Bioequivalency of Doxycycline Products

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Abstract □ The bioavailability of three different brands and three different dosage forms of doxycycline was studied in normal subjects. Single doses, equivalent to 200 mg of doxycycline, were administered to six subjects in a crossover design as the innovator's intravenous solution given orally (Treatment A), the innovator's capsule product (Treatment B), a noninnovator's capsule product (Treatment C), the innovator's oral suspension product (Treatment D), and a second noninnovator's capsule product (Treatment E). All dosage forms contained doxycycline as the hyclate, except the suspension which contained the nonhyclate form. Serum levels were determined periodically over 48 hr, and cumulative urinary excretion was measured concurrently over a 120-hr collection period. No statistically significant differences were observed in any *in vivo* indicator of bioequivalence when the three capsule products were compared. Consequently, they were judged to be bioequivalent. When these capsule products were compared to the oral solution, no statistically significant differences were observed. However, when the capsules and the suspension were compared, statistically significant differences were found in the rate of absorption. *In vitro* dissolution tests were also conducted on the three brands of capsules, and times required to achieve 50% dissolution showed rank-order correlation with corresponding absorption rate constants.

Keyphrases □ Doxycycline products—bioavailability, three different brands and three different dosage forms □ Bioavailability—doxycycline products, three different brands and three different dosage forms

In recent years, numerous products introduced by pharmaceutical manufacturers have been chemical equivalents rather than innovator products. Consequently, the biological equivalency of the available solid, oral, multiple-source dosage forms of a given drug entity has become important and comparative bioequivalency studies have become a means of evaluating the quality and acceptability of such noninnovator products. The tetracycline analog, doxycycline, recently became available on a multiple-source basis. Since cases of bioinequivalence for some chemically equivalent products of the tetracycline antibiotics have been documented (1–4), bioequivalence of different sources of doxycycline cannot be merely assumed.

Two tetracycline antibiotics for which a substantial bioequivalency literature exists, tetracycline and oxytetracycline, were recently classified as "drugs whose solid oral dosage forms it is not possible at this time to state categorically there is, or is not, a potential for bioequivalence . . . problems" in a report of the APhA Academy of Pharmaceutical Sciences (5). In a recent study (6), the 50- and 100-mg capsules of one of the two noninnovator's brands of doxycycline capsule dosage forms were compared and determined to be bioequivalent to the innovator product.

The objectives of the present study were: (*a*) to evaluate the bioequivalency of the two currently commercially available, chemically equivalent, noninnovator brands of doxycycline capsules, using the innovator's capsule product as the standard; (*b*) to study the bioavailability of all three brands of capsules in comparison to equivalent doses of an oral solution and an oral suspension of the drug; and (*c*) to determine the correlation, if any, between the dissolution profiles of the capsule dosage forms and their relative bioavailability.

EXPERIMENTAL

***In Vivo* Bioavailability Study Protocol**—Six healthy, normal, adult male volunteers, 21–30 years old, with normal creatinine clearance values were employed as subjects. Five different dosage forms or brands of doxycycline were administered with approximately 240 ml of water. Each of the five treatments provided a dose of 200 mg of doxycycline, calculated as the base.

In Treatment A, reconstituted doxycycline hyclate for injection[1] was administered as an oral solution; Treatments B[2], C[3], and E[4] consisted of different brands of capsules, each containing doxycycline hyclate equivalent to 100 mg of doxycycline base. Treatment D consisted of an oral suspension[5], which contained 25 mg of doxycycline base, present as the monohydrate, per 5 ml after reconstitution.

Each treatment was administered using a complete crossover design, which was randomized to minimize any possible sequential effects. A time interval of at least 1 week elapsed between treatments, allowing adequate time for essentially complete doxycycline elimination. The subjects fasted at least 3 hr prior to dosing and continued to fast until 3 hr after drug administration.

