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Oral Absorption of Cefoperazone Pivaloyloxymethyl Ester

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세포페라존피바로일옥시메칠에스텔의 경구 흡수

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Pivaloyloxymethyl ester of cefoperazone was synthesized by treating sodium cefoperazone with chloromethyl pivalate and its chemical structure was determined by spectroscopic trials. The pharmaceutical properties of the ester were investigated to assess its potential as a prodrug of cefo perazone. Cefoperazone pivaloyloxymethyl ester was microbiologically inactive itself *in vitro*, but hydrolyzed into the parent drug *in vivo*. After a single oral dose of each drug to rabbits, serum concentrations of cefoperazone were determined by high performance liquid chromatographic assay. The ester showed higher and more sustained blood level than cefoperazone. Therefore, the total area under the serum concentration-time curve of the derivative was 16.8 times larger than that of the parent drug.

Keywords—cefoperazone, prodrug, cefoperazone pivaloyloxymethyl ester, partition coefficient, tissue hydrolysis, oral absorption, pharmacokinetic parameter

Cefoperazone is a semisynthetic injectable cephalosporin, which is effective against a wide variety of infections caused by Gram-negative or Gram-positive aerobes, and in many anaerobic infections[1-3]. However it is poorly absorbed by the gastrointestinal tract after oral administration and its use is limited to the parenteral administration.[4] It was shown that pivaloyloxymethyl ester of cephaloglycin was well absorbed from the gastrointestinal tract and after the absorption was cleaved enzymatically with liberation of cephaloglycin.[5-7]

The present study was undertaken to examine the possibility of improving the oral absorption of cefoperazone by the synthesis of its pivaloyloxymethyl ester. We now report the synthesis of the ester and the results of comparative absorption and hydrolysis studies between the ester and cefoperazone.

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EXPERIMENTAL

Materials

Sodium cefoperazone and cefoperazone dihydrate were kindly provided by Samsung Pharm. Co., Korea. Methanol and acetonitrile were of HPLC grade from Baker Co., and all other chemicals and solvents used were of reagent grade and were used as received.

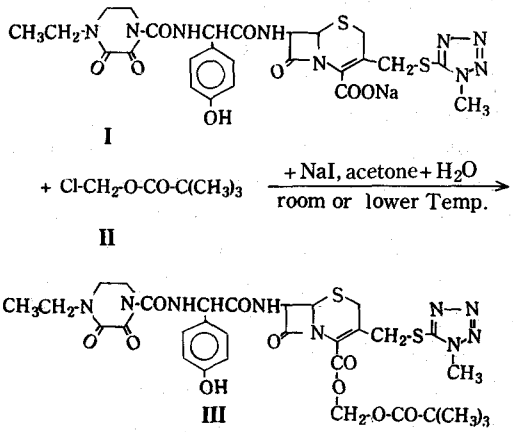
Apparatus

Spectral measurements were performed with PMR(Varian FT-80A NMR Spectro., U.S.A.), IR

(Perkin-Elmer model 783, U.K.) and UV/VIS spectrophotometer (Hitachi model 200-20, Japan). HPLC analyses were conducted with a UV detector operated at an analytical wavelength of 254nm (Waters model 510, U.S.A.). Treatments of biological samples were carried out using high speed refrigerated centrifuge (Beckman model J2-21, U.S.A.).

Synthesis of Cefoperazone Pivaloyloxymethyl Ester[8-14]

Sodium cefoperazone (**I**, 6.67g, 0.01M) was dissolved in a cosolvent of 45m*l* of acetone and 20m*l* of water, and sodium iodide(2.0g, 0.013M) was added to the solution as a catalyst. Then a solution of chloromethyl pivalate(**II**, 1.65, 0.011M) was added dropwise. The reaction mixture was cooled in an ice bath and stirred for 7hr at below 5 °C, and then added dropwise while stirring onto 130m*l* of ice water. After standing for 1hr at 0 °C, the mixture was filtered to obtain the residues. Ultrasonic extraction of the resulting residues with 10m*l* of chloroform, followed by filtration and evaporation of the filtrate under reduced pressure, gave crude products which were finally purified by following procedures. The sample was dissolved in the least amount of acetone, and added dropwise while stirring onto twice amount of ice water. After standing for 1hr at 0 °C, filtration and elutriation of the residue with petroleum ether gave pure and near white product (Scheme 1).



Scheme 1—Synthesis of cefoperazone pivaloyloxymethyl ester.

