COMPARATIVE PHARMACOKINETICS OF RIFAMPICIN AND 25-DESACETYL RIFAMPICIN IN HEALTHY VOLUNTEERS AFTER SINGLE ORAL DOSE ADMINISTRATION

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Abstract

The *in vivo* evaluations were conducted as part of an open label, analytical blinded, single dose, randomized, two treatments, two periods, two sequences, fast state, crossover bioequivalence study. Plasma levels of rifampicin and des-ethylrifampicin were determined using a validated HPLC method.

Non-compartmental analysis for determination of peak drug concentration (Cmax), time to reach maximum plasma concentration (Tmax), the plasma half-life (t1/2), the area under curve of measured plasma concentrations to the last sampling point (AUD) and total area under curve (AUC) were performed with the software Kinetica 3.1 Compartmental analysis was performed using Topfit 2.0 software. Hierarchy of the performance of compartmental models was established using Akaike, Schwarz and Imbimbo criteria. Significance of difference between the values of the criteria was tested using F-test.

The concentrations of metabolite were much smaller compared to the parent drug. Absorption was very rapid, maxim concentration being reached in less than one hour and the metabolism was fast. Twelve hours after administration, the plasma levels became low, much under the therapeutic level. Tmax values were 2.2 h for parent drug and 3.8 for metabolite. The difference was statistically significant and the result was the expected one. The metabolite appeared in plasma later, consecutive to absorption and metabolism of the parent drug. The correlation coefficient was poor. The cause of this lack of correlation could be the high variability (CV%=49 %) of the parameter corresponding to the parent drug. This result was considered as a consequence of the fact that rifampicin is mainly excreted through the feces and only a small part is excreted through the urine. Variability of the two compounds was similar, lower than the variability of all the other parameters. Correlation of the values for parent drug and active metabolite was better than in case of Tmax, but lower than in case of AUC and Cmax. The AUC values for metabolite were one order of magnitude smaller, but it is important to note a better correlation between the two active entities (r=0,66). The variation coefficients indicated that it is the case of a high variability drug.

Since the parent drug has a great value of partition coefficient (logP=2,77), its solubility in deep compartment has to be significant and the expected result was at least a two-compartment model. The pharmacokinetic model which described the evolution of

plasma level of both parent drug and metabolite was the mono-compartmental one. Increasing the number of compartments didn't improve significantly the modeling performance.

The pharmacokinetics of rifampicin and its active metabolite after a single administration in healthy volunteers does not impose an adjustment of therapeutic schedule following a variable metabolism. High variability of plasma levels of rifampicin could arise mainly from a high variability of absorption, distribution and elimination.

Rezumat

Evaluările *in vivo* au fost efectuate, ca parte a unui studiu de bioechivalenţă încrucişat, deschis, analitic orb, cu doza unică, randomizat, cu două tratamente, două perioade, două secvenţe de stat, *a jeun*. Nivelurile plasmatice de rifampicină şi dezacetil rifampicină au fost determinate folosind o metoda HPLC validată.

Analiza non-compartimentală pentru determinarea concentraţiei maxime de medicament (C_{max}), timpul pentru a atinge concentraţia plasmatică maximă (T_{max}), timpul de înjumătăţire plasmatică ($t_{1/2}$), aria de sub curba concentraţiilor plasmatice măsurate la ultimul punct colectat (AUD) şi aria totală de sub curbă (AUC) au fost efectuate cu software-ul Kinetica 3.1. Analiza compartimentală a fost efectuată cu ajutorul software-ului TopFit 2.0. Ierarhia performanţelor modelelor compartimentale a fost stabilită cu ajutorul criteriilor Akaike, Schwarz şi Imbimbo. Semnificaţia diferenţelor dintre valorile de criterii a fost testata cu ajutorul testului F.

Concentraţiile de metabolit au fost mult mai mici decât cele ale medicamentului părinte. Absorbţia a fost foarte rapidă, concentraţia maxima fiind atinsă în mai puţin de o oră, şi metabolismul a fost rapid. După douăsprezece ore de la administrare nivelurile plasmatice au devenit mici, mult sub nivelul terapeutic.

