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Evaluation of Effect of Food Ingestion on Bioavailability of Cephalexin by Moment Analysis

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The effect of food ingestion on the bioavailability of cephalexin in humans was investigated. The amounts of urinary excretion following single oral doses to healthy male subjects were determined by high-performance liquid chromatography, and the bioavailability was evaluated by means of moment analysis of excretion rate-time curves. The ingestion of test meals (high carbohydrate, high protein and high fat) delayed the excretion rate with the peaks approximately two-thirds lower and 1—1.5 hr later than that in fasting subjects, but did not affect the total excretion amounts. No significant difference was found among the test meals. The moment analysis indicated that the rate of bioavailability increased by an average of 71% following food ingestion without significant change in the extent of bioavailability.

Keywords——cephalexin; bioavailability; food ingestion; moment analysis; urinary excretion rate-time curve; HPLC

The bioavailability of drugs is a matter of vital importance in clinical chemotherapy. Cephalexin, a semisynthetic derivative of cephalosporin C, is a very widely used antibiotic which has a broad spectrum of antimicrobial activity, rapid absorption through the intestinal tract, weak binding to plasma protein, no metabolites, and low toxicity.[2-4] The bioavailability of cephalexin as well as other oral drugs, therefore, depends largely on the extent and rate of its absorption in the human body, and food ingestion is an important factor affecting absorption. Previous investigations showed that food ingestion delayed the onset of cephalexin absorption, resulting in low and prolonged blood levels;[5,6] however, the total urinary recovery was not appreciably different in fasting and nonfasting states.[7] These reports, however, did not describe the nature of the meal and the time relationship between eating and dosing.

Our previous paper showed that the extent and rate of bioavailabilities can be expressed by the statistical moments of a time course curve.[8] This paper demonstrates the utility of the moment analysis method in examining the effect of food ingestion on the bioavailability of cephalexin. The assay method employed was a reversed phase high-performance liquid chromatography,[9] which had been found to give more reliable results than the conventional biological method used in previous studies on cephalexin bioavailability.[5-7]

Experimental

Reagents and Materials——Cephalexin used as a standard material and cephalexin capsules (Keflex®) administered to volunteers were gifts from Shionogi and Co. (Osaka, Japan). Distilled water and methanol

1) Location: *Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto 606, Japan.*

2) P.E. Gower and C.H. Dash, *Br. J. Pharmac.*, 37, 738 (1969).
3) R.L. Perkins, H.N. Carlisle, and S. Saslow, *Am. J. Med. Sci.*, 256, 122 (1968).
4) R.S. Griffith and H.R. Black, *Postgrad. Med. J.*, 47S, 32 (1971).
5) C.H. O'Callaghan, J.P.R. Tootill, and W.D. Robinson, *J. Pharm. Pharmacol.*, 23, 50 (1971).
6) R.S. Griffith and H.R. Black, *Clin. Med.*, 75, 14 (1968).
7) T.S. Thornhill, M.E. Levison, W.D. Johnson, and D. Kaye, *Appl. Microbiol.*, 17, 457 (1969).
8) K. Yamaoka, T. Nakagawa, and T. Uno, *J. Pharmacokin. Biopharm.*, 6, 547 (1978).
9) T. Nakagawa, J. Haginaka, K. Yamaoka, and T. Uno, *J. Antibiot.* (Tokyo), 31, 769 (1978).

were filtered through a 0.45 μm pore size triacetylcellulose membrane (Fuji Photo Film Co., Tokyo, Japan), and degassed before preparing the mobile phase. Hydrochloric acid of analytical reagent grade was used as supplied.

Subjects and Treatments——Four healthy male volunteers, 23 to 29 years old, weighing 58 to 60 kg participated in this study. The subjects had no past histories of allergic reaction to penicillin and showed normal renal function, the values for creatinine clearance according to the Cockroft equation[10] ranging from 94.3 to 119.8 ml/min. They took no drugs other than cephalexin during the study. The fasting subjects received two 250 mg capsules together with 200 ml of water after 12 hr fasting, and the nonfasting subjects, who had also fasted for 12 hr before taking test meals, received the same doses with 50 ml of water 15 min after ingestion. The subjects were not permitted to eat until 4 hr after dosing. The test meals were (1) a high carbohydrate meal consisting of toast with strawberry jam, ham, and milk (this meal contained approximately 70.8% carbohydrate, 18.8% protein, and 10.4% fat), (2) a high protein meal consisting of toast, roast pork, egg white, and skimmed milk (this meal contained 55.5% carbohydrate, 38.8% protein, and 4.6% fat), and (3) a high fat meal consisting of toast with butter, bacon, egg yolk and milk (this meal contained 44.3% carbohydrate, 15.7% protein, and 40.0% fat). All meals weighed about 400 g. The dosing experiments were conducted by a crossover technique such that the four subjects received the same treatment at the same time and the same four subjects received different treatments at least one week apart. The fasting data were taken from the same four subjects.

