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Antibiotic Penetrance of Ascitic Fluid in Dogs

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Antibiotic concentrations in ascitic fluid after parenteral therapy may be important in the treatment of peritonitis. We have created ascites in dogs by partial ligation of the inferior vena cava. Ascitic fluid volume was measured at the time each antibiotic was administered. Nine antibiotics were studied in the same three dogs. Antibiotic concentration in ascitic fluid was found to vary inversely with ascites volume. Percentage of penetration (ratio of ascites peak to serum peak $\times 100$) ranged from 5.8 to 65% among the drugs studied. Only metronidazole showed a statistically significant higher percentage of penetration than other antimicrobials. Concentrations in ascitic fluid after single doses of cephalothin (15 mg/kg) and the aminoglycosides (2 mg/kg, gentamicin and tobramycin; 7.5 mg/kg, amikacin and kanamycin) did not exceed the minimum inhibitory concentration of many gram-negative rods and may justify the use of higher than usual initial parenteral doses, or possibly initial intraperitoneal administration in seriously ill patients.

Patients with hepatic cirrhosis and ascites are at increased risk of developing spontaneous bacterial peritonitis (2, 3). Successful antibiotic therapy of this highly fatal disease is likely to depend upon both the antimicrobial spectrum of the drugs selected and their ability to penetrate into ascitic fluid. Previous studies of peritoneal transport of antibiotics in man have been performed during peritoneal dialysis (6, 17, 18). Such studies, utilizing a protein-free fluid that is rapidly exchanged, may not accurately reflect the kinetics of antibiotics in ascitic fluid arising as a result of cirrhosis. Furthermore, patients with cirrhosis frequently have low serum albumin levels, which may alter the serum binding of certain antibiotics and change their pharmacokinetics. There is little information on ascitic fluid penetration of newer antibiotics, particularly those of the cephalosporin and aminoglycoside groups. The only paper directly addressing this topic suggested that cephalothin should be administered by both the intravenous and intraperitoneal routes in the initial treatment of peritonitis in cirrhotics with ascites (24).

Because the literature data concerning antibiotic penetration of ascitic fluid does not seem to be adequate, we elected to study this problem utilizing a previously described model (13) for the creation of ascites in dogs. In this paper we report the ascitic fluid penetrance of single doses of nine antibiotics in this dog model. In addition, ascitic fluid volume was measured and compared with ascites concentrations for several of the antibiotics studied.

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MATERIALS AND METHODS

Creation of ascites in dogs. Mongrel dogs weighing 18 to 22 kg were anesthetized with intravenous pentobarbital, and the inferior vena cava was ligated through a right thoracotomy according to the technique of McKee et al. (13). An umbilical tape was used to ligate the vena cava by tying down loosely on a 1.5-mm-diameter rigid tube that was then removed. Three of the first seven dogs prepared in this manner developed ascites and were used for the antibiotic studies. Two of the three dogs required 15 g of sodium chloride daily in their diets periodically to maintain the ascites. All dogs were housed in separate pens, fed commercial dry dog food, and given free access to water.

Large collateral veins developed on the abdomen and thoracic wall, and the animals showed obvious loss of muscle mass after the procedure. Previous microscopic studies by McKee et al. (13) have shown that the procedure results in passive congestion of the liver with marked dilation of subcapsular lymphatics and venous sinusoids. Ascites formation occurs primarily by means of fluid escape from the capsular surface of the liver (7).

Ascitic fluid volume and characteristics. The volume of ascitic fluid was measured before each antibiotic administration according to the method of Luttwak (11) using ^{125}I-labeled human serum albumin. Five microcuries of ^{125}I-labeled albumin was injected into the peritoneal cavity, and the volume

of fluid was determined by counting a 10-ml sample of ascitic fluid obtained 30 min after injection. Stability of the volume determination at 10, 20, and 30 min as shown by Luttwak (11) was confirmed.

Cell counts were determined on a Coulter counter (Coulter Electronics, Inc., Hialeah, Fla.). Differentials were hand counted. Total protein, albumin, creatinine, blood urea nitrogen, bilirubin, serum glutamic-oxalacetic transaminase, and alkaline phosphatase were determined using the Auto Analyzer (Technicon, Terrytown, N.Y.).

