Pharmacokinetics of Ampicillin and Methoxymethyl Ester of Hetacillin in Dogs

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Abstract □ Concentrations of ampicillin and the methoxymethyl ester of hetacillin were measured in plasma, prostatic fluid, and spinal fluid of dogs receiving ampicillin or the hetacillin ester by continuous intravenous infusion. The ratios of ampicillin in prostatic fluid relative to plasma levels were higher after dosing with the hetacillin ester. Pharmacokinetic parameter values were consistent with urinary excretion characteristics. Ready diffusion of the ester across biological membranes may facilitate eradication of pathogenic organisms in prostatic and spinal fluids.

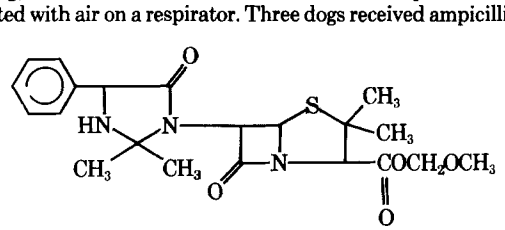
Keyphrases □ Ampicillin—pharmacokinetics, plasma, prostatic fluid, and spinal fluid levels, dogs □ Hetacillin methoxymethyl ester—pharmacokinetics, plasma, prostatic fluid, and spinal fluid levels, dogs □ Pharmacokinetics—ampicillin and hetacillin methoxymethyl ester, plasma, prostatic fluid, and spinal fluid levels, dogs □ Antibacterials—ampicillin and hetacillin methoxymethyl ester, pharmacokinetics, plasma, prostatic fluid, and spinal fluid levels, dogs □ Distribution, drug—ampicillin and hetacillin methoxymethyl ester, plasma, prostatic fluid, and spinal fluid levels, dogs

The methoxymethyl ester of hetacillin[1] (I) is a new lipophilic prodrug of ampicillin, with distribution and kinetic properties different from other ampicillin precursors (1, 2). These properties include rapid absorption from the GI tract and extensive distribution into body tissues, particularly prostatic and central nervous system (CNS) tissue. The hetacillin ester appears to be absorbed after oral doses largely as the intact ester. Once absorbed, the ester undergoes hydrolysis in the blood and other tissues and body fluids to yield ultimately free ampicillin. Distribution characteristics of the unchanged ester and ampicillin are functions of the relative rates of tissue uptake and enzymatic and chemical hydrolyses.

The entry of ampicillin and the hetacillin ester into prostatic fluid and tissue of dogs during continuous intravenous infusion was described previously (2). The purposes of the present study were to examine these data from a pharmacokinetic point of view and to describe the tissue distribution characteristics of the hetacillin ester after continuous intravenous infusion in dogs.

EXPERIMENTAL

Full details regarding animal preparation, dosing, sampling, and assay procedures were given elsewhere (2). In summary, eight male beagle dogs, 17–21 kg, were anesthetized with intravenous thiopental sodium[2] and ventilated with air on a respirator. Three dogs received ampicillin sodium[3]



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[1] BL-P1761.
[2] Pentothal sodium, Abbott.
[3] Polycillin-N, Bristol Laboratories, Syracuse, N.Y.

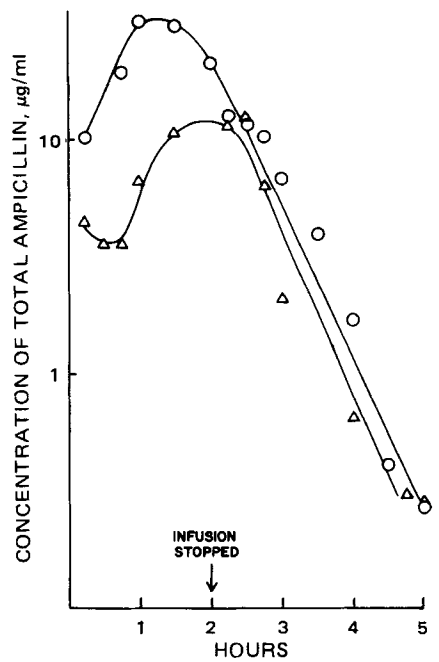


Figure 1—*Concentration of total ampicillin activity in plasma (○) and prostatic fluid (△) during and after infusion of the hetacillin ester (Dog 10).*