Blood samples (4 ml) were collected in evacuated glass containers[6] just prior to dosing and at 1, 2, 3, 4, 7, 12, 24, 28, 34, and 48 hr after drug administration. Urine voids were collected at specific intervals and pooled for 120 hr postadministration.

***In Vitro* Dissolution Studies**—*In vitro* dissolution determinations were conducted on the three brands of capsules. Although doxycycline has no compendial monograph dissolution requirement, the general Method I of NF XIV (7) was used to establish and compare dissolution profiles. The mesh screen basket[7] was rotated at 25 rpm in a dissolution fluid of 0.1 *N* HCl. Samples were taken at 3-min intervals, filtered through a 0.22-μm membrane filter[8], and analyzed spectrophotometrically[9] at a wavelength of 268 nm. Doxycycline concentrations in solution were determined from a standard calibration plot prepared using doxycycline base[10] as a standard.

Potency Determinations of Test Products—Maximum concentrations of doxycycline achieved in the dissolution medium were employed as an indicator of the actual potency for each brand of capsule, thus providing information concerning content uniformity. Six oral suspension and intravenous solution products of identical lot number were analyzed by the method used in serum

[1] Vibramycin Intravenous, Lot 28286, Pfizer Laboratories. (This solution also contained 480 mg of ascorbic acid.)
[2] Vibramycin Hyclate Capsules, Lot 31570, Pfizer Laboratories.
[3] Doxychell Hyclate Capsules, Lot 55301E3, Rachelle Laboratories.
[4] Doxy II Hyclate Capsules, Lot 5570563, USV Pharmaceutical Corp.
[5] Vibramycin Monohydrate for Oral Suspension, Lot 27387, Pfizer Laboratories.
[6] Vacutainers, silicone-coated interior, Becton, Dickinson and Co., Rutherford, N.J.
[7] Model 53 stirring motor, rotating-basket assembly, 65-212, Hansen Research Corp., Van Nuys, Calif.
[8] Millex disposable filter unit, SLGS02805-0.22, Millipore Corp., Bedford, Mass.
[9] DB-G spectrophotometer, Beckman Instruments, Fullerton, Calif.
[10] A sample of doxycycline base, Lot 70681-S8052, was generously donated by Pfizer Laboratories.

Table I—Derived Pharmacokinetic Parameters from Both Serum Level and Urinary Excretion Data

Parameter	Treatment					Statistics[a]
	A	B	C	D	E	
Peak value of mean serum concentration–time curve, μg/ml	3.65	3.57	3.53	4.12	3.51	N.S.[b]
Mean value of peaks of individual serum concentration–time curves, μg/ml	3.86	3.68	3.55	4.29	3.69	$p < 0.05$
Mean time of peak values of individual serum concentration–time curves, hr	2.50	2.83	2.83	2.67	3.00	N.S.
Mean of area under individual serum concentration–time curves, μg/ml × hr	80.89	79.08	93.39	94.96	77.97	N.S.
Mean of individual total urinary excretion of unchanged drug, mg	97.69	92.93	85.20	100.16	90.21	N.S.

[a] Analysis of variance for repeated measures design. [b] N.S. = not statistically significant at 0.05 level of significance ($p > 0.05$).

and urine concentration determinations because of excipient interference in the UV spectral analysis.

Fluorometric Determination of Doxycycline in Biological Samples—The analytical technique employed for the determination of doxycycline in urine and serum was based on the fluorometric[11] method of Kohn (8). This method yields results comparable in accuracy and reproducibility to microbiological methods (9).

Statistical Evaluation of Results—Serum concentrations at each time period, peak serum concentrations, areas under the serum level–time curves, and total urinary excretion of doxycycline were analyzed by analysis of variance for a crossover design, using a computerized statistical program (10). This program utilizes a repeated measures design to allow for treatment interactions in the same subject, which would not be seen if each treatment was given separately to different subjects (11).