Antibiotic administration. Antibiotics were administered intramuscularly in the gluteus muscle, except for metronidazole, which was given orally. Each antibiotic was administered to the same three dogs. Dosages are given in Table 2 and are based on the dry weight of each dog before the creation of ascites. Serum and ascites specimens were obtained at zero time, 0.5, 1 through 6, and 24 h after antibiotic administration. Blood was obtained from the jugular vein and ascites fluid was obtained by midline abdominal wall aspiration. Dogs were fasting 12 h at the onset of each study and were allowed water ad libitum but no food for the 24 h of an experiment.

Antibiotic assays. Antibiotic assays were performed in triplicate using a microbiological disk-plate method with a 24-h incubation at 37°C (10, 16). *Bacillus subtilis* ATCC 6633 was used for the assay of cephalothin and all the aminoglycosides. *Sarcina lutea* was the indicator organism for cephaloridine and clindamycin. *Staphylococcus aureus* 6538P was used for cefazolin. Metronidazole was assayed by the method of Levison (9), modified by using a strain of *Clostridium perfringens* obtained from the University of Minnesota. One molar phosphate buffer (pH 8.0) was added to the clindamycin specimens immediately after drawing to interrupt further hydrolysis from ester to active clindamycin. Specimens were placed on 6.35-mm paper disks (Schleicher and Schuell, Inc., Keene, N.H.) using disposable 20-μl pipettes (Unopette, Becton-Dickinson and Co., Rutherford, N.J.). For each antibiotic, the initial standard curves were prepared in both pooled dog ascitic fluid and serum. No differences were noted between standard curves obtained from serum or ascitic fluid for any of the antibiotics, and subsequent assays were done with dog serum only for the standard curve. All antibiotics were supplied as sterile dry powders or solutions. Tobramycin was furnished through the courtesy of Eli Lilly and Co., Indianapolis, Ind., and amikacin was supplied by Bristol Laboratories, Syracuse, N.Y.

Statistical analysis. Standard error of the mean was determined by the method of Mantel (12). Antibiotic serum half-life ($t_{1/2}$) was calculated from the standard formula using the slope of the regression line determined by the method of least squares (5). The nonparametric Mann-Whitney U test was used to determine statistical significance (1).

RESULTS

Serum and ascitic fluid characteristics. Ascitic fluid chemical and cellular characteristics are shown in Table 1 for the three dogs studied and six additional dogs. Leukocyte and erythrocyte counts tended to be slightly higher for specimens obtained after repeated paracenteses during the course of an antibiotic study. Maximum hematocrit was 1.2%.

Serum total protein and albumin were reduced in these animals, ranging from 3.7 to 4.8 g/100 ml (mean, 4.5) for protein and from 1.5 to 2.7 g/100 ml (mean, 2.2) for albumin. Blood urea nitrogen, serum creatinine, bilirubin, serum glutamic-oxalacetic transaminase, and alkaline phosphatase were normal. Protein electrophoresis in two animals revealed qualitatively similar patterns for ascitic fluid and serum, but quantitatively both globulins and albumin were lower in ascites.

Ascites volume and antibiotic concentration in ascitic fluid. Ascitic fluid volume ranged from 2.2 to 10.8 liters in the three dogs during the course of the study (Table 2). Volumes for an individual dog changed slowly and did not vary by more than a liter from week to week unless sodium chloride was withheld or added to the diet. The variation of ascitic fluid antibiotic concentration with ascites volume for cefazolin is shown in Fig. 1. Peak concentrations of cefazolin in serum were similar for the three dogs (22, 24, and 29 μg/ml). Other antibiotics showed a similar variation of ascites concentration with volume, but ascitic fluid volumes often were more closely grouped and the range of ascites antibiotic concentrations was not as wide as with cefazolin. During the first few hours after administration, the relationship of ascitic fluid antibiotic level to ascitic fluid volume is linear when plotted on a semilogarithmic graph, as shown in Fig. 2 for amikacin and cefazolin. After 2 to 3 h, ascitic fluid peak concentrations begin to occur and the relationship becomes nonlinear.

