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Correlation between microbiological and chemical assay of ampicillin in bioequivalence studies in man

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Summary

Using the urinary excretion method, the bioavailability of ampicillin from two commercially available brands was investigated. The amounts of ampicillin in urine were determined chemically and microbiologically. The results indicate good agreement between the chemical and microbiological assay methods. The chemical assay was recommended as the method of choice for the assay of ampicillin in bioequivalence studies. Using this method the two brands were found not to be bioequivalent.

Introduction

Chemical and microbiological assay methods were previously described and good agreement between the two methods was reported (Smith et al., 1967; Angelucci and Baldieri, 1971). The chemical method was based on the copper-catalyzed hydrolysis of the intact ampicillin molecule to produce a copper–penicilloic acid complex which was then measured spectrophotometrically at 320 nm. The microbiological method was based on measuring the growth inhibition zones produced by ampicillin on an ampicillin-sensitive organism, e.g. *Staphylococcus aureus* (Kavanagh, 1972). In the determination of ampicillin in biological fluids, the microbiological method was mostly used (Jusko and Lewis, 1973). However, the chemical method has certain advantages over the microbiological one, e.g. simple, fast and economical. These advantages make the chemical method very valuable in routine analysis where large numbers of samples are usually expected. In the present work the urinary excretion of ampicillin was studied using both methods of assay.

Numerous reports on the bioequivalence of different ampicillin brands have been

published (Macleod et al., 1972; Mayersohn and Endrenyi, 1973; Whyatt et al., 1976). The results were conflicting. We now report a comparative bioavailability study on two commercially available brands of ampicillin.

Materials and methods

Materials

Two brands of 500 mg ampicillin capsules were designated as brands O and P: brand O, Omnipen capsules, Lot 1171883, Wyeth Labs., PA, U.S.A. and brand P, Pentrexyl capsules, Lot MB 8011, Bristol Italiana (Sud.), S.P.A. Italy, under authority of Bristol Labs., U.S.A. Anhydrous ampicillin (WHO Reference) was used as the standard.

Assay

(i) Chemical method

Urine samples, obtained from subjects taking ampicillin capsules, were treated as follows: a certain volume (1–10 ml) of urine was made to volume (50–100 ml) with copper sulphate buffered solution (pH 4.9–5.2; Smith et al., 1967). Three aliquots (10 ml each) were separately pipetted into 20 ml glass test tubes labelled a, b and c. Samples a and b were heated in a waterbath (75°C) for 30 min and then immediately cooled in ice together with sample c (control) for 10 min. The 3 samples were allowed to attain room temperature. The absorbance of each sample (a and b) was measured at 320 nm using a Beckman DB-GT grating spectrophotometer. Calibration curves were prepared in urine (0.01–0.04 mg/ml) by repeating the above procedure. The data were subjected to linear regression analysis to obtain the appropriate calibration factors.

(ii) Microbiological method

A method similar to that described by Kavanagh was used (Kavanagh, 1972). Ampicillin solutions were prepared in sterile phosphate buffer (pH = 7.9) immediately before use. The test organism was *Staphylococcus aureas* (NCTC 6751). The Grove and Randall agar medium No. 11, consisting of a 20-ml base layer and a 5-ml seed layer and a 5-ml seed layer, was used. The drug solutions were applied to the inoculated agar surface. The plates were then incubated at 33°C for 17 h. Four replicates were made for each sample. The diameter of each growth inhibition zone was measured and the mean value was recorded. The calibration curves were drawn by plotting the mean diameter of the growth inhibition zone against log drug concentration.

Two sets of calibration curves (6 each) were prepared from an aqueous solution of ampicillin using: (i) the chemical method (0.01–0.04 mg/ml); and (ii) the microbiological method (0.25–2.00 μg/ml). The data were statistically analyzed for linearity and reproducibility. The mean calibration factor obtained in each case was used to determine the amount of ampicillin in an aqueous solution of unknown concentration. Six replicates of this solution were analysed by each of the two assay methods.

Bioavailability studies

Five healthy male volunteers participated in the trials; average age 36 years and average body weight 68 kg. The participants did not take any other medication for one week before or during the trial. The ampicillin brands O and P, among 4 different brands showed the largest difference in dissolution rates (unpublished data) and were therefore chosen for these studies.

