SIMULATION OF BIOEQUIVALENCE STUDY ON THE BASE OF DISSOLUTION CURVES

Grezal, Gy.
Grezal G.
Toth T.
Dinya, E.
Health Informatics Department, Semmelweis Medical University, Budapest, Hungary

Abstract

A computer method and software based on the in vitro dissolution of drug preparations has been elaborated for the estimation of bioequivalence using Microsoft Excel 2007, Visual Basic programming language. The method generates a „dissolution surface" from the parameters of time (X-axis), from pH (Y-axis) and from the dissolved amount (A) in % of the drug. This dissolution surface allows the determination of the general dissolution curve of the test and reference preparations. By supposing that the absorption rate constant is known from the literature, the change of the amount of dissolved drug as the function of time can be determined. On the base of this function the maximum amount of the dissolved drug in the gastrointestinal tract and the AUC can be calculated and the test/reference ratio can be determined. In the case of linear pharmacokinetics these ratios are identical to the ratios of parameters that can be calculated in the circulation. By generating parameters between the allowed biological limits the dissolved drug – time curves of „volunteers" in the necessary number are created with the randomly generated „residence times" and their confidence intervals can be determined, i.e. on the base of dissolution curves bioequivalence can be estimated.

Keywords: In vitro, dissolution, bioequivalence, simulation

Introduction

A drug preparation is regarded as a substitute – containing an active agent of the same quantity and quality defined by the pharmacopoeia – for other preparation (Test-Reference) as far as the statistic difference - (the confidence interval of T/R ratio of values measured in volunteers) of the

main pharmacokinetic parameters (C_{max}, AUC) measured in tests on healthy volunteers - falls between certain limits [Grezal and Vereczkey, 2012].

The preparations besides their active agents (described above) differ in the ingredients of their vehicles making up the greatest majority of their mass. This difference may result in different solution of the preparations in the intestines, consequently the quantity of drug entering the circulatory system may differ more than allowed.

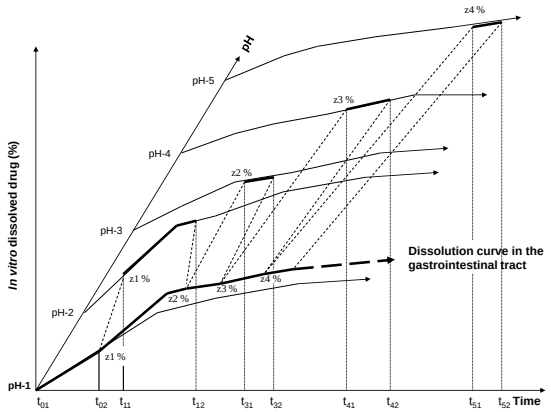
This risk can be reduced by the (in vitro) "dissolution" test previously performed. The dissolution check/determination is carried out in solutions in which the preparations spend supposedly "longer" time while passing through the gastrointestinal tract and so, based on this, we can form a picture about the dissolution properties of the different drug formulations. The joint evaluation (the statistic analysis used- F1 and F2 values) of mostly different dissolution curves of the preparations and pH values in the different (from neutral to acidic) media result in –many times – not quite well established values in terms of dissolution "comparability".

The simulation technique introduced under makes the decision regarding equivalence more reliable by processing the information from the dissolution tests. The method rests on the reference to the in vitro assessments of bioequivalence tests.

Results

Representing dissolution in a three-dimension space (X-axis = time, Y-axis = pH values, Z-axis = amount of dissolved substance A in %) we are given a "dissolution surface" between the dissolution points (Fig.1). By using this "dissolution surface" the "mean" dissolution curve of the preparation passing through the gastrointestinal tract is determined.

