Studies on Synthesis, Hydrolysis and Oral Absorption of Piperacillin Phthalidyl Ester

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(Received September 9, 1988)

피페라실린프탈리딜에스텔의 합성, 가수분해 및 경구흡수에 관한 연구

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(1988년 9월 9일 접수)

Piperacillin phthalidyl ester was synthesized by reacting piperacillin with triethylamine and bromophthalide in acetone and its chemical structure was determined by UV, IR, and PMR. The partition coefficient of the ester was increased and the ester was more lipophilic and less water soluble than piperacillin. The ester did not show the antimicrobial activity against *Bacillus subtilis* ATCC 6633 *in vitro*, but when hydrolyzed, the parent drug of ester, piperacillin, revealed antimicrobial activity *in vivo*. After a single oral dose of both piperacillin and the ester to rabbits, the serum piperacillin concentration was measured by bioassay. The ester exhibited improved pharmatokinetic characteristics: T_{max} of 2hr, C_{max} of $4.26\mu g\cdot ml^{-1}$, K_{el} of $0.057hr^{-1}$, and total AUC of $85.42\mu g\cdot hr\cdot ml^{-1}$. Piperacillin on the other hand, did not exhibit any gastro-intestinal absorption.

Keywords—piperacillin, piperacillin phthalidyl ester, prodrug, solubility, dissolution, partition coefficient, hydrolysis, oral absorption, pharmacokinetic parameter

Piperacillin (PIP) is a novel semisynthetic pencillin analog having a broad spectrum of antibacterial activity and has been shown to be effective in the treatment of serious infections caused by *Pseudomonas aeruginosa, Klebsiella pneumonia, Serratia marcescens* and other Gram-negative bacteria.[1-5] However the compound is very acid-unstable and has low lipid solubility, and thus it is poorly absorbed by the GI after oral administration and its use is limited to parenteral administration. Therefore piperacillin phthalidyl ester (PIP-PT) was synthesized in an attempt to overcome these disadvantages in the physiochemical properties of parent drug and to increase oral bioavailablity.[6-9]

To obtain relevant information for designing orally active prodrug of parenteral PIP, the physicochemical properties such as solubility, dissolution behavior and partition coefficient, and oral bioavailability were investigated.

EXPERIMENTAL

Materials and Apparatus

Piperacillin and bromophthalide were kindly given by Sam-Sung Pharm. Co., and Dong-A Pharm. Co., respectively. Methanol, triethylamine, acetone, ethyl acetate, *n*-octanol and dimethyl sulfoxide were analytical grade and were used without further purification. IR and UV

spectra were recorded with a Perkin-Elmer model 783 infrared spectrophotometer and Hitachi 200-20 spectrophotometer, respectively. NMR spectra were recorded on a Hitachi R-600 spectrophotometer.

Experimental Animal

Male New Zealand white rabbits weighing 2.0-2.5kg were used for oral absorption studies, and male Sprague Dowley rats weighing 200-230g were used for hydrolysis studies *in vitro*.

Synthesis of Piperacillin Phthalidyl Ester

5.2g (0.01M) of piperacillin was dissolved in the mixture of acetone and previously added triethylamine while stirring for 2hr. To this solution was added 2.1g (0.01M) of C_3-bromophthalide which was reacted at 0-5 °C for 5hr. The mixture was poured into ice water. After cooling to 0 °C for 1hr, the crystalline precipitate was collected and dissolved in chloroform. The combined chloroform extracts were washed with sodium bicarbonate solution (100ml × 3), then dried over calcium chloride and evaporated under reduced pressure. After recrystallization from ethyl acetate (100ml × 3), the crystalline powder was obtained as piperacillin phthalidyl ester and the yield was 20% (Scheme 1). m.p.: 174-175 °C. Anal. calcd. for $C_{31}H_{31}O_9N_5S$: C:57.31; H: 4.8; N:10.78; S: 4.94— found; C; 57.36; H: 4.92; N: 10.48; S; 4.72. IR ν_{max}^{KBr} cm^{-1}; 3300cm^{-1} (aromatic, C-H), 1775cm^{-1} (aromatic, C=0 ester). UV λ_{max} (methanol): 224.8 nm. PMR (CDCl$_3$); δ: 1.2 (3H, t, **CH$_3$** CH$_2$), 1.5 (6H, s, 2 –CH$_3$), 3.2–4.1 (6H, m, CH$_3$ **CH$_2$**, piperazine ring CH$_2$ CH$_2$), 4.53 (1H, s, 3 –H), 5.5–5.9 (3H, m, 5H, 6H, α–H), 7.3–7.9 (10H, m, C$_6$H$_6$, C$_6$H$_4$ ester CH), 9.4 (1H, d, NH), 9.9 (1H, d, NH)
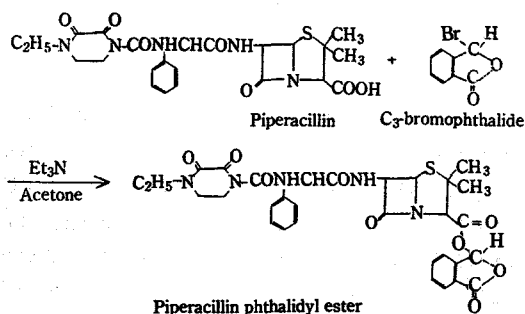
Scheme 1—Synthesis of piperacillin phthalidyl ester.

