In Vivo vs In Vitro Bioequivalence Saeed A. Qureshi, Ph.D. (www.drug-dissolution-testing.com)

In vivo bioequivalence, or simply bioequivalence, is commonly referred to as an evaluation study conducted to establish equality of mostly two oral products such as tablet or capsule. Equality of two products (test *vs* reference) is established by comparing their blood drug concentration-time (C-t) profiles. The reason for selecting C-t profiles for such comparison is that as therapeutic effects depend on drug concentrations in blood i.e., if two or more products provide similar C-t profiles then they will provide similar therapeutic effects as well, thus they will be considered therapeutically bioequivalent, or simply bioequivalent.

The similarity, or bioequivalence, of two C-t profiles is established based on two parameters: (i) area under the curve (AUC), the curve being the C-t profile, which reflects the extent of drug absorbed into blood circulation, and (ii) maximum observed drug concentration (C_{max}) of the curve or profile, representing rate of drug absorption. If these two parameters (AUC and C_{max}) are within the accepted standards, then profiles are considered equal and corresponding products as bioequivalent.

The similarity or difference in the profiles in fact represents the availability or release of a drug in solution form (which is dissolution) in the GI tract, in particular the intestine. Availability of a drug in solution is generally essential for drug absorption and appearance in the blood to result in a C-t profile. Therefore, in reality, similarity or differences in C-t profiles reflects *in vivo* dissolution characteristics of the products. Thus, a bioequivalence study is no more than a study to evaluate, or compare, the *in vivo* dissolution characteristics of two products. It is this fundamental and important concept that forms the basis for bioequivalence assessment, and by extension the evaluation of *in vivo* dissolution.

Often it is assumed that a bioequivalence study assesses absorption and/or the pharmacokinetic characteristics of one or more products, which is not accurate. The pharmacokinetic studies are generally conducted for drugs (not for products) using a drug in a solution. It can be argued that if a solid oral dose/product would be used for pharmacokinetic study or evaluation, then it may not be considered to accurately reflect the pharmacokinetics of the drug as product attributes (formulation/manufacturing) would affect these. The C-t profiles of a product are linked to *in vivo* dissolution through pharmacokinetic parameters of the drug, as described in Equation 1 below.

In vivo drug dissolution (profile) + Pharmacokinetic parameters = C-t profile ... [Eq. 1]

To obtain drug levels, or C-t profiles, one requires pharmacokinetic parameters of a drug which are usually determined from a separate study following administration of a drug in solution. The required parameters for establishing C-t profiles are the elimination rate constant or equation, volume of distribution and absolute bioavailability of a drug [1]. These parameters are often described in pharmacology books.

If the C-t profiles of two products are compared that have the same drug in the same amount, then the pharmacokinetic parameters will remain the same or constant and the above equation will be reduced to:

In vivo drug dissolution (profile) = C-t profile … [Eq. 2]

This forms the basis of linking *in vivo* dissolution to C-t profile for product evaluations. It is critical to note that Equation 2 will be valid only if products tested have the same drug in the same amount. What may cause the differences in C-t profiles, if the drug in the products is the same and in the same amount? It is formulation and/or manufacturing differences.

Therefore, from an *in vivo* bioequivalence study, one determines the C-t profiles of two products having the same drug in the same amount and compares the in vivo dissolution of the two products. There are standards/guidances, in particular from the US FDA, which are followed

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around the world for bioequivalence evaluation. In short, two products are administered to usually healthy human volunteers, in a cross-over (statistical) design, i.e. half the volunteers received one product (reference) and the other half receive the other product (test) in the first period. Then, this sequence is switched (crossover) in the second period. Blood samples are withdrawn from volunteers at specific time intervals and drug levels are determine using a method validated analytical usually chromatographic. AUC and C_{max} parameters are calculated and compared. If these parameters meet the criteria are they then declared bioequivalent, otherwise not. In this regards, two things should be kept in mind: (i) bioequivalence is a pass/fail type test; (ii) if the test fails, and one desires to show bioequivalence, the only option available is to rework the formulations and test the revised product following the same standard protocol for the test. The standard protocol or experimental procedure remains constant and does not change with products.

In vitro bioequivalence: The link between *in vivo* dissolution and a C-t profile is then extended by considering that if one obtains dissolution results *in vitro* and combine them with the pharmacokinetic parameters of the drug then one should be able to predict or determine C-t profiles. The process of this conversion is known as convolution and has been described in detail in other publications [1, 2].