Ascitic fluid levels of antibiotics. Mean peak levels and ranges of antibiotic concentrations and percentage of penetration (ratio of ascites peak to serum peak $\times 100$) are shown in Table 2. Typical pharmacokinetic curves for the study are shown for cefazolin and cephalothin (Fig. 3) and for metronidazole and clindamycin (Fig. 4). Kinetic curves for cephaloridine and

TABLE 1. *Characteristics of ascitic fluid from nine dogs*

Determination	Mean	Range
Leukocyte count	975	110–2,000
% Polymorphonuclears (%)	50	16–77
Erythrocyte count	7,500	300–20,000
Total protein (g/100 ml)	3.1	1.9–3.8
Albumin (g/100 ml)	1.7	1.2–1.9
pH	7.62	7.5–7.7

TABLE 2. *Mean and range of ascites volume, peak serum level, peak ascites level and percentage of penetration (ratio of ascites peak to serum peak ×100) for nine antibiotics administered to the same three dogs*

Antibiotic	Dose (mg/kg)	Ascites volume (liters)		Serum peak (μg/ml)		Ascites peak (μg/ml)		% Penetration	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Cephaloridine	15	6.2	3.7-9.4	27.7	23-35	5.3	3.5-6.8	19.1	15.2-24.3
Cefazolin	15	5.8	2.2-10.8	24.0	22-29	6.2	2.7-9.0	25.8	11.3-32.7
Cephalothin	15	5.1	3.3-8.3	14.5	10-18	3.1	1.3-5.4	21.4	8.4-30.0
Kanamycin	7.5	4.6	3.2-6.4	21.3	19-25	2.4	1.1-4.3	11.3	5.8-21.5
Amikacin	7.5	5.6	3.3-9.1	19.4	17-23	3.3	1.9-4.6	17.0	8.6-25.0
Gentamicin	2	3.5	3.0-3.8	8.9	5.4-15	1.7	0.6-3.7	19.1	11.1-24.7
Tobramycin	2	4.6	2.3-7.5	6.4	4-8.2	1.1	0.6-1.5	17.2	13.4-21.5
Clindamycin	8.6	5.8	2.8-10.5	7.8	4.8-10.5	2.1	1.4-3.3	26.9	13.3-39.3
Metronidazole	250[a]	5.9	4.2-9.1	12.1	9.8-15.5	5.7	4.2-6.7	47.0	38.2-65.0

[a] Dose in milligrams.

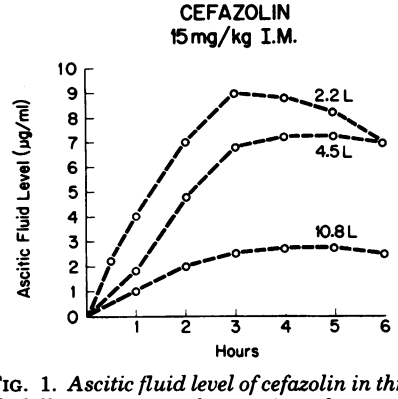

FIG. 1. *Ascitic fluid level of cefazolin in three dogs with different ascites volumes given the same dose of cefazolin based on dry weight. Peak serum concentrations were similar in the three animals.*

all the aminoglycosides were similar to those in Fig. 3. The kinetics of metronidazole (oral administration) showed a later serum peak and a longer $t_{1/2}$ than the other drugs tested. Clindamycin, administered as the phosphate, requires hydrolysis to the active form of the drug, resulting in later serum and ascites peaks. There were no statistical differences in percentage of penetration among the cephalosporins, aminoglycosides, and clindamycin. Metronidazole percentage of penetration was significantly higher ($P < 0.05$) than all cephalosporins and aminoglycosides, but was not statistically higher ($P < 0.10$) than clindamycin. Peak concentration of antibiotic in ascitic fluid occurred 4 h after administration for all drugs, except cephalothin (2 h), kanamycin (3 h), and clindamycin (5 h). Detectable concentrations of antibiotic were present in ascitic fluid at 24 h for clindamycin, metronidazole, amikacin, cefazolin, and cephaloridine. Ascitic fluid clindamycin mean concentration at 24 h was 1.1 μg/ml (range, 0.9 to 1.3) and was the highest of the nine drugs tested.

Serum $t_{1/2}$ for the aminoglycosides was 0.9 h for kanamycin, 1.3 h for amikacin and tobramycin, and 1.7 h for gentamicin. Percentage of penetration (Table 2) closely paralleled serum $t_{1/2}$ for the aminoglycosides. Cefazolin had the longest $t_{1/2}$ of the cephalosporins (1.5 h); this compared with 1.1 and 1.2 h for cephalothin and cephaloridine, respectively. Clindamycin (3.3 h) and metronidazole (4.2 h) had a considerably longer serum $t_{1/2}$ than the other drugs tested. In general, the percentage of penetration of the antibiotics (Table 2) paralleled the serum $t_{1/2}$.

