

EVALUATION OF ANTIBACTERIAL ACTIVITY IN EXPERIMENTAL
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Mice infected intracerebrally (ic) or intraperitoneally (ip) with *Pseudomonas aeruginosa* were treated subcutaneously with graded doses of five antibiotics all of which displayed antipseudomonal activity *in vitro*. The analysis of MIC, ED₅₀ in the ip infection model (ED₅₀-ip) and that in the ic system (ED₅₀-ic) for each agent showed: a) gentamicin, the most active agent *in vitro*, was also active *in vivo*, with an ED₅₀-ic nearly six-fold higher than ED₅₀-ip; b) ceftazidime, considerably active both *in vitro* and *in vivo*, showed comparable values of ED₅₀ in the two systems; c) ceftriaxone, also active *in vitro*, showed limited activity *in vivo*, its two ED₅₀ values being similar; d) aztreonam, moderately active both *in vitro* and *in vivo*, showed comparable activity in the two infection models; e) carbenicillin, the least active agent *in vitro*, gave poor results *in vivo*, with an ED₅₀-ic 2.6-fold higher than ED₅₀-ip.

Despite the introduction of new antimicrobial agents and a more thorough understanding of the pathology of infections of the central nervous system, the mortality and morbidity of bacterial meningitis and brain abscess remain high. In this respect, *Pseudomonas aeruginosa* is undoubtedly a Gram-negative pathogen of major importance as it frequently causes fatal sepsis and/or localized lesions in areas or individuals particularly devoid of defense¹⁾. Because of the varied results obtained with aminoglycoside treatment in Gram-negative bacillary meningitis^{2,3)}, there is a need for continued development and screening of new antimicrobial agents for therapy of *Pseudomonas* infections.

In the present study we analyzed the antipseudomonal activity of different agents in mice and, for each antibiotic, the therapeutic efficacy (ED₅₀) in experimental meningo-encephalitis was compared to that in peritonitis. This type of analysis was found to be suitable for identifying different patterns of activity among the tested drugs.

Materials and Methods

Mice

Balb/c female mice, 6~10 weeks of age, were obtained from the Mammalian Genetics and Animal Production Section, Division of Cancer Treatment, National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD, USA.

Organism

A single strain of *P. aeruginosa*, immunotype 11^{4,5)}, isolated from a clinical specimen was supplied by Dr. CASSONE, Istituto Superiore di Sanità, Roma. The organism was routinely cultured in nutrient broth (Difco) and incubated at 37°C for 18 to 24 hours with constant aeration.

Preparation of Inoculum

The cultures were centrifuged at 15,000 rpm for 15 minutes in a Sorvall RC 2B centrifuge, the supernatant was discarded, and the soft pellet of cells was resuspended in sterile saline. Viable cell

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count was performed by the colony-forming unit method using Tryptose agar. A standard curve, based on turbidity as read on a photoelectric colorimeter, was also prepared to correlate turbidity with viable colony counts, and was used subsequently to determine challenge concentrations.

Induction of Meningo-encephalitis

The intracerebral (ic) inoculum procedure was that described by CAMPANILE *et al.*^{4,5}. *P. aeruginosa* cells were suspended in 0.03 ml saline aliquot and injected under sterile conditions through the central-middle area of the frontal bone to 2-mm depth by means of 0.1 ml glass microsyringe and 27-gauge disposable needle. All animals developed meningo-encephalitis as proved by clinical signs and histopathological examination. Control mice receiving saline with no bacteria fully recovered from trauma within 30~60 minutes. Surgical mortality was less than 3% and always occurred within 1~2 minutes from injection.

Induction of Peritonitis

The animals were injected intraperitoneally (ip) with 0.2 ml of the appropriate dilution of *P. aeruginosa* in saline. All animals developed peritonitis as proved by clinical signs and histopathological examination.

Determination of LD₅₀

Fifty percent lethal dose (LD₅₀) values were determined from the results of ip or ic administration of graded doses of live organisms and calculated by the method of LITCHFIELD and WILCOXON⁶.

