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SUSTAINED RELEASE NIFEDIPINE FORMULATIONS: MOMENT, MODELLING AND SIMULATION AS PHARMACOKINETIC ANALYSIS APPROACH

C. Remuñán[1], A. Mrhar[2], S. Primozic[2], R. Karba[3], J.L. Vila-Jato[1]

[1] Laboratory of Pharmaceutical Technology. Faculty of Pharmacy. University of Santiago de Compostela (Spain) (correspondence)

[2] Department of Pharmacy (Faculty of Natural Sciences and Technology) and [3] Faculty of Electrical and Computer Engineering. Edvard Kardelj University of Ljubljana (Yugoslavia).

ABSTRACT

The bioequivalence of three sustained release nifedipine formulations designed in this laboratory, was assayed in rabbits by comparing their plasma time profiles to a reference (Adalat^{R} retard).

The analysis of data was performed by using the statistical moments as a measure of drug release. Results show a slowler absorption rate in the experimental formulations than in Adalat^{R} retard, and not differences in the absorption extent.

The composed one-compartment model, described for humans, showed to be also succesfull for rabbits.

INTRODUCTION

Nifedipine is a calcium channel antagonist originally introduced for the treatment of angina pectoris and more recently for hypertension. Nifedipine is a slightly water-soluble drug whose bioavailability is very low when it is orally administered in crystalline form (1). Its biological half-life is, on the other hand, very short (2, 3) sustaining its antihypertensive effect only for a few hours. Therefore, several studies of nifedipine have been made in order to enhance its bioavailability (1, 4) and to prolong the duration of its action (5, 6, 7, 8).

In this study, the bioequivalence of three sustained release nifedipine formulations, previously developed by us (8) was assayed in rabbits by comparing their plasma-time profiles to a reference (Adalat^R retard). The analysis of data was performed by using the statistical moments (9, 10) and a computer simulation was assesed in rabbits after single and multiple dosing simulation. With regard to this, a pharmacokinetic model of nifedipine was developed and the corresponding parameters were identified on the analog-hybrid computer (11, 12).

MATERIALS AND METHODS

Formulations

Three dosage forms were prepared by a previously described method (8) by granulating a nifedipine-polyvinilpyrrolidone (1:3) coprecipitate (90%) and Gelucire^R, as wax matrix. The dose was 20 mg in all the cases and the investigated formulations, which differed in the wax excipient (**X**) and the compression force (**Y**) were:

X	Y	Formulation
Gelucire^R 53/10	6000 N	**B**
Gelucire^R 53/10	12000 N	**C**
Gelucire^R 54/02	12000 N	**D**
Adalat^R retard	(reference)	**A**

Nifedipine Assay

Drug plasma levels in rabbits were determined by the HPLC method proposed by *Miyazaki* (13) and slightly modified by us, retaining the original performances.

Pharmacokinetic Experiments

Overnight-fasted white male rabbits weighing 2.5-3.5 Kg received one of the formulations per oral route. During all the assay time, rabbits remained fasted but was allowed the free access to water. Blood samples were collected from marginal ear vein at specified intervals of time.

Analysis of Pharmacokinetic Data

Moment Parameters: a software package implemented on an Apple microcomputer which enabled the calculation and statistical evaluation of moment parameters (14) and the Statgraphics program (Statistical Graphics Corporation) were used. Statistical analysis consists of:

1.- One-way analysis of variance by means (ANOVA) and the least-significant difference test (LSDT) for homogeneous variances (Bartlett's test).

2.- Kruskal-Wallis one-way analysis of variance by ranks (K-W), for non-homogeneous variances.

From the concentration-time data, the individual profiles of nifedipine plasma levels were performed, from which the following pharmacokinetic parameters were obtained:

1.- $AUC_{(0-\infty)}$: the area under the plasma concentration (c) versus time ($0<t<\infty$) curve.

2.- C_{max}: the observed peak plasma concentration of nifedipine.

3.- T_{max}: the observed time of the peak (Cmax).

4.- $T_{1/2\ \beta}$: the half-life elimination.

5.- Cl/F: the plasma clearance, where F is the systemic availability.

