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Chemotherapy of an Experimental *Bacteroides fragilis* Infection in Mice

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The efficacies of five common antimicrobial agents were determined for a pure *Bacteroides fragilis* infection in mice. Therapy was begun 4 h after bacterial injection and given every 8 h thereafter for 5 days. Blood levels were determined over an 8-h period for each concentration of antibiotic tested. Clindamycin and tetracycline were the most effective in preventing the formation of abscesses. Chloramphenicol, penicillin G, and cephalothin were not effective in protecting the mice from infection.

Bacteroides fragilis is an anaerobic, gram-negative, nonsporeforming bacillus frequently encountered in human clinical specimens. Although the in vitro susceptibility of this organism has been determined to many antimicrobial agents, the lack of a suitable experimental animal model has prohibited the testing of the efficacies of these agents in vivo.

We have recently described the production of a pure *B. fragilis* infection in mice that results in an easily visible subcutaneous abscess (12). This model provides the first opportunity for the testing of antimicrobial agents against a pure *B. fragilis* infection in an experimental animal. Using this model, we have tested the efficacies of five common antimicrobial agents: clindamycin, chloramphenicol, tetracycline, penicillin G, and cephalothin.

MATERIALS AND METHODS

Source of organisms. *B. fragilis* VPI 9032 was originally isolated from a human clinical specimen.

Bacterial inoculum. Before this series of experiments, a chopped-meat broth culture of *B. fragilis* VPI 9032 was lyophilized in a number of ampoules. One ampoule was opened for each experiment into chopped-meat broth. The culture was grown overnight (18 to 20 h), and 0.1 ml was inoculated into 10-ml volumes of chopped-meat-carbohydrate semisolid medium (12). The chopped-meat-carbohydrate semisolid culture was incubated for 18 to 20 h and then injected into mice.

Injection of bacteria. One-half milliliter of the chopped-meat-carbohydrate semisolid culture was injected subcutaneously under the loose skin of the groin on the left side of the mouse as previously described (12).

Source of antibiotics. The antibiotics used and the sources were: penicillin G, potassium salt (1,595 U/mg), and tetracycline-hydrochloride, Sigma Chemical Co., St. Louis, Mo.; chloramphenicol-sodium succinate, and chloramphenicol-hydrochloride, Parke-Davis, Detroit, Mich.; cephalothin, Eli Lilly and Co., Indianapolis, Ind.; clindamycin phosphate and clindamycin-hydrochloride, The Upjohn Co., Kalamazoo, Mich.

Injection of antibiotics. All antibiotics were dissolved in sterile distilled water and given in 0.1-ml volumes. All solutions were made up fresh daily and stored at 4°C with the exception of tetracycline-hydrochloride, which was made up fresh for each injection time. The antibiotics were given intraperitoneally (i.p.) on the animal's right side. Tetracycline-hydrochloride was also given by oral gavage. Two groups of approximately 25 mice each were tested with each concentration of antibiotic. For each experiment, at least 25 mice were used as a control group. The mice received the bacterial injection before being randomly selected for treatment or as controls; the latter received 0.1 ml of sterile distilled water in place of the antibiotic.

Schedule of injections. Antibiotic therapy was begun 4 h after the bacterial injection and then given every 8 h thereafter for a period of 5 days.

Determination of MICs. Minimal inhibitory concentrations (MICs) were determined by the agar dilution procedure (13) with the medium of Wilkins and Chalgren (14). Chloramphenicol-hydrochloride and clindamycin-hydrochloride were used for determinating of MICs to chloramphenicol and clindamycin.

