CHAPTER III

MATERIALS AND METHODS

Materials

Test Products

Five commercial brands of cimetidine, 400 mg film-coated tablets and 200 mg cimetidine injection, were provided by local manufacturers and/or distributors. The letters (A,B,C,D and E) are given to represent the brand names of the tablets. Information of these products were accessible in Appendix A.

Reagents

1. Standard cimetidine powder (Smith, Kline and French Lab., U.S.A.) Lot No. 914CETG-36
2. Internal standard ; Procainamide Hydrochloride, powder (E.R.Squibb and Sons, U.S.A.) Lot No. 78438
3. Acetonitrile HPLC grade (Merck, Damstadt, West Germany) Lot No. 31086094
4. Heparin 5,000 i.u./ml (Leo Pharmaceutical Products, Ballerup-Denmark) Lot No. A 36 B
5. Dichloromethane AR (Carlo Erba, Italy) Lot No. 1488L100
6. Phosphoric acid AR (Merck, Damstadt, West Germany) Lot No. 308903

7. Sodium hydroxide AR (Eka Kemi, Sweden) Lot No. S44501

8. Sodium metabisulfite AR (Fisher Scientific Company, U.S.A.) Lot No. 744354

9. Sulfuric acid AR (Merck, Damstadt, West Germany) Lot No. 2563000

10. Triethylamine AR (Merck, Damstadt, West Germany) Lot No. 2070408

Apparatus

1. Analytical Balance (August Sauter KG D-7470, West Germany)

2. Disintegration Tester (Manesty machines Ltd., Liverpool 24, England)

3. Dissolution Apparatus (72 RL, Hanson Research Corp., Northridge, Calif., U.S.A.)

4. Spectrophotometer (Spectronic 2000, Bausch & Lomb, N.Y., U.S.A.)

5. High Pressure Liquid Chromatrography (LC-3A, Shimadzu, Japan)

6. Digital Computer (IBM Compatible 16 Bit, Micro Source)

Method

In Vitro Studies

Five brands of cimetidine, 400 mg film-coated tablets were evaluated using the official and non-official

test of U.S.P. and/or B.P. for film-coated tablets. The test included :

1. Uniformity of Weight B.P. 1973 (34)

Twenty tablets of each of the five brands of cimetidine tablets were sampled and accurately weighed tablet by tablet. The average weight and standard deviation were calculated.

2. Assay for Content of Active Ingredient

Cimetidine Tablets :-

The amount of cimetidine in tablet was determined by the method of Leonard et al. (35) which was described as follows : Not less than 20 cimetidine tablets were weighed and finely powdered. A portion of the powder, equivalent to about 200 mg of cimetidine was accurately weighed, and transferred to a 250 ml volumetric flask. About 150 ml of 0.1 N sulfuric acid was added and shaken for 15 minutes, 0.1 N sulfuric acid was adjusted to volume. The solution was then mixed using a mixer and filtered through filter paper. A 2 ml of clear solution was withdrawn and diluted with 0.1 N sulfuric acid to 200 ml. The diluted solution was concomitantly determined for its absorbance at the wavelength of maximum absorbance about 219 nm, in a spectrophotometer using 0.1 N sulfuric acid as the blank.

Cimetidine Injection :-

To 2 ml of cimetidine injection, sufficient 0.1 N sulfuric acid was added to produce 250 ml. A portion of 2 ml of the diluted solution was sampled and adjusted to 200 ml with 0.1 N sulfuric acid. The solution was measured for its absorbance at about 219 nm , in a spectrophotometer, using 0.1 N sulfuric acid as the blank.

The amount of cimetidine in the samples were calculated employing a standard curve (Appendix B).

3. Disintegration Test

The disintegration tests for five brands of cimetidine tablets were studied according to the B.P. 1980 method for coated tablets (36).

Procedure :

Individual tablet was introduced into each of the six tubes of the basket. A disk was then added to each tube, and the apparatus was operated using water maintained at 37 $\pm$ 1°C as the immersion fluid. The tablets pass the test if all six have disintegrated within one hour. (If any of the tablets have not disintegrated, the test was repeated on a further six tablets. Water in the beaker was replaced with 0.1 N hydrochloric acid. The tablets then pass the test if all six tablets have disintegrated in the acid medium within one hour.) The mean disintegration time of each brand was calculated.

4. Dissolution Test

According to the U.S.P.XXI 3rd supplement (37), the dissolution rate of cimetidine tablet was determined in nine hundred millilitres of CO_2-free deionized water at 37 ± 0.5° C by the rotating basket method of the U.S.P. (38), with a stirring speed of 100 ± 5 rpm . Three millilitres of samples were taken at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes intervals. The same quantity of dissolution medium was added immediately after each sampling to keep the volume of the dissolution medium constant during the experiment. The amount of drug dissolved was determined in a UV spectrophotometer at 217 nm, in comparison with a standard curve.

Standard Curve

Standard cimetidine solutions with known concentrations in CO_2-free deionized water were prepared and analyzed using a UV spectrophotometer at 217 nm . Absorbances obtained versus known concentrations were fitted to a straight line using linear regression.

In Vitro Evaluation

The characteristics of all five commercial brands of cimetidine tablets were examined and evaluated, using general standard of U.S.P. and/or B.P. to determine which brand passed the requirement. Analysis of variance and Student's t-test were performed to assess the differences between the original and local brands for the disintegration times and dissolution values.

In Vivo Studies

Test Products

Injections : The original brand of cimetidine injection was selected. The concentration of cimetidine in each ampoule was 200 mg/2 ml.

