ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1981, p. 76-81
0066-4804/81/010076-06$02.00/0

Vol. 19, No. 1

Efficacy of Ampicillin Therapy in Experimental Listeriosis in Mice with Impaired T-Cell-Mediated Immune Response

IRMA A. J. M. BAKKER-WOUDENBERG,* P. DE BOS, W. B. VAN LEEUWEN, AND M. F. MICHEL

Department of Clinical Microbiology, Antimicrobial Therapy, Erasmus University, Rotterdam, The Netherlands

The importance of intact host defense mechanisms for successful antimicrobial therapy was investigated via a comparison of the activities of ampicillin against experimental *Listeria monocytogenes* infections in normal mice and congenitally athymic (nude) mice. Nude mice were used for these experiments because recovery from infection with this organism depends on development of cellular immunity induced specifically by a T-cell-mediated reaction. When infections were induced with 5×10^5 colony-forming units, a total dose of 800 mg of ampicillin per mouse (32 doses of 25 mg each), which is twenty times the dose required for a cure of infections in normal mice (8 doses of 5 mg each), would not cure infections in nude mice. With a reduction in inoculum to 10^5 colony-forming units, cures were obtained with a total ampicillin dose of 800 mg (32 doses of 25 mg each), but not with 400 mg (16 doses of 25 mg each). These studies show clearly that the efficacy of ampicillin against infections with *L. monocytogenes* is dependent upon intact host defense mechanisms.

Data from several studies suggest that ampicillin is the drug of choice for treatment of infections due to *Listeria monocytogenes* (7, 11, 17). Therapeutic failures not related to the resistance of the organism to ampicillin have been reported, however, in patients with defects in cell-mediated immune mechanisms associated with malignancies of the lymphoreticular system such as Hodgkin's disease or the use of immunosuppressive drugs (8, 15, 16, 21). Reasons for such therapeutic failures have been discussed by Watson et al. (21). In an attempt to illuminate this problem at the experimental level, we have compared the activities of ampicillin against infections with *L. monocytogenes* in normal mice and nude mice with impaired T-cell-mediated immunity.

MATERIALS AND METHODS

Animals. Female B10LP nude mice, homozygous for the mutation "nude" (nu/nu), and their age-matched immunologically normal controls, heterozygous for the nude gene (nu/+), were used in all experiments. Mice in both lines were specific pathogen free, were 10 to 12 weeks old, and had been bred in the Laboratory Animals Centre TNO Zeist, The Netherlands. The absence of thymus glands in the nu/nu mice was checked by dissection.

Bacteria. A clinical isolate of type 4b *L. monocytogenes* was used in these experiments. The minimal inhibitory concentration of ampicillin for this isolate was 0.16 μg/ml by the tube dilution test. The virulence of the isolate was maintained by passage into the yolk sac of 10-day-old chicken embryos. With death of the embryo at 3 to 5 days, yolk sac material was inoculated onto a blood agar plate, with incubation for 16 h at 37°C. Beef broth was inoculated from this plate and incubated for 16 h at 37°C. This culture, the stock, was stored in small portions at −70°C. Portions were thawed as needed for each animal inoculation and used to seed a fresh culture which was incubated for 16 h at 37°C and then stored at 4°C. This stationary-phase culture contained 2×10^9 viable organisms per ml (range, 1.8×10^9 to 2.2×10^9). Inocula were prepared from these 16-h cultures by appropriate dilution with physiological saline so that the requisite numbers of colony-forming units (CFU) were contained in a 0.5-ml volume. The numbers of viable organisms inoculated in each experiment were determined by plate counts made just before and after animal inoculation.

Experimental infection. Infections were induced by injection of 5×10^4 (4.5×10^4 to 5.5×10^4), 10^5 (0.9×10^5 to 1.1×10^5), or 5×10^5 (4.5×10^5 to 5.5×10^5) CFU of *L. monocytogenes* into the tail vein. The numbers of viable *L. monocytogenes* recovered from the spleen, liver, and blood were used as indices of the severity of the infection. Mice were sacrificed at different intervals after inoculation and at least 5 h after dosage with ampicillin. Spleens and livers were removed and homogenized separately in 20 ml of physiological saline (VirTis homogenizer; 30 s at 10,000 rpm). Tenfold serial dilutions of homogenates in saline were prepared, and 0.2-ml volumes of each dilution were spread on blood agar plates. The residual particulates in the undiluted homogenate were tested for viable bacteria by the pour plate method. The Wilcoxon rank-sum test was used to identify results significantly different from those in control groups.