Determination of antibiotic blood levels. The amount of antibiotic present in the blood was determined by the method described by Sabath and Toftegaard (11). For each concentration of antibiotic tested, blood levels were determined in two separate groups of five mice each. Blood was collected from each mouse by tail bleeding at 0.5, 1, 2, 3, 4.5, 6, and 8 h after the last injection of the antibiotic. Ten microliters of blood from each mouse was placed on a 6.35-mm paper disk (Schleicher and Schuell, no. 740-E). The disks were placed on the assay medium (11) and incubated in an anaerobic chamber similar to that described by Aranki and Freter (2). The assay plates were incubated at 37°C for 3 to 5 h until

hemolysis of the blood had occurred. The assay medium was made 1 day before use and stored at 4°C. The levels of antibiotics in the blood were determined by comparison with standard curves constructed by exponential regression with a computer. Clindamycin-hydrochloride and chloramphenicol-hydrochloride were used to construct the standard curves for clindamycin and chloramphenicol. Standard curves were constructed by dissolving known amounts of antibiotic in whole sheep blood. Whole sheep blood was used routinely for the standard curves because preliminary trials indicated that no significant differences were detectable between the quantitation of the antibiotics in sheep blood and in mouse blood.

Assay organisms. The strains of *Clostridium perfringens* used as assay organisms for the different antibiotics are given in Table 1.

Anaerobic techniques and media. The media for the cultivation of *B. fragilis* were prepared prereduced (5), and all culture manipulations were performed as described in the *VPI Anaerobe Laboratory Manual* (5).

Mice. Swiss white mice, ICR strain, 18 to 20 g (Flow Laboratories, Dublin, Va.), were used for all experiments. The mice were caged in groups of 25 with a light-dark cycle of 12 h and were allowed to stabilize for at least 48 h before being used in any experiment.

Determination of effects of therapy. The mice were examined daily beginning on the 4th day after bacterial injection and continuing through the 14th day for the presence of a localized subcutaneous abscess. Maximum abscess formation was found to occur by the 7th to the 10th day (11). The mice were not kept past the 14th day.

RESULTS

MICs. The MICs for the five antibiotics tested are given in Table 1. *B. fragilis* VPI 9032 was susceptible to clindamycin, tetracycline, and chloramphenicol and relatively resistant to both penicillin G and cephalothin in vitro.

Blood levels. The antibiotic concentrations in the blood are given in Table 2 for an 8-h period after the final injection of the antibiotic. For all of the antibiotics tested except tetracycline, the peak blood level occurred within the first 0.5 h after injection.

Clindamycin. Intraperitoneal injections of 37.5, 75, 150, or 300 mg of clindamycin per kg were given every 8 h for 5 days. Table 3 gives the percentage of mice protected from infection by each dosage and the peak blood levels obtained. Peak blood levels for clindamycin in humans are generally considered to fall within a range of 3 to 14 μg/ml (7). To obtain comparable blood levels in mice, we found it necessary to give 75 to 150 mg/kg. These dosages gave peak blood levels of 3 and 11 μg/ml and protected 69 and 74% of the mice, respectively. Increasing the dosage to 300 mg/kg yielded peak blood levels of 32 μg/ml and protected 84% of the mice. Although the lowest dosage tested, 37.5 mg/kg, gave a peak blood level of only 0.44 μg/ml, it protected 45% of the mice. This result is not surprising since the MIC for the test organism was only 0.015 μg/ml.

Chloramphenicol. Intraperitoneal injections of 75, 150, or 300 mg of chloramphenicol per kg were given as described for clindamycin. The 150-mg/kg dosage gave peak blood levels of 25 μg/ml, which were comparable to blood levels of 22 μg/ml usually achieved in humans (6). The test organism had an MIC of 4 μg/ml. This dosage protected 43% of the mice that were examined on the 7th day after bacterial injection. By the 10th day, however, the number of mice protected had dropped to 23%. Similar results showing an increase in the number of visible abscesses after cessation of therapy were also observed with dosages of 75 and 300 mg/kg (Table 3).

