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DEVELOPMENT OF AN ORAL SUSTAINED-RELEASE ANTIBIOTIC MATRIX TABLET USING IN-VITRO/IN-VIVO CORRELATIONS

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ABSTRACT

A sustained release (SR) cephalexin tablet formulation containing xanthan gum and sodium alginate as matrix formers was evaluated in human volunteers. The formulation was optimized based on response surface analysis and computer simulation of cephalexin plasma levels versus time curves. The optimized formulation was tested in-vivo in human volunteers along with a fast release (FR) capsule formulation. The SR matrix formulation prolonged the cephalexin blood levels up to 8 hours in humans. The matrix formulation reduced variations in cephalexin plasma levels in individual subjects without any dose dumping as compared to the FR formulation. The plasma levels predicted by the computer program using in-vitro release data and the drug's pharmacokinetic parameters showed excellent correlation with in-vivo data. Using the Wagner-Nelson method, there was good correlation between in-vitro dissolution and in-vivo absorption in individual subjects. The relative bioavailability of cephalexin was reduced by about thirty percent. Very little absorption was seen after six to eight hours. The SR matrix formulation is an alternative delivery method to produce prolonged concentrations.

INTRODUCTION

During the development of a sustained release (SR) dosage form it is very important to establish a meaningful correlation between the in-vitro and in-vivo rate of drug release (1,2). Relating the in-vitro dissolution behavior of a SR dosage form to the complete plasma level time curve represents the type of correlation that would be of interest to both the formulator and clinical pharmacologist. The ability to predict the in-vivo performance of a dosage form based upon in-vitro release, in combination with the drug's pharmacokinetic parameters offers several advantages. This kind of approach eliminates unnecessary, time consuming, costly human studies with formulations having less than desirable in-vivo performance. This approach was used in formulating cephalexin as SR matrix tablets. Xanthan gum and sodium alginate were used as matrix-forming polymers.

Cephalexin is a cephalosporin antimicrobial agent with activity against gram-positive bacteria and modest activity against gram-negative microorganisms (3). Oral therapy with cephalexin results in peak concentrations in plasma of 16 μg/ml after a dose of 0.5 g. Cephalexin has high solubility at low pH (120 mg/ml in simulated gastric fluid) and low solubility at high pH (13-24 mg/ml at typical gut pH, 5 to 8).

The usual parenteral or oral antibiotic regimen results in high peak blood levels that fall well below therapeutic concentrations before administration of the next dose. The commonly accepted optimum approach to the administration of many β-lactams including cephalexin, involves frequent dosing in quantities that will maintain the plasma concentration above the minimum inhibitory concentration (MIC) for the duration of the dosing interval. For cephalexin, a plasma concentration above 1.0 μg/ml is above the minimum inhibitory concentration in-vitro for most susceptible microorganisms (4).

These characteristics coupled with the short half-life (0.9 hours) of cephalexin suggest that cephalexin is a rational candidate for a sustained release dosage form. The currently recommended dosing regimens for the commercially available fast release (FR) oral capsule formulation require 1-4 grams/day in four divided doses. These regimens result in unnecessarily high peak plasma concentration and undesirably low (usually subtherapeutic) plasma concentrations during the later portion of the dosing interval. Several theoretical advantages are associated with a sustained release dosage form for this category of antibiotic: (a) more constant plasma levels resulting in shorter treatment periods, (b) less frequent dosing leading to better patient compliance, (c) overall reduced cost of therapy.

Schneider et al. (5) described an experimental oral prolonged release cephalexin tablet formulation containing 40% carboxymethyl cellulose obtained prolonged blood levels after oral administration for at least six hours.

In this study, a novel cephalexin 750 mg matrix tablet formulation is selected based on computer simulated cephalexin blood levels and tested in-vivo to establish an in-vivo in-vitro correlation. Earlier experiments showed that xanthan gum is an efficient matrix former for sustained release tablets (6), as will be demonstrated, optimum release profiles were not obtained using xanthan gum alone, prompting exploration of xanthan gum in combination with sodium alginate.

