Evaluation of an Oral Prolonged-Release Antibiotic Formulation

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Abstract □ The antibiotic cephalexin was formulated as an oral prolonged-release tablet and evaluated by *in vitro* dissolution testing as well as *in vivo* in 10 human subjects. Comparisons were made of the time course of the blood levels among the prolonged-release formulation, the commercially available capsule, and intravenous administration. Even though lower peak blood levels were attained in the prolonged-release tablet, absorption continued for at least 6 hr. Comparison with *in vitro* dissolution data showed that absorption was dissolution rate limited. Bioavailability comparisons showed that the prolonged-release formulation was completely available, as was the commercial oral capsule.

Keyphrases □ Cephalexin—prolonged-release tablet compared to capsule and intravenous administration, dissolution *in vitro* and bioavailability in humans □ Dissolution, *in vitro*—prolonged-release cephalexin tablet □ Bioavailability—prolonged-release cephalexin tablet compared to capsule and intravenous administration in humans □ Antibacterials—cephalexin, prolonged-release tablet compared to capsule and intravenous administration, dissolution *in vitro* and bioavailability in humans

A prolonged-release oral antibiotic dosage form has several potential advantages compared to nonprolonged release formulations: better patient compliance, more constant blood levels resulting in shorter treatment periods, and reduced cost. 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. Although it is generally believed that antibiotic efficiency is enhanced by wide excursions of the blood levels, which periodically fall below the minimum inhibitory concentration (MIC), this hypothesis has not been demonstrated clinically.

One study (1) indicated that the antibiotic levels must be above the MIC for a specified period to eradicate an infection and that this action is relatively independent of whether the level is achieved by constant infusion or intermittent bolus dosing. Since antibiotic levels may be below the MIC for significant periods after parenteral or oral bolus dosing, there may be unnecessary prolongation of treatment and excess drug consumption. In certain circumstances, a prolonged-release antibiotic regimen may allow outpatient treatment of diseases like bacterial endocarditis, where continuous therapy is indicated after disappearance of clinical manifestations to ensure against relapse.

This paper reports studies conducted with cephalexin to show that prolonged-release oral dosage forms can give reasonable blood levels for extended periods. Studies also were conducted with human volunteers after intravenous and oral administration of commercially available capsules. A comparison was made between the commercial oral capsule and the prolonged-release formulation.

EXPERIMENTAL

In vitro release studies were conducted by placing the test dosage form in a USP dissolution basket and rotating the basket at 120 rpm in the release medium. The release medium was maintained at 150 ml and was thermostated to 37° in a jacketed beaker. Samples were removed at regular intervals and assayed for cephalexin by UV spectrophotometry at 260 nm.

To approximate the pH change encountered as the dosage form travels along the GI tract, the release medium was changed at definite times. Dissolution studies were begun in 0.1 *N* HCl for 1 hr and continued for 1 additional hr in 0.01 *N* HCl; finally, the medium was changed to pH 7 phosphate buffer or distilled water for 3–4 hr. Distilled water was preferred because less degradation of cephalexin occurred in water than in phosphate buffer and little difference in release rate was observed between the two media.

The *in vivo* studies were performed using 10 healthy volunteers (seven males and three females, average age of 25 years). Cephalexin was given on 4 experimental days, with a 1-week interval between each administration. Various blood samples were drawn at 0.5-hr intervals for 3 hr and then hourly for a total of 8 hr after drug administration.

Initially, each subject received 500 mg of cephalexin orally after an overnight fast. The dosage form was two commercial 250-mg capsules[1]. One week later, each subject received a rapid intravenous injection of 500 mg of cephalexin[1] dissolved in 9.6 ml of sodium bicarbonate (8.4%). The solution was administered within 2 min of preparation to minimize degradation. In the 3rd and 4th weeks of the study, each subject received two 250-mg prolonged-release tablets; cephalexin was mixed with the other components as powders and compressed into tablets (12.7 mm in diameter and 6 mm thick) with a hydraulic press[2] at 2500 kg of total pressure for 5 min.