6.- Vss/F: the volume of distribution at steady state.

7.- MRT: the mean residence time, calculated by the equation MRT = AUMC/AUC, where AUMC is the area under the first moment curve of plasma concentration versus time.

8.- VRT: the variance of the MRT.

9- MAT: mean absorption time. MAT of the drug after the noninstantaneous input (n.i.) was calculated according to *Riegelman* (15): $MAT = MRT_{(n.i.)} - 1/\beta_{(n.i)}$

Modelling and Analog-Hybrid Simulation: simulation and identification, carried out with an analog-hybrid computer (Electronics Associates Inc. mod. EAI-580) was used to generate plasma profiles of nifedipine after single and multiple dosing.

The identification procedure with an adaptive model was used for verification of the pharmacokinetic model structure. Parameters were modulated manually on the computer in order to obtain an optimal accordance between the model response and the experimental data. Integral square error was used as a criterion function (16).

RESULTS AND DISCUSSION

The mean nifedipine plasma levels following single administration of formulations A, B, C and D are shown in Table 1 and depicted in Figure 1. The concentrations exhibit in all cases a substantial intersubject variation. These differences may be attributed, as in humans, to the variability in the rate of drug absorption (2, 17, 18) and/or the extent of first-pass hepatic extraction and metabolism (19, 20, 21, 22, 23).

The mean pharmacokinetic parameters obtained from individual experimental data are given in Table 2 and the results of the statistical analysis performed on them in Table 3.

As formulation D shows a plasma profile very different from the Adalat^R retard one, it has only been included as an example in modelling and simulation approach and not in the moment parameters analysis.

TABLE 1

Plasma Levels of Nifedipine (μg/ml) Following Single Oral Administration of Formulations A, B, C and D to Rabbits (Mean ± S.D.).

	TIME (hours)							
	0.5	1	2	4	6	8	12	16
A (n=10)	4.19±4.56	6.80±4.95	8.98±3.22	8.49±4.63	6.48±2.81	3.71±2.02	1.81±2.08	0.85±1.21
B (n=11)	1.25±0.99	2.73±3.18	5.65±7.91	8.23±8.48	10.22±5.86	8.73±5.07	5.12±3.14	2.66±1.93
C (n=12)	0.76±0.65	2.52±1.80	5.94±4.83	8.32±4.96	8.43±4.66	6.54±5.78	3.59±3.51	1.67±1.35
D (n=5)	0.34±0.54	1.26±0.97	1.59±1.22	0.91±0.67	4.89±6.49	13.52±2.11	3.65±3.78	0.25±0.32

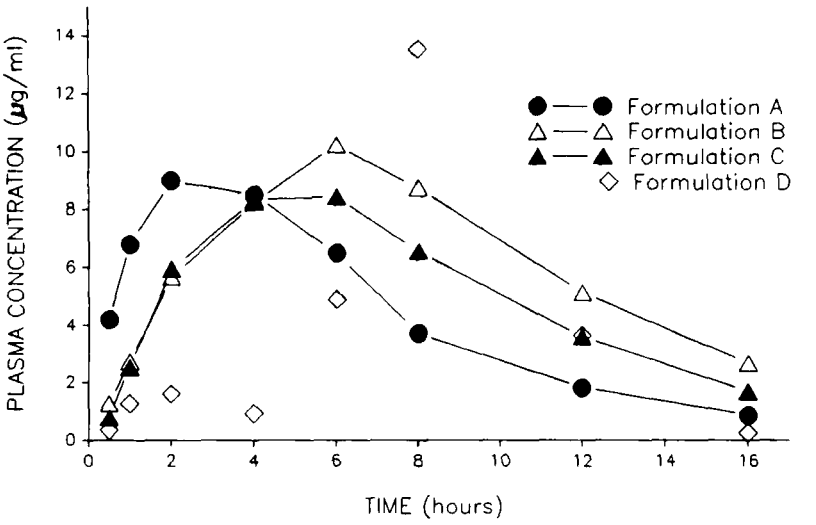

FIGURE 1

Mean Plasma Profiles of Nifedipine Following Single Oral Administration of Formulations A, B C and D to Rabbits.