Tablets : It was desired to select two commercial brands of 400 mg cimetidine tablets. One was the original brand which to be assigned as the reference standard against another local manufactured brand with maximum dissolution value and minimum retail price.

Subjects

Nine healthy volunteers (1 female, 8 male,average 21.2 years; range 19-27 years) gave their informed written consents to participate in this study. All subjects had normal physical examination and laboratory test results including liver and renal functions (Appendix C). The methods of the study were fully explained to all subjects. They were permitted to take no medication for at least one week preceding the study and during the experiment period.

Dose and Drug Administration

For oral study : A 400 mg cimetidine tablet was given orally in a single dose. The doses were administered with 200 ml of water after an overnight fast. The subjects were allowed to have breakfast 2 hours after dosing.

For parenteral study : A 200 mg/2 ml intravenous dose of cimetidine solution was made up to 20 ml with Normal Saline and administered over a 2-minutes period. The dose was administered after an overnight fast, 2 hours before breakfast.

Experimental Design

Both cimetidine injection and tablet were given to the subjects following a crossover experiment. Administration of each product was separated by a washout period of one week (Table 1).

Table 1 Dosing Schedule :

Subject	Week 1	2	3
1 - 3	A	B	C
4 - 6	B	C	A
7 - 9	C	A	B

A and B represent the brand names of cimetidine tablets
C represents the brand name of cimetidine injection

Samples Collection

For oral study : Blood samples (5 ml) were drawn from the antecubital vein prior to dosing and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 hours after drug administration.

For parenteral study : Blood samples (5 ml) were taken prior to dosing and at 0, 0.25, 0.50, 0.75, 1.0, 1.50, 2.0, 3.0, 4.0 and 6.0 hours after drug administration.

All blood samples were collected in centrifuge tubes containing heparin. After centrifugation (2500 rpm for 15 minutes) the plasma samples were collected and kept at -20 °C until subsequent analysis.

Determination of Cimetidine in Plasma

Concentrations of cimetidine in plasma samples were determined using the modified high pressure liquid chromatographic method described by Mihaly et al. (39-40). The procedure was developed as follows:

Plasma sample 1 ml

- add Internal standard* 100 µl (20 µg/ml in 0.09% Sodium metabisulfite sol.)
- add 2 M Sodium hydroxide 0.5 ml
- mix
- extract with 20 ml Dichloromethane (mix 60 seconds and centrifuge 3000 rpm for 10 minutes)

transfer the organic layer into a second tube

- evaporate under a gentle stream of nitrogen at 45° C

residue

- reconstitute in 1 ml of the chromatographic mobile phase

inject 200 µl sample solution into the HPLC

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* Procainamide Hydrochloride

HPLC Conditions for Cimetidine Analysis in Plasma

Apparatus : HPLC LC-3A, Shimadzu, Japan

Column : μ-Bondapak C_{18} , stainless steel column, Water Associates Pty. Ltd., U.S.A.
pre-column 5 cm x 2.0 mm i.d.
analytical column 30 cm x 3.9 mm i.d.

Mobile phase : 0.9 % triethylamine and 5 % acetonitrile in water, adjusted to pH 3 with phosphoric acid (85 % v/v)

UV detector : 228 nm

Flow rate : 1.4 ml/min

Attenuation : 2^1 mv/full scale

Pressure : 100 kg/cm^2

Retention time : Cimetidine 6.4 minutes
Procainamide 4.7 minutes

Standard Curve

Certain amount (0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 μg) of standard cimetidine were added to 1 ml of pooled drug-free plasma. These samples were analyzed following the same procedure as described above (37-38). The ratio of area under the peak of cimetidine and internal standard obtained versus the known cimetidine concentrations were fitted to a straight line using linear regression.

Bioavailability and Statistical Analysis

Individual plasma cimetidine profile from each treatment was analyzed according to noncompartment (model independent)(41-42) estimating program. The area under the curve (AUC), the area under the moment curve (AUMC), the mean residence time (MRT) from the intravenous plasma concentration-time data were obtained directly from the output. For oral plasma concentration-time data, the area under the curve and the area under the moment curve were calculated by the trapezoidal method and extrapolated to infinite time.

The absolute and relative bioavailabilities, the half-life, and the first order absorption rate constant were calculated using the following equations :

$$F_{ab} = \frac{[AUC]_{o\ oral}^{\infty}}{[AUC]_{o\ i.v.}^{\infty}} \cdot \frac{Dose_{i.v.}}{Dose_{oral}} \qquad \text{Eq. 1}$$

$$F_{rel} = \frac{[AUC]_{o\ oral(test)}^{\infty}}{[AUC]_{o\ oral(ref)}^{\infty}} \cdot \frac{Dose_{oral(ref)}}{Dose_{oral(test)}} \qquad \text{Eq. 2}$$

$$t_{1/2} = 0.693 \times MRT_{i.v.} \qquad \text{Eq. 3}$$

$$K_a = 1/MAT \qquad \text{Eq. 4}$$

$$MAT = MRT_{oral} - MRT_{i.v.} \qquad \text{Eq. 5}$$

$$MRT = \frac{[AUMC]_0^\infty}{[AUC]_0^\infty} \qquad \text{Eq. 6}$$

where;

F_{ab} = absolute bioavailability

F_{rel} = relative bioavailability

$[AUC]_0^\infty$ = area under the plasma concentration-time curve from zero time to infinity

$[AUMC]_0^\infty$ = area under the (first) moment curve from zero time to infinity

MAT = mean absorption time

MRT = mean residence time

K_a = first order absorption rate constant

$t_{1/2}$ = effective half-life