DESETHYL PIPERACILLIN, A NEW ACTIVE METABOLITE OF PIPERACILLIN IN HUMAN

YOSHINORI MINAMI, MASAHITO KOMURO, KUMIKO SAKAWA and NAOBUMI ISHIDA

Biological Research Laboratory,
Taiho Pharmaceutical Co., Ltd.,
Kawauchi-cho, Tokushima 771-01, Japan

KEIZO MATSUMOTO and KAZUNORI OISHI

Department of Internal Medicine,
Institute for Tropical Medicine,
Nagasaki University,
Sakamoto-machi, Nagasaki 852, Japan

(Received for publication September 19, 1990)

Piperacillin (PIPC) is a broad-spectrum penicillin[1] and has been one of the most useful β-lactam antibiotics over the past 10 years. An active metabolite of PIPC previously reported is a ring-opening product at 2,3-dioxopiperazine moiety (T-1220A) in rat[2], but there is no report on active metabolites in human.

In the course of clinical study for the co-administration of PIPC with a β-lactamase inhibitor, tazobactam[3], an active metabolite of PIPC (**1**) which is distinct from T-1220A was found in human plasma and urine. The metabolite **1** was also found in the same manner on the administration of PIPC alone, suggesting that the metabolism of PIPC was not affected by the co-administration of tazobactam. The pharmacokinetic properties of **1** in human will be reported elsewhere. Herein we wish to describe the isolation, structural determination and antimicrobial activity of **1**.

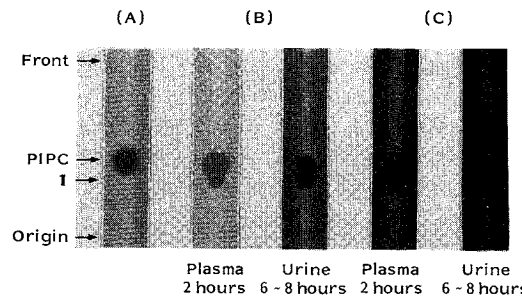
Plate 1 shows the thin-layer bioautograms of plasma and urine from healthy volunteers after 1 hour infusion with 2.5 g of YP-14 (PIPC-tazobactam, 4:1, Taiho Pharm. Co.) or 2.0 g of PIPC (Toyama Chem. Co.). Plasma was deproteinized with acetonitrile-0.5 N formic acid (99:1) and concentrated to dryness. After suitable dilution with distilled water, plasma or urine were applied to TLC plates (Silica gel 60W, Merck) which had been dried at 110°C for 30 minutes after a soak in 0.1 M phosphate buffer (pH 7.0). The plates were developed with ethyl acetate-acetone-acetic acid-water (30:10:6:5) followed by air-drying, and laid on agar plates inoculated with *Micrococcus luteus* ATCC 9341 at 4°C for 30 minutes. The agar plates were incubated at 37°C for 20 hours. In both forms of YP-14 and PIPC, the bioautograms of plasma and urine apparently showed an unknown active spot (Rf 0.32), together with that of PIPC (Rf 0.42).

The isolation of **1** was carried out from human urine collected after administration of YP-14. Urine adjusted to pH 5 was passed through a Diaion CHP-20 column and **1** was adsorbed. After washing with water, active fraction containing PIPC and **1** was eluted with water-methanol (1:1). The fraction was concentrated and further separated by preparative HPLC under the following conditions: column, YMC D-10 (250 × 20 mm, i.d., Yamamura Chemical Co.); mobile phase, 25% acetonitrile-10 mM KH_2PO_4; flow rate, 9.9 ml/minute; detector, UV 220 nm. Active peak portion at Rt 8 minutes (PIPC at Rt 16 minutes) was collected repeatedly and concentrated. After removal of inorganic salts by Diaion CHP-20 column chromatography, the fraction was lyophilized to give pure **1**, which revealed the same Rf value on bioautogram with the unknown active spot described above.

1 showed a β-lactam absorption at 1772 cm^{-1} in IR spectrum, and gave *quasi*-molecular ions at *m/z* 490 $(M+H)^+$ and *m/z* 528 $(M+K)^+$ which were 28 mass units less than those of PIPC in FAB-MS. ^1H NMR data of **1** and PIPC are summarized in Table 1. The proton signals at penicillanic acid moiety showed good similarity in both compounds, but an amido signal at δ 8.90 was newly found in the spectrum of **1**, instead of ethyl signals in that of PIPC. Furthermore, the 6'-CH_2 signal at the 2,3-dioxopiperadine moiety was shifted up-field to

Plate 1. Typical bioautograms of plasma and urine after 1 hour infusion of YP-14 or PIPC to healthy volunteers.

(A) Authentic PIPC, (B) YP-14 (2.5 g) infusion group, (C) PIPC (2.0 g) infusion group.

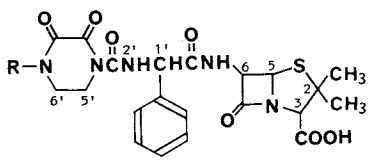


Plasma were obtained at 2 hours, and urine were collected during 6~8 hours period after start of infusion.

Table 1. ^{1}H NMR chemical shifts of PIPC and **1**.

