. Level C correlation is a weak single point relationship having no reflection of dissolution or plasma profile and compares the amount of drug dissolved at one dissolution time-point to one pharmacokinetic parameter like $t_{60\%}$ to AUC. Due to the involvement of multiple time points multiple times, multiple level C is more producible than level C. It relates one or many pharmacokinetic parameters to the quantity of drug dissolved at various time points. Level D correlation is helping in the development of a formulation. It is a rank order analysis which is not considered as a formal correlation (U.S. Department of Health and Human Services, 1997).

Over the last one decade, the establishment and validation of IVIVC has extensively been studied (Ahmad et al., 2009; U.S. Department of Health and Human Services, 1997; Khan et al., 2010). A validated IVIVC not only plays an important role in formulation development but can also be used to predict the *in vivo* performance of a dosage form that has been developed according to a predefined methodology. Thus the validation of an IVIVC is as critical as the finding of a discriminating and specific

Table 2. Calculation of percentage drug released (PDR) and percentage drug absorbed (PDA) forming fast release formulation using Wagner-Nelson equation.

Time (h)	Fast release formulation					
	Plasma drug concentration (C, mg/L)	AUC	K× AUC	C_p +(K× AUC)	PDA	PDR
0	0	0	0	0	0	0
0.5	0.18	0.045	0.006	0.19	9.5	39.62
1	0.35	0.178	0.025	0.38	19	48.91
2	0.66	0.683	0.096	0.75	38	59.07
3	0.91	1.468	0.210	1.12	56	67.74
4	1.12	2.483	0.350	1.47	78	79.64
5	1.27	3.678	0.520	1.79	90	90.71
6	1.28	4.953	0.693	1.97	99	93.98
8	0.99	7.223	0.85	2.00	100	98.36
10	0.75	8.963	1.01	2.00	100	99.07
12	0.57	10.283	1.71	2.01	100	99.60
15	0.38	11.708	1.75	2.01	100	99.61
24	0.11	13.912	1.40	2.05	100	99.86

dissolution method. FDA has introduced the guidelines, how to develop IVIVC and validate it by internal and external approaches (Khan et al., 2010). This article gives an overview of pharmaceutical, experimental, mathematical and statistical concepts for establishing *in vitro-in vivo* correlation.

METHODOLOGY

Formulations

To develop IVIVC, commonly three formulations with different release rates are used; the formulations are abbreviated as follows:

1. Fast release formulation
2. Medium release formulation
3. Slow release formulation

In case of tablets, the quantity of polymer and/or the binder, compression force and the blending efficiency are usually varied to design formulations with different release rates (U.S. Department of Health and Human Services, 1997). Subsequently, it is mandatory to evaluate the formulations for elaborating whether their physicochemical properties like formulation assay, flow characteristics and compressibility, fulfill their compendial requirements (Shargel et al., 2005).

For the establishment of an IVIVC, the release governing excipients in the formulations should be similar. Preferably, *in vitro* dissolution profiles should be determined with different dissolution test conditions. The *in vitro* dissolution profiles leading to an IVIVC with the smallest prediction error (%) is then opted for further use as it is believed most bio-indicative (Rasool et al., 2010).

RESULTS

To illustrate the statistical and mathematical concept of IVIVC development and its validation, the following simulated data has been adopted (Tables 2 to 4). The values of determination coefficients for IVIVC level A for fast, medium and slow release formulations were 0.9273, 0.9616 and 0.9641, respectively, while 0.9885 and 0.9996 were the values of determination coefficients in case of IVIVC level B and C, respectively.

DISCUSSION

Obtention of *in vitro* dissolution data

A sensitive and reliable *in vitro* dissolution method is used for determining the quality of a formulation. Since the dissolution test is emerging as a surrogate for *in vivo* bioequivalence study rather than a traditional quality control tests (Rasool et al., 2010). Therefore a reliable *in vitro* dissolution test for a drug substance is necessary for the development of IVIVC. Such a dissolution method can be developed by serial modifications in dissolution medium like the addition of a surfactant. After development of a reasonable dissolution medium, the *in vitro* dissolution testing is performed for suitable time period on the formulations selected. The dissolution samples are analyzed spectrophotometrically or chromatographically, to find out the amount of drug released. Consequently, the drug release versus time profiles are obtained for correlating with *in vivo* data. This data exhibits the fraction of drug dissolved at different time points.

Analysis of dissolution data

For the analysis of dissolution data, various model

Table 3. Calculation of percentage drug released (PDR) and percentage drug absorbed (PDA) forming medium and slow release formulations using Wagner-Nelson equation.