Serum was removed from blood samples after centrifugation and stored at −20° until assayed. Assays were performed by an agar–diffusion method with *Sarcina lutea* (ATCC 9341). Seeded agar plates were prepared as follows. One liter of molten medium[3] was inoculated with 1.0 ml of an overnight growth of *S. lutea*. Aliquots of 9 ml were then dispensed into sterile plastic petri dishes (100 × 15 mm) and allowed to solidify. The samples (15 μl) were spotted on paper disks and incubated at 37° for 24 hr, the inhibition zone was measured, and the samples were compared to standards. All assays were performed in triplicate.

A few urinary excretion studies were used as a screen to establish *in vivo* drug release from the products. Commercial cephalexin capsules and the prolonged-release tablets were administered after an overnight fast with 200 ml of water to two subjects, and drug excretion was monitored as a function of time. Urine samples were obtained initially, every 30 min after dosing for 2 hr, and then every hour thereafter for 3 hr; cumulative urine was collected for a total of 24 hr. The urine samples were centrifuged at 5000 rpm for 5 min and assayed microbiologically.

The serum levels obtained after intravenous and oral (commercial capsule) administration were fit to a two-compartment open model with the aid of a nonlinear least-squares regression program, NONLIN (2). The results were reported previously (3), with NONLIN fits giving $R^2 \geq 0.92$ for all subject data (intravenous and oral). The serum levels obtained after administration of the prolonged-release tablet were not modeled but were graphically compared to the oral capsule data.

Absorption rate constants for the capsule data were calculated from the slopes of semilogarithmic plots of the percentage unabsorbed *versus* time (4) as well as from nonlinear least-squares fits (3). The absorption rate constants obtained by these two methods did not differ significantly (*i.e.*, 1.93 ± 0.91 hr^{-1} from percentage unabsorbed plots and 1.90 ± 0.68 hr^{-1} from NONLIN fits). Bioavailability was determined as the ratio of

[1] Eli Lilly & Co., Indianapolis, Ind.
[2] Model C laboratory press, F. S. Carver, Summit, N.J.
[3] Antibiotic Medium No. 1, Difco Laboratories, Detroit, Mich.

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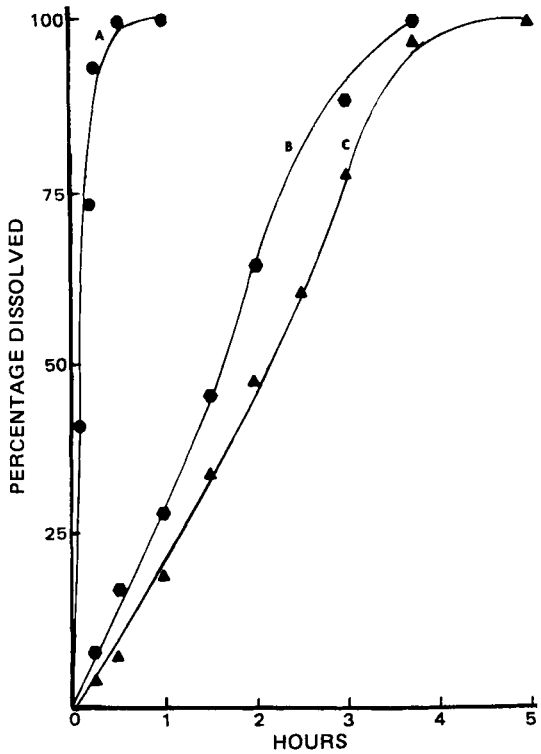

Figure 1—*Dissolution results for cephalexin dosage forms. Key: ●, commercial capsule; ●, prolonged-release tablet containing 50% carboxymethylcellulose and 50% cephalexin; and ▲, prolonged-release tablet containing 40% carboxymethylcellulose, 10% dicalcium phosphate, and 50% cephalexin.*

the total area under the serum concentration–time curve (obtained graphically) for the oral preparations compared to the intravenous administration.