Yield: 1.14g(15%). m.p.: 98.4 °C. TLC (silicagel $60F_{254}$, EtOH: AcOEt: H_2O = 4:14:3): Rf: 0.82. UV λ_{max}(methanol): 270 and 230nm. IR(ν_{max}^{KBr} cm^{-1}: 2980-2800 ($-CH_3$ stretch), 1780 and 1750-1740 (C = O, ester), 1465 ($-CH_2$, bend), 1450, 1390 and 1370(t-butyl, bend), and 1170(C-O, ester). NMR ($CDCl_3$) δ (ppm): 7.30(d, J10, 2H), 6.82(d, J10, 2H), 4.98(d, J6, 1H), and 1.35(s and t, 12H). Anal. calcd. for $C_{31}H_{37}N_9O_{10}S_2 \cdot 2H_2O$: C; 46.78, H; 4.69, N; 15.84, S; 8.05—Found: C; 47.1, H; 4.95, N; 15.2, S; 7.50

Determination of Partition Coefficient

In order to evaluate the lipophilic character, the partition coefficients were determined by the conventional method. A known amount of solute was dissolved in water-saturated octanol or octanol-saturated water. The two phases were shaken for 3hr and then allowed to equilibrate at 36.5 ± 1 °C for 6hr. An aliquot was taken and diluted with methanol, filtered through 0.5μm Millipore filter, and then analyzed HPLC.

Tissue Hydrolysis Experiment *in Vitro*[15,16]

Male Spraque-Dawley rats weighing over 200g, fasted overnight prior to the experiment, were used to identify the hydrolysis of the ester in the body. The rat was anesthetized with ethyl ether, and a midline abdominal incision was made. The liver was perfused with saline solution through the portal vein until it came to be pale, and then removed immediately. After severing the intestine at the duodenal junction, the upper 30cm proteion of the small intestine was separated and washed with saline solution. Tissue samples were cut into small pieces and suspended in KCl/0.01M phosphate buffer to form a 25% suspension. The mixture was homogenized and centrifuged at 10,000g for 20 at 4 °C. The blood was taken by heart puncture and centrifuged at the same condition. The suspernatant fraction obtained was refrigerated and used in 12hr.

Cefoperazone pivaloyloxymethyl ester was dissolved in dimethyl sulfoxide and then added to the supernatant tissue or serum fraction. followed by maintenance at 37 ± 0.5 °C. Test samples were withdrawn at appropriate intervals, diluted with

methanol, vortexed, centrifuged, and filtered according to the procedures described in oral absorption experiment. The quantity of the ester remained in the fraction was analyzed by HPLC in following conditions: column; Radial-Pak C_{18} cartridge, mobile phase; methanol/water (60/40), detector; 254nm, sensitivity; 0.01 aufs, and flow rate; 2 ml/min.

Oral Absorption Experiment[17-23]

Healthy male New Zealand white rabbits weighing 2.0-2.5kg, fasted for 16hr prior to the experiment, were used. Cofoperazone and its ester were administered orally in 5m*l* of 0.1% Tween 80 suspension at a dose of 150mg/kg as cefoperazone, respectively. Venous blood of 1.5-2m*l* was taken at appropriate time intervals, and allowed to stand for 15min at room temperature. The sample, was centrifuged for 15min at 1,400g and then serum was obtained. 1m*l* of methanol was added to 0.5m*l* of serum, and the mixture was vortexed for 3min and recentrifuged for 15min at 1,400g. The supernatant fraction was filtered through 0.5μm millipore, and analyzed to quantify cefoperazone by HPLC in following conditions: mobile phase: mixture*/1N acetic acid/acetonitrile/water (1.2/2.8/180/816). *Mixture consisted of triethylamine/glacial acetic acid/water (14/5.7/80.3). The other HPLC conditions were same as those in tissue hydrolysis experiment.

All of 6 rabbits were divided into two groups and cross-overed after a week.

RESULTS AND DISCUSSION

Table I—*Partition Coefficients (P_c) of Free Cefoperazone (CPZ) and Cefoperazone Pivaloyloxymethyl Ester (CPZ-PV) in Octanol Buffer System at 36.5 ± 1 °C.*

Materials	P_c				
	pH 1.2	pH 2.3	pH 3.0	pH 4.0	pH 5.6
Free CPZ	0.041	0.038	0.021	—*	—
CPZ-PV	2.618	4.630	49.240	4.427	2.304

Data are the average of four experiments. *Not observed.

