약제학회지 (1987), 제 17권 제 4호
J. Kor. Pharm. Sci., Vol.17, No.4, 183~188 (1987)

Pharmaceutical Studies of Cefoperazone Phthalidyl Ester, a Novel Prodrug of Cefoperazone

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(Received November 19, 1987)

세포페라존프탈리딜에스텔의 약제학적 연구

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(1987년 11월 19일 접수)

A new cephalosporin derivative, cefoperazone phthalidyl ester, were synthesized and investigated in terms of dissolution and absorption properties. In comparison with cefoperazone, its phthalidyl ester showed the following characteristics. The mean dissolution time and variance of retention time were more significantly prolonged in simulated intestinal fluid than those in simulated gastric fluid. After a single oral dosing of both cefoperazone and its ester to rabbits, serum concentrations of cefoperazone were measured by bioassay, and the results showed that the ester exhibited much higher and more sustained blood level than the parent drug. The total area under the curve of cefoperazone phthalidyl ester were 10.8 times greater than that of cefoperazone.

Cefoperazone is one of the semisynthetic cephalosporins, which is used as injectable forms only. It has a broad spectrum of antibacterial activity against *Pseudomonas aeruginosa,* unlike older cephalosporins, along with the *Enterobacteriaceae* and other Gram-negative bacteria, Gram-positive bacteria and anaerobic bacteria.[1]

In spite of their inherent stability towards acids, semisynthetic cephalosporins are generally not absorbed from the gastro-intestinal tract,[2-5] and it has long been an objection for research in this field to provide orally active derivatives.

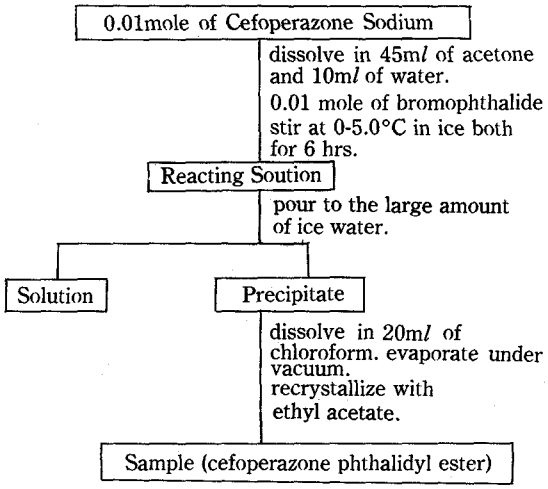
On the other hand, for the past several years, a number of penicillin derivatives have been synthesized. Among them, phthalidyl ester of ampicillin was found to be absorbed more readily than the parent compound, ampicillin and to yield much higher levels in blood and tissues.[6-10]

As a step towards this goal, a novel cefoperazone phthalidyl ester was synthesized by reacting cefoperazone sodium with bromophthalide in acetone.[11-13] Dissolution profiles, total amount of dissolved ester, dissolution parameter and variance of retention time were studied. The pharmacokinetic parameters of oral cefoperazone phthalidyl ester were compared with those of cefoperazone in rabbits.

EXPERIMENTAL METHODS

Materials and Apparatus

Cefoperazone and bromophthalide were kindly given by Sam-Sung Pharm. Co., and Dong-A Pharm. Co., respectively. Cefoperazone phthalidyl ester was prepared by our laboratory as shown in Scheme 1, and the ester was identified with spectrometric trials such as UV, IR and PMR spectroscopy. Acetone, methanol, acetonitrile, chlo-



Scheme 1—Preparation of cefoperazone phthalidyl ester.

roform, ethyl acetate and pyridine were of analytical reagent grade or extra pure grade. Dissolution tester (Eyela, DE-1S) was used for dissolution test.

Experimental Animals

Male New Zealand white rabbits weighing 2.0-2.5kg were used in all experiments. Water and commercial chow were given *ad libitum* in the same condition for a week before the experiment.

Dissolution Test

The *in vitro* dissolution test of the ester was performed by the constant surface area method.[14] A disk containing 100mg of the drug as cefoperazone was prepared from the 40-50 meshed powder using the punch-die assembly with diameter of 10mm and pressure of 10 ton/inch^2 for 10 seconds.