MATERIALS AND METHODS

Materials

The following chemicals were used as is to prepare the formulations: Cephalexin monohydrate, USP (Sigma Chemical Co., St.Louis, MO), Xanthan gum (Keltrol TF^R, Kelco, Division of Merck & Co., Clark, NJ), Sodium alginate (Keltone LV^R, Kelco, Division of Merck & Co., Clark, NJ), Lactose (Foremost Whey Products, Wisconsin Dairies, WI), All chemicals used in the in-vivo study met the specifications in the official compendia (USP XXII/NF XVII,1990). Other chemicals used in the analysis of cephalexin were: Sodium acetate, 8-Chloro- theophylline (Sigma Chemical Co., St.Louis, MO), Acetonitrile, Methanol (Fischer Scientific, Fairlawn, NJ), Standard Cephalexin (US Phamacopeial Convention, Inc., Rockville, MD).

Methods

1. Preparation of tablets: Cephalexin powder (750 mg) was mixed with xanthan gum (or xanthan gum and sodium alginate) and lactose and compressed into 1 gram tablets with a hydraulic press (Model C Laboratory Press, F.S Carver, Inc., Menomonee Falls, WI) at a compression force of 3000 pounds for 30 seconds. A two factor central composite design was used to study the effects of xanthan gum and sodium alginate on cephalexin release (7).

The design layout is shown in Table 1. The two factors selected for this study were:

- Concentration of xanthan gum - X1
- Concentration of sodium alginate - X2

A total of eleven formulations were prepared and tested for in-vitro release.

Table 1

Factorial Design: Variable Levels

Independent Variables	-1.414 eu[a]	-1 eu	0 eu	+1 eu	+1.414 eu
X1: Xanthan gum (mg/tab[b]), 1eu=50mg	49.3	70	120	170	190.7
X2: Na Alginate (mg/tab[b]), 1eu=80mg	6.9	40	120	200	233.1

[a] eu=Experimental unit.
[b] 1000 mg tablet contains 750 mg cephalexin, matrix forming polymers and lactose.

2. In-vitro dissolution studies: The dissolution technique utilized a USP apparatus 1, basket with 900 ml of dissolution medium with a rotation speed of 100 rpm. The pH change method, in which simulated gastric fluid (SGF) was used for the first 2 hours and simulated intestinal fluid (SIF) for 10 hours, was employed.

3. Pharmacokinetic simulation: A Computer Program, Pharmacokinetics Simulater (PKS), Rutgers University, College of Pharmacy, NJ was used to simulate plasma drug concentrations from in-vitro data based on published mean pharmacokinetic parameters of cephalexin (8).

4. Analytical methodology: (a) In-vitro samples - Analysis of cephalexin dissolution and assay samples were done by a HPLC method (9). (b) In-vivo samples - Serum and urine samples were also assayed for cephalexin according to a sensitive and specific HPLC assay method (10). The method was validated to establish linearity, precision, accuracy and recovery.

5. Selection of subjects: The study was approved by the Institutional Review Board of Rutgers University, and all subjects gave written informed consent prior to participation. Eight healthy, non-smoking male volunteers 18 years of age or older were selected for participation. All subjects denied hypersensitivity to cephalosporins and/or penicillins. All subjects were screened for normal health status prior to enrollment. Restrictions - Food or beverages containing alcohol or caffeine were not allowed 48 hours prior to and 24 hours during each study period. All subjects fasted for 10 hours prior to receiving the standard or test formulation on each test day.

Clinical Trial

The study involved a two period crossover, open label single dose design with a one week washout interval. Subjects were randomized to group A or group B. Group A received three 250 mg capsules of the standard fast release (FR) cephalexin (Eli Lilly & Co., Indianapolis, IN) in trial period 1 and one 750 mg test matrix tablet in trial period 2. Group B received the alternate formulation in the respective trial periods. At scheduled intervals for a 12 or 16 hour period following drug administration, 10 ml of blood was taken for each sample analysis. Blood samples were treated and assayed for cephalexin. Each volunteer collected urine at scheduled intervals. Urine samples were assayed for cephalexin.

Data Analysis

Mean values for maximum serum concentration (Cmax), time to maximum serum concentration (Tmax), and area under the concentration-time curve (AUC $0-\infty$) were calculated for the matrix tablet and for the fast release capsules. The AUC ($0-\infty$) for each subject was calculated using the trapezoid method. Individual terminal elimination rate constants (K) were calculated from the slope of the terminal portion of a semilogarithmic plot of the serum cephalexin concentration versus time curve from FR capsule data. An exponential curve stripping program, JANA (Statistical Consultants Inc., KY), was used to calculate the pharmacokintic rate constants K_1 and K_2.