Antimicrobial treatment. Individual doses of sodium ampicillin (Beecham Farma B.V., The Netherlands) amounting to 5, 10, 25, and 50 mg per mouse were administered subcutaneously every 12 or 8 h for

totals of 8, 12, 16, or 32 doses, the first of which was delivered 36 h after inoculation.

Antibiotic assay. Blood specimens for assay of ampicillin were obtained by orbital puncture under light ether anesthesia. Three blood samples were collected from each mouse at different times after ampicillin injection. The standard large-plate agar diffusion procedure with Oxoid diagnostic sensitivity test agar and a *Staphylococcus aureus* strain (ATCC 9144 strain Oxford) as the indicator organism (1) was used for the ampicillin assays, using 50 μl of mouse serum per test. The assay system was sensitive to 0.25 μg of ampicillin per ml. The coefficient of variation of 12 determinations on solutions containing 0.5 to 16 μg of ampicillin per ml was 1 to 3%.

RESULTS

Courses of infections in untreated mice. Table 1 summarizes the results obtained in mice inoculated with 5×10^5 CFU. This inoculum was lethal for normal mice within 48 to 72 h. At 36 h, blood contained 380 CFU/ml. Nude mice inoculated with 5×10^5 CFU did not recover from the infection, but they did not die. Although spleens were enlarged from the start of the infection, there was no evidence of splenic necrosis or other lesions. Only 1 of the 45 nude mice inoculated with 5×10^5 CFU had bacteria in the blood (819 CFU/ml) on day 6.

Table 2 summarizes the results obtained in mice inoculated with 10^5 CFU. In normal mice there was a progressive increase in bacterial populations of the spleen and liver for the first 36 h, followed by decreases in number between days 3 and 5. By day 16, spleens and livers were sterile, even though spleens were enlarged and exhibited signs of necrosis. The blood was sterile throughout the observation period. Of the 55 mice, only 2 died, 1 on day 4 and the other on day 5. Events in nude mice inoculated with 10^5 CFU were essentially identical to those inoculated with 5×10^5 CFU.

With inoculation of 5×10^4 CFU, normal mice recovered quickly (data not shown). Spleen and liver cultures were positive up to day 10 but were negative thereafter. Blood was always sterile. Events in nude mice inoculated with 5×10^4 CFU were identical with those in recipients of 5×10^5 CFU.

Efficacy of ampicillin therapy. (i) Infections with 5×10^5 CFU. In normal mice first treated 36 h after inoculation, administration of 5 mg of ampicillin every 12 h for eight doses resulted in sterile cultures of the spleen, liver, and blood on day 14 (Table 1). This dosage was without effect on the course of disease in nude mice in whom CFU from spleens and livers of treated and untreated mice were the same. Increasing the daily dose of ampicillin to 10, 25, or 50 mg, decreasing the interval between doses from 12 to 8 h, and increasing the number of doses from 8 to 32 effected only a marginal improvement in results (Table 1). With the most intensified treatment schedules, bacteria recovered from the spleen and especially the liver decreased initially but increased with termination of ampicillin therapy. Of 105 mice given eight doses of 10, 25, and 50 mg of ampicillin every 12 h, 6 died during the experimental period, possibly from ampicillin toxicity. Administration of 25 mg of ampicillin every 8 h for 12 doses led to significant reductions in number of CFU recovered from spleens and livers, but recovery was incomplete. Of 35 mice, 3 died within 20 days, and numbers of CFU in spleens and livers on day 21 were not significantly different from those in untreated controls. Administration of 25 mg of ampicillin every 12 h for 32 doses effected temporary sterilization of spleens and livers; however, these organs were again culture positive 5 days after the last dose of ampicillin. *L. monocytogenes* organisms recovered at this time were fully susceptible to ampicillin.