The disk in the basket was placed into apparatus and apparatus was operated immediately at 100rpm. Samples of 3 m*l* were taken at 5, 10, 20, 30, 60, 90 and 120 min from starting the test. The same volume of the fluid removed was replaced with fresh fluid and 100 m*l* of dissolution medium was equilibrated to 37 ± 0.5°C. The amount dissolved was determined from the ultraviolet absorbance at 280nm of the filtered portion of the solution under test, suitably diluted with dissolution medium, in comparison with the solution having a known concentration of the ester in the same medium. Dissolution test deals with the dissolved percent, mean dissolution time, variance of retention time, and total amount of dissolved ester by the basket method in simulated gastric and intestinal fluicds.

Absorption Experiment

Cefoperazone and its ester suspension were orally administered to 6 rabbits, respectively at a dose of 150mg/kg as cefoperazone. Blood samples of 1.5-2.0 m*l* were collected at 0.5, 1, 2, 4, 5, 6, 8, 12, 16, and 24hr after drug administration, respectively. The blood samples were centrifuged immediately at 14,000g and 4°C and then serum was obtained. Blood levels were determined by bioassay using the strain of *Escherichia coli* in the nutrient agar medium, and calibration curve of the standard cefoperazone.

The experiment was performed onto crossover test. Rabbits were fasted for about 16 hrs before the experiment, but water was freely provided during the fast.

RESULTS AND DISCUSSION

Dissolution Behaviors

In order to find out the proper dissolution behaviors of the ester, the dissolution test was carried out and dissolution profiles were obtained from the test in simulated gastric fluid (pH 1.2) and intestinal fluid (pH 6.8).

Fig. 1 shows the dissolution profiles for the ester in simulated gastric and intestinal fluids, respectively. Dissolution parameters of cefoperazone phthalidyl ester are listed in Table I. Dissolution parameters were calculated by the following equation and computer programs were used for it.[17,18]

$$m_\infty = \int_0^\infty \left(\frac{dm}{dt}\right) \cdot dt \quad (1)$$

$$MDT = \int_0^\infty t\left(\frac{dm}{dt}\right) \cdot dt \Big/ \int_0^\infty \left(\frac{dm}{dt}\right) \cdot dt \quad (2)$$

$$VRT = \int_0^\infty (t - MRT)^2 \cdot Cp \cdot dt \Big/ \int_0^\infty Cp \cdot dt \quad (3)$$

where t is time, m is the total amount of dissolv-

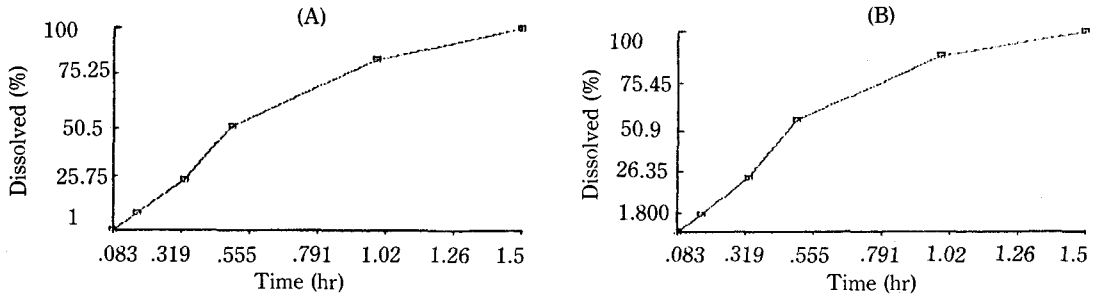


Figure 1—Dissolution profiles for cefoperazone phthalidyl ester in simulated gastric (A) and intestinal (B) fluids at 37±0.5°C.