RESULTS AND DISCUSSION

Formula Optimization

When xanthan gum alone was used as the matrix polymer, cephalexin release occured more rapidly in acid than in alkaline pH. A burst effect was observed in acid (35 % released in the first hour) which is related to the high solubility of the cephalexin in acid and the time required for hydration of xanthan gum. Increasing the levels of xanthan gum resulted in a decrease in release rate in both dissolution media, more so in simulated intestinal fluid (Fig. 1).

If this type of response carried through in-vivo, absorption would be rapid at first but too slow at later times for complete absorption. To overcome this, a pH dependent polymer, sodium alginate was added to the formulation. As shown in Figure 1, this combination retarded cephalexin release from acid medium somewhat during the first two

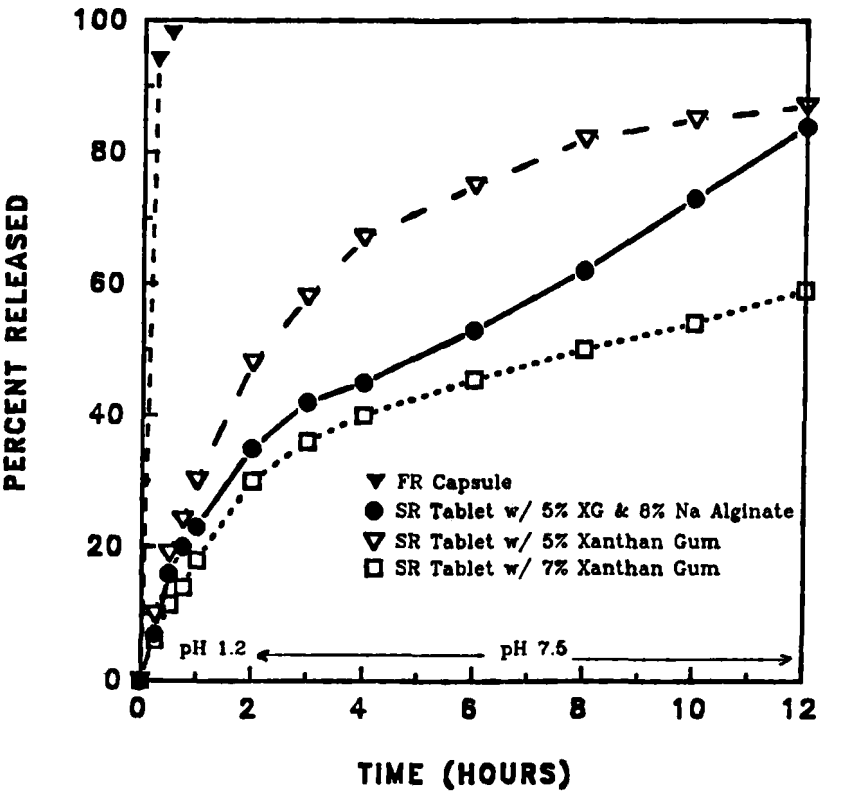

FIGURE 1

In-vitro release of cephalexin from the fast release (FR) capsule and slow release (SR) matrix tablet. (First two hours in simulated gastric fluid, pH 1.2 and ten hours in simulated intestinal fluid , pH 7.5.)

hours, while the release rate in simulated intestinal fluid was more rapid. The combination of polymers produced a dissolution curve that was closer to a linear pattern than that obtained using xanthan gum alone.

A factorial design was used to optimize cephalexin formulation. The following three responses were measured:

1. Time for 50% dissolution (T50%).
2. Time for 90% dissolution (T90%).
3. Apparent cube root release rate constant K.

The data was collected for each formulation trial. A commercially available statistical analysis package, Design-Expert (Stat-Ease, Inc. Minneapolis, MN), was used

to perform regression analysis. All the models generated have confidence values for regression coefficients above 90% indicating good. The Hixson-Crowell cube root dissolution model was used to calculate T50% and T90% release (11).

Contour diagrams were generated by plotting the entire concentration range of xanthan gum (49.3 to 190.7 mg) and of sodium alginate (6.9 to 233.1 mg) to demonstrate the effect of the two variables on the cephalexin release rate. T50% and T90% increased with an increase in concentration of xanthan gum as expected. Only T90% data is shown in figure 2. The figure 2 also depicts the effect of sodium alginate on cephalexin release from matrix tablets. As the concentration of sodium alginate increased, T90% decreased indicating that sodium alginate accelerated the release of cephalexin in simulated intestinal fluid. The rate of dissolution or erosion of tablets containing sodium alginate was faster in SIF due to the high solubility of sodium alginate in this medium. This effect was less significant at higher concentrations of xanthan gum. For example the T90% for tablets containing 190 mg of xanthan gum was not reduced significantly at higher concentration (230 mg) of sodium alginate (Fig. 2).

