Dissolution of Pentoxifylline from Extended Release Formulations. Researches Concerning Development of a Biorelevant Test

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ABSTRACT This paper presents results of a pharmacokinetics study concerning pentoxifylline and its main metabolites after administration of extended release formulation of Trental 400 mg and correlation of this pharmacokinetics with in vitro dissolution test results of parent drug. In order to establish most relevant in vitro test, dissolution was performed in different experimental conditions (stirring rate and dissolution medium). Correlation was linear and very good. Generalization of correlation to rate of appearance of metabolites in plasma proved that this process could be well correlated with dissolution. Most relevant test was finally found to be the release in water medium, in conditions of a high stirring rate – 100 rpm.

KEY WORDS biorelevant test, pentoxifylline and its principal metabolites, dissolution, in vivo–in vitro correlations, apparent absorption

Introduction

Dissolution testing played for long time an important role as a quality control tool in production, being a significant maker of batch-to-batch stability of technology processes. More recently it was tried an expansion of the role of dissolution as predictor of in vivo absorption. Since the new objectives were much more complex, the initially developed tests proved to be frequently non-adequate, a series of non-correlations being reported [1]. Main reason of these non-correlations were connected with the great difference between desire for reproducibility and standardization of quality control oriented methods on one hand and more variable and complex physiological parameters connected with in vivo dissolution on the other hands. Research for more biorelevant tests and substituting dissolution tests for clinical studies became a constant concern of biopharmaceutical research in the last years. Development of the Biopharmaceutics Classification System (BCS) [2, 3], clarified the problems revealing that only for a part of active substances is possible. Biorelevant dissolution testing was proved valuable in predicting of in vivo behaviour of lipophilic, poorly water-soluble drugs [4–6] and the absorption of BCS class III compounds. A further restriction was observed, correlation being good enough mainly for extended release (ER) formulations. Correlation between in vitro dissolution and in vivo absorption, at least for extended release formulations became a compulsory test [7]. Present paper concerns the development of a biorelevant dissolution test for in vitro–in vivo correlations (IVIVC) for an extended release formulation containing pentoxifylline. Research was considered important following high variability of pentoxifylline absorption and metabolism.

Material and Methods

Clinical method

Pentoxifylline tablets were administered to healthy subjects in a cross-over two-period, two sequences bioequivalence study. Tested and reference drugs were administered in two period of five days, separated of seven days wash-out period. Plasma levels were measured in the first and the fifth day of each period. The study was approved by National Ethics Committee and National Medicines Agency.

Analytical method

Plasma levels of pentoxifylline and its M1 (1-(5’-hidroxyhexyl)-3,7-dimethylxanthine) and M5 (1-(3’-carboxypropyl)-3,7-dimethylxanthine) metabolites were determined using a Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC/MS/MS) validated method.

Computerized methods for estimation of parameters

Estimation of parameters was performed using the subroutines of the software KINETICA 3.1 and TOPFIT 2.0 [8].
Dissolution methods
Dissolution equipment was the USP Apparatus 2 with paddles. Dissolution medium was 900 ml water or phosphate buffer pH 6.8 at 37±0.5°C. Stirring rates were 50, 75 and 100 rpm. A sample of approximately 0.5 mL was removed from each vessel and replaced with fresh medium. Analytical assay method was spectrophotometric, determinations being performed at 274 nm.

Theory
Correlations were made between dissolution fraction FD and fractions of drug absorbed calculated using Wagner–Nelson formula [9]:

\[
FRA = \frac{C_p + k [AUC]_t}{k [AUC]_\infty}
\]

where:
- FRA – fraction of drug absorbed at time t;
- \(C_p\) – plasma drug concentration at time t;
- k – elimination rate constant;
- \([AUC]_0\) – area under the concentration time curve from time 0 to time t;
- \([AUC]_\infty\) – area under the concentration time curve from time 0 to infinity.

Neglecting concentration term it was in fact used the formula

\[
FRA = \frac{[AUC]_t}{[AUC]_\infty}
\]

which represents rather the „eliminated fraction” than „absorption fraction” but this simplification lead to a better correlation than usual formulas.

Generalized dissolution–absorption correlations
Correlations between dissolution and absorption were used also in a generalized form [10] of correlation between dissolution of parent drug and kinetics of appearance in plasma of its metabolites.

Results and Discussion
Correlations absorption–pharmacokinetics of active compounds
It was tried the correlation between dissolution of pentoxifylline in vitro and absorption of pentoxifylline. Correlation was then extended a correlation between dissolution of pentoxifylline and “apparent” absorption of metabolites M1 and M5. In fact, this “apparent absorption” is a resultant of absorption of pentoxifylline and metabolism (Figure 1).

