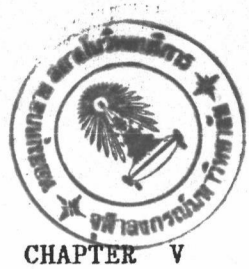

CHAPTER V

DISCUSSION

In Vitro Studies

Eight commercial brands of doxycycline capsules were first tested for uniformity of weight, content of active ingredient and content uniformity. Each of them met the BP requirement for the uniformity of weight which set the standard for capsules containing less than 300 mg as follow:- not more than two out of a sample of twenty capsules may be outside ± 10% of the mean weight, and all must be within ± 20%. Content of active ingredient of each brand was within the 90-120% limits as specified in the USP XXI monograph [49]. For content uniformity; since the content in two out of the first ten capsules of brand A were out of the range of 85.0-115.0% as shown in table 3, twenty additional capsules were required to confirm the test. However, the final result indicated that all eight commercial brands studied met the USP XXI requirement for content uniformity. These results supported the assumption that all various brands studied were pharmaceutically equivalence.

At the same time, each of the eight brands of doxycycline capsules studied met the requirement for disintegration tests according to USP XXI under capsules. All capsules disintegrated in water within 15 minutes. The rank order in terms of the mean disintegration time was brand B <E <F <H <A <D <G <C as shown in table 2. There were statistically significant difference among brands as described by the analysis of variance and pairwise statistical comparison of the disintegration time reported in table 5. These

differences may be due to the differences in type and amount of the excipient filled in different brands of doxycycline capsules since the rate-controlling step in capsule disintegration is the formulation of the fill rather than the gelatin shell itself [48].

From the compendial monograph dissolution requirement of USP XXI [40], the amount of drug which is not less than 85% of the labelled amount must be dissolved in medium in 60 minutes. All eight commercial brands studied passed this requirement.

The dissolution rate constants of each brand were reported in table 6. Rank order in term of dissolution rate constants was brand H > A > B > E > G > F > D > C. Analysis of variance indicated that there were statistically significant difference among eight different brands as shown in table 7 and the Student's t-statistics showed that brand C, D and F were significantly different form brand A [$P<0.05$] while brand B, E, G, H were not. Besides, no significant difference was found among brand C, D, E, F and G. The difference in dissolution rate constants was due to the variation in the excipients and from the process of formulations. The excipients in the drug product could affect dissolution kinetics of the drug by either altering the medium in which the drug is dissolving or by reacting with the drug itself [6].

In Vivo Studies

The eight commercial brands of doxycycline capsules studied were classified into three groups according to their dissolution rate constants. A representative from each group was chosen to assess the bioavailability of the local products relatively to the original product.

1. Analytical Method

From the validation of analytical method shown in appendix I, the % C.V. values obtained from the between-run precision are rather high. These might be because of the crowded use of HPLC apparatus and the experience of the investigator.

The percent recovery of doxycycline in plasma is rather scattered among the concentrations used. This shows that doxycycline recovery in plasma is independent upon its concentrations. Although the recovery values of tetracycline [internal standard] are rather low, they are also independent upon its concentrations.

The low % recoveries of internal standard was compensated by using the higher concentration of internal standard in analysis in order to keep the peak height ratio of the calibration curve within the range of 0.1-10.

2. Plasma Doxycycline Level

The mean plasma doxycycline concentrations of each brand [A, B, C and D] were illustrated in figure 5 while the individual plasma doxycycline concentrations versus time profile was illustrated in appendix J. Several individual plasma concentration-time profile [figures 9-28] showed the secondary peak approximately ranged from 7 to 12 hours after the intake of doxycycline capsule. This secondary peak would probably be due to the enterohepatic recycle of the drug as has been previously reported.

3. Bioavailability of Doxycycline

The Pharmacokinetic parameters were obtained from both compartmental and noncompartmental methods. In compartmental method, both CSTRIP and PCNONLIN programs were applied to analyze the pharmacokinetic parameters. From CSTRIP program, the adequancy of the simple one-compartment open model to describe the serum level profile was indicated by its high coefficients of determination, r^2. When the PCNONLIN program was applied, all plasma data were assumed to follow the classical one compartment model with or without a lag time.

In the present study, the pharmacokinetic parameters and statistical results, calculated by different methods and programs, described a few unlike results as shown in table 46, 47, and 48 respectively. These may be due to the differences in the assumptions and the methods of calculation of the programs. The values obtained from CSTRIP program calculated by stripping while those obtained from PCNONLIN program calculated by estimating from initial pharmacokinetic paramaters. In addition, AUC_o^∞ obtained from noncompartmental program were calculated by trapezoidal rule, but AUC_o^∞ of CSTRIP program were obtained using the values from stripping and then calculated according to the compartment proposed.

Although statistical results of AUC_o^∞ calculated from different programs showed a few unlike, but the values of AUC_o^∞ of brand A, B, C, and D showed no significant difference [$P > 0.05$] among programs as presented in table 49, 50, 51, and 52 respectively. These indicated that pharmacokinetic parameters obtained from different programs were not statistically significantly different.