A Grid search was performed using the computer program toselect the optimized formulation. The program was able to generate 8 sets of conditions for xanthan gum concentration and sodium alginate concentration which will give the most desired release profile using the minimum concentration of xanthan gum and sodium alginate. To test the validity of these predictions, one of the sets of conditions, xanthan gum 49 mg per tablet and sodium alginate 80 mg per tablet was chosen and tablets were prepared using the hydraulic press. The actual and predicted responses are compared in Table 2. As seen from Table 2, these responses are in good agreement, thus confirming the validity of this model.

The FR capsules dissolved completely in less than 45minutes, whereas the matrix tablets containing both polymers released 90% of the cephalexin in about 12 hours (Fig. 1).

Pharmacokinetic Simulation

Based on the pharmakokinetic parameters of cephalexin and the dissolution profiles of three matrix formulations, blood level curves were simulated using the computer program as shown in Figure 3. For the tablets containing 5% XG, there was a sharp rise in cephalexin blood level (open triangles) to about 6 μg/mL at 2.4 hours which then declined rapidly. The curve for the tablet with 7% XG was more even, but projected

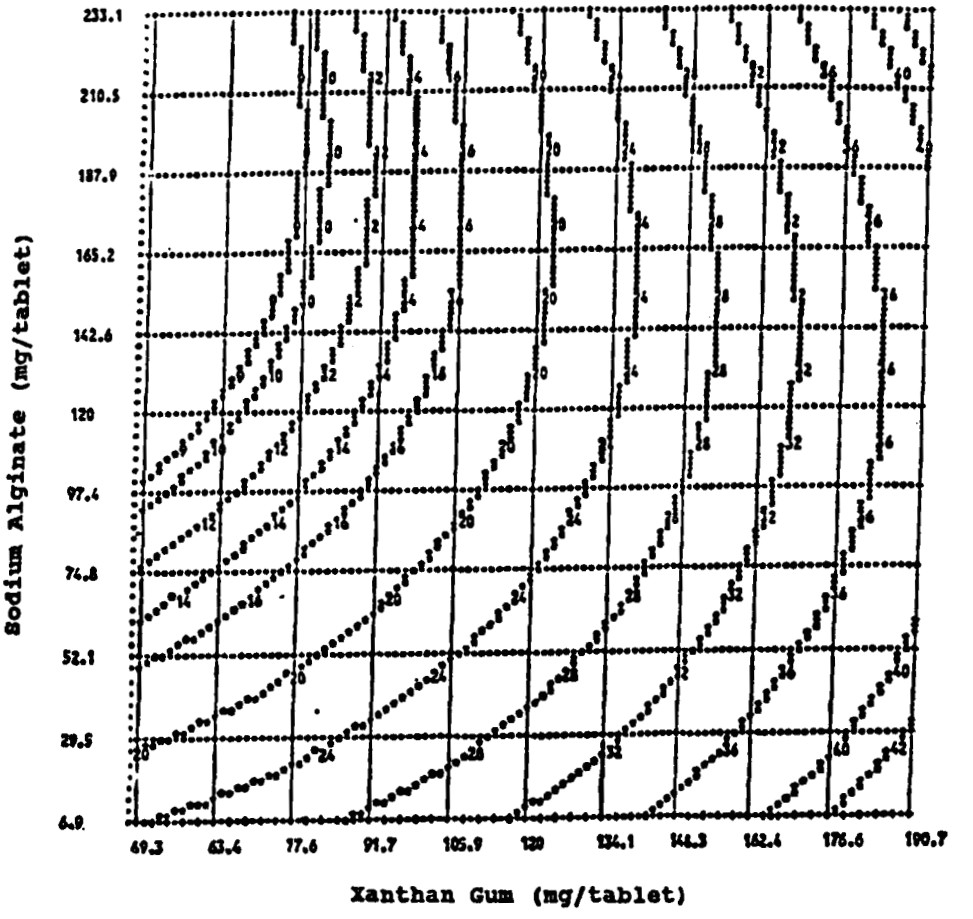

FIGURE 2

Interaction effects of xanthan gum and sodium alginate on time for 90% (T90%) cephalexin release.

