Effect of Dexamethasone or HWA-138 in Combination with Antibiotics in Experimental \textit{Haemophilus influenzae} Type b Infection

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Modulation of the host's inflammatory response in bacterial meningitis may be beneficial. In this study, the effects of dexamethasone and HWA-138, an analog of pentoxifylline, on CSF cultures and cochlear inflammation in an infant rat model of \textit{Haemophilus influenzae} type b were studied. Five-day-old infant rats were inoculated once intraperitoneally with 1 × 10^4 to 10 × 10^4 CFU of \textit{H. influenzae} type b (strain 1406). Twenty-four hours later, infant rats were treated intraperitoneally with one dose of ampicillin (0.1 mg/g of body weight), cefotaxime (0.05 mg/g), or cefuroxime (0.05 mg/g) alone or in combination with one dose of dexamethasone (0.00015 mg/g) or HWA-138 (0.005 mg/g). Twenty-four hours after treatment with cefuroxime plus dexamethasone, animals had a significantly (P < 0.04) greater incidence of bacteremia and meningitis (eight of nine animals) than that in animals of the other treatment groups. Overall, dexamethasone was associated with less inflammation (P < 0.04) of the cochlear nerve compared with that from antibiotic treatment alone. In this model, when suboptimal antimicrobial therapy is administered, anti-inflammatory agents may be beneficial with respect to reducing cochlear inflammation. However, dexamethasone and cefuroxime lead to a higher rate of positive blood and cerebral spinal fluid cultures than cefuroxime alone.

Modulation of the host's inflammatory response during bacterial meningitis may be beneficial since the pathogenesis of the neurologic sequelae relates, in part, to this reaction (14). In experimental animals, dexamethasone and other anti-inflammatory agents administered adjunctively with antibiotics may result in diminished brain water content (cerebral edema), intracranial pressure, and lactate concentrations in cerebral spinal fluid (CSF), among other effects (19–22). Simultaneous administration of dexamethasone and ceftriaxone leads to decreased activity of tumor necrosis factor (TNF) and indices of meningeal inflammation in the CSF of rabbits with experimental \textit{Haemophilus influenzae} type b meningitis compared with that in the CSF of animals treated with ceftriaxone alone (12). In a randomized prospective study of dexamethasone versus placebo adjunctive therapy along with cefuroxime in childhood meningitis, a significant reduction in bilateral moderate or more severe hearing loss was demonstrated in children with \textit{H. influenzae} type b meningitis who received dexamethasone (5). A similar but nonsignificant reduction was observed when ceftriaxone was used with dexamethasone in a second study. In those clinical studies, cytokine concentrations in CSF were also reduced in the dexamethasone-treated children (11). Presumably, a reduction in inflammation within the cochlea or surrounding the eighth cranial nerve results in less likelihood of deafness in the children to whom dexamethasone is administered.

Pentoxifylline is a xanthine derivative which inhibits phosphodiesterases and is used widely to improve microcirculation in patients with chronic occlusive arterial disease. Several investigators have described the anti-inflammatory properties of pentoxifylline, such as modulation of neutrophil adherence to endothelial cells as well as a decrease in superoxide production by neutrophils (9, 18). Pentoxifylline also inhibits TNF activation of neutrophils, in addition to decreasing TNF production by macrophages stimulated by endotoxin (17). In experimental models of gram-negative infection or endotoxemia, pretreatment or early treatment with pentoxifylline increases survival and decreases lung injury (6, 16, 23). In the rabbit model of \textit{H. influenzae} type b meningitis, pentoxifylline decreased protein and lactate concentrations in CSF when it was administered 30 min prior to ceftriaxone infusion (13).

In a previous study (4), we were unable to demonstrate that dexamethasone added to antibiotic therapy resulted in any less inflammation within the cochlea than did antibiotic therapy alone. In that study, two doses of the antibiotics were administered prior to sacrifice of the animals. In the present study, we modified our treatment protocol so that only one dose of antibiotic was administered prior to sacrifice to determine whether there is any difference in inner ear pathology when dexamethasone or HWA-138, a derivative of pentoxifylline with properties similar to those of the parent compound, is added to antibiotic therapy.