Solubility Test

The solubility of PIP and PIP-PT was tested hydrochloric acid buffer (pH 1.2 and 2.0), McIlvaine buffer (pH 2.3, 3.0, 5.6 and 7.0) and alkaline borate buffer (pH 8.0) in accordance with solubility test of KP IV.

Dissolution Test [10-11]

100ml of dissolution medium (simulated gastric and intestinal juice) was equilibrated to 37 ± 0.5 °C. The sample containing 100mg of PIP or PIP-PT as piperacillin was dried sufficiently and prepared from 50 meshed powder. Each sample was poured into the disk and the disk in the basket was placed into apparatus. Apparatus was operated immediately at 100 rpm. Samples of 5 ml were taken at 5, 10, 20, 30 and 60 min from starting the test and filterated by 0.45 μm Millipore filter. The filtered portions were diluted suitably and the amount dissolved was determined from ultraviolet absorbances at 224.8nm. Dissolution test was dealt with the dissolved percent, mean dissolution time, variance of retention time, and total amount of dissolved ester.

Partition Coefficient [12-13]

The sample containing 100mg of PIP or PIP-PT as PIP was added to 25ml of n-otanol. After stirring at 37 ± 0.5 °C for 30 min, the saturated n-octanol was filtered by 0.45 μm membrane filter. The filtered portion was added to 25 ml of simulated gastric and intestinal juices, respectively and stirred to reach equilibration. n-Octanol phase and aqueous phase were shaken mechanically for 15 min. at 3,000 rpm and then centrifuged. Each phase was diluted with methanol and ultraviolet absorbances were determined at 224.8nm. The concentration of each phase was calculated using a calibration curve obtained with standard solutions having known concentrations of the drug.

Hydrolysis Experiment *in Vitro* [14-16]

Male Sprague-Dowley rats were starved, except free access to water for 16-18 hr before the experiments. The liver and small intestine were removed immediately, and washed several times with saline. Samples were cut into small pieces and homogenized with potassium chloride— 0.01M phosphate buffer [1.15g of potassium

chloride was added to 0.01 M potassium phosphate monobasic solutions (pH 6.0) adjusted with 0.01 M NaOH solution] using an tissue homogenizer. Blood samples were taken from live rats and healthy human volunteer. After centrifugation at 1,000g for 20 min, the supernatant was kept frozen and used within 12 hrs. 10 mg of PIP-PT was dissolved in 0.2 ml of dimethyl sulfoxide and added to 5 ml of the supernatant. Sampling was carried out at 5, 10, 20, 30, 60 and 120 min after incubation at $37 \pm 0.5\,°C$. 0.5 ml of hydrolysate was added to 1 ml of methanol and shaken vigorously for 30 min using the vortex mixer. After centrifugation at 1,400g for 15 min, the supernatant was filtered with 0.45 μm Millipore filter and determined by high performance liquid chromatography in following conditions: column; μ-Bondapak C_{18} cartridge, mobile phase; methanol/0.1M-Na_2HPO_4 solution/water (45/10/45), detection; 254 nm, and flow rate; 1ml /min.

Oral Absorption Experiment[17-19]

Male New Zealand white rabbits weighing 2.0-2.5kg were starved except free access to water for 16-18hr before the experiment. PIP and PIP-PT was suspended in 15ml of 0.1% polysorbate 80 solution, and administered orally to a group of 3 rabbits at a dose of 300mg/kg as PIP by intubation. Blood was taken from the ear vein of rabbit at 0.5, 1, 2, 4, 5, 6, 8, 12, 16, 20 and 24hr after dosing. The blood samples were centrifuged at 1,400g for 15 min and then serum was obtained. The plasma concentration of parent PIP was measured by bioassay[20-21] using *Bacillus subtilis* ATCC 6633 in the Mueller Hinton medium, which strain resulted in good calibration curve between drug concentration and the diameter of inhibition zone.