Figure 1.
Dissolution in gastrointestinal tract



According to the structure of the gastrointestinal tract the preparation passes through the oesophagus into the stomach. (This passage due to the shortness of time can be neglected). The pH value of the gastric juices is pH1, and in this range the preparation stays for t_{01} time. Then, the first phase of general dissolution curve will be the phase pH1 curve of the dissolution surface extending to t_{02} point, at the end of which the quantity of dissolved substance is the z_1%. Then we identify the t_{11} point of time belonging to the z_1% in the curve of the pH2 dissolution surface (this was already been dissolved in the previous process), then we place the dissolution phase from t_{11} to t_{12} to the part of the general curve having already been framed up. (Until t_{12} point of time z_2% has been dissolved). In other words the section of pH2 dissolution curve is applied to the already devised general dissolution curve, which starts at dissolution % i.e. at (z_1%) –at the t_{11} point of time - in the general curve and lasts until the t_{12} point of time. The preparation passes through pH3, pH4 media entering the duodenum which is supposed to be of pH5 value. The process in continued as written above through the media of pH6 and pH7 value until the end of the gastrointestinal tract (pH7).

If the dissolving media applied in "in vitro" dissolution tests are identical with the composition of the dissolving media of the gastrointestinal tract, the general dissolution curve of the gastrointestinal tract can be regarded as "realistic". As far as the solvents used for dissolution do not meet this requirement, the simulation does not necessarily lead to correct results.

Volunteers presented different reactions as to the passage of the preparation through the gastrointestinal tract (duration in the intestines varies according to the sections of the gastrointestinal tract - dissolution spaces). This difference can be modelled in the simulation by using the general dissolution curve of the gastrointestinal tract in the following way.

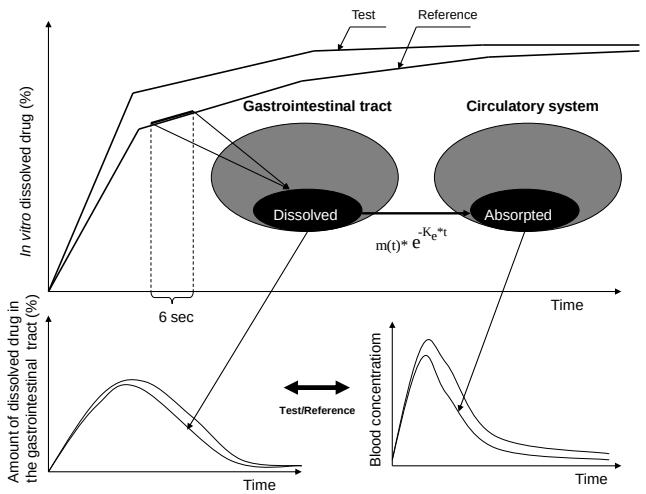
It may more or less be predicted how long (minimum and maximum) time a preparation is staying (without meal) in each organ (e.g. in the stomach minimum 10 and maximum 30 minutes, but an arbitrary combination can be tested). Then, a random number is generated in this designated interval by a random function of even distribution and in this t_z range, this value is regarded as the duration time of the preparation (The designated durations correspond to the t_{11}-t_{12} intervals. Consequently, "generating" a new volunteer is carried out by the generation of the random number at each section of the general dissolution curve (within the prescribed limits of the given sections, which can also be zero). The duration times obtained from the series of numbers generated in this way represent an individual volunteer. By means of this method arbitrary number of volunteers - accidently differing from each other - can be "generated" and in their gastrointestinal tracts varying quantities of active agent of different

distribution are dissolved under the limits of reality ensured at the adequate level of dissolution test.

The next step is the calculation of the quantity of active agent measurable in the gastrointestinal tract of the "volunteers" generated like this. Once the "general" dissolution curve has been known in a volunteer, the quantity of the active agent entering the gastrointestinal tract in short period of time (e.g. in case of the present software six seconds) can be calculated. These quantities are continuously calculated, then - by the analogy of repeated dosing of deferent quantities of active agent - this is added to the quantity having already been in the gastrointestinal tract and in the meantime the active agent - dissolved in the gastrointestinal tract in this way - is being absorbed into the circulatory system. As the kinetics of referent preparations used in the bioequivalence tests is known, the absorption rate constant of the active agent absorbed from the gastrointestinal tract into the circulatory system can be determined. This absorption rate equals the absorption rate of active agent "disappearing" from the gastrointestinal tract. (The accuracy of this constant in the simulation is highly robust, which by test calculations with different values or – directly – analytically can be appreciated.