**MATERIALS AND METHODS**

**Animals.** Each experiment was performed with outbred pathogen-free 5-day-old Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Ind.) from two litters randomly distributed in equal numbers between each of two mothers. The animals were housed and fed under standard conditions.

**Bacteria.** \textit{H. influenzae} type b 1406, which was isolated from the CSF of a child with deafness following meningitis at Texas Children's Hospital, was frozen at −70°C in Trypticase soy broth with 10% glycerol (pH 7.3) after a single passage from the original clinical culture. This isolate has been used in our previous studies of labyrinthitis in infant...
rats (4, 24). The MICs and MBCs of the antibiotics used in this study for this isolate were as follows: ampicillin, <0.125 and <0.125 μg/ml; cefotaxime, <0.125 and <0.125 μg/ml; and cefuroxime, 0.5 and 0.5 μg/ml, respectively. For each experiment, *H. influenzae* type b was grown overnight in brain heart infusion broth supplemented with hemin and NAD (10 μg/ml each) at 35°C. One milliliter of the overnight culture was inoculated into 10 ml of supplemented brain heart infusion broth and incubated for 3 h at 35°C on a rotating shaker. Centrifugation at 10,000 × g for 10 min was used to obtain the pellets. The pellets were washed twice in phosphate-buffered saline (PBS; pH 7.4) containing 0.1% gelatin (PBS-G) and resuspended in PBS-G, adjusting the optical density at 540 nm to obtain 1 × 10^5 to 10 × 10^5 CFU/ml, which was confirmed by colony count.

**Animal inoculation.** Five-day-old infant rats were inoculated once intraperitoneally (i.p.) with 1 × 10^5 to 10 × 10^5 CFU of *H. influenzae* type b. At 48 h, the infant rats were sacrificed with pentobarbital given i.p. In previous experiments, cochoral inflammation was found to be maximal at 48 h after inoculation (24). CSF was collected by cisterna magna puncture, and blood was collected by cardiac puncture. CSF cell count was determined with a Spencer hemacytometer; CSF and blood were cultured on chocolate agar without the addition of cephalosporinase.

In order to determine antibiotic concentrations in CSF and serum, 5-day-old healthy infant rats were inoculated i.p. with 10^5 CFU of *H. influenzae* type b. Two days later, rats were treated with single i.p. doses of the following: (i) ampicillin at 0.1 mg/g of body weight (100 mg/kg); (ii) ampicillin at 0.1 mg/g plus dexamethasone at 0.00015 mg/g (0.15 mg/kg); (iii) ampicillin at 0.1 mg/g plus HWA-138 at 0.0005 mg/g (5 mg/kg); (iv) cefotaxime at 0.05 mg/g (50 mg/kg); (v) cefotaxime at 0.05 mg/g plus dexamethasone at 0.00015 mg/g; (vi) cefotaxime at 0.05 mg/g plus HWA-138 at 0.0005 mg/g; (vii) cefuroxime at 0.05 mg/g (50 mg/kg); (viii) cefuroxime at 0.05 mg/g plus dexamethasone at 0.00015 mg/g; and (ix) cefuroxime at 0.05 mg/g plus HWA-138 at 0.0005 mg/g.

One hour after the administration of the medications listed above, animals were sacrificed and CSF and sera were obtained.

**Bioassay for antibiotic concentrations.** Ampicillin, cefotaxime, and cefuroxime were measured in sera and CSF by a modification of the bioassay method of Bennett et al. (2). In antibiotic medium no. 1 (Difco Laboratories, Detroit, Mich.), ampicillin and cefuroxime were measured by using *Bacillus subtilis* (Subtilis Spore Suspension; Difco). Cefotaxime was measured by using *Escherichia coli* (MIC of cefotaxime, <0.1 μg/ml).
TABLE 3. Positive CSF cultures 24 h after treatment in infant rats inoculated with *H. influenzae* type b

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. of positive CSF cultures/total no. tested after treatment with antibiotic</th>
<th>Alone</th>
<th>+Dexamethasone</th>
<th>+HWA-138</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>1/7, 1/9</td>
<td>1/7</td>
<td>1/9</td>
<td>0/8</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0/7, 0/7</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>2/8, 8/9*</td>
<td>2/8</td>
<td>8/9*</td>
<td>3/8</td>
</tr>
</tbody>
</table>
* Only one dose of antibiotic with or without the anti-inflammatory agent was administered.