RESULTS AND DISCUSSION

Solubility

The solubility of PIP and PIP-P at pH 1.2 − 8 is shown in Fig. 1. PIP showed high solubility above pH 4 as pH increased. But PIP-PT did not show
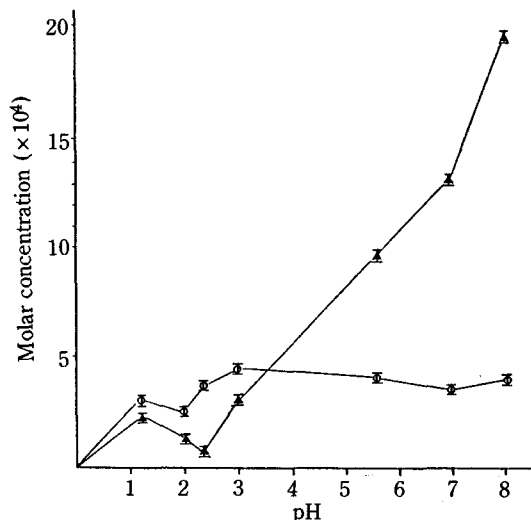

Figure 1—Solubility profiles for piperacillin (▲) and piperacillin phthalidyl ester (○) at $25 \pm 1.0\,°C$ under various pH conditions.

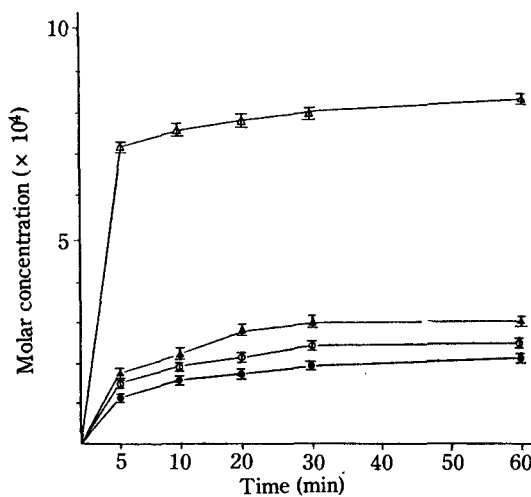**Figure 2**—Dissolution profiles for piperacillin and piperacillin phthalidyl ester in simulated gastric and intestinal juices at $37 \pm 0.5\,°C$.
Key: gastric juice − PIP (●), PIP-PT(○), intestinal juice − PIP (△), PIP-PT(▲)

the change of solubility due to pH.

Dissolution Behavior

Fig. 2 shows the dissolution rates of PIP and PIP-PT in simulated gastric and intestinal juices. In order to interpret dissolution behaviors pharmaceutically, total M (total amount of dissolved PIP-PT), MDT (mean dissolution time) and VRT

Table I — *Dissolution Parameters until Infinite Time for Piperacillin Pathalidyl Ester in Simulated Gastric and Intestinal Juices.*

Medium	Parameter		
	Total M	MDT	VRT
Gastric juice	2.287	0.116	0.027
Intestinal juice	2.853	0.111	0.019

Table II — *Partition Coefficients of Piperacillin and Piperacillin Phthalidyl Ester at 37±0.5°C (Mean ± S.E.).*

Material	Partition coefficient*	
	Gastric juice	Intestinal juice
PIP	0.7881 ± 0.6	0.7398 ± 0.8
PIP-PT	4.5806 ± 1.2	10.5455 ± 0.9

*Each value represents the ratio of drug concentration between *n*-octanol and aqueous phases.
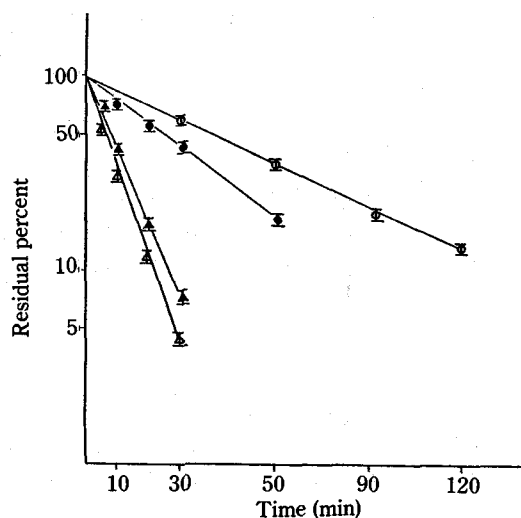

Figure 3—First-order plots of piperacillin phthalidyl ester remaining in rat liver homogenates (△); blood (▲), intestine homogenate (○), and human blood (●) at 37 ± 0.5°C.