(ii) Infections with 10^5 CFU. In normal mice first treated 36 h after inoculation, 5 mg of ampicillin administered every 12 h for eight doses effected sterilization of spleens and livers by day 6 (Table 2). The same dosage led to an initial decrease in CFU in spleens and livers of nude mice, followed by a rise to pretreatment levels. Increasing the ampicillin dose to 25 mg, decreasing the intervals between doses from 12 to 8 h, and increasing the total numbers of doses from 8 to 32 effected some improvement in results. Thus, 25 mg of ampicillin administered every 8 h for 12 doses effected temporary sterilization of spleens and livers of most nude mice. Fifteen days after termination of therapy, *Listeria* cells with unimpaired susceptibility to ampicillin were recovered from spleens and livers of all mice. Of 35 mice, 1 died within 19 days after inoculation. Administration of 25 mg of ampicillin every 12 h for 32 doses appeared to be fully curative.

Comparison of concentrations of ampicillin in serum of normal and nude mice. The concentrations of ampicillin in sera of normal and nude mice after subcutaneous administration of 5- and 25-mg doses are shown in Fig. 1. The levels of ampicillin in sera of nude mice and their normal littermates were essentially identical. Peak concentrations were 180 μg/ml with the 5-mg dose and 550 μg/ml with the 25-mg dose. Concentrations in excess of 0.16 μg/ml, the minimal inhibitory concentration for the *Listeria* isolate used in this study, were present for about 4 and 5 h at the respective doses. The concentrations of ampicillin attained after administration of 5- and 25-mg doses during the

TABLE 1. *Effects of various ampicillin dosage regimens on the numbers of L. monocytogenes in spleens and livers of normal and nude mice at different intervals after inoculation with 5 × 10^5 CFU*

Organ	Host[a]	Dosage regimen			Log no. of CFU (±SE)[b] per organ at day after infection:					
		mg per mouse	No. of doses	Interval between doses	1.5	4	10	18	21	23
Spleen	Normal	0	0	0	7.48 ± 0.09					
		5	8	12		5.88 ± 0.13	0.88 ± 0.54	0	0	0
	Nude	0	0	0	5.78 ± 0.38	5.23 ± 0.27	5.54 ± 0.09	4.97 ± 0.17	5.34 ± 0.12	5.20 ± 0.10
		5	8	12		4.34 ± 0.11	4.94 ± 0.21	4.90 ± 0.15	4.74 ± 0.09	
		10	8	12		4.41 ± 0.27	4.44 ± 0.13	4.85 ± 0.11	4.19 ± 1.47	
		25	8	12		4.06 ± 0.21	4.20 ± 0.21	3.90 ± 0.48	4.48 ± 0.74	
		50	8	12		4.10 ± 0.21	4.09 ± 0.25	4.68 ± 0.13	4.48 ± 1.00	
		25	12	8		0	3.04 ± 0.79	1.18 ± 0.77	4.41 ± 0.61	
		5	16	12		4.34 ± 0.11	2.78 ± 0.26	4.78 ± 0.07		5.01 ± 0.11
		25	16	12		4.06 ± 0.21	0.78 ± 0.58	5.11 ± 0.18		5.10 ± 0.16
		25	32	12		4.06 ± 0.21	0.58 ± 0.40	0		2.37 ± 0.35
Liver	Normal	0	0	0	7.05 ± 0.19					
		5	8	12		5.81 ± 0.13	0.57 ± 0.35	0	0	0
	Nude	0	0	0	4.94 ± 0.33	5.43 ± 0.53	4.84 ± 0.17	4.78 ± 0.31	4.65 ± 0.10	5.21 ± 0.10
		5	8	12		4.22 ± 0.12	4.59 ± 0.29	4.56 ± 0.04	4.62 ± 0.03	
		10	8	12		3.75 ± 0.51	4.27 ± 0.03	4.27 ± 0.28	4.14 ± 0.67	
		25	8	12		3.63 ± 0.39	4.13 ± 0.03	3.73 ± 0.57	4.43 ± 0.98	
		50	8	12		3.04 ± 0.76	3.88 ± 0.12	3.93 ± 0.28	3.97 ± 0.41	
		25	12	8		0.40 ± 0.40	0	0.86 ± 0.86	3.47 ± 0.24	
		5	16	12		4.22 ± 0.12	1.49 ± 0.41	4.74 ± 0.08		4.84 ± 0.11
		25	16	12		3.63 ± 0.39	1.09 ± 0.65	4.61 ± 0.22		4.56 ± 0.29
		25	32	12		3.63 ± 0.39	1.08 ± 0.64	0		1.86 ± 0.46