Penicillin. High dosages of penicillin have been reported by some workers to be effective against *B. fragilis* infections (E. J. Benner, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother, 17th, San Francisco, Calif., Abstr. 396, 1974). We tested the efficacy of very high dosages of penicillin by injecting 500 and 1,000 mg/kg i.p. every 8 h for 5 days. These doses corresponded to 797,500 and 1,595,000 U/kg. The MIC for the test organism was 32 μg/ml. At 500 mg/kg, 84 to 86% of the mice developed abscesses (Table 3), which was not significantly

TABLE 1. *Susceptibilities of various strains of C. perfringens* and *B. fragilis* (VPI 9032) *to diverse antimicrobial drugs*

Organism and strain	MIC (μg/ml)				
	Clindamycin	Chloramphenicol	Cephalothin	Tetracycline	Penicillin G
C. perfringens					
ATCC 13124	0.015				
SAL 249[a]		4–8			
VPI 8050A			0.25		
SAL 19				0.125–0.25	0.125
B. fragilis VPI 9032	0.015	4	64	0.5	32

[a] SAL strains were obtained from F. Tally, Tufts Medical Center, Boston, Mass.

TABLE 2. *Concentrations of various antimicrobial agents in whole blood of normal mice after administration of doses used in treating infected mice*

Drug administered	Route	Concn (mg/kg)	μg of drug/ml of whole blood ± SD[b]						
			0.5[c]	1	2	3	4.5	6	8
Clindamycin	i.p.	37.5	0.44 ± 0.17	0.40 ± 0.16	0.16 ± 0.08	0.13 ± 0.1	<0.1	<0.1	<0.1
		75.0	2.9 ± 1.1	0.8 ± 0.2	0.2 ± 0.07	0.1 ± 0.03	<0.1	0.15 ± 0.02	<0.1
		150.0	11.0 ± 7.0	3.0 ± 0.5	0.6 ± 0.03	0.5 ± 0.3	<0.1	0.16 ± 0.1	<0.1
		300.0	32.0 ± 6.0	11.0 ± 3.0	2.9 ± 0.9	2.7 ± 1.0	2.0 ± 1.0	0.5 ± 0.4	0.3 ± 0.3
Chloramphenicol	i.p.	75.0	12 ± 6	9 ± 6	4 ± 2	3 ± 1	ND[d]	ND	ND
		150.0	25 ± 8	18 ± 8	14 ± 12	5 ± 3	<3	ND	ND
		300.0	48 ± 34	34 ± 24	15 ± 11	7 ± 4	<3	<3	<3
Penicillin G	i.p.	500.0	114 ± 40	44 ± 18	1.3 ± 0.25	0.3 ± 0.09	0.2 ± 0.05	0.4 ± 0.2	ND
		1,000.0	228 ± 64	543 ± 104	10 ± 4	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.6 ± 0.8
Cephalothin	i.p.	1,000.0	170 ± 73	70 ± 42	18 ± 16	6 ± 4	4 ± 3	<3	ND
Tetracycline	i.p.	62.5	34 ± 28	35 ± 26	9 ± 9	6 ± 5	1.3 ± 0.9	0.7 ± 0.5	0.5 ± 0.2
		125.0	90 ± 41	92 ± 20	41 ± 13	55 ± 20	28 ± 11	28 ± 13	43 ± 26
	o.p.	125.0	1.6 ± 1.1	1.8 ± 0.6	0.9 ± 0.7	0.5 ± 0.3	<0.2	ND	ND
		250.0	2.1 ± 0.6	2.3 ± 1.1	1.0 ± 0.5	0.7 ± 0.3	0.3 ± 0.05	<0.2	1.2 ± 0.9
		500.0	3.3 ± 1.7	6.6 ± 0.8	4.1 ± 2.3	3.3 ± 2.2	1.0 ± 0.5	1.2 ± 0.9	0.3 ± 0.07

[a] i.p., Intraperitoneal; o.p., oral gavage.
[b] SD, Standard deviation.
[c] Time in hours after drug administration.
[d] ND, None detected.