RESULTS AND DISCUSSION

The results from the *in vitro* dissolution studies are shown in Fig. 1. The carboxymethylcellulose formulations (curves B and C) released cephalexin much more slowly than the commercial capsule formulation (curve A), which dissolved completely in 20 min. The retarding effect of the dicalcium phosphate (dibasic calcium phosphate) is seen when curve C is compared to curve B.

The dissolution rate differences between the commercial capsule and the prolonged-release formulation also were reflected in the urinary excretion of cephalexin after oral administration (Fig. 2). These studies (*in vitro* dissolution and urinary excretion) served as a screen to test formulations for the best candidate for further study. Such a formulation contained cephalexin, carboxymethylcellulose, and dicalcium phosphate dihydrate in the ratio of 5:4:1, respectively.

The average blood level–time curves are shown in Fig. 3[4]. The curve for the prolonged-release formulation was constructed without excluding the results from any individual subject. Thus, this curve includes the results from two subjects that showed little prolongation of blood levels and one subject that exhibited significantly delayed absorption. By comparing the average blood level–time curves obtained for the three formulations, three observations can be made:

1. With the commercial oral capsule, absorption was rapid, being essentially complete in 2 hr as signified by the parallel β-phases for the blood levels after intravenous and oral administration.
2. The prolonged-release formulation produced significantly lower blood levels than the commercial capsule.
3. Absorption continued for at least 6 hr for the prolonged-release formulation.

The lower peak blood level with the prolonged-release formulation is not surprising with a well-absorbed antibiotic like cephalexin, because it would be impossible to prolong blood levels and also to achieve the same peak levels as the commercial capsule without increasing the amount of drug in the prolonged-release formulation. Upon multiple dosing at 6-hr intervals, the peak levels for the prolonged-release formulation would rise 1–2 μg/ml, but no significant accumulation would occur upon multiple dosing with the commercial capsule.

[4] Individual subject data are available from the authors upon request.

Table I—Average Percentage Unabsorbed for Oral Cephalexin Dosage Forms

Hours	Commercial Capsule[a]	Prolonged Release
0.50	87.5 (4.0)[b]	92.1 (5.0)[b]
0.75	54.4 (10.6)	78.7 (8.6)
1.00	33.9 (10.9)	85.5 (6.5)
1.50	13.1 (6.9)	63.7 (13.7)
2.00	5.1 (3.4)	49.8 (18.0)
2.50	2.0 (1.5)	38.8 (18.7)
3.00	0.3 (0.2)	28.9 (18.0)
4.00	—	15.6 (14.2)
5.00	—	5.1 (4.0)

[a] Calculated using k_a = 1.9 hr^{-1} with an absorption lag time (t_0) of 0.43 hr (3). [b] Values within parentheses are standard deviations.

Besides prolonging the blood levels, another important consideration with prolonged-release formulations is the extent of absorption or bioavailability. When formulated as prolonged-release products, many well-absorbed drugs exhibit significant reductions in bioavailability. For cephalexin, this formulation was essentially completely bioavailable when compared to the intravenous administration (96 ± 20% with the intravenous taken as 100%). The commercial capsule exhibited 120 ± 16% availability when compared to the intravenous administration. This high value may be accounted for by overfill on the part of the manufacturer and/or loss of potency in the cephalexin powder used to prepare the intravenous and prolonged-release formulations as well as microbiological assay standards. Nevertheless, the prolonged-release formulation is completely available, although with more variability, suggesting that all drug is released from the formulation and available for absorption. Therefore, either the formulation does not travel far down the GI tract in 6 hr or cephalexin can be well absorbed for a long distance along the