Antibiotics

Gentamicin (Gentalyn, Essex) and carbenicillin (Geopen, Pfizer) were purchased. Ceftazidime (RG 159, Glaxo), aztreonam (SQ 26776, Squibb) and ceftriaxone (Rocefin, Roche) were kindly supplied by their respective manufacturers. Ceftazidime was prepared as the pentahydrate and converted to the sodium salt by dissolution in sodium carbonate. Aztreonam was supplied in vials as a sterile powder blend of aztreonam and L-arginine for reconstitution with 10 ml sterile water for injection (weight ratio of aztreonam to L-arginine 1.0/0.78). Ceftriaxone was provided as the disodium salt to be reconstituted with sterile water for injection.

Susceptibility Testing

Minimum inhibitory concentrations (MICs) were determined by a broth microdilution method using nutrient broth (Difco). 10⁴ organisms/0.1 ml (four replicates) were placed in the wells of microdilution plates and brought to a final volume of 0.2 ml by addition of serial two-fold dilutions of antibiotics in nutrient broth (range of final concentrations: 0.03~125 µg/ml). The plates were incubated for 24 hours at 37°C. The MIC was taken as the highest dilution of antimicrobial agent in which no growth appeared.

Mouse Protection Test

The infected animals were treated subcutaneously (sc) at 1 and 5 hours from bacterial challenge with serial two-fold dilutions of each antibiotic. 6~8 mice were used at each dose level and deaths were recorded for 5 days. Mean effective doses of antibiotics (ED₅₀) in the protection experiments were calculated according to the method of LITCHFIELD and WILCOXON⁶, using experimental groups of 6~8 animals.

Results

Meningo-encephalitis Model

The object of the present study was to analyze the penetration of different antibiotics into the cerebrospinal fluid (CSF) of mice infected ic with *P. aeruginosa* by comparing the mean effective dose of each agent in the ic infection model with that in the ip system. The working hypothesis was that, provided the two models were similar with respect to mortality criteria, the ED₅₀-ic would relate to

Table 1. Mortality data of mice challenged with *Pseudomonas aeruginosa*.

Mice injected	Route	Inoculum size ($\times 10^9$ cfu)	Mortality (%)	MST ^c (range)
44 ^a	ic	1.5	94.4	1~3
68 ^b	ip	100	96.6	1~3

^a Data from 7 experiments. ^b Data from 10 experiments. ^c Median survival time (days).

Table 2. Mouse protection test with different antibiotics in *Pseudomonas aeruginosa* infections.

Antibiotic	MIC ^a	ED ₅₀ -ic ^b	ED ₅₀ -ip ^b	ED ₅₀ -ic/ED ₅₀ -ip
Gentamicin	<0.03	90 (82~99) ^c	16 (11~24)	5.6 ^d
Ceftazidime	0.97	32 (22~49)	37 (26~52)	0.8
Ceftriaxone	0.97	500 (370~675)	450 (281~720)	1.1
Aztreonam	3.9	152 (101~227)	106 (68~165)	1.4
Carbenicillin	31.2	1,285 (955~1,728)	480 (266~864)	2.6

^a $\mu\text{g/ml}$, ^b mg/kg , ^c 95% confidence intervals, ^d numbers are ED₅₀-ic to ED₅₀-ip ratios.

ED₅₀-ip in a fashion largely conditioned by the agent pharmacokinetics, regardless of its absolute anti-*Pseudomonas* activity. When Balb/c female mice were injected ic with graded numbers of *P. aeruginosa* cells, the mean lethal dose (LD₅₀) was found to be 3×10^6 cells/mouse. At least 50 LD₅₀ were needed to obtain a percent mortality of nearly 95% (data pooled from 7 experiments, Table 1). In individual experiments, the median survival time (MST) of mice receiving 50 LD₅₀ was 1.5~2 days. When *P. aeruginosa* was injected into the peritoneal cavity, the LD₅₀ was found to be 10^9 cells/mouse. Ten LD₅₀ would result in a percent mortality of approximately 97% with an MST in most experiments of 2 days (Table 1). In both models, all deaths resulting from infection occurred within 5 days from microbial challenge.

