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Rifampicin + ceftriaxone versus vancomycin + ceftriaxone in the treatment of penicillin- and cephalosporin-resistant pneumococcal meningitis in an experimental rabbit model

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Abstract

This study was planned to compare the efficacy of ceftriaxone + vancomycin with ceftriaxone + rifampicin in a rabbit model of penicillin and cephalosporin-resistant *Streptococcus pneumoniae* meningitis. Meningitis was induced by intracisternal inoculation of *S. pneumoniae*. After 18 h of incubation, Group 1 was given saline solution (control group), whilst Groups 2 and 3 were given ceftriaxone + vancomycin and ceftriaxone + rifampicin, respectively. Cerebrospinal fluid bacterial concentrations were measured at 0, 2, 12, 14 and 24 h after therapy was initiated. In the control group, bacterial growth was present at all time points, whereas no growth was observed in either the ceftriaxone + vancomycin group or the ceftriaxone + rifampicin group after 2 h of therapy. Ceftriaxone + rifampicin was found to be as effective as ceftriaxone + vancomycin in the treatment of penicillin-resistant *S. pneumoniae* meningitis in experimental rabbit model.

Keywords: Experimental meningitis; Penicillin-resistant *Streptococcus pneumoniae*; Pneumococcal meningitis; Vancomycin; Rifampicin

1. Introduction

Streptococcus pneumoniae is one of the most common causes of pyogenic meningitis. *Streptococcus pneumoniae* strains resistant to penicillin and extended-spectrum cephalosporins have led to treatment failures in patients with meningitis [1–3]. High-level penicillin resistance rates among *S. pneumoniae* in Turkey range between 1.3% and 17% [4,5]. For treatment to be successful, cerebrospinal fluid (CSF) drug concentrations need to be 10–100-fold higher than the minimum inhibitory concentration (MIC). Penicillin and extended-spectrum cephalosporins do not routinely achieve adequate CSF levels to treat meningitis caused by intermediately susceptible strains reliably. Optimal therapy for meningitis caused by *S. pneumoniae* resistant to penicillin and extended-spectrum cephalosporins is still not established. The aim of this study was to compare the efficacy of ceftriaxone + vancomycin with ceftriaxone + rifampicin in a rabbit model of meningitis caused by a high-level penicillin and cephalosporin-resistant strain of *S. pneumoniae*.

2. Materials and methods

2.1. Bacterial strain

A high-level penicillin-resistant (MIC, 2 μg/mL) and cephalosporin-resistant (ceftriaxone MIC, 1.0 μg/mL) strain of *S. pneumoniae* was used as the infecting bacteria. This strain was isolated from an adult patient with pneumococcal meningitis. It was grown overnight on blood agar (Oxoid, Basingstoke, UK) and the plate was then flooded with phosphate-buffered saline and aliquots were frozen

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at −70 °C. Aliquots were thawed on the experiment day and a bacterial solution was prepared using these bacteria in 0.9% NaCl (Eczacibasi, Istanbul, Turkey) by adjusting to 1.0 McFarland standard. This solution was further diluted 1/300 to gain a concentration of 10^6 colony-forming units (CFU)/mL [6]. Then, 0.5 mL of this bacterial solution was injected intracisternally into each rabbit. The bacterial count of the solutions used during the experiment contained $10^6 \pm 10^5$ CFU/mL. The MICs of antibiotics were determined by broth microdilution with Mueller–Hinton broth supplemented with 3% lysed horse blood [6].

2.2. Rabbit meningitis model

Each rabbit was anaesthetised intramuscularly with ketamine (35 mg/kg) and xylazine (5 mg/kg) during the induction of meningitis and during each sampling of CSF. Animals were not anaesthetised between the procedures and they were kept in their cages. Fifteen minutes after anaesthesia, a spinal needle (Hayat Ticaret, İstanbul, Turkey) was introduced into the cerebellomedullary cistern of each rabbit to collect 300 μL of CSF. Equal amounts of suspension containing 10^6 CFU/mL *S. pneumoniae* and 0.9% NaCl were then injected. Eighteen hours after the inoculation, meningitis criteria were investigated and antimicrobial agents were administered. A CSF white cell count of more than 1000/mm^3 and a bacterial count >10^2 CFU/mL were accepted as the indications of meningitis. Rabbits in Group 1 were given only sterile saline solution (control group), whilst Groups 2 and 3 received ceftriaxone + vancomycin and ceftriaxone + rifampicin, respectively. Drugs used were pharmaceutical products: vancomycin (Lilly, Indianapolis, IN, USA), ceftriaxone (Roche, Neuilly-sur-Seine, France) and rifampicin (Aventis-Pharma, West Malling, UK). Ceftriaxone and vancomycin were administered via the marginal vein of the rabbit's ear. Rifampicin was given via nasogastric tube. Meanwhile, a second dose of vancomycin and rifampicin was given 12 h later. The doses of the antimicrobial agents were: ceftriaxone 125 mg/kg, vancomycin 20 mg/kg and rifampicin 15 mg/kg.