TABLE 2

Pharmacokinetic Parameters of Nifedipine Following Single Oral Administration of Formulations A, B and C to Rabbits (Mean ± S.D.).

	AUC (μg/ml.h)	C_{max} (μg/ml)	T_{max} (h)	$T_{1/2\beta}$ (h)	Cl/F (l/h)	Vss/F (l)	MRT (h)	VRT (h^2)	MAT (h)
A	80.80 ± 29.85	10.81 ± 4.09	2.60 ± 1.58	2.96 ± 0.88	0.28 ± 0.12	1.70 ± 0.78	6.07 ± 1.67	9.11 ± 2.7	1.80 ± 1.48
B	103.57 ± 60.31	12.81 ± 8.15	5.18 ± 2.54	3.35 ± 1.52	0.31 ± 0.24	2.39 ± 1.69	8.34 ± 2.52	10.32 ± 5.4	3.89 ± 1.78
C	99.36 ± 64.14	11.22 ± 5.99	5.00 ± 2.16	4.34 ± 2.31	0.30 ± 0.22	2.35 ± 1.46	8.91 ± 3.88	15.5 ± 13.4	2.64 ± 1.11

TABLE 3

Statistical Analysis of Nifedipine Pharmacokinetics Parameters Corresponding to Formulations A, B and C.

TEST	AUC*	C_{max}*	T_{max}*	$T_{1/2\beta}$**	Cl/F*	Vss/F*	MRT*	VRT**	MAT*
ANOVA*	NS	NS	S		NS	NS	NS		NS
LSD*			A BC						
K-W**				NS				NS	

* Homogeneous variances, ** non-homogeneous variances; S: significant, NS: non significant. All test performed at 0.05 significance level

The value of AUC, which is indicative of the relative *extent of absorption*, is greater in Formulation B and C than in A but the statistics shows that this difference is not significant ($p = 0.601$).

The parameters C_{max} and T_{max} describe the *rate of absorption*. This also varies widely in humans. Several investigations have shown in relation to the time required to reach maximun plasma levels, *fast* and *slow absorbers* of nifedipine (2, 17, 18) situation that could be consistent with duration of gastric retention time. At least one of these investigations has been conducted using rabbits (6). The value of C_{max} is similar in all the cases and the experimental formulations show a somewhat slowler absorption phase with consequently delayed peak (greater T_{max}) than the reference, which was confirmed by means of the ANOVA ($p = 0.0154$). The LSDT shows that formulations B and C differ from the reference regarding to the absorption rate. But MAT analysis don't show differences between the three formulations ($p = 0.0637$). In this kind of studies, the differences in MAT between

formulations present a direct meassure of *in vivo* drug release retardation. Our findings suggest that the proposed formulations utilizing Gelucires^{R} provides an improved sustained release vehicle for nifedipine.

The elimination half-life of nifedipine is apparently dependent upon the dosage form in which it is administered and the prolonged half-life after oral tablets are administered may reflect more the absorption half-life than the elimination half-life of the drug (24). Looking at Figure 1, we can see that the absorption of nifedipine from formulations B and C is practically slower than the elimination, producing a type of Flip-Flop phenomenom. It has been reported that 24 to 32 hours after tablet administration to humans, nifedipine may still be in process of being absorbed, which may occur due to its precipitation in the intestinal mucosa (3).

The elimination half-life of nifedipine after administration of oral tablets has been found to vary between 6 and 11 hours in humans. In rabbits, we have found that the $T_{1/2\beta}$ moves in a large range, between 1.24 and 11.31 hours. The drug remained longer in the organism in the case of the studied formulations but this difference is not significant because of the great variability found. Neither the differences concerning the MRT are significant ($p=0.0756$).

Only three articles have been published about administration of nifedipine to rabbits: *Nielsen-Kudsk et al* (25) investigate the myocardial accumulation and pharmacokinetics disposition of nifedipine in isolated, perfused and spontaneously beating rabbit hearts. *Umeda et al* (7) administered to the rabbit fast release and sustained release suppositories previously developed by them, and solutions by intravenous route as well, but they do not report the distribution volume values.

