Beta-Lactamase Effect on Ampicillin Treatment of *Haemophilus influenzae* B Bacteremia and Meningitis in Infant Rats

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Intraperitoneal injections of 250 mg of ampicillin per kg every 6 h for 30 h sterilized the blood and cerebrospinal fluid of infant rats infected with either a β-lactamase-containing strain of *Haemophilus influenzae* type b or a strain lacking the enzyme. However, a single injection of 100 mg/kg sterilized the blood and cerebrospinal fluid of significantly fewer of those rats infected with the β-lactamase-producing strain. The results suggest that resistance of β-lactamase-containing strains of *H. influenzae* in vivo may be inoculum dependent, as demonstrated previously in vitro. The infant rat model appears suited for the quantitative delineation of the effect of β-lactamase on the treatment of *H. influenzae* bacteremia and meningitis with β-lactamase antibiotics.

Since 1973, *Haemophilus influenzae* b strains resistant to ampicillin have been isolated from patients in many parts of the world (2–4, 14, 15). Their resistance has been found to be due to a triethylene melamine (TEM) β-lactamase (6). The TEM β-lactamase, which is a cell-bound, periplasmic, noninducible, plasmid-mediated enzyme, inhibitable by cloxacillin but not *p*-chloromercuribenzoate (PCMB), has been the β-lactamase most often found in *Enterobacteriaceae* (6).

Single gram-negative bacilli exhibit high-level resistance to antibiotics hydrolyzed by the TEM β-lactamase, provided that the outer membrane of the bacillus also retards entry of the antibiotic. If the antibiotic enters freely, its minimal inhibitory concentration is only moderately elevated when the bacillus acquires the enzyme. Large inocula of bacilli containing the β-lactamase, however, are highly resistant in tests because they manage conjointly to hydrolyze all of the unreplenished antibiotic in the test tube. A conspicuous example of this has been the effect of the TEM β-lactamase, which does hydrolyze cephalothin, on the susceptibility to cephalothin of *Escherichia coli*, whose outer membrane is relatively permeable to cephalothin (5). TEM-containing *E. coli* commonly are susceptible or intermediate to cephalothin when treated, but can be resistant if

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large inocula are used (7). TEM-containing *H. influenzae* b provide a similar example, because their outer membrane is unusually permeable to ampicillin (6). The in vivo correlates of this phenomenon would be difficult to elaborate by clinical experimentation, since neither the inoculum nor the antibiotic replenishment rate can be readily determined in the tissues of the infected patient. Ampicillin therapy has failed to cure patients with meningitis due to ampicillin-resistant *H. influenzae* b, but may not always have failed (13), and its efficacy for infections other than meningitis is unknown (6).

The purpose of the studies presented here was to explore the use of an animal model for relating the in vitro and in vivo effects of ampicillin, or of other β-lactam antibiotics, on *H. influenzae* b with and without the β-lactamase. A majority of infant rats challenged intranasally with 10^7 *H. influenzae* b have been shown to develop, within 24 h, a bacteremia that persists for 6 or more days, with 10^4 to 10^5 bacteria per ml of blood. Most bacteremic infant rats, and all of those with 10^4 or more *H. influenzae* b per ml of blood, develop meningitis with leukocytes and *H. influenzae* b in their cerebrospinal fluid (CSF) as early as 18 h after challenge. CSF bacterial counts reach 10^5 to 10^6/ml by 48 h and persist at this magnitude for a week or more, although the mortality of this experimental meningitis, untreated, is only about 10% (8, 11).

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MATERIALS AND METHODS

Sprague-Dawley strain COBS/CD rats obtained from Charles River Laboratory, Inc., Wilmington, Mass., were used for these studies and were similar to those used prevously (8, 11). The strains of *H. influenzae* b were strain Eagan, previously described (1), and strain Simpkin. Strain Simpkin was obtained from the CSF of a child with meningitis. It was found to produce β-lactamase, as indicated by the chromogenic cephalosporin assay (12), which was performed by Ray Arthur of the Department of Laboratory Medicine, Johns Hopkins Hospital, Baltimore, Md. Strain Simpkin had an ampicillin minimal inhibitory concentration (MIC) of 120 μg/ml, when measured by tube dilution in Trypticase soy broth enriched with 5% Fildes (BBL, Cockeysville, Md.) and supplement B (Difco Laboratories, Detroit, Mich.) at an inoculum size of 10^5 bacteria per ml. Strain Eagan did not produce β-lactamase and had an MIC of 0.5 μg/ml. The storage methods and media used for growing these bacteria, the techniques of intranasal inoculation and of blood and CSF sampling, and quantitation of bacteria have been described (10, 11).