Time (T, h)	Medium release formulation		Slow release formulation	
	PDA	PDR	PDA	PDR
0	0	0	0	0
0.5	8	29.67	5	24.69
1	16	38.94	12	34.92
2	33	49.00	28	44.07
3	50	57.71	46	51.75
4	69	69.68	61	62.61
5	80	80.76	72	73.70
6	89	86.92	84	79.96
8	96	91.30	91	87.32
10	100	97.01	99	93.04
12	100	99.67	100	98.60
15	100	99.65	100	99.51
24	100	99.83	100	99.76

Table 4. Values of AUC, MRT, $t_{50\%}$ and MDT for fast, medium and slow release formulations.

Formulation	*In vivo* parameter		*In vitro* parameter	
	AUC (µg.h/ml)	MRT (h)	$t_{50\%}$ (h)	MDT (h)
Fast release formulation	11.998	4.91	6.987	2.12
Medium release formulation	13.009	5.10	7.112	2.92
Slow release formulation	13.912	5.31	7.216	3.51

dependent and independent approaches are utilized. Out of model independent approaches, similarity factor analysis (a pair wise procedure) is commonly chosen to compare the dissolution profiles. The similarity factor (f_2 metric) analysis is conducted using following equation (Rasul et al., 2010):

$$f_2 = 50\log\left\{\left[1+\frac{1}{p}\sum_{i=1}^{p}\left(R_i - T_i\right)^2\right]^{-\frac{1}{2}}\times 100\right\}$$

where, R_t and T_t are the cumulative percentage dissolved at time point "t" for reference and test products, respectively and "p" is the number of pool points.

The similarity factor is a logarithmic reciprocal square root transformed of the sum of the squared error and is a measurement of the similarity in the percentage dissolution between the two plots (Murtaza et al., 2009). The two compared dissolution profiles are considered similar if $f_2 > 50$, and the mean difference between any dissolution sample not being greater than 15% (U.S. Department of Health and Human Services, 1997).

Mean dissolution time, a model independent approach, would be calculated from dissolution data to determine level B correlation by the following equation (Murtaza and Ahmad, 2009):

$$MDT = \int_0^{\infty} t\left(\frac{dm}{dt}\right)dt \Big/ m_{\infty}$$

where 'm' stands for the amount of drug dissolved at time "t".

For level C correlation, time required for a specific value of mean dissolution like $t_{50\%}$ (time required to dissolve 50% of total drug) is required which is calculated from the curve of zero order equation (U.S. Department of Health and Human Services, 1997).

Obtention of *in vivo* absorption data

To establish IVIVC, *in vivo* absorption data is also required which means that pharmacokinetic and bioavailability study is also to be done. Pharmacokinetic study reflects the performance of drug in body. It involves the study of the influence of body on the drug, that is, absorption, distribution, metabolism and excretion. Where as the bioavailability indicates the extent to which a drug reaches systemic circulation and is commonly assessed

by evaluating AUC (area under plasma drug concentration-time curve), C_{max} (maximum plasma drug concentration) and t_{max} (time required to achieve C_{max}) (Murtaza and Ahmad, 2009).

Generally, single dose cross over experimental design with a washout period of one week, is adopted to obtain absorption data for establishing IVIVC. Thus for three formulations with different release rates, a three treatment cross over study design is allocated. For ethical reasons, the subject panel should be homogenous and consist of appropriate number of healthy young volunteers, not less than six (Rasool et al., 2010; Khan et al., 2010). However, the size of subject panel should depend on the difference in the biological data.

The volunteers receive formulations according to study design and blood sampling is conducted according to good clinical practice (European Medicines Agency, 2009) and Helsinki declaration for human use in experimentation (World Medical Association Declaration of Helsinki, 2008). The bio-analysis of collected blood samples is conducted using validated analytical methods. The plasma drug concentration data is plotted against time points, and subsequently this curve is used to calculate pharmacokinetic parameters such as AUC, AUMC, C_{max}, T_{max} and K_e (elimination rate constant) using an appropriate pharmacokinetic model through manual calculations or suitable software like Kinetica® (European Medicines Agency, 2009.). Kinetica® has convolution/-deconvolution modules as well as Wagner-Nelson and Loo-Riegelman modules.

Analysis of *in vivo* data

The association between the *in vitro* and *in vivo* data is stated mathematically by a linear or nonlinear correlation. But, the plasma drug concentration data cannot be related directly to the obtained *in vitro* release data; they are needed to be converted first to the fundamental *in vivo* release data using pharmacokinetic compartment model analysis or linear system analysis. Based on a pharmacokinetic compartment analysis, the in vivo absorption kinetics can be evaluated using various pharmacokinetic parameters. The linear system analysis method requires the availability of a unit input response (Rasool et al., 2010).