Euthanasia was performed on untreated and treated animals 42 h after bacterial inoculation. The study was approved by the local ethical committee on animal studies.

2.3. Measurement of bacterial concentrations

Bacterial concentrations in CSF were measured at 0, 2, 12, 14 and 24 h after therapy was initiated by plating undiluted and serial 10-fold dilutions of CSF (100 μL) on sheep blood agar and incubating in 5% CO_2 at 37 °C for 24 h.

2.4. Statistical analysis

Mann–Whitney test was used for comparison of results from the groups. A *P*-value less than 0.05 was considered significant.

3. Results

Of 36 rabbits, only 20 (6 rabbits in the placebo group, 8 rabbits in the ceftriaxone + vancomycin group and 6 rabbits in the ceftriaxone + rifampicin group, all with fever, tachycardia and opisthotonus) were evaluated. Sixteen animals were excluded since their CSF leukocyte count ranged between 20/mm^3 and 100/mm^3. Subsequent CSF culture of these 16 rabbits did not grow bacteria. CSF white cell counts were similar in the ceftriaxone + vancomycin group and the ceftriaxone + rifampicin group. Mean bacterial concentrations of the three groups as $\log_{10}$ CFU/mL at 0 h were similar (Group $1 = 5.48 \pm 0.60$; Group $2 = 4.52 \pm 1.04$; Group $3 = 4.55 \pm 1.18$; $P > 0.05$). Bacterial concentrations in the placebo group at 2, 12, 14 and 24 h were 5.2 ± 0.66, 4.2 ± 1.25, 5.07 ± 0.83 and 4.71 ± 0.77 $\log_{10}$ CFU/mL, respectively. After 2 h of therapy there was no bacterial growth in Groups 2 and 3. The difference between the control group and treatment groups was significant ($P < 0.05$) at all time points, whereas the decrease in treatment groups was similar ($P > 0.05$).

4. Discussion

The combination of ceftriaxone + rifampicin was as effective as the combination of ceftriaxone + vancomycin in this study. No growth was detected in either the ceftriaxone + vancomycin group or the ceftriaxone + rifampicin group in the CSF of rabbits after 2 h of therapy.

The combination of ceftriaxone + vancomycin had been found to be synergistic in a rabbit model [7]. Klugman et al. [8] stated that treatment of cephalosporin-resistant pneumococcal meningitis could be established by addition of rifampicin or vancomycin to ceftriaxone.

To our knowledge, there are two reported studies comparing ceftriaxone + vancomycin and ceftriaxone + rifampicin. Friedland et al. [1] found a ceftriaxone + vancomycin combination to be as effective as ceftriaxone + rifampicin in experimental rabbit meningitis induced by a *S. pneumoniae* strain that was resistant to penicillin and extended-spectrum cephalosporins. Lee at al. [9] recently compared vancomycin, rifampicin and ceftriaxone alone, in double and triple combinations, and in the presence and absence of dexamethasone in the same model. Rifampicin was as effective as the combination regimens. Moreover, regardless of dexamethasone, therapeutic efficacy of ceftriaxone + vancomycin + rifampicin and ceftriaxone + rifampicin was superior to ceftriaxone + vancomycin. Our findings are in concordance with Friedland et al., but not Lee et al. The difference may be due to the MIC of the study strains.

Excessive vancomycin usage is associated with vancomycin resistance in enterococci [10]. In addition, it is

well known that co-administration of dexamethasone with vancomycin significantly reduces the penetration of vancomycin into the CSF [11]. At present, co-administration of dexamethasone at the beginning of therapy for acute bacterial meningitis has been accepted as a standard of care to improve the outcome in adults as in children [12].

In 16 animals, meningitis criteria were absent after 18 h incubation time. This may be attributed partly to the bacterial solution. The −70 °C storage period may have affected the growth phase of the bacteria and their virulence [13], but all other study animals had >1000 leukocytes/mm^3 and positive CSF cultures. Inadequate surgical intervention may have also contributed to the lack of meningitis induction. In a fully developed form of meningitis in rabbits, CSF leukocyte counts are typically in the range 1000–10000 leukocytes/mm^3 [13], therefore >1000 leukocytes/mm^3 was used for meningitis criterion. In addition, this cut-off value was thought to help exclude the effect of the procedure itself on CSF.