Thus, the volume of active agent - entering the gastrointestinal tract (administration in every six second) by means of dissolution and disappearing at a known constant absorption rate from the gastrointestinal tract - can be described in the function of time (Fig. 2).

Figure 2.
The change of the amount of dissolved drug as the function of time



As, based on the function above, the dissolved substance volume for the test and referent preparations – the maximum of the time curve and the area under the curve – can be calculated, the test/referent ratio can be determined. Supposing linear kinetics (if kinetics is not linear, simulation cannot be applied) in case of the test/referent ratio C_{max} and AUC measured in the circulatory system equals the ratios calculated in the gastrointestinal tract. Generating the necessary number of "volunteers" confidence intervals per parameters can be calculated i.e. based on the dissolution curves bioequivalence can be simulated.

The reliability of the results obtained is determined by the adequate *in vitro* simulation of the dissolution in the gastrointestinal tract. The rate of dissolution – in case of preparation of great variability - is determined by the "variety" of the composition of the dissolving medium (greatly influencing the speed of dissolution) at the given site of the gastrointestinal tract. Furthermore, in case of a preparation sensitive to the change in dissolution conditions, the time distribution of dissolved substance may significantly be different even in case of identical preparations (in the gastrointestinal tract of the volunteer the composition of the dissolving medium may change or the absorption time of a particular preparation in a given section – dissolving medium – may also be different and this may give rise to the case that the same preparation is not equivalent with itself). This uncontrollable random factor may be eliminated by the "adequate" *in vitro* dissolution test, consequently a more reliable decision is obtained as to the bioequivalence of the preparations.

Naturally the model can further be "refined" as the dissolution rate may change in different ways in the different preparations in the function of concentration (the volume of the dissolving medium), the different qualities of the gastrointestinal tract (peristaltic), the appropriate model of which in the dissolution test is the rotation speed of the mixer.

These additional factors – by the procedures described above – may be built in the model without any difficulty, although, the need of information (the measurements carried out under different conditions) of a simulation like this is well over the data need of the previous technique. In case of preparations of great variability, in the function of dissolution conditions, the costs of invested work - due to the increased reliability of decision - would certainly return.

As far as the dissolution processes of the gastrointestinal tract could be modelled "very well", the simulation (due to its reliability) in case of preparations of great variability (where sometimes it takes place that - based on the bioequivalence test e preparation is not equivalent even with itself either) would give more reliable results than bioequivalence tests (by

ensuring the identity of the test circumstances of the two preparations by simulation – which in "*in vivo"* conditions cannot be achieved).

The two risk factors of the present practice of bioequivalence tests (on the one hand, the different dissolution rates of the two preparations in the gastrointestinal tract may differ from the in vitro data and on the on the other, we cannot control – especially not equalize – the dissolution times in the different dissolution surfaces of the gastro- intestinal tracts of the volunteers) may distort the "similarities" of the preparations in both directions. I.e. based on the bioequivalence tests in case of "really equivalent" preparations we can conclude that in-equivalence is present and vice versa.

The recommended simulation would result in a more reliable evaluation of the "similarity" and its costs – as compared to the bioequivalence tests – would be reduced by more than one order of magnitude. The table made up of two simulation calculations illustrates the risk factors of bioequivalence tests.

The simulation program used takes into account even the dissolution changes due to change in the volume of the dissolving medium and the difference in the rpm of mixer's fan. Measurement was carried out at a fixed volume and rpm. The differences due to the change in volume and rpm are fictitious as they are estimations based on chemical knowledge. (In "actual" situation they are also to be measured).