Five-day-old rats were inoculated intranasally either with the strain of *H. influenzae* b that had a β-lactamase or with the one that did not. On days 2 and 5 thereafter, their blood was cultured to identify which of the rats were bacteremic. On day 5, each was given either intraperitoneal sodium ampicillin (Omnipen, Wyeth) in the doses indicated or an equivalent volume of saline. Finally, on day 7, the rats were sacrificed, samples of their blood and CSF were cultured, and their CSF leukocytes were counted. In one experiment, meningitic rats were sacrificed at intervals of 5, 15, 30, 60, 110, 140, and 240 min after intraperitoneal injection of 100 mg of ampicillin per kg in order to determine blood and CSF concentrations of ampicillin. The ampicillin concentration was measured by agar diffusion using *Sarcina lutea* as the test organism.

RESULTS

Table 1 shows the results of treatment with 250 mg of ampicillin per kg intraperitoneally every 6 h for 30 h. All but one of the untreated rats had positive blood cultures, and all had positive CSF cultures. Bacterial counts in blood and in CSF of untreated rats infected with the strain that had the β-lactamase or with the strain that lacked it were similar, indicating similar infectivity for the two strains. None of the treated rats had positive cultures from blood or CSF. All of the treated rats had leukocytes in the CSF, indicating that they had had meningitis. Treated rats, however, had significantly fewer cells ($P < 0.01$) than untreated.

Table 2 shows the results of treatment with a single intraperitoneal ampicillin injection of 100 mg/kg. All untreated control rats had positive blood and CSF cultures and, as previously, a significantly greater pleocytosis than the treated rats. At this lower dose, some of the treated rats also had positive blood and CSF cultures. Significantly more of the treated rats infected with the β-lactamase-containing strain of *H. influenzae* b had positive blood and CSF cultures than did treated rats infected with the strain lacking the enzyme ($P < 0.01$). Six isolates from CSF and three each from blood drawn at 5 and 7 days, from rats infected with the β-lactamase-containing strain, still had the enzyme when retested for it.

TABLE 1. *Treatment of infected infant rats with 250 mg of ampicillin per kg every 6 h for 30 h*

Day 1	Day 5		Day 7					
				Blood			CSF	
(intranasal challenge strain of *H. influenzae* b)	(intraperitoneal dose every 6 h for 30 h)	No. of infant rats	No. of rats with positive cultures	Geometric mean no. of bacteria/ml (range)[a]		No. of rats with positive cultures	Geometric mean no. of bacteria/ml (range)[a]	Mean no. of leukocytes/ml (range)
Without β-lactamase	Saline	5	4	$10^{4.43}$ (4 × 10^3–1.4 × 10^5)		5	$10^{4.43}$ (7.5 × 10^3–5 × 10^5)	2163 (140–4,800)
	Ampicillin, 250 mg/kg	10	0	0		0	0	613 (440–840)
With β-lactamase	Saline	5	5	$10^{4.45}$ (2.7 × 10^3–1.7 × 10^5)		5	$10^{5.18}$ (5 × 10^3–5 × 10^5)	2482 (420–12,000)
	Ampicillin, 250 mg/kg	7	0	0		0	0	437 (250–740)

[a] Values for ranges include only positive cultures.

TABLE 2. *Treatment of infected rats with a single 100-mg/kg dose of ampicillin*

Day 1	Day 5	No. of infant rats	Day 7					
			Blood			CSF		
(intranasal challenge strain of *H. influenzae* b)	(intraperitoneal dose every 6 h for 30 h)		No. of rats with positive cultures	Geometric mean no. of bacteria/ml (range)[a]		No. of rats with positive cultures	Geometric mean no. of bacteria/ml (range)[a]	Mean no. of leukocytes/ml (range)
Without β-lactamase	Saline	9	9	$10^{3.43}$ $(9 \times 10^2 - 1.7 \times 10^5)$		9	$10^{4.60}$ $(5 \times 10^2 - 5 \times 10^5)$	2,735
	Ampicillin, 100 mg/kg	29	7	$10^{0.96}$ $(2 \times 10^2 - 2.3 \times 10^5)$		5	$10^{0.55}$ $(10^2 - 5 \times 10^6)$	573 (80–3,040)
With β-lactamase	Saline	14	14	$10^{4.03}$ $(10^2 - 1.4 \times 10^5)$		14	$10^{4.2}$ $(7 \times 10^3 - 5 \times 10^6)$	1,453 (140–4,800)
	Ampicillin, 100 mg/kg	62	38	$10^{2.35}$ $(7 \times 10^2 - 5 \times 10^4)$		30	$10^{4.4}$ $(4 \times 10^3 - 5 \times 10^5)$	827 (110–3,680)

