

## Pharmacokinetics of Bay k 4999, a New Broad-Spectrum Penicillin

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The pharmacokinetics of the broad-spectrum penicillin Bay k 4999 were studied in six healthy male volunteers. A 2-g dose was given by the intravenous route. The tissue penetration of the antibiotic was studied by both dermabrasion and blister techniques. A total of 26.4% of the drug was recovered in the urine in 24 h, 79% of this being excreted in the first 2 h. The elimination half-life in serum was 1.3 h. The dermabrasion levels of Bay k 4999 were generally similar to those in serum, but after 1 h the blister fluid levels of antibiotic were greater than those in serum. Different drug levels obtained by blister and dermabrasion techniques may be due to the different composition of the two fluids.

There are at present a number of broad-spectrum penicillins with antipseudomonal activity under investigation. Azlocillin (11), mezlocillin (1, 14), and piperacillin (12) are three  $\alpha$ -amino-substituted penicillins. Bay k 4999 is structurally related to these and has the formula 6[D-2-(3-furfurylideneamino-2-oxoimidazolidine-1-carboxamido)-2-(4-hydroxy-phenyl)-acetamido] penicillanic acid. The compound has a wide spectrum of activity, including activity against *Pseudomonas aeruginosa* (13). In this study the pharmacokinetics of Bay k 4999 were investigated in healthy volunteers, including the penetration of antibiotic into "tissue" fluid. Two tissue fluid models were used, a dermabrasion (4, 9) and a blister technique (10)

### MATERIALS AND METHODS

Six healthy male volunteers with no history of previous renal or hepatic disease and no known allergy to penicillin participated in the study. They were aged 22 to 36 years and had normal body build and mean weight of  $76 \pm 5.9$  (standard deviation) kg. Renal function was assessed by serum creatinine and blood urea concentrations and was within the normal range.

On the night before the study, the volunteers were instructed to avoid excessive fluid intake. On retiring to bed, they were instructed to strap onto the anterior aspect of the upper forearm two 0.2% cantharides plasters (1 by 1 cm) and to cover these with a gauze swab. On the morning of the study they were instructed to have only a light breakfast of toast or cereal plus one cup of beverage. A predose sample of blood was taken for assay, and then 2 g of Bay k 4999 (supplied by Bayer UK Ltd) dissolved in 20 ml of

sterile water was injected into a contralateral (to the site of the plasters) forearm vein during 2 to 3 min. Samples of blood for antibiotic estimation were taken from a forearm vein other than that used for the injection at 5, 10, 20, 30, and 45 min and 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 h after the injection; the serum was separated and assayed the same day.

Urine samples were collected at intervals during the 24 h after injection, the volumes were noted, and portions were frozen at  $-20^{\circ}\text{C}$  to be assayed the next day.

The blisters were sampled by means of a fine needle every hour during the 1 to 5 h after injection, and the blister fluid was placed upon preweighed, sterile, 6-mm-diameter assay disks. Duplicate samples were obtained in four out of the six volunteers (the volume of fluid in the blister was inadequate in two cases). The protein content of a sample of blister fluid was measured by routine electrophoresis, and a leukocyte count was determined with a Coulter Counter.

At 30 min after injection, an area (approximately 30  $\text{mm}^2$ ) of skin on the anterior aspect of the volunteer's forearm was abraded with a high-speed drill (20,000 rpm) and a sterile buff. Over this area was placed a preweighed, 6-mm-diameter assay disk. Any pinpoints of bleeding (which were few) were allowed to clot first. The disk was kept in place by means of a glass slide strapped to the skin for 1 h. It was then removed and weighed, and another disk was placed over the area for a further 1 h. At 1.5 h postinjection, another area of skin was abraded and similarly treated. Thus, four estimations with the dermabrasion technique were performed, namely, one at 30 min to 1.5 h ("1-h" sample), two at 1.5 to 2.5 h ("2-h" sample), and one at 2.5 to 3.5 h ("3-h" sample).

The samples were assayed for antibiotic activity with a large-plate agar diffusion technique, with serum and urine samples being applied randomly in triplicate. The indicator organism was *Bacillus subtilis* ATC 6633. The medium employed was Difco Penassay agar

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no. 1, pH 6.6. Serum standards were prepared in pooled human serum, and urine standards were prepared in phosphate-buffered saline, pH 6.6, in which urine was diluted when necessary. In the case of the samples obtained from the dermabrasions and blisters, antibiotic standards made up in human serum were applied in triplicate on identical disks, in the same volume as that calculated by weighing to be on the test disk.

