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# Effect of Meningitis and Probenecid on the Penetration of Vancomycin into Cerebrospinal Fluid in Rabbits

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This study examined the effects of experimental pneumococcal meningitis and probenecid administration on the penetration of parenterally administered vancomycin into cerebrospinal fluid in rabbits. Bacterial killing was also examined in infected animals. Meningitis was induced by intracisternal inoculation of *Streptococcus pneumoniae*. Vancomycin was administered in a loading dose followed by a continuous intravenous infusion for 6 h. Serum and cerebrospinal fluid samples were obtained at 0, 2, 4, and 6 h for antibiotic assays and quantitative cultures. Meningitis significantly enhanced the penetration of vancomycin into cerebrospinal fluid, but probenecid administration had no effect. In normal rabbits, at 6 h the mean percent penetration (cerebrospinal fluid concentration/serum concentration × 100%) ± the standard deviation was 1.9 ± 0.9% in the nonprobenecid group ($n = 10$) and 1.9 ± 1.1% in the probenecid group ($n = 9$). In rabbits with experimental pneumococcal meningitis, the mean percent penetration at 6 h was 3.9 ± 2.6% in the nonprobenecid group ($n = 11$) and 4.3 ± 2.1% in the probenecid group ($n = 9$). Mean bacterial titers in the cerebrospinal fluid of infected animals decreased by more than 3.0 $\log_{10}$ colony-forming units per ml in both the nonprobenecid and the probenecid groups.

The role of vancomycin in the therapy of central nervous system infections has not been clearly established. Vancomycin demonstrates potent bactericidal activity against many gram-positive pathogens, but its ability to penetrate into cerebrospinal fluid (CSF) has been questioned. Vancomycin activity has not been detected in CSF of human volunteers with normal meninges who are receiving large intravenous injections (7, 16). The more important consideration, the penetration of vancomycin into CSF in patients with meningitis, has not been examined in clinical studies. Since this issue cannot readily be studied in patients with meningitis without exposing them to unnecessary risks, this study employed a rabbit model to examine this issue (2). The effect of meningitis on the penetration of vancomycin into CSF was determined by comparing the penetration of vancomycin into CSF in rabbits with experimental pneumococcal meningitis with that in normal rabbits. Bacterial killing by vancomycin in vivo was also examined in animals with meningitis.

It was noted that the relative penetration of vancomycin into CSF increased during therapy in infected animals. This observation suggested either that permeability of the blood-CSF barrier to vancomycin was increasing during therapy or that the efflux of vancomycin from CSF was decreasing. To explore this latter possibility, we studied the effect on additional rabbits of probenecid, a compound known to inhibit the transport of penicillin and other antibiotics from CSF to blood (2-4, 6, 17).

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## MATERIALS AND METHODS

**Test organism.** A clinical isolate of *Streptococcus pneumoniae* type III obtained from the bacteriology laboratory at the University of Missouri Medical Center was used throughout this study. Virulence was maintained by serial passage in white mice. The minimum inhibitory concentration of vancomycin for this organism was 1.0 μg/ml, as determined by the broth dilution microtechnique (12).

**Rabbit model.** A total of 39, 2-kg New Zealand white rabbits were studied by the method of Dacey and Sande (2). Meningitis was established in 20 rabbits by intracisternal inoculation of 6 to 8 $\log_{10}$ colony-forming units (CFU) of *S. pneumoniae* type III. Animals were first anesthetized with 50 to 60 mg of sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, Ill.), administered intravenously. After removal of 0.5 ml of CSF, a 0.2-ml suspension of *S. pneumoniae* was slowly injected into the cisterna magna. Organisms in the suspension were cultured in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 5% defibrinated sheep's blood for 8 h, washed in 0.9% saline, and suspended in sterile 0.9% saline to a concentration of 6 to 8 $\log_{10}$ CFU per ml for inoculation. After the animals were inoculated, the cisternal needle was withdrawn; the animals were

removed from the frame and returned to their cages. Within hours, all rabbits developed acute meningitis. By the time therapy was initiated (17 to 20 h after inoculation), all inoculated animals manifested lethargy, fever (temperature of >40.0°C), CSF leukocytosis (1,450 to 19,800 leukocytes per $mm^3$; 90 to 95% polymorphonuclear leukocytes), and CSF bacterial titers ranging from 3.1 $log_{10}$ to 6.6 $log_{10}$ CFU per ml. In previous studies with rabbits inoculated with *S. pneumoniae* type III, increasing CSF titers have been observed during the first 3 days of illness (13), and increases in CSF titers have been documented under experimental conditions identical to those in this study (14).