Any statistical differences found among treatments were further compared to determine differences between treatments by Tukey's Allowable Difference Test (12). Differences in calculated absorption rate constants for each treatment were tested for statistical significance using the Student t test for each possible combination of treatment pairs.

RESULTS

A summary of the average serum concentrations of the six subjects for each treatment at each of the 10 sampling times after drug administration is shown in Fig. 1. The insert in the figure depicts these same values but on an expanded time scale from 0 to 12 hr postadministration. Derived pharmacokinetic parameters from both serum levels and urinary excretion data appear in Table I. The results of the analysis of variance among treatments are recorded in the final column of this table. A significant difference was found among treatments for the average peak values of the individual serum concentration–time curves (Table I) and also at the 2-hr postadministration time (Fig. 1) when the mean serum concentrations were evaluated.

These differences among treatments were further compared by means of a multiple-range test, Tukey's Allowable Difference Test, to determine significant differences between treatments at the 0.05 level of significance (12). At 2 hr postadministration, a statistical difference was noted between the serum levels produced by Treatment D, the oral suspension, and Treatment E, a noninnovator capsule product. A borderline difference was also observed between the suspension serum levels and Treatment C, the other noninnovator capsule product. All other paired comparisons did not produce statistically different results.

When comparing the mean peak values of the individual serum concentration–time curves, a statistically significant difference was observed between Treatments D and C. Borderline differences were noted between the suspension and both Treatment B, the innovator's capsule, and Treatment E. No statistically significant differences were observed in other paired comparisons.

Absorption rate constants, k_a, were calculated for each treatment by fitting the mean serum level data for six subjects to the classical one-compartment pharmacokinetic model (13), using a nonlinear least-squares regression computer program (14, 15) adapted for use on the PDP-10 system[12]. The k_a values determined were as follows: Treatment A, 1.38 hr^{-1}; Treatment B, 0.91 hr^{-1}; Treatment C, 0.87 hr^{-1}; Treatment D, 1.32 hr^{-1}; and Treatment E, 0.88 hr^{-1}. No statistical significance was observed when these values were compared using a two-tailed Student t test at the 0.05 level of significance.

A summary of the *in vitro* test results is provided in Table II. Six capsules of each manufacturer's product were studied, and the dissolution profiles of the three capsule products are presented in Table II. The mean cumulative amounts of doxycycline dissolved are reported for each time up to one interval past the maximum amount dissolved. The time to achieve 50% dissolution was calculated after the dissolution data were fitted to a model describing the dissolution profiles of conventional capsule dosage forms (16).

Table II also shows the actual amount of active drug present in each capsule dosage form. These values were derived from the dissolution profiles. The maximum amounts of drug in solution were averaged for each of the six capsules used to study a certain manufacturer's product. For the oral solution and suspension, six containers with identical lot numbers were assayed by the method previously described, and the average amounts of active drug present in the solution and suspension dosage forms were 107.2 ± 3.4 and 112.9 ● 3.2 mg, respectively.

DISCUSSION

Bioavailability involves both the rate and extent of drug absorption. In this study, the bioavailability of two chemically equivalent capsule formulations was compared to a recognized standard, the formulation of the innovator whose efficacy has been documented by clinical experience. Chemically equivalent products that achieve the same bioavailability profile as measured by appropriate *in vivo* parameters are generally assumed to produce the same therapeutic effects (17).

Parameters used to compare the extent of bioavailability or the amount of drug absorbed from the formulations are the area under the serum concentration–time curve and the amount of unchanged drug excreted in the urine. According to an evaluation of these two parameters, the three different brands of doxycycline capsules did not produce any statistically significant differences. Therefore, the amount of drug absorbed from each was essentially equal for the subjects tested.

[11] Model 110 fluorometer, Turner Associates, Palo Alto, Calif.

[12] Digital Equipment Corp., Maynard, Mass. (The assistance of the University of Pittsburgh computer staff is acknowledged.)