Pharmacokinetic analysis of serum data was carried out by using the iterative regression analysis program NONLIN (8) on a Univac model 418 III digital computer. For computer analysis, all serum data were weighted according to their reciprocals.

## RESULTS

The mean serum levels of Bay k 4999 together with one standard deviation and the results obtained from the dermabrasion and blister fluid studies are shown in Fig. 1. A mean peak level of 200  $\mu\text{g}/\text{ml}$  was obtained immediately after dosing. The amount of antibiotic in serum then declined rapidly to 5  $\mu\text{g}/\text{ml}$  at 5 h and to undetectable levels at 6 h postdosing. The curved nature of the decline in serum antibiotic levels suggested that drug was being taken up by other tissues or fluids at early times, a situation frequently represented in terms of a linear pharmacokinetic model containing more than one body compartment. The simplest multicompartment systems are the two- and three-compartment models, and serum drug profiles for these two models after rapid intravenous injection are described by a biexponential or a triexponential expression, respectively.

Individual serum profiles were therefore examined by means of the program NONLIN in terms of both biexponential and triexponential functions. Although the triexponential function provided a marginally better fit to the data in most cases, an F test of the respective weighted squared deviations (2) showed that in only one of the six subjects was a significantly better fit obtained with the more complex function. It was therefore concluded that the biexponential function provided an adequate description of serum levels and that the levels could be interpreted in terms of two-compartment model kinetics.

The results of this analysis are presented in Table 1. In the table the first-order rate constants and  $k_{12}$  and  $k_{21}$  represent the rate of drug transfer from the central (serum) and peripheral (tissue) compartments and from the peripheral compartment back to the central compartment, respectively;  $k_{el}$  is the first-order rate constant for loss of drug from the body by all routes of elimination; and  $t_{1/2(\alpha)}$  and  $t_{1/2(\beta)}$  are the drug disappearance half-lives from plasma during distribution and postdistribution phases, respectively. The values of  $V_1$  and  $V_{d(\text{ss})}$  represent the

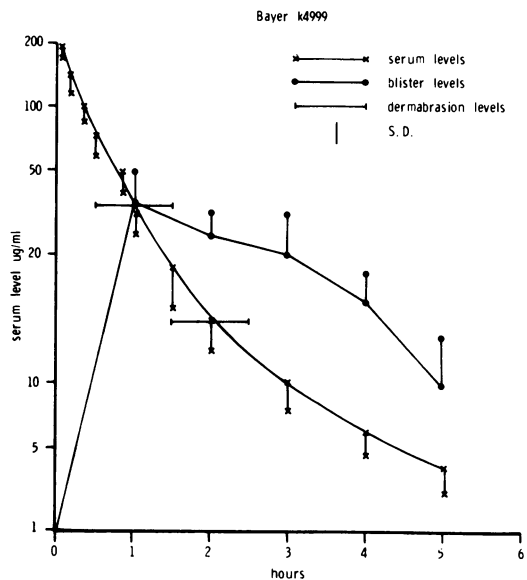


FIG. 1. Mean concentrations of Bay k 4999 (micrograms per milliliter) in serum and blister and dermabrasion fluid after a single 2-g intravenous dose to six volunteers.

apparent drug distribution volumes of the central compartment and the overall drug distribution volume once equilibration between serum and tissues has been reached. Both volumes are expressed as percentages of body weight.

The drug initially distributed into an apparent volume equivalent to 12% body weight. The subsequent distribution phase had a half-life of 0.27 h, and tissue uptake was virtually complete 1.2 h after dosing, at which time the apparent distribution volume was 20% body weight. The terminal half-life of Bay k 4999 in serum was 1.3 h. The mean serum clearance was 274 ml/min. Although this value is double the normal glomerular filtration rate, its significance is uncertain because the mean urinary excretion of unchanged compound was  $529 \pm 132$  (standard deviation) mg, or only 26.4% of the dose, 79% of this being excreted in the first 2 h.

The levels of antibiotic in the dermabrasion fluid 1 and 2 h postdosing were similar to levels in serum at those times. The 3-h level in dermabrasion fluid was below the assay sensitivity limit in four of the six volunteers.