DISCUSSION

Two physiological factors are believed to be responsible for ascites formation in cirrhosis: obstruction to hepatic venous outflow and prehepatic portal venous obstruction (8, 25). The model chosen for this study produces ascites largely by means of the former mechanism, and results in a somewhat higher ascites protein content than in cirrhosis, largely because fluid "weeps" from the liver surface (7). Secondary factors contributing to ascites formation in cirrhosis (reduced serum albumin, and salt and water retention) are also present in this model (13). Thus, the model approximates the physiological situation in cirrhosis except for the absence of portal vein obstruction.

The study was deliberately performed with single doses of each antibiotic to determine the ascites penetration of initial systemic administrations, possibly a critical factor in seriously ill patients with peritonitis. No attempt was made to establish equilibrium conditions. Previous studies of antibiotic concentrations in perito-

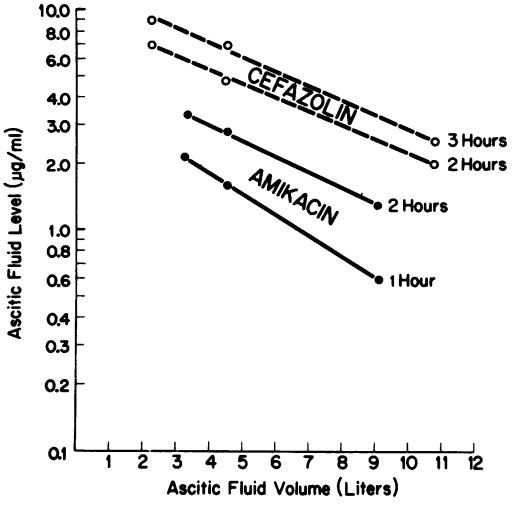

FIG. 2. *Semilogarithmic plot of cefazolin (broken line) and amikacin (solid line) ascitic fluid concentrations as a function of ascitic fluid volume for the first 3 h after intramuscular injection.*

neal dialysis fluid in humans have indicated that therapeutically adequate concentrations of methicillin and tetracycline can be expected after 1 to 2 h after systemic administration (17). In similar studies, tobramycin and gentamicin concentrations have been therapeutically inadequate (18, 23). Renal function was abnormal in all the patients studied, resulting in longer than normal serum $t_{1/2}$. Cephalothin penetrance of ascitic fluid in uninfected cirrhotic patients was studied by Wilson and associates (24). Mean peak ascites concentration was 3.22 µg/ml 1 h after a 1-g intravenous administration. Ascites volume ranged from 4 to 12 liters.

The results of the present study show that ascitic fluid concentrations of antibiotics are inversely proportional to the volume of ascites present. Percentage of penetration (ratio of ascites peak to serum peak ×100) can be expected to range from 8.4 to 32.7% for the cephalosporins, from 5.8 to 25% for the aminoglycosides, from 13.3 to 39.3% for clindamycin, and from 38.2 to 65% for metronidazole, depending upon the ascites volume present (Table 2). Among the cephalosporins and aminoglycosides studied, no single antibiotic was found to penetrate peritoneal fluid preferentially. The mean peak ascitic fluid cephalothin concentration achieved in this study (3.1 µg/ml) is virtually identical to the level achieved in the only comparable human study (24). Metronidazole was the only drug studied that showed a statistically higher percentage of penetration than the others. Metronidazole was also the only drug administered

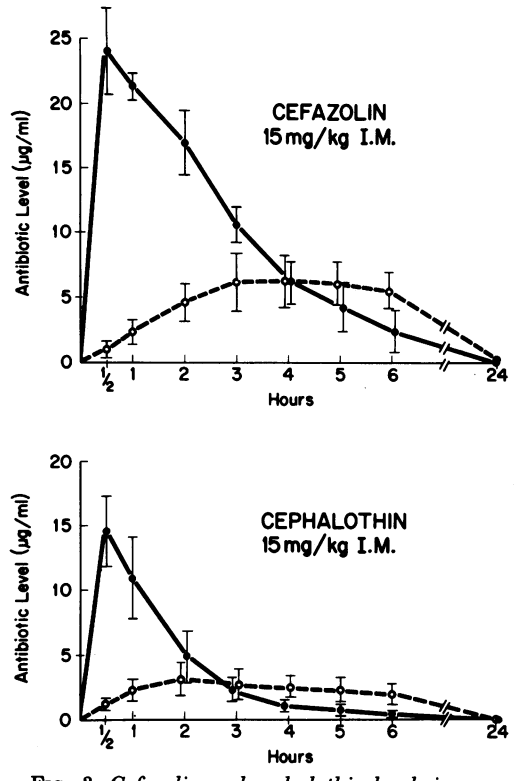

FIG. 3. *Cefazolin and cephalothin levels in serum (solid line) and ascitic fluid (broken line) in the same three dogs. Vertical bars indicate standard error of the mean.*