This, thus, forms the basis of *in vitro* drug dissolution testing, which is important and required for efficient development and evaluations of drug products as potentially many costly and time consuming *in vivo* studies can be avoided.

Establishing *in vitro* bioequivalence: As described above, the *in vivo* bioequivalence is established using AUC and C_{max} parameters obtained from the C-t profiles. How would one compare or establish bioequivalence using drug dissolution profiles. There are two difficulties: (i) What parameters and criteria are to be used to establish similarity (bioequivalence) of *in* vitro dissolution profiles to represent product bioequivalency? (ii) If the bio (physiological) aspect is to be considered then these *in vitro* results (profiles) must be somehow linked or related to the C-t profiles.

There is one parameter, sometimes referred to as f_2 or similarity factor. Apart from the limitations of the f_2 , as described earlier in a separate post [3], this parameter lacks a link to bio- or physiological relevancy. Therefore, it may not be useful for the evaluation of dissolution profiles for bioequivalency. To be relevant and useful, *in vitro* dissolution results (profiles) have to be linked or converted to a physiological response, which in this case would be a C-t profile.

In this regard, one may use of the Equation 1 as described above, i.e., if one has in vitro dissolution results and is combined it with physiological/pharmacokinetic parameters of the drug in humans then one can obtain potential C-t profiles in humans. The exact procedure to obtain C-t profiles is described elsewhere [1]. As these derived profiles would be similar to the C-t profiles obtained from bioequivalence studies these can be evaluated and/or compared as C-t profiles using AUC and C_{max} parameters. If these fall within the parameters acceptable bioequivalence standards, then the products may be considered as bioequivalent. Therefore, in short, in vitro drug dissolution testing combined with pharmacokinetic parameters by a technique known as convolution provides a powerful method for evaluating in vitro bioequivalence.

The next question would be why is such an approach not usually been considered or applied to evaluate *in vitro* bioequivalence. There appears to be two reasons for this:

1. There is a traditional view in which rather than using a convolution approach to derive C-t profiles, most often the deconvolution approach is suggested. In the deconvolution approach in which in dissolution vivo results are obtained/derived from C-t profiles and these in vivo results (profiles) are compared to in vitro dissolution results. As stated above, there is no method available to compare dissolution results (profiles) to declare bioequivalence, therefore, the deconvolution approach may not be useful or successful in evaluating in vitro bioequivalence. This may be one of the reasons for the lack of success for the

www.drug-dissolution-testing.com For simple and practical ideas assessment of *in vitro* bioequivalence. However, use of the convolution approach addresses the limitations of the deconvolution approaches and should be successful in evaluating products, as has been shown in the literature [1].

2. The other reason is that of the estimation of in vitro dissolution experimentally. It should go without saying that no matter how strong the theoretical basis is for dissolution testing or how efficient and accurate methodologies are for linking in vitro results to in vivo results, if the experimental conditions do not relate well to an vivo (physiological) in environment, in vitro dissolution results will be of limited value. This is precisely what is happening the current practices of in dissolution testing. For example, apparatuses used, in particular basket and paddle, do not provide any relevance to a physiological environment a drug/product goes through. The recommended experimental conditions, which are often product dependent, have no relationship or relevance to the physiological environment. Therefore, it should not be possible to relate or link the results to in vivo obtained using current practices. in particular basket/paddle apparatuses. One should be extremely cautious in drawing any conclusion regarding the relevance of in vitro dissolution results of a product to its in vivo results, including reference to the quality of a product as quality is also linked to the in vivo behavior. It cannot be emphasized enough that at present the use of any dissolution results obtained under current practices, in particular with Basket/Paddle the use of apparatuses, will almost always lead to false conclusions. One should be watchful of this serious situation.

The obvious next step would be to address how conduct appropriate and bio-relevant to dissolution studies. This next step is simply by addressing the above mentioned two deficiencies. There have been reports in this regard, where a simple and practical convolution procedure has been described to transfer and evaluate in vitro dissolution results. Secondly, a slight modification to the vessel based (Basket/Paddle) apparatuses has been suggested using a crescent-shaped spindle which appears to address the deficiencies of the apparatuses. These two new recent developments appear to show that indeed assessment of in vitro bioequivalence is a strong and practical option. The pharmaceutical industry and standard setting consider organizations may these new developments, which would significantly reduce the burden of development and evaluation of products as well as their regulatory assessments and monitoring.



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