The calculation was carried out in 25 volunteers in ten series (i.e. in 250 cases for each pH volume -in the hypostatised ranges – random number were generated for the duration time, the volume of the dissolved substance and rpm – discretely for the test and the referent dissolution curves).

For the dissolution curves and the referent of the volunteers the same random number was used in the first case. The confidence intervals calculated in this way are in the Table 1.

Table 1.
The simulated confidence intervals for test and reference pairs under same conditions

Cases	Amax		AUC	
	Lower	Upper	Lower	Upper
1	1.042	1.090	0.846	0.894
2	1.033	1.086	0.813	0.872
3	1.024	1.080	0.841	0.896

4	1.024	1.076	0.806	0.868
5	1.002	1.058	0.837	0.895
6	1.030	1.073	0.811	0.871
7	1.041	1.084	0.833	0.882
8	1.010	1.068	0.814	0.881
9	1.051	1.092	0.859	0.912
10	1.021	1.070	0.826	0.875

In the second case randomly (for the previous simulation) generated series was used in the test in each volunteer, whereas new, random numbers were generated for the referent dissolution curves. The results are contained in Table 2.

Table 2.
The simulated confidence intervals for test and reference pairs under not same conditions

Cases	Amax		AUC	
	Lower	Upper	Lower	Upper
1	1.003	1.128	0.824	0.943
2	0.952	1.109	0.807	0.913
3	0.944	1.078	0.786	0.869
4	0.995	1.117	0.804	0.892
5	0.942	1.076	0.811	0.896
6	1.020	1.156	0.848	0.960
7	0.930	1.060	0.826	0.916
8	0.967	1.112	0.846	0.952
9	1.067	1.283	0.846	0.938
10	1.017	1.188	0.841	0.944

By comparing the tables it is evident that under not same conditions the two preparations are “steadily” equivalent, the confidence interval falls

between the ranges of 0.8-1.25 meaning acceptance [EMA 2010] whereas under the same conditions, in pairwise running non equivalent results are present, too. So the risk between the two treatments is due to the occasionally uncontrollable difference in physiological change and/or the passage through the gastrointestinal tract of the volunteer.

Naturally by increasing the number of cases the length of confidence interval is decreasing, so the distribution centre of the test/referent ratio per volunteer can get close to the safety borders, consequently ratios calculated for many volunteers do not fall **between the safety intervals.**

Finally it should be emphasized that, based on the method, by means of the amount of substance dissolved and absorbed in the intestines and the time curve, the time – when maximal amount of substance (T_{max}) is present in the gastrointestinal tract - can be estimated. This is by no means identical with the C_{max} time found in plasma after absorption, but it supplies a good estimate if the speed of dissolution is different in case of the two preparations. The 6 seconds "sampling" results in a more correct assessment than the *in vivo* 15-30-60 minute sampling.

Conclusion

The simulation technique described does not appreciate bioequivalence or difference on the basis of the substance volume having entered the blood but on the basis of the quantity of medicine dissolved in the gastrointestinal tract, which the bioequivalence tests are directed at. The method allows avoiding the greatest "in vivo" problem of bioequivalence tests: the inevitable "intra-individual variability" in the volunteers, which in case of the so called medicines of great variability is a fairly high number and may need an involvement of more than sixty volunteers [Tóthfalusi and Endrényi, 2012].

References:

EMA European Medicines Agency, 2010. Guideline on investigation of bioequivalence. London, UK, January 20. 2010, accessed 14.05.2013 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500070039.pdf

Grezal Gy, Vereczkey L, 2012. Bioekvivalencia vizsgalat szimulacioja kioldodasi gorbek alapjan. *Acta Pharm Hung* 82:55-59 (in Hungarian)

Tóthfalusi L, Endrényi L, 2012. Sample sizes for designing bioequivalence studies for highly variable drugs. *J. Pharm. Pharm. Sci.* 15:73-84