Before therapy, infected and uninfected rabbits were anesthetized with 25 to 60 mg of sodium pentobarbital, administered intravenously. Indwelling femoral arterial and venous catheters (Intramedic polyethylene tubing 7420) were inserted. All animals were then placed in a stereotaxic frame, and a spinal needle was positioned without trauma in the cisterna magna. All animals received an intravenous loading dose (10 mg/kg) of vancomycin (Vancocin; Eli Lilly & Co., Indianapolis, Ind.), which was followed by a constant intravenous infusion (8.3 mg/kg per h) for 6 h. Probenecid (supplied courtesy of E. J. Cragoe, Jr., Merck Sharp & Dohme, West Point, Pa.) was administered by a continuous intravenous infusion (10 mg/kg per h) through a separate femoral catheter. Small doses of sodium pentobarbital (<10 mg/kg per h) were used as needed to maintain anesthesia, and 0.9% saline (10 to 20 ml/h) was administered intravenously throughout the 6-h experimental period. During therapy, samples of blood (2 ml from the femoral arterial catheter) and CSF were obtained at 0, 2, 4, and 6 h for the determination of vancomycin concentrations. Bacterial titers were also determined on CSF samples from infected animals with serial 10-fold dilutions in Trypticase soy broth and with pour plates with Trypticase soy agar supplemented with defibrinated sheep blood.

**Assays for vancomycin.** Concentrations of vancomycin in serum and CSF were determined by an agar well diffusion method, with *Bacillus subtilis* as the test organism (1, 18). All samples were tested in triplicate. Samples of serum were diluted in pooled normal rabbit sera, and CSF samples were tested without dilution. Neither serum nor CSF from infected animals produced zones of inhibition in this assay system. Vancomycin standards for assays of serum were diluted in pooled normal rabbit sera. Vancomycin standards for CSF assays were diluted initially in 0.9% normal saline (NaCl), normal rabbit CSF, and infected rabbit CSF. After zone sizes were found to agree within ±5%, standards diluted in normal saline were used exclusively.

**Analysis of data.** Antibiotic penetration into CSF at each sampling time was expressed as the percent penetration: percent penetration = CSF concentration/serum concentration × 100%. The effect of probenecid, meningitis, and sampling time on serum and CSF concentrations of vancomycin and on percent penetration values was analyzed for statistical significance by a two-way analysis of variance for three repeated measures (19). This analysis employed the results from the nine animals in each treatment group that had complete sets of data at each sampling time.

## RESULTS

Serum concentrations of vancomycin increased during therapy in all treatment groups (Table 1). These concentrations were augmented by the presence of experimental pneumococcal meningitis but were unaffected by probenecid administration. Mean serum concentrations of vancomycin ranged from 33.3 μg/ml at 2 h to 51.1 μg/ml at 6 h in uninfected animals and from 64.7 μg/ml at 2 h to 108.4 μg/ml at 6 h in infected animals. Differences between normal

TABLE 1. *Effect of experimental meningitis and probenecid administration on penetration of vancomycin into CSF in rabbits*

| Rabbit group | Time (h) | Vancomycin concn + SD[a] (μg/ml) | | Percent penetration ± SD (%)[b] |
|---|---|---|---|---|
| | | Serum | CSF | |
| Normal | | | | |
| Nonprobenecid ($n$ = 10) | 2 | 33.3 ± 9.4 | 0.59 ± 0.03 | 1.7 ± 0.4 |
| | 4 | 41.8 ± 19.9 | 0.63 ± 0.12 | 1.7 ± 0.6 |
| | 6 | 51.1 ± 37.5 | 0.71 ± 0.28 | 1.9 ± 0.9 |
| Probenecid ($n$ = 9) | 2 | 34.7 ± 8.4 | 0.55 ± 0.10 | 1.7 ± 0.5 |
| | 4 | 42.5 ± 11.5 | 0.66 ± 0.20 | 1.7 ± 0.7 |
| | 6 | 46.6 ± 17.2 | 0.75 ± 0.30 | 1.9 ± 1.1 |
| Infected | | | | |
| Nonprobenecid ($n$ = 11) | 2 | 64.7 ± 21.7 | 1.2 ± 1.0 | 1.9 ± 1.3 |
| | 4 | 80.6 ± 25.5 | 2.8 ± 2.3 | 3.3 ± 2.4 |
| | 6 | 80.0 ± 38.7 | 3.0 ± 2.7 | 3.9 ± 2.6 |
| Probenecid ($n$ = 9) | 2 | 75.0 ± 14.8 | 1.4 ± 0.70 | 1.8 ± 0.8 |
| | 4 | 99.7 ± 28.6 | 2.7 ± 1.50 | 2.6 ± 1.2 |
| | 6 | 108.4 ± 43.2 | 4.8 ± 3.20 | 4.3 ± 2.1 |