T90% (hours): 9,10, 12, 14, 16, 20, 24, 28, 32, 36, 40, 42.

Table 2

Comparison of Actual and Predicted Responses[a]

Y_1 = T50% (Hrs)		Y_2 = T90% (Hrs)		Y_3 = K($\%^{1/3}$/hr)	
Actual	Predicted	Actual	Predicted	Actual	Predicted
5.0	4.4	12.5	11.7	0.18	0.23

[a] For optimized tablet formulation containing xanthan gum, 49 mg/tablet, and sodium alginate, 80 mg/tablet.

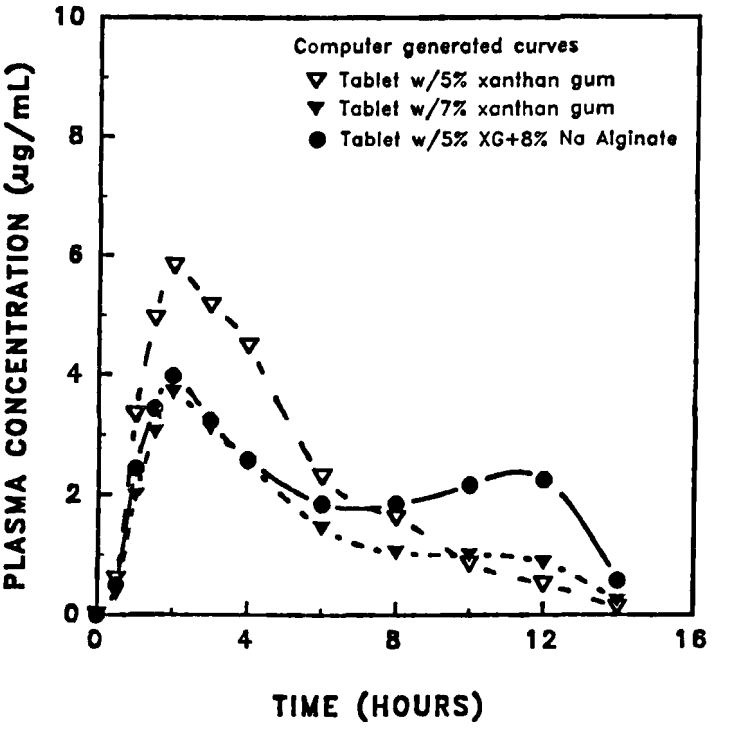

FIGURE 3
Computer generated curves of cephalexin plasma concentrations based on in-vitro data.

levels after 8 hours were marginal. The most desirable profile was obtained with the combination of gums: Cmax was projected to be about 4 μg at 2 hours and then decline slowly to 2 μg and remain at this level for up to 12 hours. The simulated blood level suggested that the optimized formulation containing 5% xanthan gum and 8% sodium alginate would release the drug slowly for at least 8 to 12 hours without causing dose dumping. This tablet formulation was used in the clinical trial.

Pharmacokinetic Analysis

1. Cephalexin plasma data: The mean cephalexin blood level-time curves are shown in Fig. 4. The SR tablet formulation produced significantly lower blood levels in all eight subjects than the FR capsule formulation. Absorption from the SR tablet formulation was continued for at least 6 hours. The FR capsule formulation exhibited Cmax value (mean