Valorile medii ale timpului concentraţiei maxime (T_{max}) au fost de 2.2 h pentru medicamentul parinte şi 3,8 ore pentru metabolit. Diferenţa a fost statistic semnificativă şi rezultatul a fost cel aşteptat. Metabolitul a apărut mai târziu în plasmă, consecutiv cu absorbţia şi metabolismul medicamentului părinte. Coeficientul de corelaţie a fost slab. Cauza acestei lipse de corelaţie ar putea fi variabilitatea mare (CV = 49%) a parametrului corespunzător medicamentului parinte. Variabilitatea în cazul metabolitului a fost de aproximativ jumătate din valoarea medicamentului părinte. Timpii de înjumătăţire au fost mai mari în cazul metabolitului decât în cazul medicamentului părinte. Acest rezultat a fost considerat ca o consecinţă a faptului că rifampicina este excretată în principal prin fecale şi numai o mică parte eliminată prin urină. Variabilitatea celor doi compuşi a fost similară, mai mică decât variabilitatea tuturor celorlalţi parametri. Corelarea valorilor medicamentului părinte şi a metabolitului a fost mai bună decât în cazul T_{max}, dar mai slabă decât în cazul ariilor de sub curbe şi al C_{max}. Ariile de sub curbe (AUC) pentru metabolit au fost cu un ordin de mărime mai mică, dar s-a constatat o mai bună corelare între cele două entităţi active (r = 0,66). Coeficienţii de variaţie au indicat faptul că este vorba de un medicament extrem de variabil.

Din moment ce medicamentul părinte are o valoare mare a coeficientului de partiţie (logP = 2,77), solubilitatea în compartimentul profund trebuie să fie semnificativă şi rezultatul aşteptat a fost de cel puţin de un model cu două compartimente. Modelul farmacocinetic care a descris evoluţia nivelurilor plasmatice ale medicamentului părinte, cât şi a metabolitului a fost unul mono-compartimental. Creşterea numărului de compartimente nu a îmbunătăţit în mod semnificativ performanţele modelului.

Farmacocinetica rifampicinei şi a metabolitului său activ, după administrarea unei doze unice la voluntari sănătoşi, a arătat că nu suntem în cazul unui metabolism variabil și nu se impune o ajustare a schemei terapeutice în funcție de estimările acestuia. Variabilitatea mare a concentraţiilor plasmatice de rifampicină ar putea apărea, în principal de la o variabilitate mare a absorbţiei, distribuţiei şi eliminării.

Keywords: rifampicin, 25-desacetyl rifampicin, pharmacokinetics.

Introduction

Antituberculosis therapy commonly consists in the association of four drugs (isoniazid, rifampicin, pirazinamide, ethambutol) and may induce clinically important adverse effects such as hepatotoxicity, neurotoxicity, skin eruptions, gastro-intestinal symptoms etc. The risk of these adverse effects is increased in patients with associated diseases (hepatic failure, renal failure, diabetes, epilepsy, psychiatric diseases etc.).

The revival of tuberculosis has been accompanied by amplification of drug resistance [1-5].

Variability of response after administration of drugs represents a significant problem in therapy. High frequency of adverse and toxic effects after the administration of therapeutic doses of some drugs increases the cost of health insurance services.

A great part of variability in both therapeutic and adverse effects of rifampicin arises from variability in pharmacokinetics.

Low rifampicin bioavailability was attributed to several factors including malabsorption, increased hepatic clearance due to autoinduction, enhanced intestinal metabolism and drug interactions [6-9].

Metabolism of rifampicin is very complex. Oxidation of Rifampicin is realized by cytochromes CYP3A4 and CYP2E1. Rifampicin has the capacity to determine the induction of CYP3A4, CYP1A2, CYP2C8, CYP2C9, and CYP2C19. The main metabolite of Rifampicin is 25-deacetyl rifampicin, but there are also other metabolites: 3-formyl rifamycin, Rifampicin quinine, N-demethyl rifampicin, 3-formyl-25-deacetyl rifampicin, desacetyl rifampicin quinine.

In patients without associated diseases, autoinduction of rifampicin's metabolism is expected to result in lower levels after repeated doses and the levels of isoniazid, pyrazinamide, and ethambutol for the majority of patients are expected to be within the expected ranges. Practically it was noticed a substantial variability of antituberculosis drug concentrations in plasma at steady state. Only a minority of patients presented very low Rifampicin plasma concentrations [10].