The control group had eight of eight positive CSF cultures 48 h after inoculation with *H. influenzae* type b.

P < 0.04 compared with the other groups.

Treatment experiments. Five-day-old infant rats were inoculated with *H. influenzae* type b 1406. At 24 h, the infant rats were treated with one dose of ampicillin (0.1 mg/g), cefotaxime (0.05 mg/g), or cefuroxime (0.05 mg/g) alone or in combination with one dose of dexamethasone (0.00015 mg/g) or HWA-138 (0.005 mg/g). The dose of HWA-138 was determined based on the results of experiments conducted by Luke et al. (7) in adult rats infected with *Candida albicans*. All medications were given i.p. Each of the treatment options was administered to at least one infant rat in each litter.

Histologic examination. For light microscopy, one half of each rat head was placed in 10% buffered formalin. As described previously (24), hematoxylin- and eosin-stained tissues were scored for any degree of inflammation of the inner ear by one investigator (E.P.H.), who was unaware of which treatment regimen was administered. The second half of each head was frozen at −70°C immediately after the rats were killed and was subsequently stained with a fluorescein-labeled rabbit antiserum to whole *H. influenzae* type b. Sections were counterstained with 0.1% Evans blue dye and examined with a fluorescence microscope. Only three heads per group were selected for histologic examination.

Analysis. Differences among the groups for inflammation and bacterial detection in the cochlea, blood, and CSF cultures were determined by Fisher's exact test. The leukocyte counts in CSF were compared by using the analysis of variance on logarithmically transformed data.

RESULTS

Antibiotic concentrations or penetrations were not diminished in CSF when dexamethasone or HWA-138 was added to the treatment in infected animals (Table 1).

All untreated animals (control) had positive blood and CSF cultures at 48 h. The cefuroxime and dexamethasone group had significantly (P ≤ 0.04) more positive blood and CSF cultures 24 h after treatment than any other group (antibiotic alone or antibiotic plus dexamethasone or HWA-138) (Tables 2 and 3). No significant differences were noted in the leukocyte count in CSF among the groups treated with antibiotic alone or antibiotic plus dexamethasone or HWA-138 (Table 4).

Histologic analysis of the inner ear. The groups treated with antibiotic and dexamethasone showed a significant decrease in the incidence of inflammation at the nerve level when compared with the incidence in the groups treated with antibiotic alone (P ≤ 0.04), but it was no different than that in the groups treated with antibiotic plus HWA-138. There was a tendency for the antibiotic plus dexamethasone therapy to induce less of an inflammatory response of the cochlea at all levels. The presence of bacteria was similar whether or not the animals received dexamethasone or HWA-138 (Table 5).

Analysis of the cochlear inflammation according to the specific antibiotic received with or without dexamethasone or HWA-138 showed no significant differences among the different groups. No cochlear inflammation was detected in two of three animals with positive CSF cultures treated with cefuroxime plus dexamethasone. None of the animals studied histologically had evidence of inflammatory cells or bacteria in the scala media and organ of Corti.

DISCUSSION

The experimental and clinical studies performed by McCracken and colleagues (5, 11–13) as well as others have greatly enhanced our understanding of the pathogenesis of the inflammatory reaction during bacterial meningitis. As a result, investigations have focused on modulating this response which hopefully would result in less neurologic damage as a result of meningitis. In several studies, dexamethasone was shown to be beneficial in decreasing the parameters of meningeval inflammation in experimental models of bacterial meningitis. In the one major clinical trial conducted in the United States which has been published (5), dexamethasone administration was associated with a significantly decreased incidence of hearing loss in children with *H. influenzae* type b meningitis treated with cefuroxime. It should be noted that for one child treated with dexamethasone and cefuroxime, a culture of CSF obtained at the end of a 10-day course of therapy was positive for *H. influenzae* type b. In addition, delayed sterilization of CSF appears to occur more frequently in children treated with cefuroxime compared with those treated with ceftriaxone or ampicillin and chloramphenicol (1, 10, 15).