[a] SD, Standard deviation.

[b] Percent penetration = CSF concentrations/serum concentration × 100%.

rabbits and rabbits with experimental pneumococcal meningitis were highly significant ($F = 30.9$; $P < 0.0001$), as were differences between mean values at the different sampling times ($F = 14.5$; $P < 0.0001$).

Vancomycin concentrations in CSF were also higher in rabbits with experimental pneumococcal meningitis but were unaffected by probenecid administration. In normal animals mean CSF concentrations ranged from 0.55 μg/ml at 2 h to 0.75 μg/ml at 6 h, whereas in infected animals they ranged from 1.2 μg/ml at 2 h to 4.8 μg/ml at 6 h. These differences between normal rabbits and rabbits with meningitis were highly significant ($F = 21.4$; $P < 0.0001$). Increases in CSF concentrations observed during therapy were also statistically significant ($F = 15.5$; $P < 0.0001$), but CSF concentrations in infected animals increased more during therapy than did those in normal rabbits ($F = 12.2$; $P < 0.0001$, for the interaction between infection status and sampling time).

The higher CSF concentrations in infected rabbits were not due to higher serum concentrations. Analysis of percent penetration values demonstrated that increases in CSF concentrations in animals with meningitis were disproportionately greater than the increases in serum concentrations. Mean percent penetration values ranged from 1.7% at 2 h to 1.9% at 6 h in normal rabbits, whereas in rabbits with experimental pneumococcal meningitis, mean percent penetration values ranged from 1.8% at 2 h to 4.3% at 6 h. These differences between infected and uninfected animals were highly significant ($F = 7.2$; $P < 0.01$). Mean percent penetration values increased significantly during therapy in all animals ($F = 14.7$; $P < 0.0001$), but a significantly greater increase was observed in rabbits with meningitis ($F = 11.9$; $P < 0.0001$, for the interaction between infection status and sampling time). The higher percent penetration values in infected animals indicated that in the presence of meningitis substantially more vancomycin crossed the blood-CSF barrier. Probenecid, however, had no effect on percent penetration values in either normal rabbits or rabbits with experimental pneumococcal meningitis. The results obtained in the probenecid groups paralleled those in the nonprobenecid groups in every respect.

Vancomycin demonstrated potent bactericidal activity in CSF of rabbits with experimental pneumococcal meningitis (Table 2). Probenecid did not affect its bactericidal activity in vivo. Before vancomycin therapy was started, the mean CSF titers of *S. pneumoniae* were 5.07 $\log_{10}$ CFU per ml in the nonprobenecid group and 4.50 $\log_{10}$ CFU per ml in the probenecid group. After 6 h of vancomycin therapy, the mean CSF titers had dropped to 1.92 $\log_{10}$ and 0.31 $\log_{10}$ CFU per ml, respectively. At the end of the 6-h treatment period, CSF cultures were sterile in five of the animals not receiving probenecid and in seven of the animals receiving probenecid.

TABLE 2. *Effect of vancomycin therapy on CSF bacterial titers in rabbits with experimental pneumococcal meningitis*

| Time (h) | Mean CSF titer ± SD[a] ($\log_{10}$ CFU per ml) in following group: | |
|---|---|---|
| | Nonprobenecid ($n = 11$) | Probenecid ($n = 9$) |
| Before therapy | 5.07 ± 1.14 | 4.50 ± 0.96 |
| 2 | 3.75 ± 1.61 | 2.84 ± 1.27 |
| 4 | 2.52 ± 2.53 | 1.58 ± 0.88 |
| 6 | 1.92 ± 2.21 | 0.31 ± 0.62 |

[a] SD, Standard deviation.