Only *Kohri et al* (6) administered orally nifedipine to rabbits (granules with pH-dependent and pH-independent release and commercial fine granules). They found, once more, that in the commercial fine granules, the plasma levels varied notably among the rabbits and were reduced to undetectable levels at 8 h in all cases. Furthermore, the plasma levels following administration of granules from pH-independent sustained release formulation were less variable as compared with the other two formulations. It has been reported that the gastric pH values vary from about 1 to 8 in dogs and humans (26, 27). Such a physiological condition may well be expected in rabbits.

Bioequivalence trials most frequently employ parameters AUC, C_{max} and T_{max} which represent the extent and the rate of absorption. In many cases, these parameters fail to describe absorption process sufficiently. Analog-hybrid simulation was used in order to investigate the influence of certain physiologic and formulation factors on the processes of dissolution, absorption and elimination.

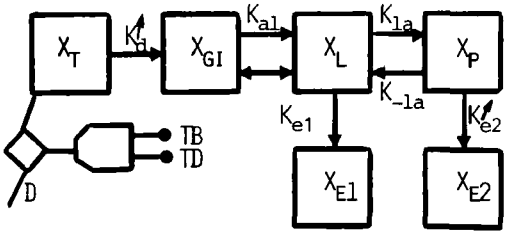

FIGURE 2

Nifedipine Non-Linear Pharmacokinetic Model with Time Varying Parameters and Initial Time Delay. Legend: X_i = *Nifedipine Quantity in i-th Compartment where subscript i means: T = Dosage Form, GI = Gastrointestinal tract, L = Liver, P = Plasma,* E_1 = *Faeces,* E_2 = *Urine;* K_i = *First Order Rate Constant where subscript i means: d = Dissolution, al = Portal Absorption, la and -la = Systemic Absorption,* e_1 = *Elimination Characterizing First-Pass Effect,* e_2 = *Systemic Elimination. D = Dose, TB = Time Base, TD = Time Delay. Sign* $\nearrow$ *means time varibility.*

Considering the literature data about the fate of nifedipine in the body and experimental average plasma concentrations/time curves, a *six compartment non linear model with incomplete dissolution, time varying dissolution and elimination constants and initial time delay* was constructed for humans (28). This model enabled successful fitting also for the individual concentration profiles after single and multiple dosing and, as such, represented a good tool for individual dosage regimen design.

The rabbit is an appropiate experimental animal in pharmacokinetic studies in certain situations (29, 30). Thus, this animal can be used to compare the absorption of a new dosage form to the absorption of a product on the market.

There is almost nothing published regarding pharmacokinetics of nifedipine in rabbits (6, 7, 25) and little on other experimentation animals (31, 32, 33, 34, 35) but it seems that the pharmacokinetics behaviour in species other than humans is similar.

We have modified the pharmacokinetic model developed by *F. Kozjek et al.* (28) for nifedipine in humans because we found in the *in vitro* assays that the dissolution of nifedipine from investigated dosage forms was complete (8).

In Figure 2 is shown the structure of the modified model for rabbits.

This *composed one-compartment model* includes distinct compartments, i.e. gastrointestinal, liver, plasma (central) and two elimination compartments, thus providing the means for better simulation of the physiological phenomena such as presystemic metabolism in the liver and systemic disposition (elimination from the central compartment).

The non linear pharmacokinetics of nifedipine with Michaelis-Menten kinetics (presystemic and systemic elimination) was approached, therefore, to a non lineal model with time varying parameters.

The corresponding mathematical model is given in the form:

$$dX_T/dt = -Kd(t).X_T$$

$$dX_{GI}/dt = Kd(t).X_T - Kal.X_{GI}$$

$$dX_L/dt = Kal.X_{GI} + K\text{-}la.X_P - Kla.X_L - Ke_1.X_L$$

$$dX_P/dt = Kla.X_L - K\text{-}la.X_P - Ke_2(t).X_P$$

$$dX_{E1}/dt = Ke_1.X_L$$

$$dX_{E2}/dt = Ke_2(t).X_P$$

Time delay is given through the following condition:

$D = 0, \ T_B \leq T_D$
$D = D, \ T_B > T_D$ (D = dose)

Time variability of dissolution and systemic elimination constants are defined by the conditions:

$Kd = Kd_1, \ t \leq T_{CD}$ $\qquad$ $Ke_2 = Ke_{21}, \ t \leq T_{CE2}$
$Kd = Kd_2, \ t > T_{CD}$ $\qquad$ $Ke_2 = Ke_{22}, \ t > T_{CE2}$

T_{CD} and T_{CE2} are the moments when the value change of Kd and Ke_2 respectively, occurs.