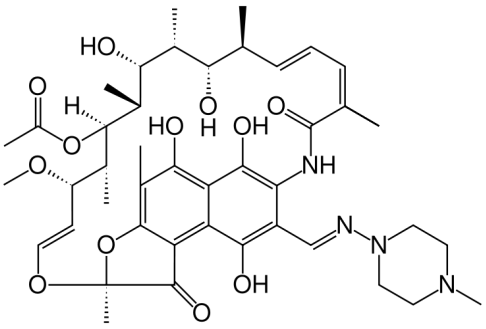

Figure 1
The chemical structure of rifampicin

Solubility and permeability data indicate that rifampicin (Figure 1) is a BCS (Biopharmaceutical Classification System) Class II drug being a candidate for approval of bioequivalence based only on *in vitro* dissolution studies. But there are many reports of failure of IR solid oral dosage forms of rifampicin to meet BE have been published and the reasons for these failures are not clear. Moreover, no reports were identified in which *in vitro* dissolution was shown to be predictive of nonequivalence among products. Therefore, a biowaiver based approval of rifampicin containing IR solid oral dosage forms, cannot be recommended [11].

There is a clear dose-response relationship for isoniazid, rifampicin and pyrazinamide, so low plasma concentrations may correlate with poorer treatment outcomes. Therapeutic relationships between Rifampicin plasma concentrations and treatment response in tuberculosis patients are not well defined, especially in patients with associated diseases. In this context, the aim of this paper was to compare pharmacokinetics of Rifampicin in healthy volunteers.

Materials and Methods

Clinical study. The *in vivo* evaluations were conducted as part of an open label, analytically blinded, single dose, randomized, two treatments, two periods, two sequences, fast state, crossover bioequivalence study. Thirty healthy subjects, both genders, were screened according to the inclusion / exclusion criteria, at least one week before the enrolment decision. The screening included standard clinical laboratory evaluations, as well as the assessment of medical history and other aspects relevant for the health status. The study medication (300 mg, two capsules containing 150 mg rifampicin each) was administered in two consecutive sessions,

separated by at least seven days wash-out period. Blood samples of 5 mL were collected in Sarstedt^{TM} vials containing as anticoagulant potassium ETDA before dosing (0.0 hours) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0 and 24.0 (second day) hours after the drug administration. Blood samples have been centrifuged at 5° C for 6 minutes at approximately 3000 rotations/min. Plasma was separated in 2 aliquots, transferred into labeled 1.5 mL polypropylene tubes in equal volume of at least 1.2-1.3 mL / each and immediately frozen and stored at a temperature under -20°C until delivery to analytical facility. Prior to implementation, the clinical study protocol was approved by the Romanian National Medicine Agency (letter no. 6630/July 20th, 2006) and Romanian National Ethics Committee (letter no. 2648/July 30th, 2007).

Analytical method. Plasma levels of rifampicin and des-ethylrifampicin were determined using a validated HPLC method [12, 13].

Mathematical modeling and statistical analyses. Non-compartmental analysis for determination of the peak drug concentration ($C_{max)}$, time to reach maximum plasma concentration (T_{max}), the plasma half-life ($t_{1/2}$), the area under curve of plasma measured concentrations to the last collected point (AUD) and total area under curve (AUC) were determined with software Kinetica 3.1. Compartmental analysis was performed using TopFit 2.0 software. Hierarchy of the performances of compartmental models was established using Akaike, Schwarz and Imbimbo criteria. Significance of differences between values of criteria was tested using F-test.

Results and Discussion

Data obtained in the bioequivalent study concerned a reference and a tested drug. In the following, the comparisons of pharmacokinetic and metabolism are performed starting from data of reference drug. Since the formulations were bioequivalent, testing of normality of distribution of pharmacokinetic parameters was undertaken using the pooled data.

Plasma levels of Rifampicin and 25-Desacetyl Rifampicin are presented in figure 2. Concentrations are given in μg/mL for rifampicin and in ng/mL for metabolite.

Analysis of space distribution of sets of curves

Examinations of figure 1 with the naked eye revealed that most curves were homogeneously distributed in space. Some curves were situated outside, above or under the other curves. There were one or two cases of

higher time of maximum concentration (maybe delayed absorption) or very low concentrations (maybe malabsorption).

One natural question which arises from examination of sets of curves concerns the space distribution of curves. Curves are "normally distributed" around a mean curve and some subjects are outliers or it is a problem of differences in rate of metabolism and data are shared in function of genes controlling metabolism?