## DISCUSSION

Vancomycin does not readily cross the intact blood-CSF barrier in humans. Geraci and his colleagues detected no vancomycin activity in the CSF of 11 normal volunteers who had received 500-mg intravenous infusions (7). Likewise, Spears and Koch measured CSF vancomycin concentrations of <0.8 μg/ml in samples from seven patients with uninflamed meninges who were receiving intravenous vancomycin therapy (16). Kirby and Divelbiss detected no vancomycin activity in the CSF of one patient after a 2-g intravenous injection, even though the simultaneously measured serum concentration was 50 μg/ml (10). In an azotemic patient, however, they measured a CSF vancomycin concentration of 10 μg/ml and a concurrent serum concentration of 100 μg/ml after a 2-g dose. The penetration results obtained in normal rabbits with uninflamed meninges in this study paralleled those reported in those clinical studies. In normal rabbits, mean percent penetration values ranged from 1.7% at 2 h to 1.9% at 6 h. Even though serum concentrations were in the human therapeutic range, CSF concentrations of vancomycin in these normal rabbits were invariably subtherapeutic; only 5 of the 57 CSF samples contained concentrations higher than 1.0 μg/ml.

The penetration of vancomycin into CSF in patients with meningitis has not been examined. Several case reports have demonstrated that parenteral vancomycin therapy alone may be effective in the treatment of bacterial meningitis, but CSF concentrations were not measured in these patients. Ehrenkranz reported a case of staphylococcal meningitis that was cured with a 14-day course of intravenous vancomycin ther-

apy (5). Similarly, Hawley and Gump reported one case of staphylococcal meningitis and one case of *Flavobacterium* meningitis that were also treated with parenteral vancomycin therapy (8). Both patients defervesced after 1 day of therapy, and their follow-up CSF cultures on day 3 of treatment were sterile. The results of this study provide pharmacological and microbiological support for these clinical observations. The penetration of vancomycin into CSF increased significantly in rabbits with experimental pneumococcal meningitis. The average percent penetration values were 3.9 and 4.3% at 6 h in infected animals. Moreover, therapeutic concentrations of vancomycin in CSF were obtained, and potent bactericidal activity was demonstrated in these animals.

The percent penetration values for vancomycin in the present study were not as high as those observed in rabbits with experimental staphylococcal meningitis and reported in a previous study. In those animals, the percent penetration values ranged from 8.4% at 2 h to 11.7% at 8 h (18). The higher values observed in animals with staphylococcal disease may have reflected differences in microbial virulence. Previous studies have demonstrated that microorganisms vary in their ability to effect changes in the permeability of the blood-CSF barrier (11, 17). Nevertheless, the results in rabbits with experimental pneumococcal and staphylococcal meningitis indicate that the penetration of vancomycin into CSF is enhanced by both types of meningitis.

Various organic acids, cations, and quaternary ammonium compounds are removed from CSF by an active transport mechanism located in the choroid plexus (6). Under normal conditions, this mechanism transports penicillin and cephalosporin derivatives from CSF (2–4, 6, 17). In the presence of meningitis, however, this mechanism breaks down, thus allowing CSF concentrations of these antimicrobial agents to increase (2, 17). Probenecid also inhibits the function of this transport mechanism and augments CSF concentrations of various penicillin and cephalosporin antibiotics (2–4, 6, 17). In this study, meningitis augmented CSF concentrations of vancomycin, but probenecid administration had no effect. If vancomycin is actively transported from CSF to blood, these data on probenecid-treated animals indicate that the mechanism differs from that affecting penicillin and cephalosporin derivatives.

The dosages of vancomycin used in this study were selected to produce serum concentrations within the human therapeutic range. In clinical studies, the intravenous injection of 1 g every 12 h produces peak serum concentrations of vancomycin ranging from 25 to 40 μg/ml (7, 10). In patients with endocarditis who are receiving vancomycin therapy, peak serum concentrations have ranged up to 50 μg/ml (9). With the vancomycin dosages used in this study, mean serum concentrations of vancomycin ranged from 33.3 μg/ml at 2 h to 51.1 μg/ml at 6 h; these values correspond well to those observed in human patients. Surprisingly, serum concentrations in rabbits with experimental pneumococcal meningitis were significantly higher than those observed in uninfected animals. The cause of these higher serum concentrations was not investigated, but impaired renal excretion of vancomycin in infected animals is a likely explanation. The hypotension induced by the experimental infection would be expected to diminish renal clearance of vancomycin and to augment serum concentrations (15).

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