This model enables the study of nifedipine pharmacokinetics after single and multiple dose application in sustained release formulations. The following phenomena concernig the structure of the model must be clearly explained:

1.- Regarding to time delay, the switch in Figure 2 regulates the set-in of initial condition (D) according to the relation between TB and TD

2.- Time variability of dissolution rate constant is particularly actual in the case of sustained release formulations that may release the drug with the larger constant at the beginning and with the smaller one at the end of the observing interval.

3.- The influence of the first-pass effect is evaluated by the introduction of compartments X_L and X_P, linked to present dynamic equilibrium (36, 37). It is well known that presystemic and systemic metabolism occur in the liver and that systemic elimination constant, Ke_2, characterizes the rate of formation of metabolites. As metabolic processes often obey to enzyme kinetics and we can not determine Michaelis-Menten constants due to having access only to central compartment, it is justified to introduce Ke_2 as time dependent parameter.

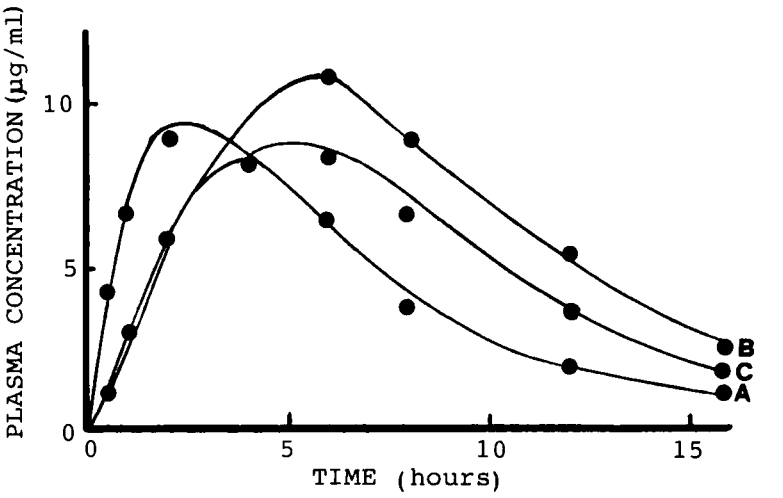

FIGURE 3

Mean Plasma Profiles of Nifedipine Following Single Oral Administration (20 mg) of Formulations A, B and C to Rabbits. Curve Depicts "Model Response" and Symbols "In Vivo" Measured Concentrations.

According to the form of the mathematical model, the analog-hybrid computer was used to perform the simulations. The method assures a straightforward solution of the problem and adequate interpretation of results together with the possibility of modification and completion of the model during the process of simulation.

In the single dose simulation studies, the mean and all individual plasma concentrations/time profiles for the three formulations under study and the reference, were simulated. For all the formulations, a satisfactory agreement between model response and the experimental plasma concentrations, was attained. The mean curves and two representative individual profiles are shown in Figures 3 to 5. In these pictures, curves depict model responses and symbols experimental nifedipine concentrations.

This procedure allowed us to aknowledge the time profile evolution of nifedipine in other compartments different than the sampled (plasmatic) one, i.e. gastrointestinal, liver, and the evolution of pre-systemic elimination and in vivo dissolution on time as well (Figures 5 and 6).