Normality of distribution of global parameters associated to curves will be checked further, but replacement of sets of curves with sets of associated global parameters means a lot of information.

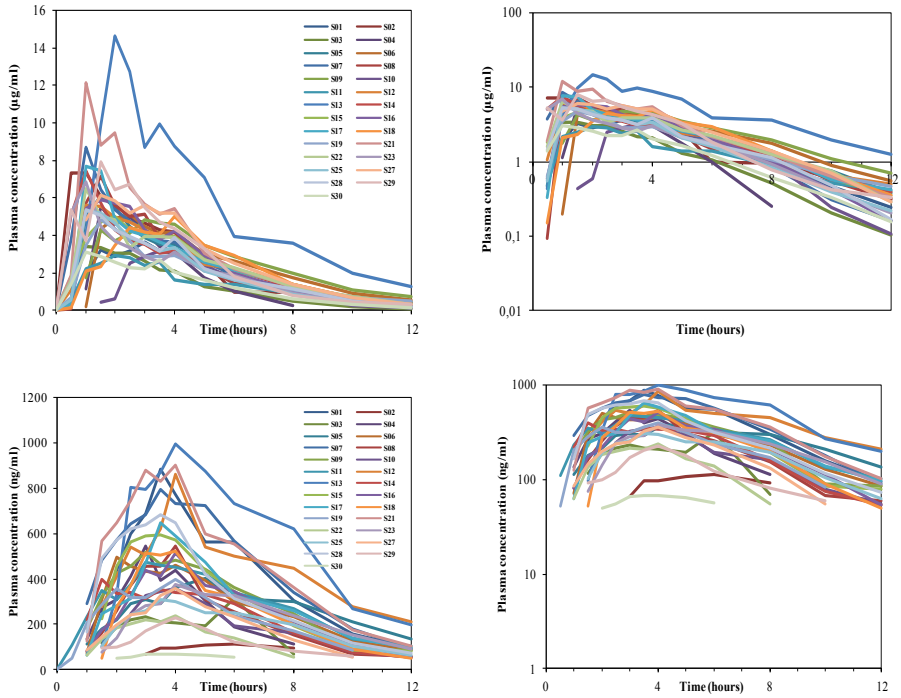

Figure 2

Individual plasma profiles of rifampicin and 25-desacethyl-rifampicin after oral administration of 300 mg rifampicin (normal and semi-logarithmic representation)

Distributions of the pharmacokinetic parameters deduced from the non-compartmental analysis of curves.

In this respect main pharmacokinetic parameters, Area Under Data (AUD, AUC_{0-T}), Area Under Curve (AUC, AUC_{total}, $AUC_{0-\infty}$), extrapolated area ($AUC_{T-\infty}$) maximum concentration (C_{max}), time of maximum concentration (T_{max}), elimination constant (k_{el}), half-time ($T_{1/2}$) were

calculated using non-compartmental analysis and compared by statistical methods.

The calculated values for main pharmacokinetic parameters are presented in table I.

Table I

The individual and mean values of the main pharmacokinetic parameters for rifampicin (PD) and its 25-desacethyl metabolite (MET)