There are many studies in the literature on the relationship of pro-inflammatory cytokine release and antimicrobials. Nau et al. [14] found that antibiotics which inhibit bacterial protein synthesis, such as rifampicin, release smaller quantities of pro-inflammatory bacterial compounds than cell wall active drugs. Bottcher et al. [15] demonstrated that β-lactam antibiotics release much more reactive oxygen species from *S. pneumoniae* than rifampicin in meningitis. Gerber et al. [16] stated that initiation of therapy with a protein synthesis-inhibiting antibacterial and continuation of therapy with a combination that includes a β-lactam may be a strategy to decrease neuronal injury in bacterial meningitis. All of these studies indicate that pro-inflammatory cytokine concentration in the CSF is an important factor determining the prognosis of meningitis. In the present study, bacterial concentration in CSF of the ceftriaxone + vancomycin group was similar to the ceftriaxone + rifampicin group.

It is concluded that rifampicin may be an appropriate alternative to vancomycin, especially when dexamethasone therapy is indicated and the pro-inflammatory cytokine levels are considered to be one of the deteriorating factors in meningitis with *S. pneumoniae* resistant to penicillin and extended-spectrum cephalosporins.

References

[1] Friedland IR, Paris M, Ehrett S, Hickey S, Olsen K, McCracken Jr GH. Evaluation of antimicrobial regimens for treatment of experimental penicillin- and cephalosporin-resistant pneumococcal meningitis. Antimicrob Agents Chemother 1993;37:1630–6.
[2] Paris MM, Hickey SM, Uscher MI, Shelton S, Olsen KD, McCracken Jr GH. Effect of dexamethasone on therapy of experimental penicillin- and cephalosporin-resistant pneumococcal meningitis. Antimicrob Agents Chemother 1994;38:1320–4.
[3] Tan TQ. Antibiotic resistant infections due to *Streptococcus pneumoniae*: impact on therapeutic options and clinical outcome. Curr Opin Infect Dis 2003;16:271–7.
[4] Gur D, Tunckanat F, Sener B, Kanra G, Akalin HE. Penicillin resistance in *Streptococcus pneumoniae* in Turkey. Eur J Clin Microbiol Infect Dis 1994;13:440–1.
[5] Cavusoglu C, Ozinel MA. Pnömokoklarda antibiyotik direnci ve dirençli pnömokokların sağaltımı. Turk J Infect 1997;11:107–17.
[6] Bilgehan H, editor. Klinik Mikrobiyolojik Tanı. İzmir, Turkey: Fakulteler Kitapevi; 2002.
[7] Cabellos C, Fernandez A, Maiques JM, et al. Experimental study of LY333328 (oritavancin), alone and in combination, in therapy of cephalosporin-resistant pneumococcal meningitis. Antimicrob Agents Chemother 2003;47:1907–11.
[8] Klugman KP, Friedland IR, Bradley JS. Bactericidal activity against cephalosporin-resistant *Streptococcus pneumoniae* in cerebrospinal fluid of children with acute bacterial meningitis. Antimicrob Agents Chemother 1995;39:1988–92.
[9] Lee H, Song JH, Kim WS, et al. Evaluation of a triple drug combination for treatment of experimental multidrug resistant pneumococcal meningitis. Int J Antimicrob Agents 2004;23:307–10.
[10] Gultekin M, Gunseren F. Vankomisin dirençli enterokoklar. Turk J Hosp Infect 2000;4:195–204.
[11] Andes DR, Craig WA. Pharmacokinetics and pharmacodynamics of antibiotics in meningitis. Infect Dis Clin North Am 1999;13:595–618.
[12] de Gans J, van de Beek D; European Dexamethasone in Adulthood Bacterial Meningitis Study Investigators Dexamethasone in adults with bacterial meningitis. N Engl J Med 2002;374:1549–1556.
[13] Koedel U, Pfister HW. Models of experimental bacterial meningitis. Infect Dis Clin North Am 1999;13:549–77.
[14] Nau R, Wellmer A, Soto A, et al. Rifampin reduces early mortality in experimental *Streptococcus pneumoniae* meningitis. J Infect Dis 1999;179:1557–60.
[15] Bottcher T, Gerber J, Wellmer A, et al. Rifampin reduces production of reactive oxygen species of cerebrospinal fluid phagocytes and hippocampal neuronal apoptosis in experimental *Streptococcus pneumoniae* meningitis. J Infect Dis 2000;181:2095–8.
[16] Gerber J, Pohl K, Sander V, Bunkowski S, Nau R. Rifampin followed by ceftriaxone for experimental meningitis decreases lipoteichoic acid concentrations in cerebrospinal fluid and reduces neuronal damage in comparison to ceftriaxone alone. Antimicrob Agents Chemother 2003;47:1313–7.