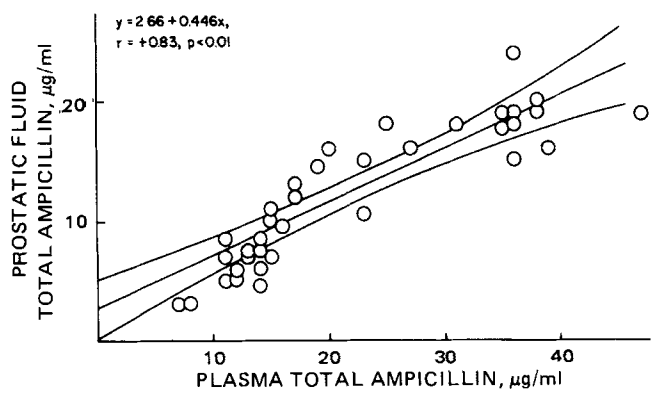


Figure 2—*Linear regression with 95% confidence limits of total ampicillin concentration in prostatic fluid against total ampicillin concentration in plasma during hetacillin ester infusion.*

as an initial bolus injection of 3 (Dogs 1 and 2) and 10 (Dog 3) mg/kg and then as a constant infusion into a femoral vein in sterile lactated Ringer's solution at a rate of 10 mg/kg/hr for 4 hr. The infusion was started simultaneously with the bolus injection. Five dogs (Dogs 4–8) were similarly treated with the hetacillin ester[4], receiving a bolus injection of 1.8 mg of ampicillin equivalents/kg followed by a constant infusion of 10 mg/kg/hr iv.

All dogs were sacrificed after a 4-hr infusion. Two other dogs (Dogs 9 and 10) were similarly infused with the hetacillin ester for 2 hr, and plasma and prostatic or spinal fluid were obtained both during and fol-

[4] Sterile bottles containing 50 mg of ampicillin equivalents/bottle, Bristol Laboratories.

Table I—Plasma and Prostatic Fluid Levels of Ampicillin during Infusion of Ampicillin

Dog		Concentration, μg/ml							
		30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
1	Plasma	50	38	33	31	29	—[a]	23	18
	Prostatic fluid	0.5	1.0	1.0	1.2	0.9	1.1	1.0	0.2
	Prostatic fluid / Plasma	0.01	0.03	0.03	0.04	0.03	–	0.04	0.01
2	Plasma	22	25	17	23	–	30	18	19
	Prostatic fluid	0.3	0.5	0.7	0.8	1.1	0.7	0.9	0.8
	Prostatic fluid / Plasma	0.01	0.02	0.04	0.03	–	0.02	0.05	0.04
3	Plasma	19	24	23	26	–	38	33	23
	Prostatic fluid	0.2	0.2	1.9	1.4	0.9	1.9	2.0	2.2
	Prostatic fluid / Plasma	0.01	0.01	0.08	0.05	–	0.05	0.06	0.10

[a]Not determined.

lowing the infusion. Infusion bottles containing the hetacillin ester were kept in ice water, and fresh solutions were prepared hourly to minimize ester hydrolysis during administration.

Prostatic fluid was obtained by urethral intubation in the perineum just distal to the prostate gland. Urine was collected by cannulation of both ureters, thus preventing contamination of prostatic fluid with urine. The vas deferens was ligated. Prostatic fluid secretion was stimulated by constant infusion of pilocarpine, 0.25 mg/kg/hr iv for 4 hr.

Blood samples were collected from a femoral artery in heparinized tubes at 30-min intervals, and plasma was separated. Prostatic fluid was collected at hourly intervals in vessels contained in an ice bath. Spinal fluid was obtained by suboccipital puncture. All samples were deep frozen immediately after collection until assayed.