The ampicillin capsules (2 × 500 mg) were taken after an overnight fast. Breakfast was allowed 4 h after drug administration. Complete urine collections were made just before drug administration and hourly for 12 h post-administration. After each urine collection the volunteers drank 200 ml of water to effect diuresis. Urine samples were kept at 4°C for 24 h before analysis. The ampicillin contents were determined chemically (Smith et al., 1967) and microbiologically (Kavanagh, 1972) as described earlier. A wash-out period of 7 days was allowed between trials.

Results and discussion

Assay methods

Straight line calibration curves, passing through the origin, were obtained for ampicillin using the chemical and the microbiological assay methods (Tables 1 and 2). The correlation coefficient ranged between 1.00 and 0.999 in all cases. When 6 calibration curves were prepared at different occasions, both assay methods gave reproducible results. The standard errors (S.E.) around the mean ($\bar{X}$) absorbance and diameter of growth zone for the different concentrations ranged between 0.0042–0.0084 (Table 1) and 0.2236–0.3096 (Table 2), respectively. The reproducibility was further demonstrated as shown in Table 3. The mean amount (mg) was 3.80 ± 0.0517 and 4.00 ± 0.0305 using the chemical and microbiological methods, respectively (Table 3). The lower limits of detection were 5 and 2 μg for the chemical and microbiological assay methods, respectively. Therefore, both methods were sensitive enough for the detection and determination of ampicillin urinary levels which might be expected in normal clinical practice and in pharmacokinetic evaluation.

The chemical and microbiological assay methods gave similar results with respect to the determination of ampicillin in aqueous solutions (Table 3) and in urine (Fig. 1). When the amount of ampicillin in aqueous solution was determined chemically and microbiologically, the mean value was 3.80 ± 0.0517 and $4.00 \pm$

TABLE 1

Calibration curves of ampicillin prepared from aqueous solutions using the chemical assay

Concentration (mg/ml)	Absorbance (320 nm)						
	1	2	3	4	5	6	$\bar{X} \pm$ S.E.
0.01	0.28	0.28	0.33	0.28	0.30	0.31	0.296 ± 0.0084
0.02	0.58	0.58	0.60	0.58	0.61	0.60	0.592 ± 0.0054
0.03	0.90	0.89	0.90	0.89	0.92	0.90	0.900 ± 0.0045
0.04	1.18	1.18	1.18	1.17	1.20	1.19	1.183 ± 0.0042

TABLE 2

Calibration curves of ampicillin prepared from aqueous solutions using the microbiological assay

Concentration (μg/ml)	Diameter of inhibition zone (mm)						
	1	2	3	4	5	6	$\bar{X} \pm$ S.E.
0.25	14.5	13.0	13.0	13.5	13.5	13.5	13.50 ± 0.2236
0.50	19.0	19.0	18.0	18.5	18.0	17.0	18.25 ± 0.3096
1.00	23.0	22.5	23.0	22.0	22.0	21.0	22.25 ± 0.3096
2.00	26.5	26.0	26.5	25.0	27.0	27.0	26.33 ± 0.3073

TABLE 3

Amounts (mg) of ampicillin in aqueous solution as determined by (a) the chemical and (b) the microbiological assay methods

Experiment	mg of ampicillin	
	a	b
1	3.80	3.88
2	3.80	3.97
3	3.80	4.06
4	4.00	4.06
5	3.60	3.97
6	3.80	4.06
$\bar{X} \pm$ S.E.	3.80 ± 0.0517	4.00 ± 0.0305
$\bar{X} \pm$ S.E. (a and b)	3.90 ± 0.0999 *	

* There is no significant difference ($P = 0.01$).

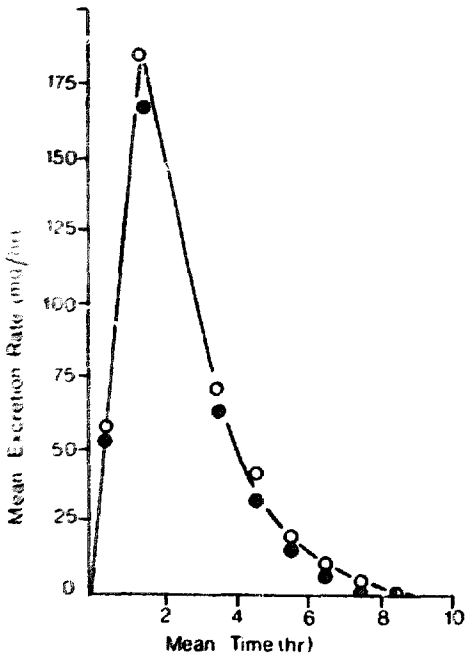

Fig. 1. Mean urinary excretion rates after oral administration of ampicillin capsules (brand O) as determined by (○) microbiological and (●) chemical methods. Mean of 3 subjects.