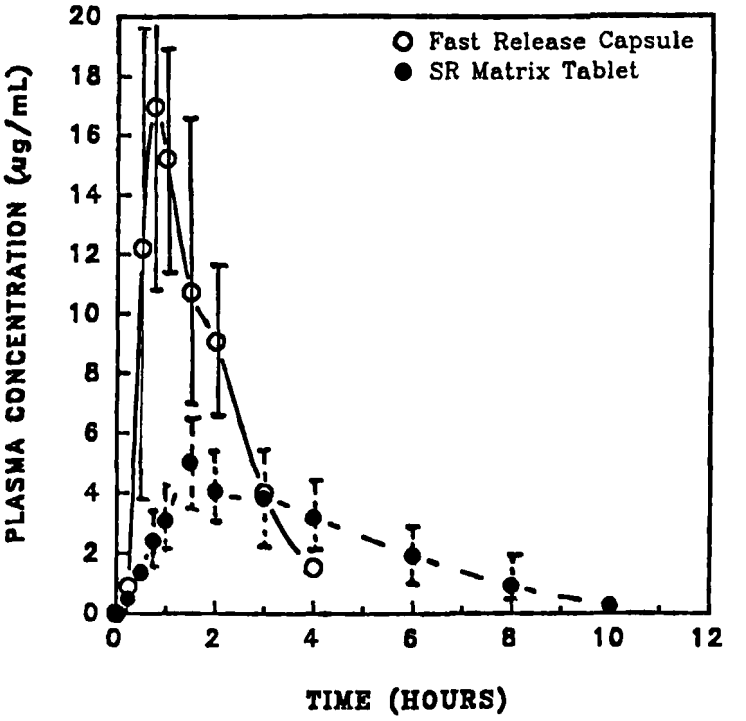

FIGURE 4
Average plasma concentrations of cephalexin as a function of time. (n = 8 human subjects)

21.19±6.06 µg/ml) four times higher than the SR formulation (Table 3). Cmax from the FR formulation was reached in less than 1.5 hours in all subjects and declined rapidly.

Drug liberation from a sustained or controlled drug delivery system usually occurs over a longer period of time than the duration of the distribution phase. In other words, if the release rate constant $<<$ intrinsic absorption and distribution rate constant, a two or higher compartmental model can be collapsed to a simple one compartment model. In doing so, the apparent absorption rate constant is overlapped by and approaches the distribution rate constant (12). Thus, in the case of cephalexin only two exponential terms could be obtained for the blood data and the curves were analyzed using a one compartment model.

The Wagner-Nelson method (13) was used to evaluate theabsorption data from individual subjects. Percent unabsorbed versus time plotted on semilogarithmic coordinates approximated a straight line in each subject. This suggested that cephalexin

Table 3

Cephalexin plasma pharmacokinetic parameters
(Mean of 8 subjects)

	FORMULATION	
Parameter[a]	Fast Release Capsule	Matrix Tablet
Cmax	21.19 ± 6.06	5.65 ± 1.32
Tmax	0.97 ± 0.39	1.75 ± 0.53
AUC 0 to 16	31.07 ± 4.18	22.08 ± 4.69
AUC 0 to ∞	33.26 ± 5.04	23.24 ± 4.71

[a] Units: Cmax-μg/mL, Tmax-hour, AUC 0 to 16 μg.hr/ml, AUC 0 to ∞- μg.hr/mL

was absorbed by an apparent first-order process from the matrix tablet. Table 4 shows the pharmacokinetic rate constants K_1 and K_2. In the case of FR capsules, K_1 is the first order absorption rate constant (Ka) and K_2 is the elimination rate constant (K). But in the case of matrix tablets, continued slow absorption and simultaneous elimination of drug makes it difficult to estimate Ka and K. The rate of absorption of cephalexin from the matrix tablet was much slower than the rate of elimination in all subjects. K_1 for the matrix tablet is close in value to K for the capsule, suggesting that for the tablet, K_1 represents the elimination rate constant and K_2 the absorption rate constant.

Since the drug was not given intravenously, the relative bioavailability was calculated by comparing AUC (from zero to infinity) from the matrix tablet with that of the FR capsule formulation. The ratio of average AUC from zero to infinity was 0.72, whereas the ratio of average AUC from 0 to 16 hours was 0.71.

Incomplete relative bioavailability (less than 75%) was seen in 6 out of 8 subjects. Assuming the FR capsules had good absolute bioavailability (5), the incomplete relative bioavailability shown by the matrix tablet formulation is probably the result of incomplete absorption. This is postulated to be due to release of the drug slowly over an extended period of time in the lower part of the intestine, where absorption may be limited (14-17). Incomplete bioavailability from a SR dosage form may also result if drug was not released within the 12 hour period and voided in the feces or degraded by intestinal bacteria.

Table 4
Summary of pharmacokinetic rate constants

Pharmacokinetic Constant[a]	FR[b] Capsule	Matrix[c] Tablet
K_1/hr	3.3038	0.8121
K_2/hr	0.8342	0.4087

[a] K_1 and K_2 - calculated using an exponential curve stripping program.
[b]For fast release K_1=Ka (first order absorption rate constant) and K_2=K (elimination rate constant)
[c] For matrix tablet, refer text.