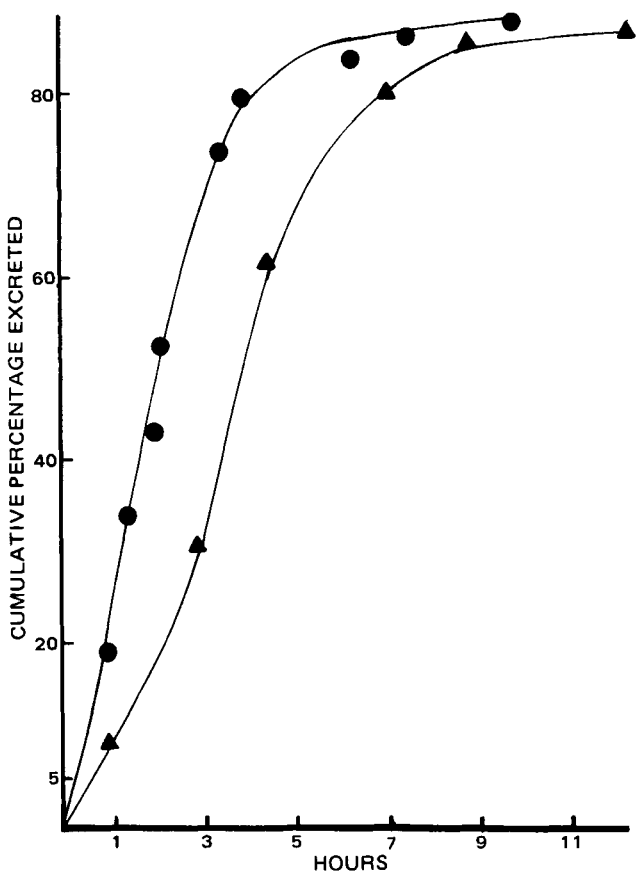

Figure 2—*Urinary excretion data for cephalexin dosage forms. Key: ●, commercial capsule; and ▲, prolonged-release tablet containing 40% carboxymethylcellulose, 10% dicalcium phosphate, and 50% cephalexin.*

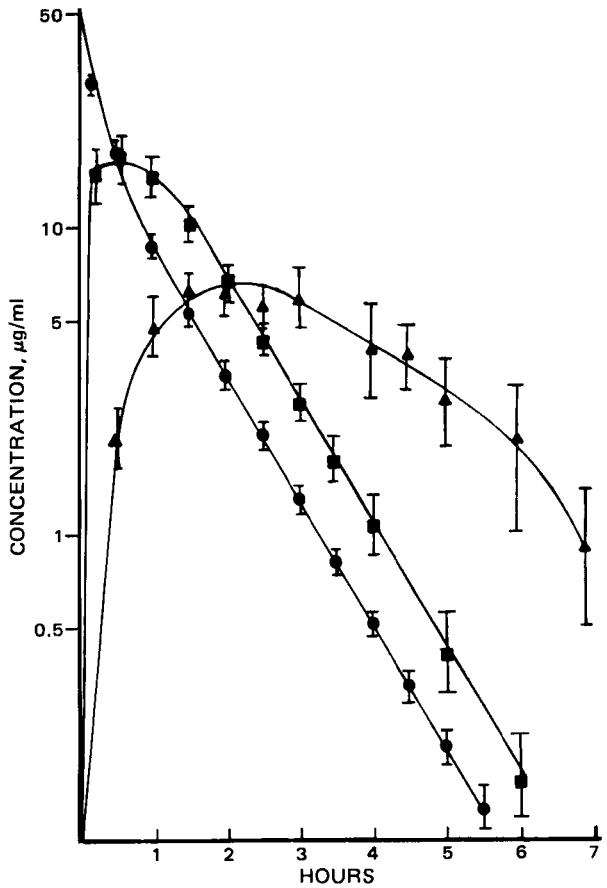

Figure 3—*Average blood level* versus *time data for cephalexin dosage forms. Key:* ●, *intravenous administration;* ■, *commercial capsule; and* ▲, *prolonged-release tablet containing 40% carboxymethylcellulose, 10% dicalcium phosphate, and 50% cephalexin.*

GI tract. Further studies will be required to differentiate between these two possibilities.

Table I shows the average percentage unabsorbed as a function of time for both oral dosage forms. Semilogarithmic plots of these data show that while the absorption of cephalexin from the capsule followed first-order kinetics, the absorption from the prolonged-release formulation followed no single kinetic order. Initially, the log percentage unabsorbed–time plot curves downward with apparent linearity at later times. This nonlinear behavior is most likely the result of the complex release pattern from the dosage form.