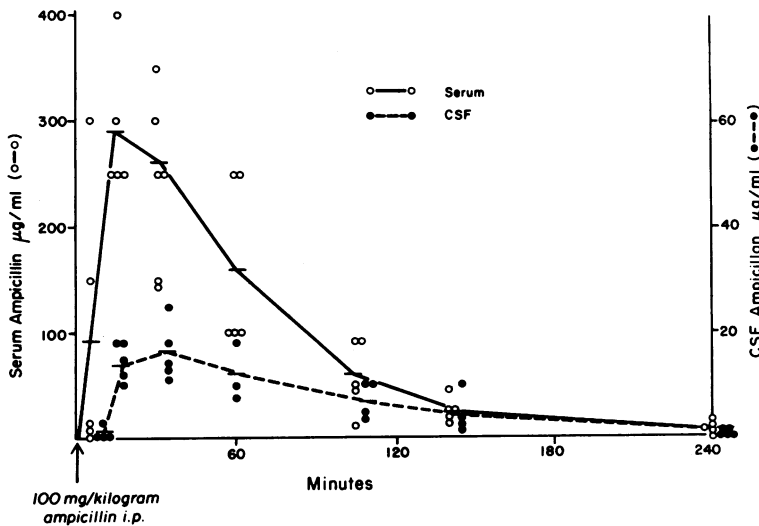[a] Values for ranges include only positive cultures.

FIG. 1. *Serum and CSF concentrations of ampicillin in infant rats with H. influenzae b meningitis.*

In a separate experiment, concentrations of ampicillin in blood and CSF were measured at intervals of time after a single intraperitoneal ampicillin dose of 100 mg/kg. As shown (Fig. 1), CSF concentrations were about one-tenth to one-twentieth of those in serum. Peak serum and CSF ampicillin concentrations were 290 and 27 μg/ml, respectively, and the serum half-life was 56 min.

DISCUSSION

The following studies explored the use of an animal model for relating the in vitro and in vivo effects of ampicillin on bacteremia and meningitis caused by *H. influenzae* b that either contained or lacked β-lactamase. Ideally, such a model should simulate, as closely as possible, the disease as it occurs in humans. It should also display both treatment success and failure within a pharmacologically reasonable dosage range so that the critical variables can be discriminated. The results of these studies indicate the suitability for these purposes of the infant rat model. The sequential occurrence of nasopharyngeal colonization and bacteremia and the appearance of positive CSF cultures and pleocytosis resemble human infection. The range of bacterial counts and the range of ampicillin concentrations in the CSF are also similar

to those reported in *H. influenzae* meningitis in humans (14).

The present studies indicate that ampicillin was sometimes effective in sterilizing the blood and CSF in infant rats with meningitis caused by β-lactamase-containing *H. influenzae*. It was possible to establish for the model an ampicillin treatment dosage that sterilized blood and CSF of all rats. However, with limiting amounts of antibiotic, the blood and CSF of significantly fewer rats infected with the β-lactamase-containing strain were sterilized than was the case for rats infected with the strain lacking the enzyme. It has previously been shown that the MIC of ampicillin in vitro for strains of TEM-containing *H. influenzae* rises steeply as the inoculum tested is increased above 5×10^4 colony-forming units/ml (6). Bacterial counts in the blood and CSF of untreated rats in these studies ranged above and below this value, making it at least plausible that the excess of treatment failures with the β-lactamase-containing strain occurred in the animals with the higher counts at the time of treatment. It was not, however, possible to determine this here, since only one CSF tap per infant rat has been technically possible. These observations are also consistent with the favorable responses to ampicillin observed in some children with meningitis caused by β-lactamase-containing *H. influenzae* (13).

ACKNOWLEDGMENTS

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