Parameter	T_{max} (h)		$T_{1/2}$ (h)		AUD (µg·h/mL)		AUC (µg·h/mL)		C_{max} (µg/mL)	
Entity / Subject	PD	MET	PD	MET	PD	MET	PD	MET	PD	MET
1	1.5	3.5	2.0	2.3	29.2	4.5	0.8	0.3	6.3	0.9
2	4	6	2.7	7.1	8.9	0.6	0.9	1.0	1.3	0.1
3	1.5	6	1.7	3.8	13.1	1.4	0.4	0.6	2.9	0.3
4	3	3	1.3	2.0	21.9	2.1	0.3	0.3	5.3	0.5
5	1.5	5	3.5	3.5	20.3	3.0	2.8	0.7	2.7	0.4
6	1.5	2	2.5	3.3	29.0	3.1	1.6	0.4	6.1	0.5
7	1	3.5	1.9	2.3	32.1	5.0	0.7	0.3	7.4	0.8
8	3	4	1.9	2.5	25.2	2.5	0.6	0.2	5.2	0.5
9	2	3	2.5	2.8	30.9	3.3	1.6	0.3	5.6	0.5
10	2.5	4	1.8	2.9	16.9	1.9	0.4	0.2	4.5	0.4
11	0.5	3	2.8	3.3	21.3	3.3	1.3	0.5	4.7	0.5
12	4	4	3.5	5.0	27.3	4.8	3.6	1.6	4.9	0.9
13	2.5	4	2.9	3.3	63.8	6.2	6.1	1.0	14.3	1.0
14	1	1.5	2.4	2.5	29.5	2.6	1.1	0.2	9.0	0.4
15	2	3.5	2.3	2.6	27.6	3.3	1.2	0.3	6.2	0.6
16	3	4	1.9	2.5	22.8	2.8	0.7	0.2	4.4	0.5
17	3.5	3.5	2.5	3.1	27.0	3.3	1.6	0.4	5.1	0.6
18	3	4	2.3	2.2	20.2	2.8	1.0	0.2	4.3	0.5
19	1	4	2.3	2.8	24.4	2.3	0.9	0.4	4.9	0.4
21	1.5	4	2.4	2.4	39.5	5.2	1.7	0.4	10.0	0.9
22	1	4	1.5	1.9	17.3	1.1	0.4	0.2	4.0	0.2
23	4	4	2.4	3.2	17.1	2.5	1.1	0.4	3.1	0.4
25	2.5	3.5	2.9	2.9	17.2	2.1	1.6	0.3	2.9	0.3
27	1	4	1.5	2.3	23.1	1.9	0.3	0.2	7.8	0.4
28	1.5	3.5	2.5	2.8	30.7	3.7	1.4	0.3	7.4	0.7
29	4	4	4.1	3.3	51.5	1.1	1.0	0.3	7.6	0.2
30	3	3.5	2.6		2.6	0.3	0.6	0.0	0.5	0.1
Mean	**2.2**	**3.8**	**2.4**	**3.0**	**25.6**	**2.9**	**1.3**	**0.4**	**5.5**	**3.0**
R		**0.28**		**0.47**		**0.66**		**0.68**		**0.68**
Stdev	**1.10**	**0.93**	**0.65**	**1.05**	**12.13**	**1.42**	**1.22**	**0.32**	**2.81**	**0.24**
CV(%)	**49**	**25**	**27**	**35**	**47**	**50**	**92**	**80**	**51**	**8**

Times of maximum concentration (T_{max}). Mean values were 2.2 h for parent drug and 3.8 h for metabolite. The difference is statistically significant and the result is the expected one. Metabolite appears in plasma

later being consecutive to absorption and metabolism of parent drug. Correlation coefficient was poor. The cause of this lack of correlation could be the high variability (CV=49 %) of parameter corresponding to parent drug. Variability in case of metabolite was approximately half of the value for parent drug.

Half - times were greater in the case of metabolite than in the case of parent drug. This result is a consequence of the fact that rifampicin is mainly excreted through the feces and only a small part excreted through the urine. Variability of the two compounds is similar, lower than variability of all other parameters. Correlation of the values for parent drug and metabolite is better than in case of T_{max} but lower than in case of areas under curves and C_{max}.

Areas Under Curves (AUC) for metabolite are one order of magnitude smaller, but it is to note a better correlation between the two active entities (r =0.66). Variation coefficients indicate that we are in the case of a highly variable drug.

Maximum concentration (C_{max}) is ten times smaller for metabolite than that of parent drug. Variability is huge for both compounds but correlation is good.

Comparison of mean curves

The most used and easy method to undertake is the comparison of mean curves instead of individual data. This approach is associated with renouncement to all the information concerning variability, which is all the time significant in biology.

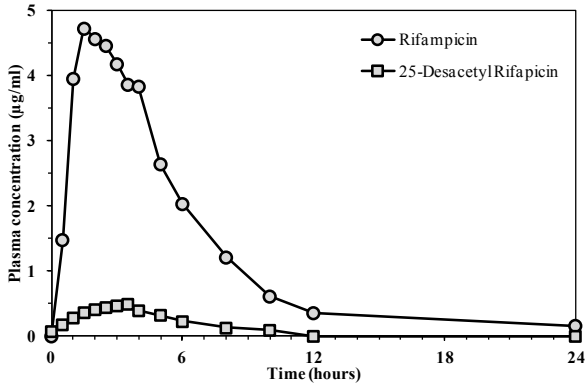

Figure 3

Mean plasma concentration of rifampicin and 25-desacethyl rifampicin

First observation is that concentrations of metabolite are much smaller than that's of parent drug (Figure 3). Absorption is very rapid,

maximum concentration being reached in less than one hour and metabolism is fast. Twelve hours after administration plasma levels become low, under the therapeutic level.