Nonsteroidal anti-inflammatory agents have also been proven useful in animal studies of bacterial meningitis. Pentoxifylline is a drug which has been used for many years in the treatment of occlusive arterial disease, but more recently, it has been evaluated as an anti-inflammatory agent. Two studies have examined the effect of pentoxifylline in experimental meningitis. Saez-Llorens et al. (13) inoculated rabbits intracisternally with *H. influenzae* type b. Six hours later animals were treated intravenously with saline, dexamethasone (1 mg/kg), pentoxifylline (20 mg/kg dose followed by a continuous infusion of 6 mg/kg/h), or a combination of pentoxifylline and dexamethasone. All animals received ceftriaxone as well. Compared with the animals that received saline, pentoxifylline-treated rabbits had a significant decrease in protein and lactate concentrations in CSF. Lower but nonsignificant leukocyte counts and con-
centrations of TNF in CSF were observed in the group treated with pentoxifylline as well. In adult rats, pentoxifylline administered at a 20-μg/kg dose i.p. 1 h after intracis-
ternal inoculation of *H. influenzae* type b lipooligosaccharide resulted in a significant reduction in blood-brain barrier permeability compared with that in controls (3). Pentoxifylline and HWA-138 both protected neonatal mice from experi-
mental *Staphylococcus aureus* infection, although high doses of either were detrimental to the animals (8).

Pentoxifylline has been studied in adult volunteers infused intravenously with 100 mg of endotoxin prepared from *Salmonella abortus equi* on two occasions (25). Saline or pentoxifylline (500 mg) was started 30 min prior to endotoxin administration, and the infusion was continued for 4 h. Pentoxifylline blunted the rise of TNF concentrations in serum, which peaked at 2 h in the saline treatment experi-
ments. Interleukin-6 concentrations were not affected. These findings are consistent with those of in vitro experiments that indicated that pentoxifylline blocks TNF production in macrophage cultures.

We examined the effects of dexamethasone and HWA-
138, an analog of pentoxifylline with similar activity but with a more prolonged half-life in neonatal mice compared with the half-life of the parent compound, in an infant rat model of *H. influenzae* type b meningitis that focused on inner ear inflam-
matation. In this model, when two doses of antibiotics were administered 24 h after bacterial inoculation and on the day prior to sacrifice, we were unable to demonstrate less inflam-
mation of the cochlea in animals treated with dexam-
ethasone plus antibiotics compared with that in animals receiving antibiotics alone (4). This was due to the fact that two doses of antibiotics virtually eliminated cochlear inflam-
mation in treated animals compared with that in untreated animals 48 h into the infection. In the experiments described here, only one dose of an antibiotic was administered, so that some difference as a result of administration of an anti-inflammatory agent may have been detected more read-
ily.

The antibiotic concentrations achieved in the serum of infected infant rats were roughly equivalent to those ob-
served in children following intravenous infusion. However, we found that for infant rats treated with cefuroxime and dexamethasone, significantly more blood and CSF cultures were positive for *H. influenzae* type b than was found for animals treated with ampicillin or cefotaxime plus dexameth-
asone. Animals receiving cefuroxime alone or with HWA-
138 also were more likely to remain bacteremic or have positive CSF cultures 24 h after treatment than ampicillin-or cefotaxime-treated animals were, but these differences were not significant. Three animals per treatment group underwent histologic examination of the inner ear. In aggregate, there was less cochlear inflammation detected in the animals receiving the combinations of antibiotics plus an anti-inflam-
atory agent compared with that detected in animals receiv-
ing antibiotic treatment alone; dexamethasone and HWA-
138 were equivalent in this regard.

The findings in this study are consistent with the clinical observation that delayed sterilization of CSF in *H. influn-
zae* type b meningitis is more common with cefuroxime than it is with several other antibiotics. The addition of dexamethasone to cefuroxime therapy resulted in an even more striking increase in blood and CSF cultures which remained positive, a finding again consistent with previous clinical observations. Concentrations of antibiotics in CSF were not diminished by either dexamethasone or HWA-138. When only one dose of an antibiotic was administered, both dexamethasone and HWA-138 adjunctive therapy were associated with decreased cochlear inflammation. Thus, when suboptimal antibiotic therapy is administered in this model, anti-inflammatory agents prove to be somewhat beneficial with respect to reducing inner ear inflammation. This may explain the beneficial effect of dexamethasone when it is administered to children who are treated with cefuroxime for *H. influenzae* type b meningitis (5).

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REFERENCES