Table 4 shows the means and standard deviations, calculated from individual values, of the identified parameters, manually modulated. Bioavailability and Mean Disolution Time

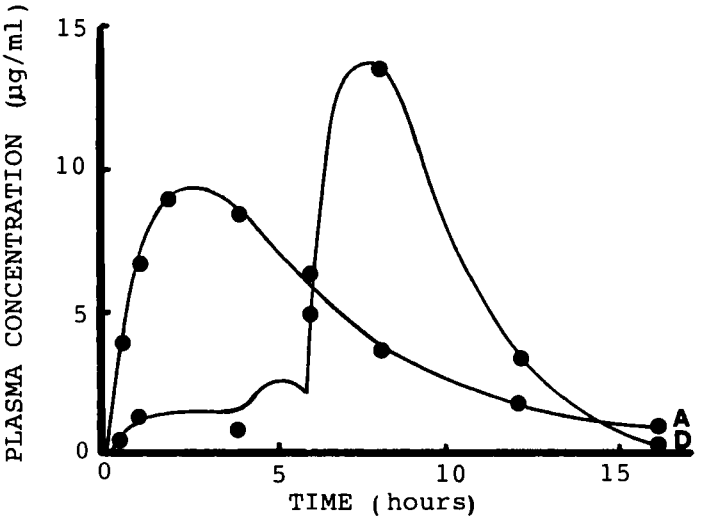

FIGURE 4

Mean Plasma Profiles of Nifedipine Following Single Oral Administration (20 mg) of Formulations A and D to Rabbits. Curve depicts "Model Response" and Symbols "In Vivo" Measured Concentrations.

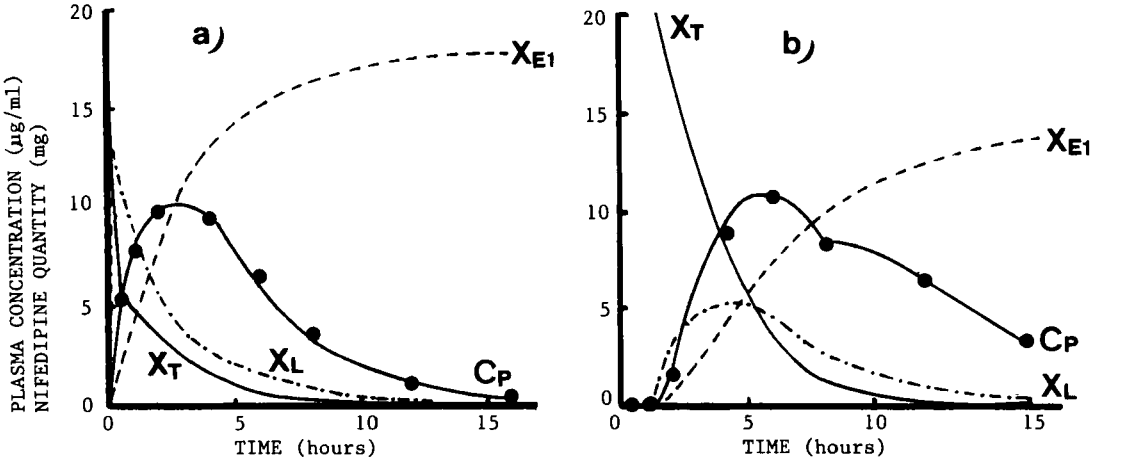

FIGURE 5

Simulated Profiles of Nifedipine in Different Compartments Following Single Oral Administration of Formulation A to Rabbit N° 9 (a) and Formulation C to rabbit N° 11 (b): C_P = Plasma Concentration, X_L = Liver Quantity, X_T = Dosage Form Quantity. X_{E1} = Nifedipine Quantity Eliminated by First-Pass Effect.

