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Pharmacokinetics of Sultamicillin in Mice, Rats, and Dogs

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The irreversible β-lactamase inhibitor sulbactam has been combined chemically via ester linkages with ampicillin to form sultamicillin. Upon oral absorption, sultamicillin is completely hydrolyzed to equimolar proportions of sulbactam and ampicillin, thereby acting as an efficient mutual prodrug. In rats, sultamicillin delivered 2 to 2.5 times greater total bioavailability for ampicillin and sulbactam than when each was used individually. Actual plasma or serum concentrations (measured in micrograms per milliliter) of ampicillin and sulbactam produced by sultamicillin were generally equivalent in rats, mice, and beagle dogs. Further studies also indicated that the components of sultamicillin were widely distributed in the various tissues of rats. These findings suggest that sultamicillin might be an effective agent against a variety of infections produced by both β-lactamase-resistant and β-lactamase-susceptible microorganisms.

Over the past 20 years, the increase in the prevalence of β-lactamase-producing strains of gram-positive and gram-negative bacteria has restricted the usefulness of β-lactam antibiotics. Previous studies have shown that penicillanic acid sulfone (sulbactam) is an irreversible inhibitor of microbial β-lactamases (8). When combined with ampicillin or other β-lactams in a physical mixture, sulbactam restores their original activity both in vitro and in vivo (5, 7). Sulbactam and ampicillin now have been combined chemically via ester linkages into the single entity sultamicillin (Fig. 1.) (J. G. Stam, E. C. Bigham, D. Hageman, V. J. Jasys, M. S. Kellogg, R. Martingano, T. C. Crawford, R. D. Carroll, M. Campbell, R. A. Volkmann, and P. D. Weeks, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 510, 1982). Sultamicillin is rapidly hydrolyzed in vivo, releasing equimolar proportions of sulbactam and ampicillin to provide effective chemotherapeutic activity (G. Foulds and D. R. Brennan, 22nd ICAAC, abstr. no. 515).

In the present study, the pharmacokinetic properties of sultamicillin were characterized after oral administration to rats, mice, and beagle dogs. Since a key determinant of chemotherapeutic activity is the concentration of antibiotic in tissues where it exerts its effect on proliferating bacteria, the tissue distribution of ampicillin and sulbactam delivered by sultamicillin in rats was also studied.

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MATERIALS AND METHODS

Antibiotics. Sultamicillin (tosylate salt), sulbactam (Na), and ampicillin (trihydrate) were prepared within Pfizer Central Research, Pfizer Inc., Groton, Conn. Weights of all antibiotics were corrected to 100% biopotency based upon molecular weights before use.

Animals. Male and female outbred mice (25 g, 5 weeks) and male rats (100 g, 5 to 6 weeks) were purchased from Blue Spruce Farms, Altamont, N.Y. Purebred male beagle dogs (14 kg) were from the colony maintained at Pfizer Inc.

Pharmacokinetic studies in rats, mice, and beagle dogs. Sultamicillin and the control agents, sulbactam and ampicillin, were administered orally in a diluent containing Methocel 15 (0.5 g), Polysorbate 80 (1.0 g), CMC 70 low (10.0 g), sodium chloride (9.0 g), and water (984 ml). Dosage volumes used were 0.2 ml for mice, 0.5 ml for rats, and 100 ml for beagles. Plasma was obtained from blood samples taken in heparinized hematocrit tubes from the orbital sinuses of mice and rats. Serum samples were obtained after centrifugation at refrigerator temperature of blood samples taken via jugular puncture of beagles.

Distribution in tissue in rats. After the oral administration of a single dose of sultamicillin or of the individual components (ampicillin or sulbactam), groups of two rats were exsanguinated at time periods from 0 through 4 h to determine the concentrations of these antibiotics in tissues. Organs were removed, weighed, and homogenized in 10 volumes of buffered saline in Potter-Elvehjem tissue grinders powered by an overhead stirrer. The concentrations of antibiotics were determined by bioassay. These experiments were replicated three times (total of six rats). Assays of plasma and tissues at six different times permitted the calculation of three key pharmacokinetic parameters. These were average peak serum concentration (C_{max}, measured in micrograms per milliliter), area under the time-serum concentration curve as a measure of total bioavailability (AUC, measured in hours times micrograms per milliliter), and half-life of the β (elimination) phase (measured in hours), calculated as described in a previous publication (4).