The levels of antibiotic in blister fluid at 1 h were similar to the levels in serum at that time. Between 1 and 3 h the levels in blister fluid decreased more slowly than those in serum, but the elimination rate increased between 3 and 5 h postdosing. During the latter time period, the mean half-life of antibiotic in blister fluid was 0.9 h. Drug levels in blister fluid were higher

TABLE 1. Pharmacokinetic constants obtained from biexponential analysis of Bay k 4999 serum data

Sub- ject	Pharmacokinetic constant								
	$k_{12}$ (h <sup>-1</sup> )	$k_{21}$ (h <sup>-1</sup> )	$k_{el}$ (h <sup>-1</sup> )	$V_1$ (%)	$V_{d(m)}$ <sup>a</sup> (%)	AUC <sup>b</sup> (μg/h per ml)	SCL <sup>c</sup> (ml/min)	$t_{1/2(\alpha)}$ <sup>d</sup> (h)	$t_{1/2(\beta)}$ <sup>e</sup> (h)
1	0.57	0.62	2.1	11.4	21.9	99.7	334.3	0.24	1.5
2	0.23	0.57	1.7	11.3	16.8	149.3	223.3	0.34	1.4
3	0.26	0.47	1.6	13.3	20.6	124.1	268.7	0.36	1.8
4	0.82	1.38	1.5	13.3	21.2	124.4	268.0	0.23	1.0
5	0.59	0.88	1.9	11.9	19.9	125.1	266.5	0.25	1.1
6	0.85	0.98	2.1	11.2	21.0	116.6	285.9	0.21	1.1
Mean	0.55	0.82	1.8	12.1	20.1	123.2	274.4	0.27	1.3
SD	0.27	0.34	0.27	1.0	2.2	16.0	36.0	0.06	0.3

<sup>a</sup>  $V_{d(m)}$ , Overall apparent distribution volume of Bay k 4999 at steady-state calculated from  $V_{d(m)} = V_1 (k_{12} + k_{21})/k_{21}$ .

<sup>b</sup> AUC, Area under the serum Bay k 4999 level-versus-time curve from zero to infinite time.

<sup>c</sup> SCL, Serum clearance of Bay k 4999, calculated from  $SCL = \text{dose} / \text{AUC}$ .

<sup>d</sup>  $t_{1/2(\alpha)}$ , Serum half-life of Bay k 4999 during the distribution (alpha) phase.

<sup>e</sup>  $t_{1/2(\beta)}$ , Serum half-life of Bay k 4999 during the post-distribution (beta) phase.

than those in serum and dermabrasion fluid at all sampling times beyond 1 h.

The mean serum protein concentration in blister fluid was 60 g/liter, of which 60% was albumin, and the leukocyte count varied from 400 to 1,600/mm<sup>3</sup>, of which 85 to 94% consisted of polymorphonuclear cells.

## DISCUSSION

The pharmacokinetics of Bay k 4999 in serum appear to be similar to those of related compounds. Azlocillin has a biological half-life of 1.3 h, mezlocillin has one of 0.94 h (7), and piperacillin has one of 1.4 h (3). However, the urinary recovery of only 26.4% Bay k 4999 as intact drug is low compared to other penicillins such as azlocillin, 60% (6), and carbenicillin, 70 to 80% (5).

The distribution characteristics of Bay k 4999 are similar to those reported for most other penicillins, the drug apparently being confined principally to extracellular water. However, the overall distribution volume may be somewhat less than that of piperacillin for which a value of 30 to 40% body weight, varying with dose size, has been reported (4). The even distribution of Bay k 4999 between central and peripheral compartments is indicated by the similar values of the rate constants  $k_{12}$  and  $k_{21}$  (Table 1).

The levels of antibiotic in dermabrasion fluids were similar to those in serum. This has been observed also with other antibiotics (4). There was also rapid equilibration of antibiotic between serum and blister fluids, and drug levels in these fluids were comparable at 1 h. At later times, however, drug levels declined more slowly in blister fluid, resulting in higher levels compared to serum.

The difference between the two tissue fluid models is of interest because estimations of the

protein content of dermabrasion fluid suggest that this is a closer representation of interstitial fluid (4). Blister fluid, on the other hand, with its higher protein and cell content, may be more closely related to an inflammatory exudate. Differences between the two tissue fluid models may also be related to dermabrasion being an "open" system, with probably very little reabsorption of drug occurring, whereas reabsorption is more likely to occur with the blister technique which has the characteristics of a "closed" system. More facile equilibration of drug between blister fluid and serum would result, however, in better agreement between blister fluid and serum levels of Bay k 4999 than between dermabrasion fluid and serum levels, and the reverse is the case. It thus appears likely that the high drug levels in blister fluid are due to trapping or binding of drug in this fluid. The similarity of dermabrasion fluid and serum levels of Bay k 4999 suggests that drug in dermabrasion fluid may provide a reasonable measure of antibiotic levels in general extravascular, extracellular fluids.

The short biological half-life of Bay k 4999 indicates that, in common with other antibiotics of this class, it should be administered every 4 to 6 h to maintain appropriate therapeutic drug levels in the body. As the renal route would appear to account for less than one-third of the administered dose, further information is needed on other possible routes of elimination.

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