Since the pharmacokinetics of rifampicin is significantly influenced by associated diseases with tuberculosis, for example type 2 diabetes [14] or renal failure [15], since patients are normal or slow acetylators the dimension of variability has to be estimated in order to be able to think to an individualized therapy.

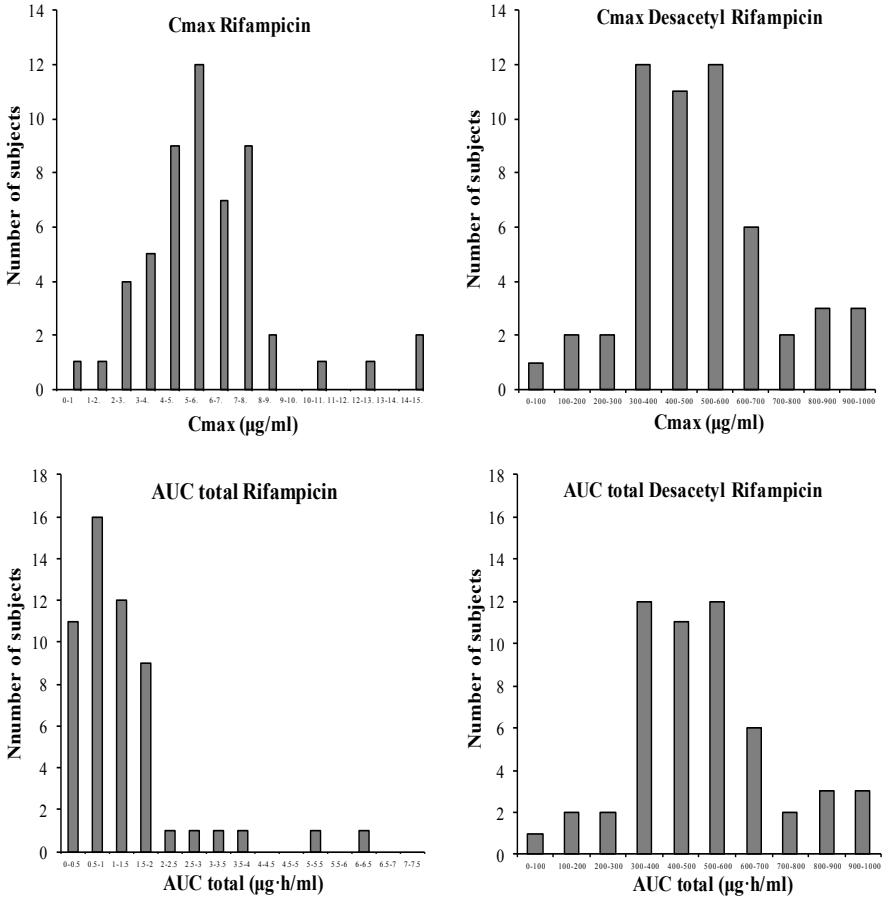

Figure 4

The distribution of the values of main pharmacokinetic parameters for rifampicin and 25-desacethyl-rifampicin

In all cases, values associated to metabolite are more closed to a normal distribution than the values corresponding to parent drug (Figure 4). The two extreme high values of AUC_{total} for parent drug, corresponding to high values of AUC and C_{max} for both, parent drug and metabolite.

Consequently, for these subjects it could be rather a problem of increased bioavailability than impaired metabolism.

Population of pharmacokinetic parameters could be considered as approximately normal distributed in case of metabolite both for C_{max} and AUC. For the parent drug, the total areas population has an asymmetrical distribution, having a long tail toward greater values. Two points are far from the rest of the group. The question arises again if these points are outliers or not.

Compartmental modelling

Since the high variability of pharmacokinetics observed in analysis of plasma levels implies also a variability in the effect, an individualization of therapy starting from individual pharmacokinetics parameters and adequate predictive models is a part of the general desire for personalized medicine [16].