Percent oral absorption of sultamicillin in rats. Percent oral absorption of sulbactam and ampicillin resulting from the administration of either sultamicillin or of ampicillin and sulbactam as individual agents was studied at doses of 10 mg/kg in rats. The weight of sultamicillin was adjusted to provide 10 mg of ampicillin per kg in one set of experiments and 10 mg of sulbactam per kg in a second set. For example, rats weighing 0.1 kg were given 1.67 mg of sultamicillin. Of this, 60%, or 1 mg, was ampicillin, thereby providing 10 mg/kg. Likewise, in a second series of rats, 2.5 mg of sultamicillin was given. Of this, 40%, or 1 mg, was sulbactam, thereby providing a dose of 10 mg/kg. When used as independent agents, ampicillin and sulbactam were administered at doses of 10 mg/kg.

Plasma samples were prepared from blood samples taken at 10 intervals from 1 through 240 min in the intravenous studies or at 7 intervals from 15 through 240 min in the oral studies. Concentrations of ampicillin and sulbactam in plasma were assayed with the differential bioassay procedure described below. Total bioavailability (0 to ∞), expressed as the AUC in hours times micrograms per milliliter, was

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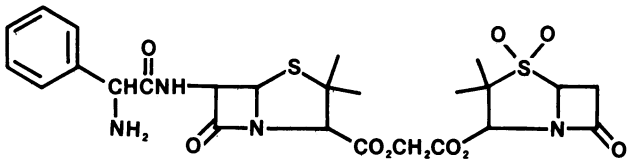

FIG. 1. Chemical structure of sultamicillin.

calculated by the trapezoidal rule. Percent oral absorption was calculated by comparing total bioavailability (AUC) resulting from intravenous and oral administration, with the former representing the 100% value. The standard formula used was % oral absorption = $(AUC_{oral}/AUC_{intravenous}) \times 100$.

Differential bioassay for ampicillin and sulbactam. The differential agar diffusion bioassay made use of *Micrococcus luteus* ATCC 9341 for the determination of ampicillin and a β-lactamase-producing strain of *Pasteurella haemolytica* (59B010) for the measurement of the β-lactamase inhibitor sulbactam. *M. luteus* is susceptible to low concentrations of ampicillin, but it is not susceptible to sulbactam in concentrations as high as 50 to 100 μg/ml. Since this organism does not contain a β-lactamase, it fails to show synergy to combinations of ampicillin plus sulbactam. *P. haemolytica* 59B010 is not susceptible to high concentrations of ampicillin and sulbactam when used singly. However, resistance to ampicillin is mediated via a β-lactamase enzyme. The inhibition of the β-lactamase by sulbactam in the plasma and tissues restores the activity of ampicillin, and quantitative zones are produced.

Standard curves of the antibiotics were prepared in the same fluid or tissue homogenate from control mice, rats, or dogs according to the experiment. The inoculum of *M. luteus* was 1 ml of a 1:100 dilution of a standardized frozen culture per 100 ml of BBL seed agar. The inoculum of *P. haemolytica* was 1 ml of an overnight culture added to 100 ml of Mueller-Hinton agar that had been adjuncted with 25 μg of ampicillin per ml and 3.75 mg of triphenyltetrazolium chloride. Incubation was at 37°C for approximately 18 h. The sensitivity of the assay procedure for ampicillin was generally 0.1 μg/ml; that for subactam was 0.3 μg/ml. The accuracy of the assay for ampicillin was 5.25%; for sulbactam, the accuracy was 6.1%.

TABLE 2. Delivery of ampicillin and sulbactam by sultamicillin and in a physical mixture[a]

Drug	C_{max} (μg/ml)	AUC (μg · h/ml)
Sultamicillin		
Ampicillin component	3.0 ± 0.62	3.3 ± 0.39
Sulbactam component	3.06 ± 0.56	3.25 ± 0.32
Physical mixture		
Ampicillin	0.86 ± 0.21	1.80 ± 0.01
Sulbactam	0.51 ± 0.17	1.28 ± 0.19

[a] Drugs were given orally to fasted rats at the following doses: sultamicillin, 20 mg/kg; ampicillin, 12 mg/kg; sulbactam, 8 mg/kg.

RESULTS

Absorption studies in rats, mice, and beagles. Sultamicillin was rapidly absorbed and produced peak concentrations of its components (ampicillin and sulbactam) in serum or plasma within 15 to 60 min after oral administration of 20 mg/kg to rats, mice, and beagles (Table 1). C_{max}s (± standard error of the mean) of ampicillin and sulbactam, as well as their AUCs (± standard error of the mean), were generally similar in the different animal species. The highest C_{max}s and AUCs for both components occurred in beagles, the next highest in mice, and the lowest in rats.