TABLE 3. *Correlation of protection achieved by administration of various antimicrobial agents with peak concentrations of the agents in whole blood*

	Route[a]	Concn (mg/kg)	Percent of mice protected		μg of drug/ml of whole blood ± SD[b] (peak blood level)
			Day 7	Day 10	
Clindamycin	i.p.	37.5	45	45	0.4 ± 0.2
		75.0	69	69	3.0 ± 1.0
		150.0	74	74	11.0 ± 7.0
		300.0	84	84	32.0 ± 6.0
Chloramphenicol	i.p.	75.0	33	17	12.0 ± 6.0
		150.0	43	23	25.0 ± 8.0
		300.0	51	36	48.0 ± 34.0
Penicillin G	i.p.	500.0	14	16	114.0 ± 40.0
		1,000.0	37	18	543.0 ± 104.0
Cephalothin	i.p.	1,000.0	26	24	170.0 ± 73.0
Tetracycline	i.p.	62.5	45	45	34.0 ± 28.0
	o.p.	125.0	55	55	1.8 ± 0.6
	o.p.	250.0	64	64	2.3 ± 1.1
	o.p.	500.0	77	77	6.6 ± 0.8

[a, b] See Table 2.

different from an 88% incidence of infection in the respective control group. The 1,000-mg/kg dosage conferred limited protection through the 7th day after bacterial injection; however, by the 10th day, 82% of these mice had developed abscesses (Table 3); this finding also was not significantly different from that in the control group.

Cephalothin. Cephalothin had an MIC of 64 μg/ml for the test organism and was tested in vivo at a very high dosage of 1,000 mg/kg i.p. every 8 h for 5 days. This antibiotic conferred only limited protection, protecting approximately one-fourth of the mice from infection (Table 3). This protection, unlike that of penicillin G, remained consistent, without the increase in the incidence of abscesses that occurred from the 7th to the 10th day with penicillin.

Tetracycline. We attempted to inject this antibiotic i.p. as we had with the previous antibiotics. Because of the toxicity of the larger dosages, however, we also gave the drug by oral gavage. The mice injected i.p. received either 62.5 or 125 mg/kg every 8 h for 5 days, and the mice treated by oral gavage received 125, 250, or 500 mg/kg. As expected, the dosages given i.p. produced much higher blood levels due to greater absorption of the drug (Table 3). The 125-mg/kg i.p. dosage killed all of the mice by the 6th day after bacterial injection. The 62.5-mg/kg i.p. dosage caused the mice some discomfort but did not result in death. These mice recovered rapidly after cessation of drug therapy on the 5th day. This dosage protected 45 to 50% of the mice. Of the oral injections, only the 500-mg/kg dosage produced any notable toxicity. These mice, like those that received 62.5 mg/kg i.p., recovered as soon as treatment was terminated. Even though the blood levels achieved with the oral dosages were much lower than those found with the 62.5-mg/kg i.p. dosage, all three of the antibiotic concentrations given by oral injection conferred a higher degree of protection than did the 62.5-mg/kg i.p. dosage. The degree of protection in the oral treatment groups ranged from 55% for the 125-mg/kg dosage to 77% for the 500-mg/kg dosage.

DISCUSSION

In the series of experiments reported here, clindamycin had the highest efficacy of the antimicrobial agents tested. The efficacy observed may be correlated, in part, with the retention of the antibiotic in the blood in concentrations exceeding the MIC of the test organism. We found that all four dosages of clindamycin tested were sufficient to maintain blood levels well above the MIC for most strains of *B. fragilis* for at least 8 h. Our results showed, however, that the larger dosage protected more mice from infection. This increase in protection cannot be explained entirely by increased blood levels since the lowest dosage (37.5 mg/kg) tested gave blood levels seven to eight times higher than the MIC as late as 8 h after administration of the drug. A twofold increase in the dosage to 75 mg/kg gave a dramatic increase in the number of mice protected. The only significant difference in the blood levels produced by these two dosages, however, occurred within 1 to 2 h after injection of the drug. One possible explanation of this observation is that higher blood levels during the 1st or 2nd hour may influence the quantity of the antibiotic that enters the abscess.