Assays for free ampicillin and the unhydrolyzed hetacillin ester were carried out by a disk diffusion method, using *Sarcina lutea* (ATCC 9341) as the test organism as described previously (2). Renal clearances were calculated from hourly urinary excretion of ampicillin or the hetacillin ester during the 2nd, 3rd, and 4th hr of infusion.

RESULTS

Concentrations of ampicillin in plasma and prostatic fluid from the ampicillin infusion and urinary excretion details are given in Tables I and II. Average plasma and prostatic fluid concentrations of ampicillin and the hetacillin ester and urinary excretion details are given in Tables III and IV.

As shown in Tables I and III, the ratio of prostatic fluid to plasma ampicillin varied from 0.1 to 0.01 during ampicillin infusion, with prostatic fluid levels ranging from 0.2 to 2.2 μg/ml. However, during hetacillin ester infusion, mean prostatic fluid levels of ampicillin ranged from 2.2 to 7.4 μg/ml, increasing from the infusion time; the ratios of prostatic fluid to plasma ampicillin were considerably increased to about 0.4.

It can be seen from Tables II and IV that the concentrations of ampicillin in urine were lower after hetacillin ester infusion but were still very high compared to plasma levels. Approximately one-half of the infused ampicillin was recovered unchanged in urine, and renal clearance was 40–80 ml/min. During hetacillin ester infusion, recovery of ampicillin in urine was considerably reduced. However, the renal clearances, except from Dogs 5 and 6 which were atypically low, were similar to those resulting from ampicillin infusion (38–74 ml/min). These values are similar to those reported previously (3). The renal clearance of the hetacillin ester was essentially zero, confirming the quantitative hydrolysis and metabolism of this agent *in vivo*.

Table II—Urinary Excretion of Ampicillin during Infusion of Ampicillin

Dog	Urine Concentration, mg/ml	Urine Volume, ml	4-hr Recovery, % of Dose	Renal Clearance, ml/min
1	4.23 (3.95)[a]	214	44.7	65.1 (25.2)[a]
2	2.44 (1.53)	252	50.1	84.8 (33.1)
3	2.86 (2.08)	157	46.3	40.7 (8.9)

[a]Mean and standard deviation from three 1-hr collections.

Spinal fluid levels of ampicillin and the hetacillin ester were obtained in Dogs 4–7 at the end of the infusion period and in Dogs 8 and 9 at 30–60-min intervals during infusion. The mean 4-hr values for ampicillin and the hetacillin ester were 11.0 ± 8.1 and 2.6 ± 1.7 μg/ml, respectively, which were both about one-third the respective plasma levels.

In Dog 8, spinal fluid concentrations of total ampicillin activity at 30, 90, 120, 180, and 240 min during infusion were 4, 12, 19, 20, and 26.5 μg/ml, respectively. In Dog 9, spinal fluid levels of total ampicillin at 30, 60, 90, and 120 min during infusion were 0.0, 1.2, 3.4, and 4.2 μg/ml, respectively. Thus, in both dogs, there appeared to be an accumulation of the antibiotic in spinal fluid during the infusion while plasma levels remained constant. Furthermore, ampicillin appeared to be cleared from spinal fluid very slowly because the levels of ampicillin in Dog 9 remained constant during 90 min postinfusion while plasma levels decreased from 3.6 to 0.8 μg/ml.

The relative rates of loss of total ampicillin activity (ampicillin plus the hetacillin ester) from plasma and prostatic fluid were determined in Dog 10 by repeated sampling during 3 hr following a 2-hr infusion of the hetacillin ester. The similarity of the rates of decline is indicated in Fig. 1. The calculated elimination half-lives in plasma and prostatic fluid were 0.46 and 0.45 hr, respectively. The linear relationship between the various combined ester and ampicillin concentrations in prostatic and spinal fluids and plasma during infusion is illustrated in Figs. 2 and 3.