(variance of retention time) were obtained by the following equation until infinite time.

$$\text{Total M} = \int_0^\infty \left(\frac{dm}{dt}\right) \cdot dt \tag{1}$$

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

$$\text{VRT} = \int_0^\infty (t - \text{MDT})^2 \cdot Cp \cdot dt / \int_0^\infty Cp \cdot dt \tag{3}$$

Table III — *Kinetic Parameters for the Hydrolysis of Piperacillin Phthalidyl Ester in Homogenates and Blood.*

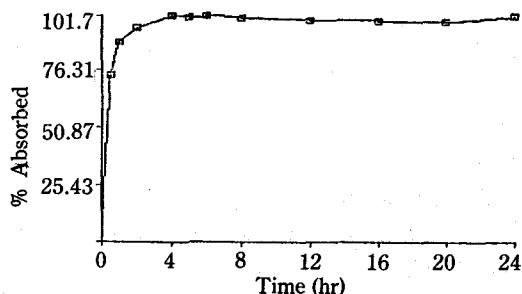| Medium	Rate constant (min⁻¹)	Half-life (hr)
Rat blood	0.0874	0.132
Rat liver homogenate	0.0993	0.116
Human blood	0.0259	0.444
Rat intestine homogenate	0.0169	0.680

Figure 4—Oral absorption profile for piperacillin phthalidyl ester by Nelson-Wagner method.

Table I shows dissolution parameters obtained computer programs.[22] As a result, the dissolution rate of PIP-PT was higher than that of PIP in simulated gastric juice. MDT and VRT of PIP-PT decreased in simulated intestinal juice more than in gastric juice while total M of PIP-PT increased in simulated intestinal juice more than in gastric juice.

Partition Coefficient

Table II shows the partition coefficients (P) of PIP and PIP-PT in simulated gastric and intestinal juices. The P values of PIP-PT were 6 times and 14 times as high as that of PIP in simulated gastric and intestinal juices, respectively.

Hydrolysis in Vitro

Fig. 3 and Table III show residual percents and half-lives of PIP-PT in homogenates of rat liver and small intestine, and blood of rat and human volunteer at 37 ± 0.5°C. The hydrolysis of PIP-PT to the parent PTP followed first-order kinetics with half lives of less than 30 min. The highest levels of hydrolysis rate were obtained with rat

Table IV—*Serum Piperacillin Concentration (μg/ml) after Oral Administration of Piperacillin and Piperacillin Phthalidyl Ester in Rabbits at a Dose of 300 mg/kg as Piperacillin.*

Material	Time (hr)										
	0.5	1	2	4	5	7	8	12	16	20	24
PIP	—	—	—	—	—	—	—	—	—	—	—
PIP-PT	3.52 ±0.67	4.18 ±0.59	4.26 ±0.57	4.06 ±0.77	3.83 ±0.63	3.65 ±0.56	3.17 ±0.62	2.49 ±0.93	1.97 ±0.67	1.57 ±0.49	1.34 ±0.32

Table V—*Pharmacokinetic Parameters for Piperacillin Phthalidyl Ester Obtained Residual and Nelson-Wagner Method in Rabbits.*

Material	K_{ab} (hr^{-1})	Abs $T_{1/2}$ (hr)	K_{el} (hr^{-1})	bio $T_{1/2}$ (hr)	C_{max} (μg/ml)	T_{max} (hr)	$(AUC)_0^\infty$ μg·hr·ml^{-1}
PIP-PT	1.118	0.1698	0.0571	12.129	4.26	2	85.42

liver followed by rat blood, human blood and rat intestine.

Oral Absorption

The data of serum PIP concentration of PIP and PIP-PT after oral administration in rabbits at a dose of 300mg/kg as PIP were presented in Table IV. PIP itself was not absorbed orally in rabbits, but PIP-PT was absorbed by the oral route to produce serum PIP concentration ranging from 1.34 to 4.26μg/ml. Fig. 4. shows absorption profile of PIP-PT by Nelson-Wagner method. Absorbed percents (% ABS) were calculated by the following equation and computer program was used for its fitting.[23]

$$\% \, ASB = \frac{A_T}{A_\infty} \times 100 = \frac{C_T + K_{el} \cdot \int_0^\infty C dt}{K_{el} \int_0^\infty C \cdot dt} \qquad (4)$$

Table V shows the parmacokinetic parameters for PIP-PT obtained by the residual method and Nelson-Wagner method. On the basis of peak serum piperacillin concentration, area under the curve from zero time to infinity $(AUC)_0^\infty$, and the ratio of K_a to K_e being over 3, we can conclude that the oral absorption properties of PIP were improved by preparing PIP-PT.

CONCLUSION

As a result of this study, following results were obtained.

1. The partition coefficients of PIP-PT were markedly enhanced in simulated gastric and intestinal juices comparing with those of PIP.

2. The hydrolysis half-lives of PIP-PT showed less thant 30 min and hydrolysis rate of PIP-PT decreased in the order of rat liver > rat blood > human blood > rat intestine.

3. PIP-PT showed the improvement of absorption in single oral dose in rabbits, however PIP did not exhibited any oral absorption.

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