Cp was below 1 μg in all subjects after 8 hours possibly due to the failure of cephalexin to be absorbed in the colon after 6 hours. Martinez-Pancheo et al. (18) evaluated Eudragit[R] based sustained release cephalexin formulations in-vivo. They found that the formulation which showed the slowest in-vitro release (about 50% in 3.5 hours) exhibited incomplete bioavailability while bioavailability was complete from the remaining two formulations.

2. Cephalexin urine data : Cephalexin urine data was obtained from only 5 subjects. Average recovery of cephalexin from the FR capsules ranged from 66.1% to 108.5% (mean = 88.9%). Average recovery of cephalexin from the SR tablet ranged from 39.7% to 89.5% (mean = 66.5%). The ratio of average recovery of cephalexin from the matrix tablets to the FR capsules (= 0.75) agrees with the blood data. Based on the plots of average excretion rates of cephalexin at mid point of time periods versus time for the two formulations and cumulative percentage excreted versus time for the two formulations, it can be concluded that cephalexin was slowly released from the matrix tablet. Thus, both plasma and urine data suggest that cephalexin was absorbed slowly from the matrix tablet.

Comparison of Computer Simulated Cephalexin Plasma Levels and In-vivo Data

The computer program was used to simulate cephalexin absorption up to various time intervals and compare it with in-vivo data as shown in Figure 5. Several

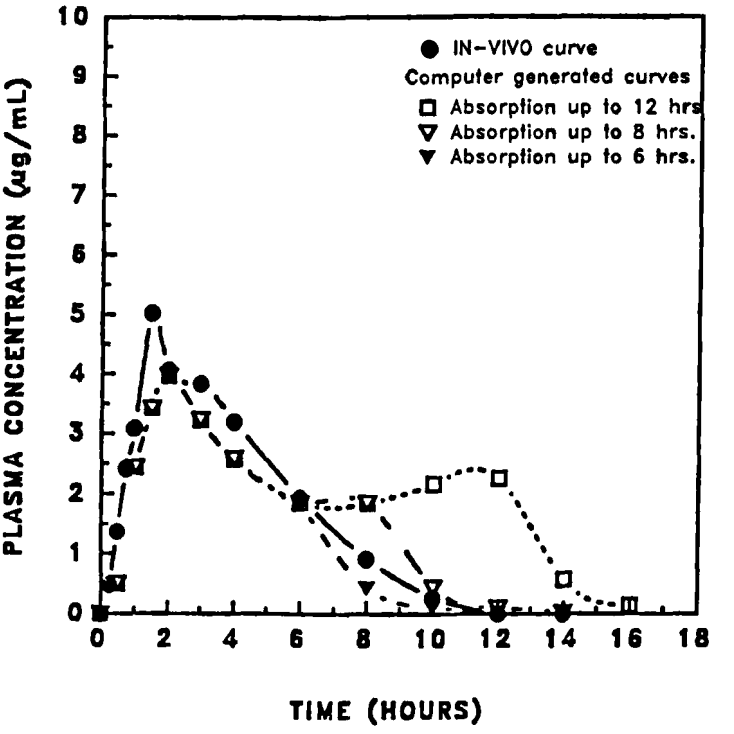

FIGURE 5
Cephalexin plasma concentrations in-vivo and generated by the computer.

assumptions were made regarding the length of time during which absorptin occured. Absorption was assumed to stop after that. The in-vivo curve falls between simulation curves for 6 and 8-hours absorption, suggesting that cephalexin absorption from the matrix tablet was continued for 6 to 8 hours.

As illustrated in Figure 1, the in-vitro dissolution profile showed that 85% of cephalexin was dissolved in 12 hours indicating that cephalexin should have been released after 6 hours. It is possible that for cephalexin the anatomical reserve length for intestinal absorption is quite small (19). It was reported that in humans, small intestine transit time is about 3 hours and 5 - 6 hours were required for pellets or tablets to arrive in the colon (20).

Based on the in-vitro dissolution profile and in-vivo release characteristic of cephalexin matrix tablet, it can be concluded that if the intestinal reserve length for

cephalexin is indeed small, then the usefulness of this kind of formulation for controlling the rate of absorption of cephalexin must depend on the rate of transit through intestine.