0.0305, respectively (Table 3). The urinary excretion rate curves drawn from data obtained following chemical and microbiological determinations, were almost superimposable (Fig. 1). These results indicate good agreement between the chemical and microbioloical assay methods similar to previous findings (Smith et al., 1967; Angelucci and Baldieri, 1971). However, in routine analysis, where large numbers of samples are expected, e.g. in pharmacokinetic studies, the chemical assay would be recommended as the method of choice for the following practical advantages: (i) it is a simple method, e.g. unlike the microbiological assay, the chemical one does not involve highly specialized techniques; (ii) the chemical assay is relatively faster. In our laboratories, using this method, we could analyse up to 50 urine samples per day on the same day of the trials whereas this would be practically impossible using the microbiological assay; and (iii) the chemical assay method is economical.

Bioequivalence studies

A previous study showed that for ampicillin capsules, good agreement was found between the total urinary recovery and the area under the plasma concentration curve (Jusko and Lewis, 1973). The urinary excretion method was, therefore, used in the present work to compare the bioavailabilities of the two ampicillin brands. The cumulative amounts of ampicillin excreted in urine and the maximum peak of excretion together with the time taken to reach that peak were used to describe the extent and rate of bioavailability, respectively (Ritschel, 1976).

Results of urinary excretion of brands O and P showed that the cumulative amounts of ampicillin excreted by each volunteers was always higher for brand O than brand P. The mean amounts recovered after 12 h ($\pm$S.E.) were 458.1 $\pm$ 28.9 and 375.0 $\pm$ 30.9 for brand O and P, respectively. The difference between the two means is statistically significant ($P = 0.05$; Table 4). Whilst the peak excretion rates of the two brands differed significantly, other pharmacokinetic parameters, e.g. the elimination rate constant and the half-life, did not change significantly for the two brands (Table 4). These results indicate that the ampicillin capsules brands O and P were not bioequivalent and that brand O was superior to brand P in this respect.

TABLE 4

Results of urinary excretion of two brands of ampicillin capsules (mean of subjects $\pm$S.E.)

Brand	Cumulative amount excreted over 12 h (mg)	Peak excretion rate ($mg \cdot h^{-1}$)	Peak excretion time (h)	Elimination rate constant, K_e (h^{-1})	Half-life $t_{1/2}$ (h)
O	458.1 $\pm$ 28.9	149.2 $\pm$ 18.1	1.9 $\pm$ 0.40	0.594 $\pm$ 0.027	1.176 $\pm$ 0.052
P	375.0 $\pm$ 30.9	110.8 $\pm$ 12.4	1.9 $\pm$ 0.25	0.561 $\pm$ 0.023	1.244 $\pm$ 0.050

References

Angelucci, L. and Baldieri, M., The spectrophotometric determination of ampicillin in body fluids. J. Pharm. Pharmacol., 23 (1971) 471–472.

Jusko, W.J. and Lewis, G.P., Comparison of ampicillin and hitacillin pharmacokinetics in man. J. Pharm. Sci., 62 (1973) 69–76.

Kavanagh, F., In Kavanagh, F. (Ed.), Analytical Microbiology, vol. II, Academic press, New York, 1972, pp. 123–145.

Macleod, C., Rabin, H., Ruedy, J., Caron, M., Zarowny, D. and Davies, R.O., Comparative bioavailability of three brands of ampicillin. Can. Med. Ass. J., 107 (1972) 203–209.

Mayersohn, M. and Endrenyi, L., Relative bioavailability of commercial ampicillin formulations in man. Can. Med. Ass. J., 109 (1973) 989–993.

Ritschel, W.A., In Ritschel, W.A. (Ed.), Handbook of Basic Pharmacokinetics, 1st edn., Drug Intelligence Publication, Hamilton, 1976, pp. 285–294.

Smith, J.W.G., DeGrey, G.E. and Patel, V.J., The spectrophotometric determination of ampicillin. Analyst, 92 (1967) 247–252.

Whyatt, P.L., Slywka, G.W.A., Melikian, A.P. and Meyer, M.C., Bioavailability of 17 ampicillin products. J. Pharm. Sci., 65 (1976) 652–656.