Mutual prodrug effect. In preliminary pharmacokinetic studies in animals, we recognized that concentrations of both ampicillin and sulbactam in serum originating from the

TABLE 1. Pharmacokinetics of sultamicillin (20 mg/kg) given orally to rats, mice, and beagles

Animal and sample time (h)	C_{max} (μg/ml)		AUC (μg · h/ml)		Half-life (h)	
	Ampicillin	Sulbactam	Ampicillin	Sulbactam	Ampicillin	Sulbactam
Rats			2.76 ± 0.11	2.28 ± 0.14	0.75	0.86
0.25	2.24 ± 0.13	1.75 ± 0.14				
0.5	1.93 ± 0.08	1.60 ± 0.12				
1	1.15 ± 0.05	0.92 ± 0.08				
1.5	0.63 ± 0.03	0.50 ± 0.03				
2	0.37 ± 0.01	0.31 ± 0.06				
3	0.16 ± 0.01	0.17 ± 0.01				
4	0.08 ± 0.01	0.09 ± 0.01				
Mice			4.39 ± 0.40	3.71 ± 0.27	1.12	1.00
0.5	2.33 ± 0.16	2.49 ± 0.24				
1	1.79 ± 0.17	1.44 ± 0.14				
2	0.82 ± 0.09	0.65 ± 0.07				
3	0.51 ± 0.07	0.36 ± 0.06				
4	0.27 ± 0.04	0.21 ± 0.02				
Beagles			19.32 ± 2.06	16.92 ± 1.12	0.98	0.76
0.5	7.03 ± 0.90	7.64 ± 0.58				
1	7.96 ± 0.91	8.11 ± 0.67				
2	5.00 ± 0.56	3.99 ± 0.50				
3	2.76 ± 0.31	2.13 ± 0.17				
4	0.98 ± 0.09	0.49 ± 0.04				
6	0.26 ± 0.02	0.10 ± 0.01				

TABLE 3. Percent oral absorption of ampicillin and sulbactam administered as sultamicillin or as single agents to fasted rats

Drug	Dose (mg/kg)	% Oral absorption[a]	
		Ampicillin	Sulbactam
Sultamicillin[b]			
Ampicillin component	10	56.7	
Sulbactam component	10		49.3
Single agents			
Ampicillin	10	23.0	
Sulbactam	10		19.0

[a] Percent oral absorption = [(AUC, oral dosage)/(AUC, intravenous dosage)] × 100.

[b] Doses of sultamicillin were adjusted to provide 10 mg of either component per kg as indicated.

oral administration of sultamicillin were greater than concentrations resulting from their administration as single agents. These observations were consistent with the effects produced by well-known prodrugs having improved oral absorption and tissue distribution, e.g. bacampicillin (3). The marked prodrug effect of sultamicillin is clearly shown by the rat data presented in Table 2. In these experiments, sultamicillin was administered orally at 20 mg/kg, ampicillin and sulbactam were administered in dosages equivalent to their molar weights in sultamicillin as milligrams per kilogram, i.e., 12 mg of ampicillin per kg and 8 mg of sulbactam per kg. The C_{max}s of ampicillin and sulbactam derived from sultamicillin were 3.5 and 6 times greater, respectively, than those derived from their administration as single agents. Similarly, AUCs for ampicillin and sulbactam arising from sultamicillin were about 2.5 times greater than those from the single agents.

The mutual prodrug effect produced by sultamicillin results from its having a more efficient oral absorption than the single agents do. Values for percent oral absorption are presented in Table 3. The percent oral absorption of the ampicillin and sulbactam components of sultamicillin were, respectively, 56.7 and 49.5. These ampicillin and sulbactam values are 2.5 times greater than comparable values resulting from their oral administration as single agents.

Distribution in tissue in rats. The distribution and concentration values of the components of sultamicillin in tissue presented in Table 4 were obtained from the rats used for the data presented in Table 2. Concentrations in tissue were determined at 0.5 through 4 h after drug administration. The data are presented as AUC (micrograms per milliliter or gram-hours). The highest concentrations in tissue for both ampicillin and sulbactam were found in the liver and kidney, followed by lung, spleen, and thigh muscle. The ratios of AUCs of ampicillin to sulbactam in the tissues, ampicillin in tissue to ampicillin in plasma, and sulbactam in tissue to sulbactam in plasma are presented in Table 5. Bergan has shown that the ability of an antibiotic to penetrate tissues is best evaluated by the use of the ratio of AUC in the peripheral focus to the AUC for the serum (1).