Figure 4 depicts the proposed model for drug released from this formulation. Initially, in the acidic conditions of gastric fluids, the dicalcium phosphate dissolves, releasing calcium, which binds to the carboxymethylcellulose. This binding, in turn, decreases the tablet's dissolution rate by decreasing its hydration rate. The cephalexin is released by dissolving in the gelatin matrix and diffusing out. As the carboxymethylcellulose matrix hydrates, the outer layers dissolve or erode from the periphery of the matrix. Thus, the initial matrix hydration, diffusion of cephalexin, and later the erosion of the matrix probably control the release rate, resulting in a system that would not be expected to exhibit a simple first-order release pattern.

To demonstrate that absorption is dosage form limited, Fig. 5 shows a plot of percentage unabsorbed *versus* percentage undissolved. While no correlation was seen between the absorption and dissolution rates of the commercial capsule, there was a high correlation between these rates for the prolonged-release formulation. Thus, for this type of formulation, *in vitro* dissolution rates are a reasonable reflection of the availability rate *in vivo*.

This study showed that cephalexin can be reasonably formulated to give prolonged blood levels after oral administration. Absorption of cephalexin apparently takes place for at least 6 hr, resulting in complete availability. Blood levels probably could be prolonged for more than 6 hr and higher levels attained if more cephalexin is incorporated into the formulation. Since the objective of this study was to determine whether 6-hr prolonged release and complete absorption could be attained, these

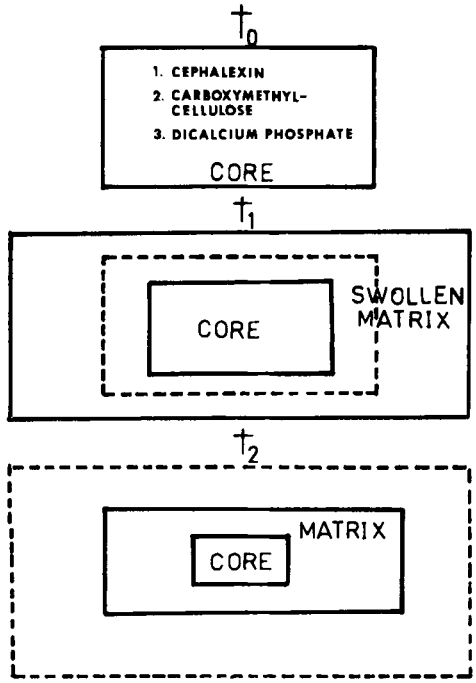

Figure 4—*Physical changes in the prolonged-release tablet at various times. Key:* t_0, *initial dosage form;* t_1, *dosage form shortly after introduction into GI fluids (dashed line represents original tablet size); and* t_2, *dosage form after matrix erosion has begun (dashed line represents swollen matrix size at largest extent).*

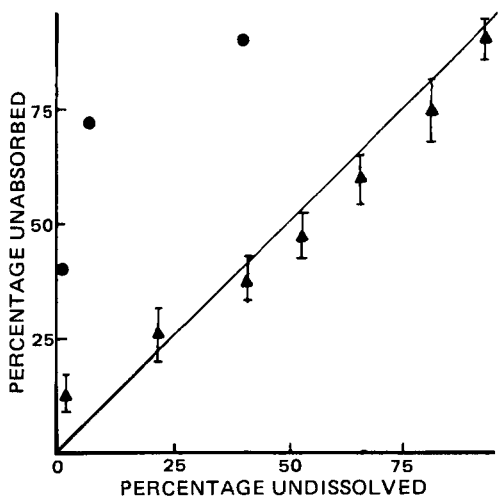

Figure 5—*Comparison of percentage undissolved with percentage unabsorbed. Key:* ●, *commercial capsule; and* ▲, *prolonged-release tablet. The line is a reference line with a slope of 1.*

possibilities were not explored. Moreover, the developed formulation controls the absorption rate of cephalexin, with a good correlation between *in vitro* dissolution and *in vivo* absorption results.

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