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.)

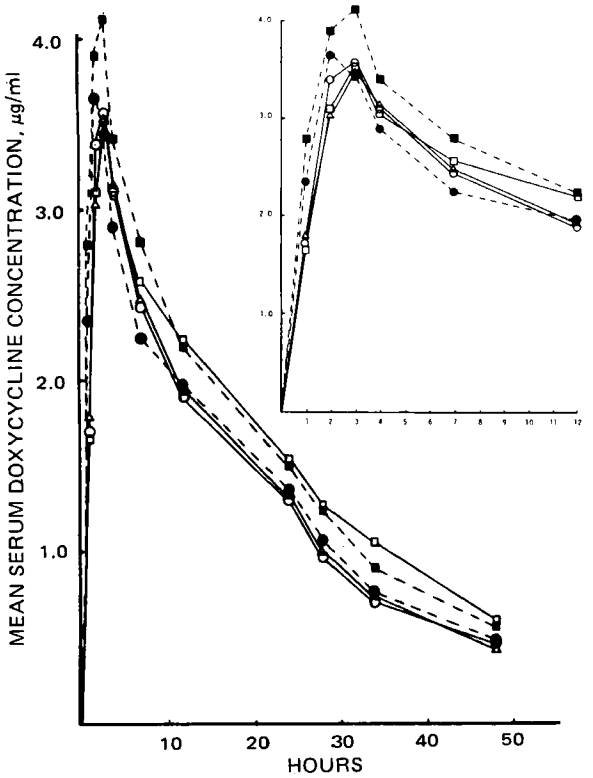

Figure 1—*Mean serum concentrations for six subjects following five treatments (0–48 hr). Insert shows expanded 0–12-hr data. Key: ●- - -●, Treatment A; ○——○, Treatment B; □——□, Treatment C; ■- - -■, Treatment D; and △——△, Treatment E.*

To be effective, doxycycline must be present in the blood and tissues at certain concentrations for certain durations of time. In comparing the three brands of capsules, no statistically significant differences were observed in serum concentrations, including the peak serum concentrations, at any sampling time period studied. The peak serum concentrations were compared both for the means of the six subjects and also on an individual subject basis, with no observed statistical difference.

Differences in rate of absorption can be compared by evaluating differences in peak times. The insert in Fig. 1 shows that mean serum concentration plots for the six subjects result in peak times of 3 hr for the three brands of capsules. Analysis of mean peak times of the individual serum concentration–time curves for the three capsule formulations tested revealed no statistically significant differences for this parameter. Therefore, on the basis of bioavailability profiles, these three capsule brands were judged to be similar in both rate and extent of absorption and may be termed bioequivalents.

A conclusion of bioequivalency for doxycycline products was also determined in another recent study (6) involving the 50- and 100-mg capsules of the innovator and one noninnovator[13]. Plasma levels were determined by a microbiological assay in 12 volunteers from 0 to 12 hr postadministration of a 100-mg dose of the drug. Each capsule was administered after a 1-hr fast in a randomized crossover design. No statistically significant differences were observed in mean plasma levels, areas under the plasma level curves, and the times for attaining the peak plasma levels (6). Although there were differences in the protocols and the observed peak times, both studies show bioequivalence for the brands of doxycycline capsule products studied. Differences in the protocols of the two studies included the number of subjects tested, assay methodology, biological samples tested, sampling times and duration, fasting period, dose of drug given, and number of different brands and dosage forms tested.

In comparing the bioavailabilities of the capsule dosage forms to those of the oral solution (Treatment A) and oral suspension (Treatment D), the parameters evaluated were the same as those used in determining the bioequivalence between capsule products. Statistically significant differences ($p < 0.05$) were observed for two of these parameters. The first parameter occurred at the 2-hr postadministration sampling time, with a statistically higher mean serum concentration produced by the oral suspension (Treatment D) as compared to one generic capsule (Treatment E). Differences at this early sampling time, which is prior to achieving the peak concentration, reflect differences in the rates of drug absorption. Since a drug must undergo dissolution before it can be absorbed, the suspension may present the drug in a form that permits it to undergo more rapid dissolution, leading to a higher serum concentration in the prepeak time periods. Another possible explanation for this result may be related to the fact that the suspension dosage form contained the nonhyclate (nonsalt) form of doxycycline, whereas all of the other dosage forms in this study contained the hyclate (hydrochloride salt) form.

The second statistically different parameter resulted from the peak serum concentrations produced in the individual subjects. Statistically higher ($p < 0.05$) concentrations were produced by the suspension (Treatment D) than by one noninnovator capsule (Treatment C). Although the peak serum levels of Treatment D showed a trend toward higher levels as compared to the other two capsule products (Treatments B and E), these differences were just short of statistical significance. These differences could be related to the different forms (salt *versus* nonsalt) of doxycycline involved or to other formulation differences between the capsules and the suspension. Since there were no differences in the compared areas under the serum concentration–time curves between capsules and suspension, a faster rate of absorption might have been responsible for producing the higher peaks, rather than differences in the active amounts of doxycycline present in the different dosage forms.