Apparatus——A high-performance liquid chromatograph (ALC/GPC 204, Waters Assoc.) equipped with a UV detector (254 nm; model 440, Waters Assoc.) was used in a reversed phase mode with a stationary phase of LiChrosorb RP-18 (E. Merck Co.) packed in a 250 mm×4.6 mm *i.d.* stainless steel column and a mobile phase of water–methanol (5 : 2, v/v) containing 0.75% 0.5 N HCl. The flow rate was maintained at 1.2 ml/min (1800 p.s.i.). A short column (20 mm×4.6 mm *i.d.*) filled with LiChrosorb RP-2 was used to guard the main column. Numerical calculations and data processing, *i.e.*, integration of chromatographic peak areas, construction of calibration equations by the least-squares method, quantification of cephalexin in urine samples, calculation of statistical moments and bioavailability from urinary excretion rate-time curves, and statistical analysis of data were all carried out on a microcomputer (PET 2001, Commodore Co., Palo Alto, Ca.) equipped with a silent printer (TSP-7706, Kanto Denshi Co., Tokyo), with programming in BASIC.

Assay Procedure——The urine specimens were collected from the subjects according to a predetermined time schedule (see Table 1). After measuring the volume, a 1 to 2 ml aliquot was passed through a 0.45 μm pore size membrane filter. A 5 μl portion of the filtrate was applied directly to the chromatograph under the conditions described above. The standard solutions for calibration were prepared by dissolving known amounts of the standard material in normal urine to give concentrations ranging from 0.05 to 4.0 mg/ml. The microcomputer was connected to the chromatograph; all the calculations and data analysis were processed and printed out in real time. The calibration plot (peak area *vs.* concentration) was linear (correlation coefficient 0.9994) and passed through the origin. The statistical treatment of data was carried out by variance analysis.

Theory

The effect of food ingestion on the absorption of an orally administered drug can be expressed by the following convolution integral for linear systems;

$$\frac{dX_u^{nf}}{dt} = \frac{D^f}{D^{nf}} \int_0^t f_m(\tau) \frac{dX_u^f(t-\tau)}{dt} d\tau \qquad \text{Eq. 1}$$

where dX_u/dt is the urinary excretion rate, D is the dose, and the superscripts, f and nf, specify fasting and nonfasting conditions. The effects of ingestion, such as delay of gastric emptying rate, and prolongation of drug absorption by food are represented by $f_m(t)$. In the previous paper,[8] the statistical moments for a urinary excretion rate-time curve were defined as

$$X_u^\infty = \int_0^\infty (dX_u/dt)\, dt \qquad \text{Eq. 2}$$

$$MRT_u = \int_0^\infty t(dX_u/dt)\, dt / X_u^\infty \qquad \text{Eq. 3}$$

$$VRT_u = \int_0^\infty (t - MRT_u)^2 (dX_u/dt)\, dt / X_u^\infty \qquad \text{Eq. 4}$$

10) D.W. Cockroft and M.H. Gault, *Nephron*, **16**, 31 (1976).

where the area under the urinary excretion rate-time curve(X_u^∞), *i.e.*, the total amount of cephalexin excreted in the urine, and the mean and variance of the urinary excretion rate-time curve (MRT_u and VRT_u) are the zero, first normal and second central moments, respectively. It is known that there is a linear relationship between urinary excretion rate and plasma concentration of cephalexin,[9] so these MRT_u and VRT_u coincide with those of the plasma concentration-time curve.

In using Eq. 2, 3, and 4, the moments were calculated by rectangular integration with extrapolation to infinite time using a monoexponential equation. The equation was determined by the least-squares method using the last five points on the urinary excretion rate-time curve. When the moments are thus obtained for fasting and nonfasting, the effect of food ingestion can be evaluated by means of the following equations;

$$F_c = \frac{X_u^{nf}/D^{nf}}{X_u^{f}/D^{f}} \qquad \text{Eq. 5}$$

$$\Delta MRT_u = MRT_u^{nf} - MRT_u^{f} \qquad \text{Eq. 6}$$

$$\Delta VRT_u = VRT_u^{nf} - VRT_u^{f} \qquad \text{Eg. 7}$$

where F_c, ΔMRT_u and ΔVRT_u, corresponding to the zero through second moments of $f_m(t)$, represent the changes in the extent of bioavailability and mean and variance of the delay time, respectively. It should be emphasized that, because the statistical moments are additive for linear systems and can be obtained without the use of a pharmacokinetic model (*i.e.* model-free parameters[8]), the change in the bioavailability due to food ingestion and/or different drug preparations can be evaluated by a simple arithmetic operation, as shown in Eq. 5, 6, and 7.

Results and Discussion

Figure 1 shows the urinary excretion rate-time curve for fasting and nonfasting conditions (average of four subjects). It was found that the maximum excretion rate for fasting subjects was 270 mg/hr at 1.5 hr after administration of cephalexin, followed by a rapid decrease to 2.84 mg/hr at 8 hr. Food ingestion markedly delayed the peak time and decreased the maximum rate; the peak values were 172.2 mg/hr at 3.0 hr in the case of the high carbohydrate meal, 181.1 mg/hr at 2.5 hr for the high protein meal, and 182.3 mg/hr at 2.5 hr for the high fat meal. The average cumulative excretion amounts are shown in Table I; the total values are almost the same in all the treatments. Extrapolation of the cumulative excretion amounts to infinite time gave predicted total excretions of cephalexin amounting to 93.3%, 93.9%, and 93.8% of the dose in the cases of the high carbohydrate, high protein, and high fat meals, respectively.