Pharmacokinetic model which described the evolution of plasma levels of both parent drug and metabolite was the mono-compartmental one (Figure 4). Increasing the number of compartments didn't improve significantly the modeling performance.

Since parent drug has a great value of partition coefficient (logP=2.77) [17] its solubility in deep compartment has to be significant and the expected result was at least two-compartment model.

Degeneration of models to pseudomonocompartmental behavior is a result, found in case of other drugs also and for many active metabolites [18-20] and can be explained by the fact that drugs though are involved in many transfer processes the final rate of change is given by a single rate / that of most slow processes.

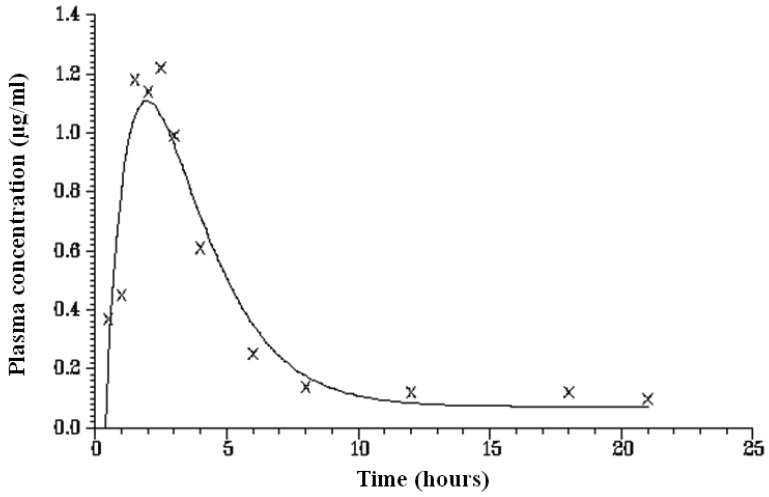

Figure 5
Mono-compartmental fitting of the mean plasma concentration profile for 25-desacethyl rifampicin

Individualization of treatment based on pharmacokinetic parameters.

Individualization of treatment is very important in the case of highly variable drugs. Mean pharmacokinetic data are useful in elaboration of general schemes for administration of drugs. Knowledge of the range of variability of individual parameters allows prediction of the possible extreme values of safety and efficacy. In order to avoid undesired effects, it is very useful to estimate the possible range of individual pharmacokinetic parameters based on one or more determination of plasma levels.

One time point sampling scheme: at 2-hours post-dose concentrations are usually less informative, but does distinguish between delayed absorption (late peak, close to normal range) and malabsorption (low concentrations at all time points) [21].

Two time points sampling scheme: at 2-hours post-dose and a second sample, at 6-hour post-dose, can differentiate between delayed absorption and malabsorption and can provide some information about clearance and half-life, assuming that drug absorption was nearly completed after 2 hours [21].

A five time points sampling scheme per patient: is sufficient to detect relevant differences in pharmacokinetic parameters, but is not sufficient to determine some pharmacokinetic parameters, like half-life, with a high degree of accuracy [22].

A six points (World Health Organization recommendation) over 8 hours, as opposed to the conventional requirements of 13 samples, proved to be sufficient to determine pharmacokinetic parameters in bioequivalence studies for fixed dose combinations [23].

Rifampicin half-lives were shorter among patients with failure or relapse compared with control cases.

Conclusions

Concentrations of metabolites are much lower than that of parent drug, the ratio of AUC being around 10% and that of maximum concentrations even lower. In these conditions, the active metabolite doesn't seem to contribute to the variability of global therapeutic effect.

The values of the pharmacokinetics parameters are approximately normal distributed. It seems that even in absence of associated diseases, i.e. in case of healthy volunteers, plasma level curves share in two clusters: very

high plasma levels and other group of rather lower, normally distributed levels.

These results support recommendations that drug concentration measurement is necessary in patients with an inadequate response to directly observed therapy. Further studies are needed to verify the findings with other patient populations, to identify further sources of variation, and to determine optimal dosing strategies.

Since the mono-compartmental models fitted well data in both, parent drug and metabolite cases, a prediction of time course of plasma levels of a subject can be made starting from individual pharmacokinetic parameters.

There is a need for more studies to have more data useful to adjust the antituberculosis therapy in order to reduce the treatment failure and the frequency of adverse effects.

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