When the dissolution rates, as measured by the time required to achieve 50% dissolution, and the corresponding absorption rate constants of each capsule formulation were compared, rank-order correlation was observed. The innovator's capsule formulation (Treatment B) corresponded to the shortest time to reach 50% dissolution and the greatest absorption rate, as indicated by the k_a. The noninnovator's formulations followed in numerical ranking, with Treatment C corresponding to the longest time to reach 50% dissolution and the smallest absorption rate constant. However, since there were no statistically significant differences among any of the *in vivo* parameters studied, including the calculated rate constants of absorption, for the three capsule products, dissolution data could presumably represent a range of acceptable values.

This study was undertaken to ascertain the bioequivalency of

Table II—*In Vitro* Dissolution Data for Three Manufacturers' Capsule Dosage Forms of Doxycycline

Minutes	Mean Cumulative Amount Dissolved for Six Capsules (mg ± *SD*)		
	Manufacturer 1[a]	Manufacturer 2[b]	Manufacturer 3[c]
3	1.5 ± 0.9	3.1 ± 1.3	1.9 ± 1.3
6	30.6 ± 2.7	40.4 ± 6.4	35.9 ± 8.8
9	50.8 ± 5.4	67.9 ± 5.3	58.2 ± 10.4
12	63.0 ± 6.7	87.7 ± 10.0	72.2 ± 13.0
15	72.3 ± 8.5	99.8 ± 7.5	81.0 ± 13.4
18	82.4 ± 9.8	104.4 ± 7.9	88.7 ± 14.1
21	88.6 ± 10.6	106.0 ± 6.1	92.7 ± 12.7
24	94.4 ± 11.2	104.9 ± 6.1	96.5 ± 11.1
27	97.7 ± 9.9	—	99.2 ± 10.2
30	100.3 ± 8.6	—	101.6 ± 8.7
33	101.1 ± 6.6	—	102.6 ± 7.0
36	102.9 ± 5.7	—	102.6 ± 6.9
39	102.4 ± 5.7	—	102.0 ± 6.1
Time (minutes) for 50% dissolution	9.33	7.12	7.77
Mean capsule potency, mg ± *SD*	103.4 ± 6.2	106.1 ± 6.0	103.3 ± 6.5

[a] Rachelle Laboratories. [b] Pfizer Laboratories. [c] USV Pharmaceutical Corp.

[13] Doxy II Hyclate Capsules, USV Pharmaceutical Corp.

the three currently commercially available capsule dosage forms of doxycycline hyclate and to compare their bioavailability profiles with those obtained from oral administration of an equivalent amount of doxycycline hyclate in solution form or an equivalent dose of doxycycline base in suspension form. Through the use of serial serum determinations and cumulative urinary excretion data collected in a single-dose study of crossover design, *in vivo* parameters were compared.

No statistically significant differences among the three capsule products were found, and the products were judged to be bioequivalent. This finding corroborates one other study (6) which concluded bioequivalency between two of the three brands employed in this study. When these same capsules were compared to the oral solution and suspension, differences were found in one prepeak mean serum level. *In vitro* dissolution tests were conducted on the three capsule products; time for 50% dissolution showed rank-order correlation with corresponding absorption rate constants.

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Stability of Aspirin in Liquid and Semisolid Bases V: Polyglycerol Esters

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Abstract □ The stability of aspirin in decaglycerol tetraoleate, decaglycerol octaoleate, and decaglycerol decaoleate was studied at 4, 26, and 45°. Degradation of aspirin in these polyglycerol esters was temperature dependent. Aspirin demonstrated the greatest stability in decaglycerol octaoleate and the lowest stability in decaglycerol tetraoleate at all temperatures studied. The hydroxyl value and the viscosity of the polyglycerol ester appeared to influence the stability of aspirin.

Keyphrases □ Aspirin—solubility and stability in polyglycerol esters, effect of temperature, hydroxyl value, viscosity □ Polyglycerol esters—as solvents for aspirin, stability, effect of temperature, hydroxyl value, viscosity □ Stability—aspirin in decaglycerol tetraoleate, decaglycerol octaoleate, and decaglycerol decaoleate

In previous reports (1–4), the stability of aspirin in various liquid and semisolid bases was investigated. The decomposition of aspirin in polyethylene glycols was due, at least in part, to a transesterification reaction between aspirin and polyethylene glycols (5). Blocking free hydroxyl groups on the polyethylene glycols retarded the decomposition of aspirin (1).

Polyglycerol esters are synthetic products and may be hydrophilic or lipophilic, depending on the number of hydroxyl groups that are reacted with the fatty acids and/or oils in question (6). The consistency of these esters varies from waxy solids and semisolids to liquids (6). Feeding studies (7) indicated that such esters are completely nontoxic and are degraded fully by the body to yield glycerol and the fatty acid. The Food and Drug Administration approved the use of polyglycerol esters ranging from 2 to 30 moles of glycerin and placed no limits on the amounts for use (6).

Decaglycerol tetraoleate, decaglycerol octaoleate, and decaglycerol decaoleate are liquid at room temperature and possess viscosity values of 6000–8000,