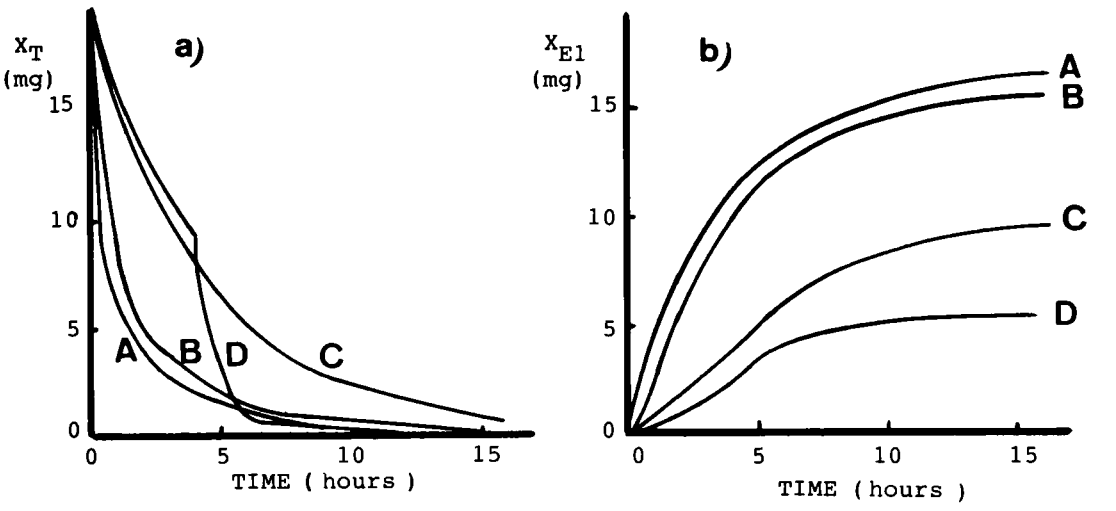

FIGURE 6

Nifedipine Mean Levels in the Dosage Form Compartment (X_T) (a) and Nifedipine Eliminated by First-Pass Effect (X_{E1}) (b) Following Single Oral Administration of Formulations A, B, C and D to Rabbits.

were graphically calculated from the presystemic metabolism and dosage form curves, respectively.

For virtually all of the bioavailability problem products observed by the FDA in the last 13 years, the bioinequivalence has been explainable in terms of poor dissolution on the part of inequivalent product (38). Regarding to nifedipine, the dissolution process is also the rate limiting step in the absorption of nifedipine from the gastrointestinal tract (34).

Our investigations were based on discovering if the reason for the differences in the shapes of the plasma curves lied mostly in the dissolution rate constants because only the adjustment of this parameter resulted in the optimal fit for all experimental curves, while Kal (very high value) remained unchanged.

In order to test possible differences in the in vivo dissolution profiles, we obtained the Mean Dissolution Time (MDT_S) for characterizing the individual curves. The MDT_S was calculated from the simulated levels of nifedipine in the dosage form compartment of the model by numeric analysis. Although the time-varying dissolution rate constant alters the *mean time* nature of this parameter, it is still a numeric measure from the shape of the curves.

TABLE 4

Identified Pharmacokinetic Parameters of Nifedipine for Formulations A, B, C y D (Mean and S.D.)

PARAMETER	F.A	F.B	F.C	F.D
T_D (h)	0.08 ± 0.25	0.22 ± 0.32	0.18 ± 0.42	0.25 ± 0.29
K_{d1} (h^{-1})	3.03 ± 3.48	2.27 ± 3.33	0.45 ± 0.23	0.10 ± 0.06
K_{d2} (h^{-1})	1.23 ± 1.20	0.75 ± 1.00	1.14 ± 1.15	1.01 ± 0.55
T_{CD} (h)	0.98 ± 0.53	1.89 ± 1.37	2.47 ± 1.45	5.17 ± 1.07
K_{e1} (h^{-1})	0.55 ± 0.15	0.43 ± 0.19	0.43 ± 0.17	0.50 ± 0.16
K_{la} (h^{-1})	0.56 ± 0.25	0.59 ± 0.29	0.26 ± 0.10	0.79 ± 0.26
K_{-la} (h^{-1})	0.24 ± 0.14	0.43 ± 0.36	0.10 ± 0.16	0.13 ± 0.25
K_{e21} (h^{-1})	1.16 ± 1.68	4.72 ± 3.52	0.80 ± 0.84	6.29 ± 0.98
K_{e22} (h^{-1})	0.20 ± 0.12	0.48 ± 0.62	0.38 ± 0.25	0.65 ± 0.47
T_{CE2} (h)	4.29 ± 1.18	3.51 ± 0.86	5.72 ± 2.18	6.05 ± 0.90
Vc (l)	0.28 ± 0.15	0.17 ± 0.06	0.21 ± 0.02	0.13 ± 0.03
MDs (h)	1.84 ± 0.82	2.59 ± 0.69	2.08 ± 0.95	2.92 ± 0.56
Fs	0.41 ± 0.11	0.49 ± 0.14	0.39 ± 0.09	0.58 ± 0.13