TABLE 5. Ratios of AUCs in rats after oral administration of 20 mg of sultamicillin per kg

Tissue	Ratio of AUCs[a]		
	Ampicillin/sulbactam	Ampicillin in tissue/ampicillin in serum	Sulbactam in tissue/sulbactam in serum
Liver	1.79	4.41	2.51
Kidney	1.01	4.03	4.06
Spleen	0.89	0.79	0.91
Lung	0.66	1.33	2.02
Muscle	1.12	0.66	0.60

[a] AUC, Total area under the serum concentration curve, expressed as hours times micrograms per milliliter.

The ratio of ampicillin to sulbactam was approximately 1 in kidney, spleen, and muscle. In the liver, however, the ampicillin content was greater than that of sulbactam; the reverse was true in lung tissue. In addition, concentrations in liver, kidney, and lung were considerably higher than levels in serum for both components.

DISCUSSION

After the oral administration of sultamicillin, the ampicillin and sulbactam components are liberated by hydrolysis mediated by the esterases in the intestinal epithelium (Foulds and Brennan, 22nd ICAAC, abstr. no. 515). The pronounced prodrug effect of sultamicillin on the oral absorption of its components, ampicillin and sulbactam, was an unexpected finding in our pharmacokinetic studies in animals. Total bioavailability in rats of both ampicillin and sulbactam derived from sultamicillin was 2.5 times greater than that arising from their administration as single agents. As expected, there was a 2.5-fold increase in the percent oral absorption in rats of ampicillin and sulbactam from sultamicillin compared with their absorption when given single agents. These animal data are supported by recent results in humans. Hartly and Wise, using oral doses of 250 and 500 mg of sultamicillin, found that levels of ampicillin in serum were double the levels of ampicillin used singly at the same dosage (6). In addition, urinary recovery of ampicillin after a 250-mg dose was 35%, in contrast to its 62% recovery observed after a 250-mg dose of sultamidillin containing only 147 mg of ampicillin. Similar comparative data are not available for the sulbacta component because this compound was not studied in human as a single agent (6).

TABLE 4. Distribution of the ampicillin and sulbactam components of sultamicillin in tissue compared with the physical mixture[a]

Fluid or tissue	Total bioavailability (AUC) (μg · h/ml or μg · h/g)			
	Sultamicillin		Physical mixture	
	Ampicillin	Sulbactam	Ampicillin	Sulbactam
Plasma	3.30 ± 0.39	3.24 ± 0.32	1.80 ± 0.005	1.28 ± 0.19
Liver	14.6 ± 0.83	8.12 ± 0.42	7.72 ± 1.25	1.87 ± 0.32
Kidney	13.33 ± 2.16	13.25 ± 2.00	5.03 ± 1.30	0.59 ± 0.07
Spleen	2.62 ± 0.57	2.94 ± 0.59	0.82 ± 0.22	0
Lung	4.38 ± 0.91	6.56 ± 1.70	1.12 ± 0.27	0.58 ± 0.58
Muscle	2.18 ± 0.24	1.95 ± 0.16	0.27 ± 0.14	0.88 ± 0.10

[a] Drugs were administered orally to fasted rats. Sultamicillin was administered at 20 mg/kg. Ampicillin and sulbactam were administered at 12 and 8 mg/kg, respectively, the equivalents to their molar weights in sultamicillin.

Ratios of ampicillin to sulbactam based on C_{max} and AUC showed nearly equal concentrations of the components present in serum or plasma and in a number of tissues (Tables 1 and 5). The highest concentrations of sulbactam and ampicillin were found in kidney and liver tissue; lesser concentrations were found in lung, spleen, and muscle. In agreement with our findings in laboratory animals, Brown et al. reported that after the intravenous administration of 500 mg each of ampicillin and sulbactam to healthy males, the concentration of sulbactam in serum and blister fluid was about 1.5 times that of ampicillin (2).

These microbiological credentials of sultamicillin, coupled with our findings of prompt and high levels in serum and a wide distribution to the various tissues and fluids of rats, suggest that sultamicillin will be a useful agent against infections produced by β-lactam-resistant as well as β-lactam-susceptible microorganisms.

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