Proton	Chemical shifts (δ) in DMSO-d_6 at 400 MHz	
	1	PIPC (Acid form)
3'-NH	9.85 (1H, d, J=7.3 Hz)	9.82 (1H, d, J=7.5 Hz)
6-NH	9.09 (1H, d, J=8.2 Hz)	9.09 (1H, d, J=8.2 Hz)
7'-NH	8.90 (1H, br s)	—
2'-Ph	7.2~7.5 (5H, m)	7.2~7.5 (5H, m)
2'-H	5.74 (1H, d, J=7.5 Hz)	5.74 (1H, d, J=7.5 Hz)
6-H	5.35 (1H, dd, J=8.1, 3.9 Hz)	5.35 (1H, dd, J=8.0, 3.9 Hz)
5-H	5.22 (1H, d, J=3.9 Hz)	5.22 (1H, d, J=3.9 Hz)
5'-H	3.89 (2H, m)	3.90 (2H, m)
3-H	3.80 (1H, s)	3.79 (1H, s)
6'-H_2	3.37 (2H, m)	3.56 (2H, m)
Et-CH_2	—	3.39 (2H, q, J=7.0 Hz)
2-CH_3	1.50 (3H, s)	1.50 (3H, s)
2-CH_3	1.37 (3H, s)	1.37 (3H, s)
Et-CH_3	—	1.09 (3H, t, J=7.0 Hz)

Fig. 1. Structures of PIPC and **1**.



PIPC R=CH_2CH_3
1 R=H

δ 3.37. These data indicate that the ethyl group in C-6 side chain of PIPC is replaced by a hydrogen atom in **1**. These assignments are also supported by the MW difference between PIPC and **1**. Accordingly, the structure of **1** was determined to be a desethyl analog of PIPC, 6-[D(−)-α-(2,3-dioxo-1-piperazinecarboxamido)phenylacetamido]penicillanic acid (desethyl-PIPC) (Fig. 1). This was confirmed by comparison with authentic desethyl-PIPC prepared according to the synthetic route of PIPC[4].

The antimicrobial activity of **1** together with PIPC determined by 2-fold serial agar dilution method are shown in Table 2. **1** was as potent as PIPC against Gram-positive bacteria, but it is several times less active than PIPC against Gram-negative becteria except *Morganella morganii* and *Proteus vulgaris*.

Acknowledgments

The authors are indebted to Dr. S. MITSUHASHI of Episome Institute for his valuable comments and suggestions. We also thank Mr. F. HIGASHITANI for antimicrobial tests and Dr. S. YAMADA for preparation of authentic desethyl-PIPC in our laboratory.

Table 2. Antimicrobial activity of PIPC and **1** against standard strains.

Test organism	MIC (μg/ml)	
	1	PIPC
Staphylocccus aureus FDA 209P JC-1	1.56	0.78
S. aureus Terajima	0.10	0.10
S. aureus MS 353	0.39	0.39
Streptococcus pyogenes Cook	0.10	0.10
Bacillus subtilis ATCC 6633	0.20	0.20
Micrococcus luteus ATCC 9341	0.10	0.05
Escherichia coli NIHJ JC-2	6.25	1.56
E. coli K-12 C600	12.5	1.56
Enterobacter cloacae 963	12.5	1.56
E. aerogenes ATCC 13048	25	3.13
Klebsiella pneumoniae PCI 602	12.5	1.56
Salmonella typhimurium IID 971	12.5	3.13
S. typhi 901	6.25	1.56
S. paratyphi 1015	0.10	0.05
S. schottmuelleri 8006	3.13	1.56
S. enteritidis G 14	0.39	0.10
Serratia marcescens IAM 1184	12.5	0.78
Morganella morganii IFO 3848	0.05	0.05
Proteus mirabilis IFO 3849	0.78	0.20
P. vulgaris OX 19	0.05	≦0.025
P. vulgaris HX 19	≦0.025	≦0.025
Providencia rettgeri IFO 3850	0.78	0.20
Pseudomonas aeruginosa IFO 3445	25	6.25
P. aeruginosa NCTC 10490	6.25	1.56
P. aeruginosa PAO 1	>25	3.13

Medium: Sensitivity Disk Agar-N (Nissui Seiyaku Co.).
Inoculum size: 10^6 cells/ml.
Incubation: 18 hours at 37°C.

References

1) UEDA, Y.: Summary of fundamental and clinical studies on T-1220, a broad-spectrum penicillin derivative. Chemotherapy (Tokyo) 25: 683~699, 1977
2) SAIKAWA, I.; A. TAKAI, Y. NAKASHIMA, C. YOSHIDA, T. YASUDA, E. SHIMIZU, H. SAKAI, H. TAKI, M. TAI & Y. TAKASHITA: Studies on β-lactam antibiotics for medicinal purpose. VI. Studies on the metabolism of 6-[D(−)-α-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)phenylacetamido]penicillanic acid (T-1220). Yakugaku Zasshi (Japanese) 97: 1071~1081, 1977
3) HIGASHITANI, F.; A. HYODO, N. ISHIDA, M. INOUE & S. MITSUHASHI: Inhibition of β-lactamases by tazobactam and in-vitro antibacterial activity of tazobactam combined with piperacillin. J. Anti-

microb. Chemother. 25: 567~574, 1990

4) SAIKAWA, I.; S. TAKANO, C. YOSHIDA, O. TAKASHIMA, K. MOMONOI, T. YASUDA, K. KASUYA & M. KOMATSU: Studies on β-lactam antibiotics for medicinal purpose. II. Synthesis of D(−)-α-[dioxo-1-piperazinecarboxamido]benzylpenicillins and structure-antibacterial activity. Yakugaku Zasshi (Japanese) 97: 980~986, 1977