The bioequivalence study of the drug product involved both the

rate and extent of drug absorption. The paramter normally used to indicate the extent of drug absorption is the area under the plasma concentration-time curve [AUC] and the parameters used to indicate the rate of drug absorption could be the absorption rate constant [Ka] and/or the time to peak plasma concentration [Tmax] while the peak plasma concentration [Cpmax] involved both the rate and extent of drug absorption.

In this bioequivalent study, the AUC_0^∞ obtained from noncompartmental program and the Ka values from either PCNONLIN or CSTRIP program were used. Since the Cpmax and Tmax values reading directly from plasma concentration-time curves were more realistic than those obtained from PCNONLIN and CSTRIP program, they are used for the interpretation of the data.

3.1 The Parameters Used to Indicate the Extent of Drug Absorption

3.1.1 Area Under the Plasma Concentration-Time Curve [AUC]

The AUC_0^∞ is related to the extent of the drug absorbed systemically. The AUC_0^∞ of brand A (original product) was less than the AUC_0^∞ of brand C, D, B [table 46] indicating that the amount of drug absorbed, when brand A [original product] was consumed, was less than those obtained from the other three brands [local manufactured products]. These may be due to the content of each capsule of brand A are rather less than those of the other brands [C, D, B] as shown in table 3.

3.1.2. The Peak Plasma Concentration

Statistical comparison indicated that the mean peak plasma levels of these four commercial brands studied were significantly different [P<0.05]. The rank order for peak plasma level obtained from reading directly and PCNONLIN program was A < D ~ C ~ B [table 46 and table 48].

The peak plasma levels obtained from the present study were within the range as those previously reported. Malmborg [20] reported the mean peak plasma concentration obtained from twelve healthy volunteers after a single dose of 100 mg doxycycline to be 1.7 µg/ml while Schreiner & Digranes [22] reported the peak level to be 2 µg/ml [n = 8]. The possibility of the difference in the level found would be due to the diffferences in the number of volunteers studied and also intersubjects variation occurred during study.

3.2 The Parameters Used to Indicate the Rate of Drug Absorption.

3.2.1. The Absorption Rate Constant [Ka]

The absorption rate constants obtained from compartmental analysis using either CSTRIP or PCNONLIN program, as reported in table 47 and 48, gave the same conclusion indicating that the absorption rate constants were not significantly different among brands [P>0.05].

3.2.2. The Time to Peak Plasma Concentration [Tmax]

In contrast to the absorption rate constant,

the time to peak plasma obtained from the method of compartmental analysis using CSTRIP program showed significant difference among brands. While the results obtained from reading directly from the plasma concentration-time curve and the PCNONLIN method showed no significant difference in peak time among brands. However, the time to peak plasma obtained from this study was in good agreement with those reported by previous investigators. Malmborg [20] reported that the time to peak plasma was ranged from 1.5 to 4 hours in healthy volunteers following a single 100-mg oral administration. The mean time to peak plasma obtained from this study using the method of reading directly from the plasma concentration -time curve, using CSTRIP and PCNONLIN program were ranged from 1.6 to 2.3 [table 46], 2.2 to 3.7 [table 47] and 1.7 to 2.4 hours [table 48] respectively.

The absorption rate constant did not show significant difference among brands and the time to peak plasma concentration obtained by reading directly from the plasma concentration-time curve also showed no significant difference while the time to peak plasma concentration calculated by CSTRIP program were significantly different among brands. Since the time to peak plasma concentration obtained by simply reading directly from the individual plasma concentration-time curve seem more realistic to the observer than those calculated by CSTRIP program which obtained after mathematical treatment of the data. Therefore, it should not be overestimated to conclude that among the four brands of doxycycline capsules studied, there was no significant difference in the rate of drug absorbed into the circulation.

4. Other Pharmacokinetic Parameters

Elimination Rate Constant and Half-life

The elimination rate constants and the plasma half-life obtained from either noncompartmental or compartmental method showed no significant differences among brands [P > 0.05]

The non-significant difference of elimination rate constants and the plasma half-life should be explained that doxycycline of every brand can be eliminated in the indifferent rate.

The pharmacokinetic parameters obtained from this study were slightly different from those reported by previous investigators. These may be due to the differences in the formulation used, the subjects participated in the studies, the method of plasma doxycycline analysis, the mathematics model applied and the assumptions in the computer program used to interpret the data.

In Vitro - In Vivo Correlation

The relationships between the in vitro characteristics, such as disintegration times and dissolution rate constants, and in vivo parameters, i.e., AUC_0^t, AUC_0^∞, and Cpmax were studied as shown in talbe 53. The in vivo parameters Ka and Tmax, were not included in the correlation studies since they showed no significant differences among brands. Disintegration time did not correlate to dissolution rate constant indicating that the disintegration time was not the rate determining step of the dissolution rate of doxycycline capsules. The in vivo parameters [AUC_0^t, AUC_0^∞, and Cpmax] were also showed poor correlation with the in vitro parameters [disintegration time and dissolution

rate constant]. Hence, this study revealed that bioavailability of doxycycline capsules was independent of its in vitro results.