Table I—*Dissolution Parameters for Cefoperazone Phthalidyl Ester in Simulated Gastric and Intestinal Fluids.*

Medium	m_∞ (mg)	MDT (hr)	VRT (hr^2)
Gastric fluid	1	0.601	0.156
Intestinal fluid	1	0.564	0.146

m_∞: total amount of dissolved cefoperazone phthalidyl ester, MDT: mean dissolution time, VRT: variance of retention time.

ed ester, $\int_0^\infty Cp \cdot dt$ is AUC, and the mean residence time of a drug in the body (MRT) is $\int_0^\infty t\ Cp \cdot dt / \int_0^\infty Cp \cdot dt$

As a result, the mean dissolution time (MDT), and variance of retention time (VRT) were more significantly decreased in simulated intestinal fluid than those in gastric fluid.

Drug Absorption

Fig. 2 is the calibration curve of cefoperazone in serum using *Escherichia coli* as the microorganism for bioassay. Relationship between the concentrations of cefoperazone and the diameters of

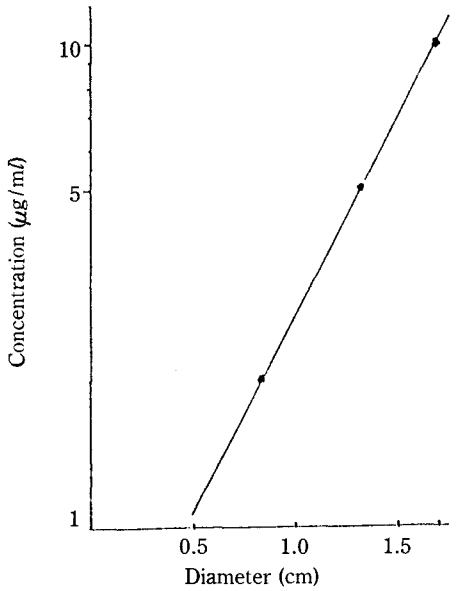


Figure 2—Calibration curve of cefoperazone in serum. log C = 0.081D−0.378, where C is concentration and D is diameter.

inhibition zone was linear over the range of 1.0 to 10 $\mu g/ml$ in serum. Therefore, this method was

Table II—*Serum Concentrations (μg/ml) of Cefoperazone after Oral Administration (150mg/kg as Cefoperazone) of Cefoperazone and Cefoperazone Phthalidyl Ester in Rabbits.*

Drug	Time (hr)										
	0.5	1	2	3	4	6	8	12	16	20	24
Cefoperazone	0.83 ±0.08	1.70 ±0.21	3.82 ±0.19	1.60 ±0.10	0.90 ±0.05	0.50 ±0.05	—*	—	—	—	—
Cefoperazone phthalidyl ester	5.85 ±0.3	6.30 ±0.2	6.60 ±0.2	6.30 ±0.4	5.90 ±0.3	5.60 ±0.2	4.90 ±0.3	3.80 ±0.4	3.10 ±0.2	2.40 ±0.4	1.85 ±0.3

Data represent mean±S.E. of 6 rabbits. * Not observed.

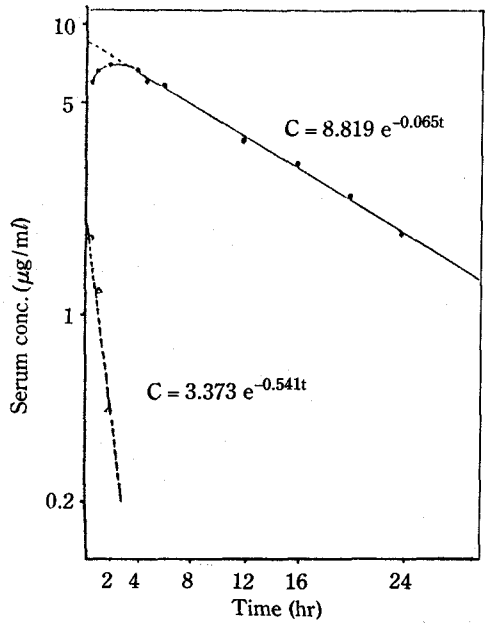


Figure 3—Serum concentration-time curve after single oral dose (150 mg/kg as cefoperazone) of cefoperazone phthalidyl ester to rabbits.

regarded as the proper assay system using the strain of *E. coli.*

Table II shows the data of the serum concentration of cefoperazone and its ester after oral administration to the fasted rabbits at a dose of 150 mg/kg as cefoperazone.