[a] Groups of five mice.
[b] SE, Standard error.

TABLE 2. *Effects of various ampicillin dosage regimens on the numbers of L. monocytogenes in spleens and livers of normal and nude mice at different intervals after inoculation with 10^5 CFU*

Organ	Host[a]	Dosage regimen			Log no. of CFU (±SE)[b] per organ at day after infection:					
		mg per mouse	No. of doses	Interval between doses	1.5	4	10	18	21	23
Spleen	Normal	0	0	0	6.15 ± 0.20	5.34 ± 0.37	1.66 ± 0.19	0	0	0
		5	8	12		2.41 ± 0.69	0	0	0	0
	Nude	0	0	0	5.21 ± 0.19	4.14 ± 0.21	4.90 ± 0.29	4.96 ± 0.13	4.60 ± 0.08	4.70 ± 0.12
		5	8	12		3.38 ± 0.06	4.95 ± 0.13	5.06 ± 0.22	4.79 ± 0.60	
		25	12	8		0	1.25 ± 0.77	1.72 ± 0.64	1.87 ± 0.14	
		5	16	12		3.38 ± 0.06	3.58 ± 0.80	4.35 ± 0.17		4.85 ± 0.19
		25	16	12		2.80 ± 0.13	0	3.30 ± 0.15		4.57 ± 0.20
		25	32	12		2.80 ± 0.13	0	0		0
Liver	Normal	0	0	0	5.51 ± 0.18	6.03 ± 0.53	1.23 ± 0.70	0	0	0
		5	8	12		1.79 ± 0.78	0	0	0	0
	Nude	0	0	0	4.81 ± 0.21	4.21 ± 0.14	3.93 ± 0.42	4.45 ± 0.23	4.52 ± 0.06	4.00 ± 0.11
		5	8	12		3.39 ± 0.10	4.45 ± 0.15	4.81 ± 0.66	4.17 ± 0.28	
		25	12	8		0	0.46 ± 0.46	2.84 ± 0.31	2.23 ± 1.07	
		5	16	12		3.39 ± 0.10	2.26 ± 0.82	2.83 ± 0.32		3.92 ± 0.06
		25	16	12		2.22 ± 0.13	0	2.95 ± 0.20		3.71 ± 0.16
		25	32	12		2.22 ± 0.13	0	0		0

[a] Groups of five mice.
[b] SE, Standard error.