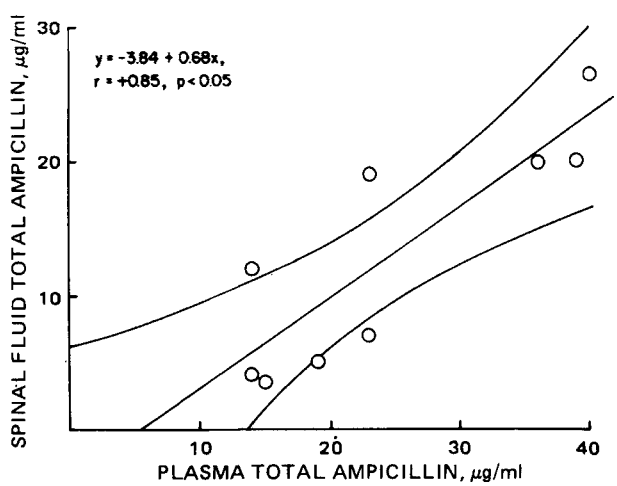


Figure 3—*Linear regression with 95% confidence limits of total ampicillin concentration in spinal fluid against total ampicillin concentration in plasma during hetacillin ester infusion.*

Table III—Mean Plasma and Prostatic Fluid Levels of Ampicillin and Hetacillin Ester during Infusion of Hetacillin Ester in Five Dogs

	Concentration, μg/ml							
	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
Plasma ampicillin	8.2 (4.7)[a]	11.6 (5.5)	12.4 (6.5)	16.0 (7.9)	17.0 (7.9)	22.6 (12.1)	22.2 (6.1)	20.0 (8.2)
Plasma hetacillin ester	5.0 (3.3)	5.0 (3.0)	5.2 (2.8)	5.8 (2.2)	6.6 (3.5)	5.2 (4.9)	7.0 (2.9)	6.4 (3.2)
Prostatic fluid ampicillin	2.2 (2.7)	3.1 (2.8)	2.6 (0.5)	5.6 (3.2)	6.7 (2.3)	5.9 (2.1)	6.2 (2.7)	7.4 (3.1)
Prostatic fluid hetacillin ester	4.6 (3.1)	6.3 (2.5)	5.8 (1.9)	7.2 (2.2)	6.4 (3.5)	8.2 (3.4)	8.3 (4.1)	8.8 (4.2)
Prostatic / Plasma ampicillin	0.23 (0.13)	0.24 (0.10)	0.28 (0.06)	0.39 (0.05)	0.42 (0.15)	0.34 (0.08)	0.31 (0.07)	0.43 (0.15)
Prostatic / Plasma hetacillin ester	0.82 (0.32)	1.50 (0.81)	1.51 (0.34)	1.53 (0.46)	1.00 (0.29)	2.70 (2.0)	1.21 (0.42)	1.51 (0.5)

[a] Mean and standard deviation from three 1-hr collections.

Table IV—Urinary Excretion of Ampicillin and Hetacillin Ester after Infusion of Hetacillin Ester

Dog		Urine Concentration, mg/ml	Urine Volume, ml	4-hr Recovery, % of Dose	Renal Clearance, ml/min
4	Ampicillin	1.17 (0.28)[a]	122	22.7	74.3 (3.9)
	Hetacillin ester	4.5 (0.6)[b]		0.1	1.0 (0.3)
5[c]	Ampicillin	0.71 (0.45)	91	7.9	20.0[d]
	Hetacillin ester	3.7 (1.5)		0.02	0.21 (0.17)
6	Ampicillin	0.70 (0.59)	218	14.3	40.8 (14.0)
	Hetacillin ester	4.5 (2.6)		0.1	0.5 (0.15)
7	Ampicillin	0.40 (0.27)	268	11.6	38.1 (15.3)
	Hetacillin ester	2.3 (0.5)		0.07	0.61 (0.34)
8[c]	Ampicillin	2.03[d]	30	6.1	21.8[d]
	Hetacillin ester	2.5		0.02	0.18

[a] Mean and standard deviation from three 1-hr collections. [b] Urinary concentration of the hetacillin ester is expressed in micrograms per milliliter in all cases. [c] Dogs 5 and 8 developed slow heart rates and low blood pressures due to pilocarpine and were generally in poor condition during the experiment. Renal excretion in these animals was low. [d] Only two collections.