TABLE 5

Statistical Analysis of MDTs and Fs

PARAMETER	ANOVA	LSD TEST
MDT_S	NS	
F_S	S	ACB D

Looking at calculated values of this dissolution parameter, we could point that dissolution of nifedipine from Formulations D and B is slower (2.92 ± 0.56 h and 2.59 ± 0.69 h) than C and the reference A (2.08 ± 0.95 h and 1.84 ± 0.82 h). But these differences were statistically not significant (for $p=0.05$) when subjected to ANOVA (Table 5), probably due to the great variability among individuals.

In relation to the simulated bioavailability, F_S, we can see after demostrating that differences are significant ($p=0.018$) and applying LSDT, that there are two homogeneous groups (Formulations A, B and C on one side and formulations B and D on another), having

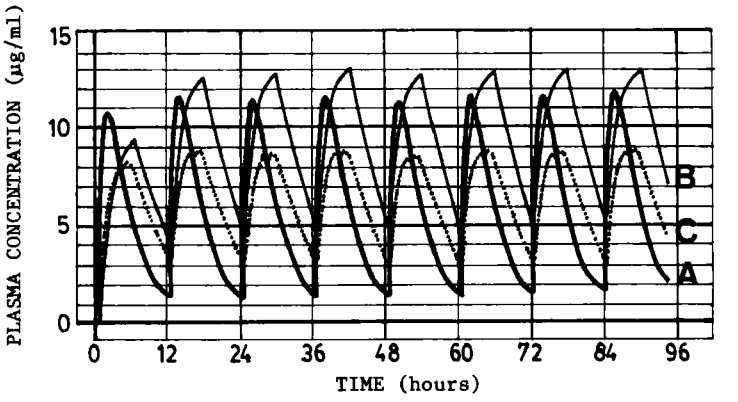

FIGURE 7

Simulated Multiple Doses Plasma Levels of Nifedipine Following Administration of Formulations A, B and C to Rabbits. Dose = 20 mg and Dose Interval = 12 hours.

the latter group greater values of F (0.49 ± 0.14 and 0.58 ± 0.13 respectively) than A (0.41 ± 0.11) and C (0.39 ± 0.09).

Looking at these results we could conclude that formulation C presents similar, and formulations B and D better bioavailability behaviour, compared to the reference regarding the absorption extent.

These findings relate well to the results of model-independent analysis of the same data, previously reported.

Multiple Dose Regimen

During the biopharmaceutical development of a controlled release product, single-dose studies are performed first. The pharmacokinetic profile of the final controlled release product and its dosage regimen should be compared under steady-state conditions with that of a conventional formulation or with another controlled release product (39).

In Figure 7 are depicted the multiple dosing simulated mean plasma profiles of nifedipine.

The structure of the model is the same and the pharmacokinetic parameters used in the simulation of multiple dosing have the same value as in the single dosing simulation. The dose is 20 mg and the dosing interval 12 hours, which is the established dosing interval for the reference Adalat^{R} Retard.

In the case of the reference, no accumulation was observed and oscillations were very big. Formulations B and C presented less oscillations than Adalat^R retard and a slight accumulation coul be appreciated. This would mean a more sustained behaviour of these formulations than the reference.

CONCLUSIONS

1.- A *composed one-compartment model*, including first-pass metabolizing effect, was developed for rabbits

2.- This pharmacokinetic model is a successfull tool for comparative bioequivalence studies using the rabbit as experimental animal.

3.- This model enables the study of nifedipine pharmacokinetics after single and multiple dose application in sustained release formulations.

4.- For all formulations, a satisfactory agreement between model response and the experimental plasma concentrations, was obtained.

5.- The assayed formulations and Adalat^R retard are not different in regarding to bioavailability and the experimental ones show a slightly slowler absorption rate than the reference.

6.- Formulations B and C presented less oscillations than the reference and a slight accumulation could be appreciated. This would mean a more sustained behaviour of these formulations than the Adalat^R retard.

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