If slower process is absorption, it is expected an “apparent absorption of metabolite” quite similar to absorption of pentoxifylline.

If on the contrary, the metabolism is slower, correlation is mainly a “dissolution-metabolism correlation” as can be seen from Figure 2 (dissolution 50 rpm Pharmacokinetics) there were obtained practically the same correlation coefficients (0.97 with differences only at third decimals) for all three active compounds.

On the contrary, the slope of the linear correlations was different (1.15; 1.38; 1.52) respectively for (pentoxifylline, M5 and M1) which support the hypotheses of an apparent dissolution metabolism correlation (Figure 3).
A further improve of the correlation \( r > 0.99 \) in all three cases) significant difference between the three active compounds, in order pentoxifylline \( \leq M5 \leq M1 \) it concerns the slopes.

Since, in fact, from the therapeutically point of view, there are no significant differences between pentoxifylline and its metabolites, a more relevant pharmacokinetic parameter could be considered the total concentration of active components, \( i.d. \) the sum of pentoxifylline, M1 and M5 plasma levels.

Correlation between \textit{in vitro} dissolution tests and “apparent absorption” deduced from total concentrations is represented in Figure 4. It can be observed that the results are essentially similar with the separate correlation for pentoxifylline, M1 and M5.

\[
\begin{align*}
\text{y} &= 0.8469x + 3.919 \\
R^2 &= 0.9765 \\
\text{y} &= 0.8779x + 3.439 \\
R^2 &= 0.9955 \\
\text{y} &= 1.0197x + 2.4909 \\
R^2 &= 0.9893
\end{align*}
\]

Figure 4. Correlation dissolution – total concentration (parent drug and metabolites)

Similarity concerns both the size and the order of correlation coefficients. Consequently, practically in all cases, dissolution at 100 rpm proved to be the most relevant, but it is to underline that the dissolutions at 50 rpm and 75 rpm correlated better with \textit{in vivo} results.

Selection of the most relevant dissolution conditions for correlation with \textit{in vivo} results

Parameters of dissolution \textit{in vitro}, considered it concerns correlation with \textit{in vivo}, where stirring rate and pH of receptor medium.

As can be seen from the Figure 5, there were obtained good correlations at all three stirring rate (50 rpm, 75 rpm, 100 rpm).

The most bio-relevant could be considered test at 75 rpm for which the correlation coefficient was greater then 0.995.

\[
\begin{align*}
\text{y} &= 0.5261x + 7.6263 \\
R^2 &= 0.9915 \\
\text{y} &= 0.5186x + 8.2422 \\
R^2 &= 0.9752 \\
\text{y} &= 0.5356x + 7.9978 \\
R^2 &= 0.9851 \\
\text{y} &= 0.4313x + 5.5258 \\
R^2 &= 0.9884
\end{align*}
\]

Figure 5. In vitro dissolution (50, 75 and 100 rpm) – “elimination fraction” correlation

It was checked also the correlation between \textit{in vitro} dissolution and an “apparent absorption” of the metabolites, in fact, the kinetics of its apparition in plasma. Surprisingly, correlation was very good. Dependence of correlation on stirring rate indicated the value of 100 rpm as the most appropriate both in cases of M1 and M5 metabolites.

\[
\begin{align*}
\text{y} &= 1.0197x + 2.4909 \\
R^2 &= 0.9893 \\
\text{y} &= 0.7063x + 1.8733 \\
R^2 &= 0.9971
\end{align*}
\]

Figure 6 Comparison of water as receptor medium with 6.8 phosphate buffer \textit{iviv} correlations

Comparison of water as receptor medium with 6.8 phosphate buffer in case of pentoxifylline, as can be seen in Figure 6, suggests a somewhat greater bio-relevant of dissolution at pH 6.8 than dissolution in water (both at 100 rpm).

Conclusions

1. Correlations were linear and very good in all tested conditions (correlation coefficient \( r > 0.97 \)) in all cases.

2. It concerns stirring rate, best correlation with \textit{in vivo} results were obtained in case of maximum speed – 100 rpm. The result is rather unexpected, since in gastrointestinal tract the peristaltic movements are thought less dynamic.

3. Stabilization of pH to 6.8 lead to modification of the slope of the line and improved the correlation in case of pentoxifylline. In case of metabolites, correlation coefficient was better for dissolution in water.

4. Generalization of correlation to metabolites rate of appearance in plasma worked also very
well. The slopes of the lines were different but in all were in the same order: pentoxifylline < M5 < M1.

5. Since pentoxifylline and its metabolites M1 and M5 are somewhat equipotent, it was considered useful to test the correlation dissolution fraction – “apparent absorption” of cumulated three active compounds. It was obtained also a good linear correlation. Results and hierarchy of dissolution parameters “bio-relevancy” was practically the same as in separate evaluations.

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