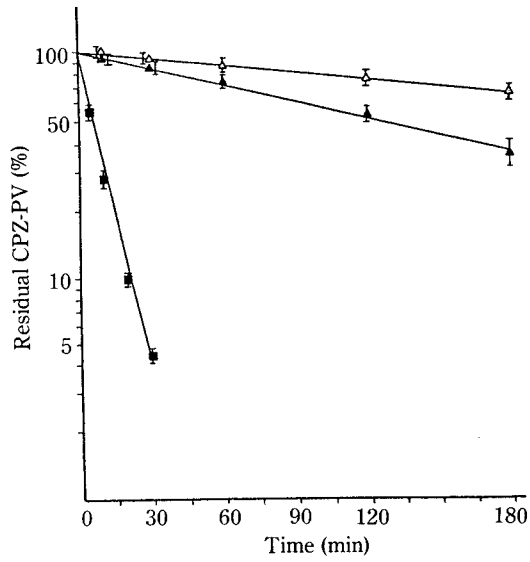


Figure 1—First-order plots of cefoperazone pivaloyloxymethyl ester (CPZ-PV) remaining in rat's liver homogenates, blood, and intestinal homogenates at 37 ± 0.5 °C.
Key: ■, liver homogenates; ▲, blood; △, intestinal homogenates.

Partition Coefficient

As shown in Table I, there is a great difference in partition coefficients of both cefoperazone (log $P_c<0$) and its pivaloyloxymethyl ester(log $P_c>0$). Therefore, it is predicted that the ester can be easily absorbed from the biological membrane after oral administration. And the value was also influenced by the pH of aqueous buffer solution.

Kinetics of Hydrolysis

It was proved that the ester was hydrolyzed and the parent drug was quantitatively liberated in the body. In liver homogenates a half of the ester was hydrolyzed in 5min and almost hydrolyzed in 30min. Therefore there is no remaining ester after 1hr. However the rate of hydrolysis was decreased in blood or intestinal homogenates. After 2hr, a half of the ester was hydrolyzed in blood and a quarter in intestinal homogenates. These results indicated that the prodrug was hydrolyzed quickly in the body, especially in liver, and hydrolyzed to small extent by the enzyme in blood, but stable against intestinal enzymes.

The semilog plotting of the residual concentra-

Table II—*Serum Concentrations (μg/ml) of Cefoperazone after Oral Administration of a Single Oral Dose (150mg/kg as Cefoperazone) of Free Cefoperazone (CPZ) and Cefoperazone Pivaloyloxymethyl Ester (CPZ-PV) to Rabbits.*

Materials	Time (hr)								
	0.5	1	2	4	5	6	8	12	24
Free CPZ	0.83	1.70	3.82	1.60	0.90	0.50	—*	—	—
	± 0.08	± 0.21	± 0.19	± 0.10	± 0.05	± 0.05			
CPZ-PV	0.56	0.90	1.55	7.12	15.51	23.63	16.23	8.20	0.86
	± 0.06	± 0.11	± 0.13	± 0.43	± 1.76	± 0.68	± 0.36	± 0.44	± 0.08

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

Table III—*Pharmacokinetic Parameters of Free Cefoperazone (CPZ) and Cefoperazone Pivaloyloxymethyl Ester (CPZ-PV) in Rabbits.*

Materials	K_{el}	$T_{1/2}$	K_{ab}	$AUC_{0\text{-}Tmax}$	$AUC_{Tmax\text{-}6\ or\ 24\ hr}$	$\frac{AUC_{0\text{-}Tmax}}{\text{Total AUC}}$	$\frac{AUC_{Tmax\text{-}6\ or\ 24\ hr}}{\text{Total AUC}}$
Free CPZ	0.5811	1.191	1.2832	3.60	7.37	0.328	0.672
CPZ-PV	0.1840	3.765	0.3168	41.29	143.08	0.224	0.776

tion of the ester versus time gave the straight line (Fig. 1). Therefore, the rate of hydrolysis in the body is of the first-order reaction and the rate constant can be calculated from the slope of the line. It was $9.919 \times 10^{-2}\text{min}^{-1}$ in liver homogenates, $5.91 \times 10^{-3}\text{min}^{-1}$ in blood, and 2.63×10^{-3} min^{-1} in intestinal homogenates.

Oral Absorption

Cefoperazone pivaloyloxymethyl ester showed higher blood level than that of the parent drug (Table II). However, the ester was slowly absorbed and the maximum concentration was obtained at 6hr after administration. It could be considered that the dissolution which is the rate-limiting step in absorption was delayed because of poor solubility characteristics of the ester.

Pharmacokinetic parameters were obtained on the basis of one-compartment open model (Table III). The AUC and maximum concentration were remarkably increased in the ester, and the rate constant for elimination was decreased. But the rate constant for absorption calculated by method of residuals was also decreased in the ester. These results strongly suggested that onset time of the ester was delayed due to limited solubility, but the effective blood level of the prodrug was more sustained than that of the parent drug.

CONCLUSION

As a result of this study, following conclusions were obtained;

1. The partition coefficients of cefoperazone pivaloyloxymethyl ester were markedly increased at pH1.2—5.6, comparing with those of cefoperazone.
2. The *in vitro* hydrolysis rates of the ester were in the order of rat liver ≫ rat blood > rat intestine.
3. The ester showed the marked and sustained oral absorption in rabbits comparing with cefoperazone.

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