In Vitro and *In Vivo* Activity of Different Antibiotics

Gentamicin, ceftazidime, ceftriaxone, aztreonam and carbenicillin are antibiotics with anti-*Pseudomonas* activity and have long been used in, or are currently proposed for, the treatment of Gram-negative bacterial meningitis. Balb/c mice were challenged ic (50 LD₅₀ units) or ip (10 LD₅₀ units) with *P. aeruginosa*. The animals were then treated twice (at 1 and 5 hours, subcutaneously) with graded doses of each antibiotic. Deaths were recorded for 5 days and fifty percent effective dose values (ED₅₀-ic and LD₅₀-ip) of each agent in the 2 systems were calculated. Minimum inhibitory concentrations *in vitro* were obtained as previously described. Table 2 summarizes the results.

Discussion

The present studies describe a model of meningo-encephalitis in mice which is a reliable means for assaying antibacterial activity in infections of the central nervous system. One advantage of this model is that, by comparatively analyzing the therapeutic activity of an agent in the ic and ip systems, it is possible to high-light the impact of pharmacokinetics in the drug activity in the ic infection. As a reference infection we selected, in this study, acute peritonitis, sustained by a high bacterial inoculum, so as to provide a disease with a course as rapid as that of the ic infection. This resulted in comparable mortality rates in the two models. In general, however, the *Pseudomonas* strain used in this study displayed a relatively low virulence, possibly because of its being cultured extensively on laboratory medium⁷). This may account for the lower LD₅₀ values reported in the past by authors who employed recent clinical isolates of *P. aeruginosa*^{8,9,10,11}). On the other hand, although in most studies lower

inocula of *P. aeruginosa* were used, there is apparently no consensus as to the best bacterial inoculum for mouse protection test⁵). Moreover, while fluctuations in the size of bacterial inoculum by a given route may result in variations of the ED₅₀ of an antibiotic^{8,12}), the difference in the bacterial load in the ic and ip models is unlikely to have played a major role in our results.

Gentamicin was highly active *in vitro* against the strain of *P. aeruginosa* used in this study, which was reflected *in vivo* by an ED₅₀-ip value of 16 mg/kg, a finding in line with previous data in the literature⁵). A recent study on the penetration of antibiotics in CFS of rabbits with experimental meningitis proved the gentamicin concentration in CFS to be 21% of that found in serum following sc administration¹³). Our results indicate that doses of gentamicin nearly six-fold higher are needed to achieve protective effects in the ic model comparable to those in the ip system. Ceftazidime was also highly active both *in vitro* and *in vivo*, a finding in agreement with previous studies¹⁴). Although little is known of the passage of ceftazidime into the CFS especially of animals with experimental meningitis, one might anticipate from our results that the percent penetration of the antibiotic should be sufficiently high. In fact, in our model the ED₅₀-ic/ED₅₀-ip ratio was 0.86. Ceftriaxone, although as effective *in vitro* as ceftazidime, gave poorer results *in vivo*. Nevertheless, the ED₅₀-ic/ED₅₀-ip ratio was 1.1, thus providing indirect evidence that ceftriaxone can penetrate cerebrospinal fluid effectively. In previous studies based on different meningo-encephalitis models, other authors reported essentially similar results^{15,16,17}). Aztreonam was moderately active both *in vitro* and *in vivo*. A recent study compared aztreonam with frequently used antibiotics in the therapy of three types of experimental bacterial meningitis in rabbits and in experimental *Escherichia coli* cerebritis in rats¹⁸). Aztreonam was more active than the comparison agent in all models of experimental meningitis tested. The concentration of the antibiotic into purulent CFS was 23% of that in serum, among the highest for β -lactam antibiotics. Our findings indicate that the ED₅₀-ic/ED₅₀-ip ratio is 1.4 for aztreonam.

Finally, carbenicillin, the least active agent *in vitro* on an absolute basis and progenitor of the β -lactam antibiotics with anti-pseudomonal activity, required doses in the ic model 2.6 times higher than those in the ip system to bring about comparable therapeutic results.

In conclusion, the studies summarized in the present paper may be useful for development of standardized models of meningo-encephalitis in mice for continued production and screening of antimicrobial agents effective in Gram-negative bacillary meningitis. Furthermore, our findings suggest that the newer β -lactam antibiotics with *in vitro* activity against members of the family *Enterobacteriaceae* may prove useful in infections of the central nervous system.

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