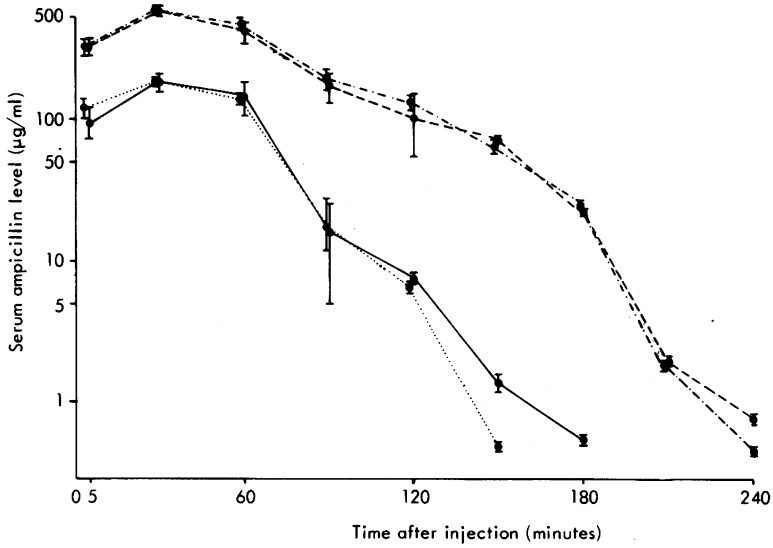

FIG. 1. *Concentrations of ampicillin in serum. Subcutaneous dosage: 5 mg to normal (——) and nude (·····) mice; 25 mg to normal (– – –) and nude (–·–) mice. Each point represents the mean of five mice (± the standard deviation).*

course of infection were the same as those in uninfected mice.

DISCUSSION

From studies by Mackaness and co-workers it is known that host resistance to listerial infection depends on a T-cell-mediated activation of macrophages, the target cells of *Listeria* spp. (2, 10, 13, 23). In our experiments, congenitally athymic nude mice (9) with impaired host defense mechanisms and their littermates were challenged with different inocula of *L. monocytogenes*, and the courses of infections were compared by determining the number of *Listeria* organisms in their spleens, livers, and blood. Whereas the mortality among normal mice was directly related to the size of the inoculum, the course of the infection in nude mice was independent of the inoculum. Nude mice were able to restrain bacterial multiplication but were unable to eradicate the organisms, thereby becoming chronically infected. These experiments confirm the findings of other investigators (4, 5, 14, 19).

Infections with *L. monocytogenes* in normal mice were cured by administration of comparatively large doses of ampicillin. Other investigators utilizing different routes of infection and inocula reached a similar conclusion (6, 18, 20, 22). Such doses did not cure infections in nude mice. Intensification of ampicillin treatment did not lead to cure of infections induced with inocula of 5×10^5 CFU. With a reduction in inoculum to 10^5 CFU it was still difficult to cure infections in nude mice. Administration of 25 mg of ampicillin every 12 h for 32 doses was required for regularly curative results. Such doses produced peak levels of ampicillin in serum in excess of 500 μg/ml.

From these experiments we may conclude that the reduced efficacy of ampicillin in nude mice was due to impaired host defense mechanisms. Killing of intracellular *L. monocytogenes* in normal mice is due to the combined activity of ampicillin and cellular defenses of the host. Lack of cellular immunity cannot be compensated by intensification of ampicillin dosage. Since the isolates from nude mice treated with ampicillin had retained their susceptibility to ampicillin, the survival of phagocytized *Listeria* cells in the presence of high concentrations of ampicillin is due either to inability of the drug to reach the bacteria in their intracellular location because of lack of penetrating capacity or to the low metabolic activity of the intraleukocytic bacteria in nude mice. In vitro studies suggest that ampicillin penetrates poorly into cells (3, 12). Our experiments also suggest that ampicillin penetrates phagocytes poorly as high doses were needed for complete recovery of normal mice. However, it must be realized that ampicillin has a short half-life in mice and that inhibitory levels were present in serum for only part of the dosage interval, with active multiplication and repopulation occurring in the drug-free interval. The

constancy of the *Listeria* populations in spleens and livers, suggesting that these organisms were in the resting state, provides support for the concept that the low metabolic activity of the intracellular bacteria was responsible for the poor activity exhibited by ampicillin in nude mice.

ACKNOWLEDGMENTS

We thank A. L. Beukelman for secretarial help and B. K. B. Reimann-Knuth Winterfeldt for critical review of the manuscript.

This study was supported in part by research grants from Beecham Farma B.V., The Netherlands.