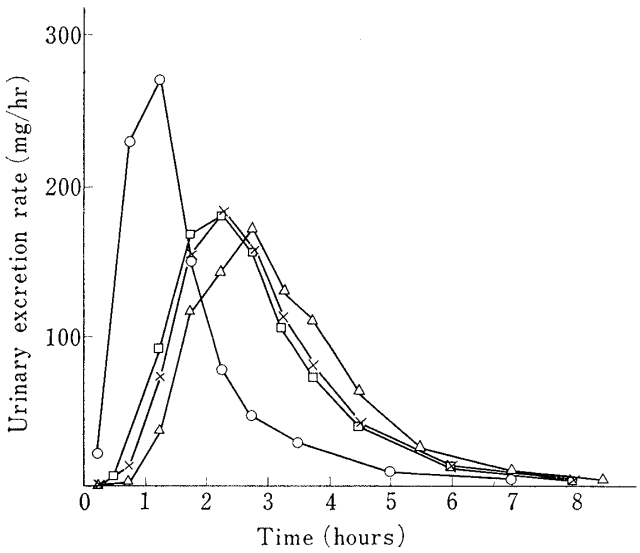

Fig. 1. Urinary Excretion Rate-Time Curve for Cephalexin[a]

○—○: fasting.
△—△: high carbohydrate meal.
□—□: high protein meal.
×—×: high fat meal.
a) Average of four subjects. S.D. values are not indicated.

The statistical moments calculated according to Eq. 5, 6, and 7 are given

in Table II. The MRT_u values increased by 85% in the case of the high carbohydrate meal, 62% for the high protein meal, and 67% for the high fat meal (average 71%), whereas F_e remained almost unchanged in all cases.

Table I. Cumulative Urinary Excretion Amounts of Cephalexin following Single Oral Doses of 500 mg (Capsules) to Fasting and Nonfasting Subjects

Time (hr)	Fasting		High carbohydrate meal		High protein meal		High fat meal	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D. (mg)
0.5	11.3	8.60	0.10	0.18				
1.0	126.4	52.0	2.36	1.08	6.32	10.2	6.75	6.25
1.5	261.6	47.1	20.5	4.13	52.3	60.2	43.8	34.0
2.0	337.0	46.2	78.9	14.4	136.6	89.0	121.6	55.4
2.5	376.3	44.0	149.6	27.6	227.2	83.2	212.7	69.8
3.0	400.1	42.5	235.7	33.6	305.4	62.1	291.6	70.9
3.5			300.9	35.3	358.3	42.0	348.6	54.9
4.0	429.1	35.3	356.5	26.2	394.8	29.2	389.0	37.9
5.0			419.9	10.8	435.2	16.5	432.4	21.1
6.0	449.1	31.8	444.4	13.1				
7.0					460.9	11.5	459.7	11.4
8.0	454.7	30.6	460.8	16.7				
9.0			464.0	17.2	467.3	11.1	466.9	8.94

Table II.[a] Effects of Food Ingestion on the Bioavailability of Cephalexin

	High carbohydrate meal		High protein meal		High fat meal	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
F_e	1.027	0.090	1.034	0.092	1.032	0.088
ΔMRT_u (hr)	1.503	0.148	1.099	0.499	1.188	0.411
ΔVRT_u (hr^2)	0.370	0.149	0.222	0.177	0.280	0.182

a) The values of X_u^∞, MRT_u and VRT_u for fasted subjects were 456.8±29.9 (S.D). mg, 1.773±0.201 hr, and 1.709± 0.258 hr^2, respectively. F_e, ΔMRT_u and ΔVRT_u were calculated according to Eq. 5, 6 and 7, respectively.

The analysis of variance indicated that there were significant differences in MRT_u and VRT_u among the treatments, but no significant difference in X_u. Subsequent t-tests of ΔMRT_u and ΔVRT_u values showed that the ΔMRT_u values for fasting compared to high carbohydrate, high protein, and high fat meals were significant at a level of 0.1%, and the ΔVRT_u values for fasting compared to high carbohydrate and high fat meals were significant at levels of 1% and 5%, respectively, other cases being insignificant.

It is interesting to compare these results with those for ampicillin and amoxicillin; Welling *et al.*[11] showed that administration of two 250 mg capsules with 250 ml of water on an empty stomach resulted in higher urinary concentrations and recovery of both antibiotics compared to fed subjects, although they failed to complete 8 hr urine sampling. Their results indicated that food ingestion significantly decreased the extents of bioavailability of ampicillin and amoxicillin. The present results show that oral administration of cephalexin following food ingestion increased the rate of bioavailability by an average of 71% without reducing the extent of bioavailability.

11) P.G. Welling, H. Huang, P.A. Craig, and P.O. Madsen, *J. Pharm. Sci.*, **66**, 549 (1977).