In-vivo/In-vitro Correlation

To demonstrate in-vivo/in-vitro correlation for the cephalexinmatrix tablet, the Wagner-Nelson method (13) was used assuming one compartment model as explained earlier. Figure 6 shows fraction of the dose absorbed versus fraction of the dose dissolved from the matrix tablet. Since the Wagner-Nelson plot does not show the extent of absorption, a second plot (shown by closed circles) was constructed to show actual dose absorbed as compared to the FR capsules. A high correlation was seen between fraction dissolved and fraction absorbed.

The Wagner-Nelson method was used to study the correlation between in-vitro dissolution and in-vivo absorption for individual subjects. All subjects showed reasonably good correlation. Thus, the data show that for this matrix tablet formulation, in-vitro dissolution rates are a reasonable reflection of the absorption rate in-vivo.

Statistical Analysis

The ANOVA for crossover studies were applied to Cmax, Tmax, interval area under the curve (0 to 16 hours) and total area under the curve (0 to infinity) (21). Cmax and Tmax are significantly different for the FR and SR formulations ($P < 0.10$) but subjects and period effects are not significant ($P < 0.10$). As discussed earlier and from the statistical analysis, it was very clear that the sustained release tablets lowered Cmax and delayed Tmax substantially as compared to those parameters for the immediate (fast) release commercial capsules.

ANOVA was used to test the hypothesis (Ho) that AUC (0 to ∞) for SR and for FR are the same at 10% level of significance. AUC from zero to infinity for the SR is not equal to FR at 10% levels of significance. This test confirms that cephalexin from the SR formulation was less bioavailable than from the FR formulation.

Since the sample size was small, it may not be possible to predict bio-equivalency of SR formulation with a high degree of power. However, plasma concentrations in all subjects, and values of Cmax and Tmax indicate that the SR matrix tablet formulation prolonged the blood levels for up to at least 6 hours. The SR formulation reduced variation in plasma levels (low standard deviation).

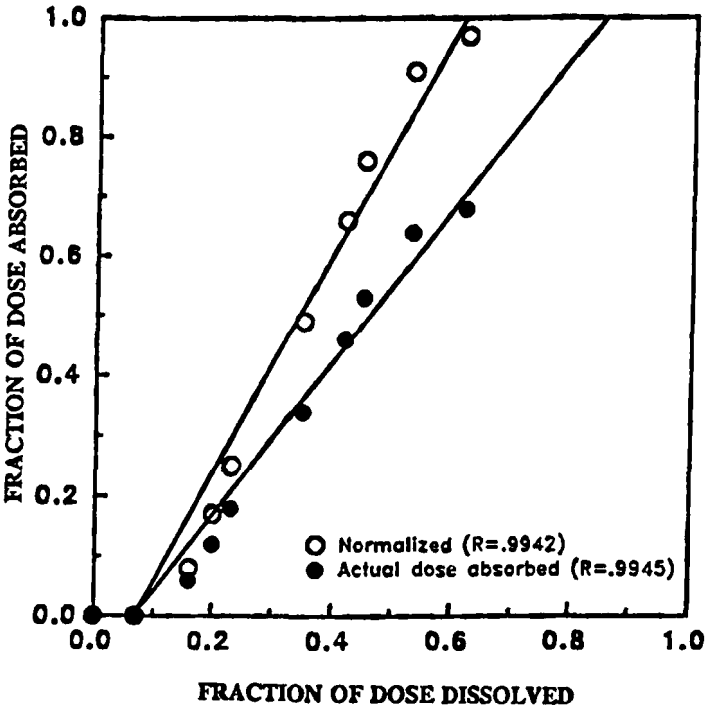

FIGURE 6
In-vitro/in-vivo correlation plot for cephalexin matrix tablet. (Average of 8 subjects)

CONCLUSIONS

A novel tablet formulation containing xanthan gum and sodium alginate as matrix formers sustained blood levels of cephalexin for up to six hours in humans. The SR formulation reduced variations in cephalexin plasma levels in individual subjects as compared to the FR commercial capsule formulation without any dose dumping. The plasma levels predicted by the computer program using in-vitro release data and the drug's pharmacokinetic parameters showed excellent correlation with in-vivo data. Using the Wagner-Nelson method, there was good correlation between in-vitro dissolution and in-vivo absorption in individual subjects. The relative bioavailability of cephalexin was reduced by about thirty percent. Very little absorption was seen after six to eight hours which could be a result of poor absorption from the colon. Other possibilities are that cephalexin was not released within the 12 hour period and the dosage form was voided in the feces, or the drug was degraded by intestinal bacteria.

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