LITERATURE CITED

1. **Bennet, J. V., J. L. Brodie, E. J. Benner, and W. M. N. Kirby.** 1966. Simplified, accurate method for antibiotic assay of clinical specimens. Appl. Microbiol. **14**: 170-177.
2. **Blanden, R. V., and R. E. Langman.** 1972. Cell-mediated immunity to bacterial infection in the mouse. Thymus-derived cells as effectors of acquired resistance to *Listeria monocytogenes*. Scand. J. Immunol. **1**:379-391.
3. **Brown, K. N., and A. Percival.** 1978. Penetration of antimicrobials into tissue culture cells and leukocytes. Scand. J. Infect. Dis. Suppl. **14**:251-260.
4. **Cheers, C., and R. Waller,** 1975. Activated macrophages in congenitally athymic "nude" mice and in lethally irradiated mice. J. Immunol. **115**:844-847.
5. **Emmerling, P., H. Finger, and H. Hof.** 1977. Cell-mediated resistance to infection with *Listeria monocytogenes* in nude mice. Infect. Immun. **15**:382-385.
6. **Emmerling, P., H. Hof, and H. Finger.** 1978. Resistance to infection with *Listeria monocytogenes* in normal and thymusless mice treated with ampicillin. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **240**:339-346.
7. **Hudgins, L. B., and S. R. Acchiardo.** 1978. Diagnosis and treatment of *Listeria monocytogenes* infection in renal transplant patients. Dialysis Transplant. **7**:1023-1027.
8. **Johnson, M. L., and E. W. Colley.** 1969. *Listeria monocytogenes* encephalitis associated with corticosteroid therapy. J. Clin. Pathol. **22**:465-469.
9. **Kindred, B.** 1979. Nude mice in immunology. Prog. Allergy **26**:137-238.
10. **Lane, F. C., and E. R. Unanue.** 1972. Requirement of thymus (T) lymphocytes for resistance to listeriosis. J. Exp. Med. **135**:1104-1112.
11. **Lavetter, A., J. M. Leedom, A. W. Mathies, D. Ivler, and P. F. Wehrle.** 1971. Meningitis due to *Listeria monocytogenes*. A review of 25 cases. N. Engl. J. Med. **285**:598-603.
12. **Lobo, M., and G. L. Mandell.** 1973. The effect of antibiotics on *Escherichia coli* ingested by macrophages. Proc. Soc. Exp. Biol. Med. **142**:1048-1050.
13. **Mackaness, G. B.** 1962. Cellular resistance to infection. J. Exp. Med. **116**:381-406.
14. **Nickol, A. D., and P. F. Bonventre.** 1977. Anomalous high native resistance of athymic mice to bacterial pathogens. Infect. Immun. **18**:636-645.
15. **Nieman, R. E., and B. Lorber,** 1980. Listeriosis in adults: a changing pattern. Report of eight cases and review of the literature, 1968-1978. Rev. Infect. Dis. **2**:207-227.
16. **Nirmul, G., S. Glabman, M. Haimov, E. Leiter, and L. Burrows.** 1971. *Listeria monocytogenes* meningitis during immunosuppression. N. Engl. J. Med. **285**:1323.
17. **Schröter, G. P. J., and R. Weil.** 1977. *Listeria monocytogenes* infection after renal transplantation. Arch. Intern. Med. **137**:1395-1399.
18. **Seelinger, H. P. R., U. Laymann, and H. Finger.** 1967. Untersuchungen zur Frage der Wirksamkeit von Ampicillin auf *Listeria monocytogenes*. Dtsch. Med. Wochenschr. **92**:1095-1097.
19. **Takeya, K., S. Shimotori, T. Taniguchi, and K. Nomoto.** 1977. Cellular mechanisms in the protection against infection by Listeria monocytogenes in mice. J. Gen. Microbiol. **100**:373-379.
20. **Vischer, W. A., and C. Rominger.** 1978. Rifampicin against experimental listeriosis in the mouse. Chemotherapy **24**:104-111.
21. **Watson, G. W., T. J. Fuller, J. Elms, and R. M. Kluge.** 1978. *Listeria cerebritis*. Relapse of infection in renal transplant patients. Arch. Intern. Med. **138**:83-87.
22. **Weingärtner, L., and S. Ortel.** 1967. Zur Behandlung der Listeriose mit Ampicillin. Dtsch. Med. Wochenschr. **92**:1098-1104.
23. **Zinkernagel, R. M., R. V. Blanden, and R. E. Langman.** 1974. Early appearance of sensitized lymphocytes in mice infected with *Listeria monocytogenes*. J. Immunol. **112**:496-501.