For many years tetracycline was the drug of choice for the treatment of anaerobic bacterial infections. Lately its use has decreased because of an increase in the number of anaerobes found to be resistant to it, in particular clinical isolates of *B. fragilis* (7). The *B. fragilis* strain used in these experiments was susceptible to tetracycline. The dosages of tetracycline sufficient to give peak blood levels equivalent to those usually obtained in humans protected the mice from infection almost as well as did clindamycin. On a weight-to-weight basis, however, higher dosages were required for tetracycline than for clindamycin. This finding resulted from the need to use the oral route of administration for tetracycline and from the limited absorption of this drug from the gastrointestinal tract (9). The differences in the amount of protection conferred by oral dosages of tetracycline correlated with the length of time that blood levels of the antibiotic were kept above the MIC. The highest oral dosage tested, 500 mg/kg, was sufficient to maintain blood levels above the MIC for up to 7 h. The lowest oral dosage, 125 mg/kg, only gave blood levels above or equal to the MIC for 4 h. The failure of the i.p. dosage of 67.5 mg/kg to give protection comparable to that of the oral dosages cannot be explained by failure to maintain adequate blood levels. This dosage gave a peak blood level of 34 μg/ml within the first 30 min and the blood level was still equal to or slightly above the MIC of the drug for the test organism 8 h later. Although toxicity was noted in these animals, it was not grossly different from the toxicity observed in the group that was given 500 mg/kg orally. It is possible that differences existed that were not noted.

Most strains of *B. fragilis* are susceptible to chloramphenicol in vitro. The strain that we used had an MIC of 4 μg/ml, which is in the range found for most strains of *B. fragilis* (15). Treatment with this antibiotic, even at levels that gave peak blood levels approximately double that seen in humans, was not very effective against the infection. This result may have been due to the fact that even the highest dosage that we tested gave blood levels that were just equal to or slightly less than the MIC within 3 to 4 h after administration of the drug. The MIC for most anaerobes is only slightly below achievable blood levels, and the antibiotic level is difficult to maintain above the MIC. In addition, the concentration achieved in the abscess may be less than the blood level. The bacteriostatic nature of this antibiotic also appeared to be reflected in the results with all three concentrations tested. Approximately 15 to 20% of the abscesses were not grossly evident during the treatment period or for up to 2 days after cessation of therapy. Previously undetected abscesses were observed as early as 3 days after termination of therapy and rapidly increased in size, so that no differences existed between the sizes of the abscesses present in the control group and in the treatment groups by the 10th day after the bacterial injection. It appeared that chloramphenicol, although present, was somewhat bacteriostatic for the test organisms; but, once therapy was terminated, the organisms were able to continue normal growth.

Penicillin G and cephalothin are both β-lactam antibiotics to which strains of *B. fragilis* are relatively resistant in vitro. Although both these antibiotics were tested at very high dosages, little efficacy was found with either. This agrees with our earlier finding that mixed infections containing *B. fragilis* could not be treated successfully with penicillin or cephalothin (3, 4). Whether this resistance of *B. fragilis* is due to production of β-lactamase or to the rapid clearance from the blood is not known. Several investigators have reported the presence of β-lactamase in *B. fragilis*; however, the levels of the enzyme detected were very low (1, 8, 10). Both these antibiotics initially gave relatively high peak blood levels; however, by the end of 1 to 2 h, blood levels of each had dropped well below the MICs of penicillin G and cephalothin. Although we initially found a higher incidence of protection with the 1,000-mg/kg dosage of penicillin during the period of therapy, there was no significant difference between the 1,000-mg/kg dosage and the 500-mg/kg dosage of penicillin a few days after termination of therapy. In this instance, it appears that the high dosage of penicillin did have a very limited effect on the infection as long as therapy was maintained.

Although caution must be exercised in the extrapolation of data obtained from experimental animals to humans, the results obtained from these experiments reflected very well the clinical findings with these antibiotics against infections in humans with *B. fragilis*. This model may be useful, therefore, for the evaluation of new antimicrobial agents by providing a means for the rapid and economical determination of their efficacies. The model infection provides a method for the testing of antibiotics against *B. fragilis* that may be more relevant to the clinical use of the drugs than the routine testing of in vitro susceptibility.

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