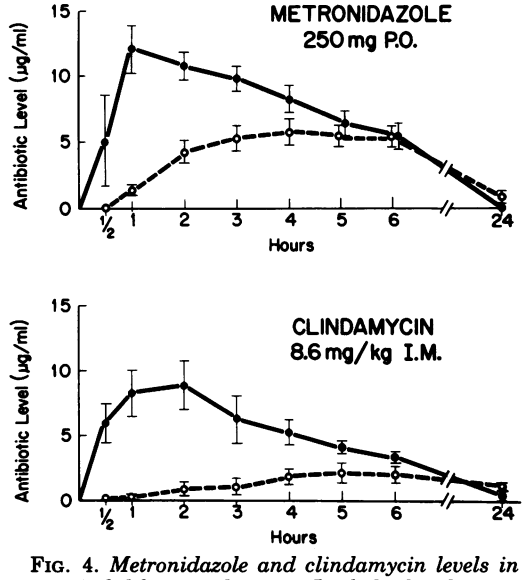

FIG. 4. *Metronidazole and clindamycin levels in serum (solid line) and ascitic fluid (broken line) in the same three dogs. Vertical bars indicate standard error of the mean.*

orally, and it had the longest serum $t_{1/2}$. Levels of metronidazole, as measured by bioassay, reflect activity of both parent drug and biologically active metabolites (19), which may account in part for the long $t_{1/2}$ and high percentage of penetration.

We find it interesting that the differences in serum protein binding of the cephalosporins in dogs (22) appear to have no affect on the concentrations achieved in ascitic fluid. It has been shown that cefazolin is 80% serum protein bound, cephalothin is 40% bound, and cephaloridine is only 10% bound; but the time required for maximum binding in vitro has been shown to be up to 50 min for cefazolin (22). A similar delay in in vivo binding of cefazolin could account for its high percentage of penetration. Since serum albumin was low in these dogs, cefazolin serum binding may have been lower than in normal canine serum. In addition, bound as well as unbound drug may diffuse from serum to peritoneal cavity from the liver surface.

The concentrations of clindamycin and metronidazole in ascitic fluid after a single dose exceed the minimum inhibitory concentration (MIC) of most anaerobic and gram-positive aerobic organisms toward which they might be directed (14, 20). In comparison to the in vitro MIC of gram-negative bacilli (15, 26), the ascitic fluid concentrations of the aminoglycosides appear to be marginal at best. This may be due to the short serum $t_{1/2}$ observed in the dog, in comparison with observations in humans. Similarly, cephalothin ascitic fluid concentrations appear to be inadequate for most susceptible gram-negative bacilli (21), at the dosage used in this study (1 g human equivalent). Concentrations of cephaloridine and cefazolin compare well with the in vitro MIC of gram-negative bacilli (21).

In the presence of peritoneal inflammation, the penetrance of antibiotics into ascitic fluid may be increased, as observed by Smithivas and associates (18) for gentamicin. Early experimental observations by David (4), however, indicate that in severe peritonitis, peritoneal absorption may be markedly inhibited.

Based on our observations in dogs, we believe that the ascitic fluid concentrations of some antibiotics (cephalothin and the aminoglycosides) may be somewhat low after a single administration, particularly if the ascitic fluid volume is large, disregarding possible enhanced penetration due to inflammation. Antibiotic kinetics in ascitic fluid suggest that accumulation will occur with subsequent doses (Fig. 3 and 4). Because initial peritoneal concentrations may be marginal, we suggest maximizing the initial systemic dose or intraperitoneal installation of the first dose of aminoglycosides or cephalothin if the patient is critically ill. Because of possible respiratory paralysis and ototoxicity, aminoglycoside dosages intraperitoneally should not exceed recommendations for systemic therapy.

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