To develop level A correlation, *in vivo* absorption profiles of the formulations from the individual plasma concentration versus time data are also evaluated using Wagner-Nelson equation (Shargel et al., 2005) as the following:

$$\text{Drug absorbed (\%)} = \left[\frac{\left[\frac{C_{(t)}}{K_e} \right] + AUC_{(o-t)}}{AUC_{(o-\infty)}} \right] \times 100$$

For the establishment of level B correlation, mean residence time (MRT) is calculated from AUC and AUMC as the following (Khan et al., 2010):

$$MRT = \frac{AUMC}{AUC}$$

MRT is the first moment of drug distribution phase in body. It indicates the mean time for drug substance to transit through the body and involves in many kinetic processes (U.S. Department of Health and Human Services, 1997).

While level C correlation is established using a pharmacokinetic parameter like AUC, C_{max} or t_{max}.

IVIVC development and its analysis

Level A IVIVC correlates the entire *in vitro* and *in vivo* profiles (Rasool et al., 2010). In order to develop level A correlation, the fraction of drug absorbed (F_a) for a formulation is plotted against its fraction of drug dissolved (F_d), where F_a and F_d are plotted along x-axis and y-axis, respectively. This curve provides basic information of the relationship between F_a and F_d, either it is linear or non-linear. Moreover, plots can also be plotted in the form of combination of two formulations like (a) slow/ moderate (b) moderate / fast and (c) slow / fast or in combination of three formulations like fast/moderate/slow. Finally the regression analysis is performed for each curve to evaluate strength of correlation. The correlation is considered as efficient as the value of determination coefficient is closer to 1 (U.S. Department of Health and Human Services, 1997; World Medical Association Declaration of Helsinki, 2008; Ahmad et al., 2008; Rasool et al., 2010; Khan et al., 2010; Aamir et al., 2010).

The predictability of the correlation is analyzed by calculating its prediction error (%). As illustrated in regulatory context, the deviation between prediction and observation is assessed either with internal validation or with external validation (U.S. Department of Health and Human Services, 1997; Ahmad et al., 2008). Internal validation elaborates the predictability of data that has been employed in the model development and is recommended for all IVIVC analysis (U.S. Department of Health and Human Services, 1997). External validation depends on how efficiently the IVIVC predicts additional data sets. This predictability is considered better than the internal one. External predictability is analyzed in the following conditions (U.S. Department of Health and Human Services, 1997):

1. When no conclusion is obtained from internal prediction.
2. When correlation is established for drugs having narrow therapeutic window.
3. When two formulations with different release rates are

involved in correlation development.

The predictability of a correlation in regulatory context is characterized in terms of the prediction error (%) using AUC and C_{max} as the following:

$$\text{Percentage Predication Error} = \left[\frac{AUC_{Obs} - AUC_{Pred}}{AUC_{Obs}}\right] \times 100$$

or

$$\text{Percentage Predication Error} = \left[\frac{C_{max\ (Obs)} - C_{max\ (Pred)}}{C_{max\ (Obs)}}\right] \times 100$$

where, $C_{max\ (pred)}$ and AUC_{pred} are calculated from the predicted plasma profiles while convolution technique is used to estimate predicted equation (Rasool et al., 2010).

$$C(t) = \int_0^t C_\delta\ (t-u) X_{vitro}(u)\, du$$

C(t) is plasma profile. C_δ is unit impulse response or concentration-time course resulting from immediate release of a unit amount of drug. It is calculated from intravenous bolus data or reference oral solution. While "u" is the variable of integration. X_{vitro} represent drug input rate of oral solid dosage form.

According to FDA guidelines, the correlation is considered predictive if mean prediction error across formulations is less than 10% for AUC and C_{max} and the prediction error (%) for any formulation is less than 15% for AUC and C_{max} (U.S. Department of Health and Human Services, 1997).

Level B correlation is developed between MDT and MRT of a formulation. The MDT and MRT are plotted along x-axis and y-axis, respectively. The regression analysis is performed for this curve also (Ahmad et al., 2008).

Level C correlation, a single point correlation, can be established by drawing a curve between $t_{50\%}$ and pharmacokinetic parameter are plotted along x-axis and y-axis, respectively following regression analysis (Ahmad et al., 2008).

Conclusion

There is extensive international harmony on the development of IVIVC and its analysis as revealed in the guidelines of FDA. There is, however, escalating awareness that some fundamentals of IVIVC establishment require to be reviewed mathematically, like equations involved in data calculations and sequence of their use is determined. In the future, there will be unremitting endeavors toward achieving *in vitro – in vivo* correlations, on a case-by-case basis for each drug or classes of drugs. A Level A IVIVC is an important mathematical tool that can help to save time and cost during and after the development of a formulation.

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