DISCUSSION

If it is assumed that the hetacillin ester obeys apparent one-compartment model kinetics, plasma data during infusion of the ester can be described by Scheme I. In this model, concentration of ester in plasma at the steady state, C_{ss}, is given by (4):

$$C_{ss} = k_0/VK \qquad \text{(Eq. 1)}$$

where k_0 is the zero-order infusion rate constant, K is the apparent first-order rate constant for loss of the ester by all processes, and V is the ester distribution volume in the body. The product VK is the ester plasma clearance (5).

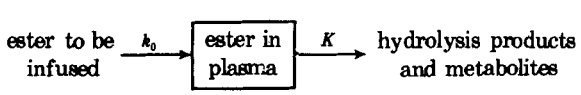
With the plasma concentrations in Table III and known infusion rates, plasma clearances of the ester for Dogs 4, 5, 6, 7, and 8 were 1087, 321, 529, 833, and 457 ml/min, respectively. The values contrast with the almost negligible renal clearances of unchanged ester.

The distribution volume of the hetacillin ester was reported to be approximately 85% of body weight (1). Substituting this value into Eq. 1 and using the relationship $t_{1/2} = \ln 2/K$ yielded a hetacillin ester half-life of 0.34 ± 0.18 hr. This value is identical to that reported for the *in vitro* degradation of the hetacillin ester in dog serum (2).

The overall ratio of ampicillin renal clearance to creatinine clearance in Dogs 1–8 was 1.31 ± 0.55, indicating some renal tubular secretion. This secretion is not as extensive as that usually observed in humans (6) but may have been influenced to some extent by the experimental conditions and surgical procedures.

The relationship between prostatic and spinal fluid levels and plasma levels of ampicillin during hetacillin ester infusion suggests that the lipophilic ester is transported readily into these fluids and that hydrolysis occurs subsequently. It is evident that drug levels in spinal and prostatic fluids can be predicted with reasonable accuracy from observed plasma levels, at least during continuous dosing. Although prostatic fluid levels of ampicillin decline with plasma levels, the antibiotic may stay within spinal fluid for longer periods.

The ability of the hetacillin ester to penetrate spinal and prostatic fluids may prove of considerable use in the eradication of pathogenic organisms resident in these sites which have been relatively inaccessible to the penicillins.



Scheme I—One-compartment open model with zero-order absorption

REFERENCES

(1) "Disposition and Safety Evaluation Studies on BL-P1761," Bristol Laboratories, Syracuse, N.Y., Jan. 1974.

(2) T. B. Kjaer and P. O. Madsen, *Invest. Urol.*, **14**, 57 (1976).

(3) B. E. Cabana, L. E. Willhite, and M. E. Bierwagen, *Antimicrob. Ag. Chemother.*, **1969**, 35 (1970).

(4) J. G. Wagner, "Biopharmaceutics and Relevant Pharmacokinetics," Drug Intelligence Publications, Hamilton, Ill., 1971.

(5) P. G. Welling, W. A. Craig, G. L. Amidon, and C. M. Kunin, *Clin. Pharmacol. Ther.*, **15**, 344 (1974).

(6) L. W. Dittert, W. O. Griffin, J. C. LaPiana, F. J. Shainfeld, and J. T. Doluisio, *Antimicrob. Ag. Chemother.*, **1969**, 42 (1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 8, 1975, from the *Section of Urology, Veterans Administration Hospital, Department of Surgery, School of Medicine, Madison, WI 53705, and the [‡]School of Pharmacy, University of Wisconsin, Madison, WI 53706.

Accepted for publication May 5, 1976.

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