The serum concentration-time curve in Fig. 3 was drawn by the residual method. For the following two reasons, we can notify that the absorption properties of the parent drug were improved. First, the two residual lines were obtained by feathering the plasma level-time curve intersect at a point where $t<0$. Second, the ratio of K_a to K_e was over 3. Besides, the absorption profiles for cefoperazone and its ester by Nelson-Wagner method[19] are showed in Fig. 4. Absorbed percents (% ABS) were calculated by the following equation and computer program was used for its fitting.[18]

$$\% \ ABS = \frac{A_T}{A_\infty} \times 100 \tag{4}$$

$$= \frac{C + K_e \int_0^T C \cdot dt}{K_e \int_0^\infty C \cdot dt} \times 100 \tag{5}$$

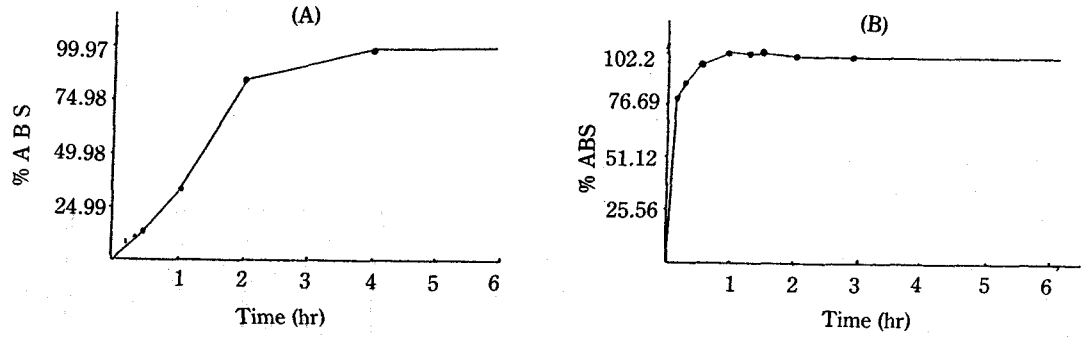


Figure 4—Absorption profiles for cefoperazone (A) and cefoperazone phthalidyl ester (B) by Nelson-Wagner method.

Table III—*Pharmacokinetic Parameters for Cefoperazone and Cefoperazone Phthalidyl Ester Obtained by Residual Method and Nelson-Wagner Method.*

Drug	K_a	$T_{1/2,ab}$	K_e	$T_{1/2,el}$	T_{max}	C_{max}	AUC_0^∞
Cefoperazone	1.283	0.540	0.582	1.19	2	3.82	11.83
Cefoperazone phthalidyl ester	0.541	0.794	0.065	10.65	2	6.6	127.74

where A_T is amount of drug absorbed to time T, A_∞ is amount of drug ultimately absorbed, C is plasma concentration of drug, and K_e is elimination rate constant of drug.

Pharmacokinetic parameters for cefoperazone and its ester obtained by the residual method and Nelson-Wagner method are listed in Table III. The maximum concentration (C_{max}) and the area under the curve from zero time to infinity (AUC) were markedly increased in cefoperazone phthalidyl ester. But the time to reach maximum concentration (T_{max}) was not different in both materials. The elimination half-life, $T_{1/2}$ of the ester after oral administration was more increased than that of cefoperazone.

Considering the above facts, although cefoperazone is hardly absorbed in gastro-intestinal tract, its phthalidyl ester showed the improvement of absorption in single oral dose.

CONCLUSION

The improvement of the absorption in single oral dose of cefoperazone phthalidyl ester, a novel prodrug of cefoperazone, was identified with the dissolution and absorption test, comparing with cefoperazone as parent drug. Dissolution of the prodrug were significantly prolonged in simulated intestinal fluid, in comparison with that in simulated gastric fluid. After a single oral dosing of both cefoperazone and its ester to rabbits, serum concentrations of cefoperazone were measured by bioassay and the ester exhibited much higher and more sustained blood level than cefoperazone.

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