Journal of Microbiology, Immunology and Infection (2017) 50, 435—439

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ORIGINAL ARTICLE

Experimental study of cerebrospinal fluid tumor necrosis factor-alpha release in penicillin- and cephalosporin-resistant pneumococcal meningitis treated with different antibiotic schedules

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Received 20 March 2015; received in revised form 25 June 2015; accepted 27 July 2015
Available online 9 September 2015

KEYWORDS
ceftriaxone;
cytokines;
daptomycin;
dexamethasone;
Streptococcus pneumoniae

Abstract *Background/Purpose:* To measure the inflammatory response in terms of tumor necrosis factor-alpha (TNF-α) levels in cerebrospinal fluid (CSF), using bacteriolytic versus non-bacteriolytic antibiotic therapy and adjunctive treatment with dexamethasone in an experimental rabbit model of pneumococcal meningitis.
Methods: In a rabbit model of pneumococcal meningitis, we tested CSF TNF-α levels in several samples from rabbits infected with the HUB 2349 strain and treated with ceftriaxone 100 mg/kg/d, ceftriaxone plus vancomycin 30 mg/kg/d, or daptomycin at 15 mg/kg or 25 mg/kg. Daptomycin schedules were compared with the same doses in combination with dexamethasone at 0.125 mg/kg every 12 hours over a 26-hour period.
Results: The ceftriaxone group had the highest levels of TNF-α. TNF-α levels were significantly higher after ceftriaxone administration than in both daptomycin groups. The high-dose daptomycin group presented the lowest inflammatory levels in CSF samples. Adjunctive treatment with dexamethasone in this group modulated the inflammatory response, bringing down CSF TNF-α levels.
Conclusion: CSF TNF-α levels were significantly lower in rabbits treated with daptomycin than in rabbits treated with ceftriaxone. Daptomycin avoided the inflammatory peak after

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http://dx.doi.org/10.1016/j.jmii.2015.07.014

administration observed in ceftriaxone-treated rabbits. The use of daptomycin plus dexamethasone achieved a significantly larger reduction in CSF TNF-α levels.

Introduction

Inflammation has been related to neuronal damage and sequelae in patients with bacterial meningitis. Inflammation generated during antibiotic therapy may be dependent on the antibiotic's mechanism of action. Finding a therapy without a significant host inflammatory cascade would be particularly useful.

Certain bactericidal antibiotics that inhibit the synthesis of the cell wall, such as β-lactams, cause bacterial lysis and the release of proinflammatory bacterial products, leading to an increased inflammatory response.[1]

Tumor necrosis factor alpha (TNF-α) is an important early proinflammatory cytokine associated with inflammation-related complications of bacterial meningitis; patients with bacterial meningitis present increased cerebrospinal fluid (CSF) TNF-α levels early in the course of the disease.[2] An early study by Saukkonen et al[3] determined the role of cytokines in the CNS, including TNF-α, using a rabbit model of meningeal inflammation. The results suggested that cytokines have multiple inflammatory activities in the CNS and contribute to tissue damage.[3]

Several reports in an infant rat model by Barichello et al[4,5] evaluated the levels of TNF-α in the hippocampus and prefrontal cortex, showing that the cytokine was produced mainly in the first 6—24 hours of the immune response. TNF-α played an important role in the pathophysiology and might be related to brain damage in the first hours of pneumococcal meningitis.[4,5]

Daptomycin is a nonbacteriolytic antibiotic with elevated bactericidal activity against a wide range of gram-positive pathogens and may have minimal effects on cytokine production.[6]

In experimental models of meningitis comparing its effectiveness with that of ceftriaxone therapy, daptomycin has already demonstrated a highly bactericidal effect and its ability to attenuate inflammation in the CSF without causing cortical damage.[7,8]

The aim of this study was to measure the inflammatory response in terms of the release of TNF-α in the CSF, comparing bacteriolytic and nonbacteriolytic antibiotic therapy and adjunctive treatment with dexamethasone in an experimental rabbit model of meningitis.

Methods

Bacterial strain

A HUB 2349 penicillin- and cephalosporin-resistant strain of *Streptococcus pneumoniae* belonging to serotype 23F and isolated from a patient with meningitis was used. Minimum inhibitory concentration (MIC) and minimum biocidal concentration (MBC; mg/L) were as follows: penicillin 4/4; ceftriaxone 2/4; vancomycin 0.25/0.25; and daptomycin 0.09/0.18.

Meningitis model

The experimental protocol was in keeping with Spanish legislation on animal experimentation and was approved by the Ethics Committee for Animal Experiments at the University of Barcelona, Barcelona, Spain. The rabbit model of meningitis described originally by Dacey and Sande[9] was used, with slight modifications. Young female New Zealand white rabbits were anesthetized intramuscularly with 35 mg/kg of ketamine (Ketolar; Parke-Davs, Prat de Ll., Spain) and 5 mg/kg of xylazine (Rompum; Bayer AG, Leverkusen, Germany). Meningitis was induced using an intracisternal injection of 250 μL of a saline suspension containing 106 colony forming units/mL of inoculum, and therapy was started 18 hours postinoculation. Rabbits were anesthetized using urethane (Sigma Chemical Company, St Louis, MO, USA) at 1.75 g/kg subcutaneously and thiopental sodium (Tiopental; B. Braun Medical S.A., Rubí, Spain) at 5 mg/kg intravenously (iv). Animals were placed in the stereotactic frame and a baseline CSF sample was taken (0 hours). A dose of dexamethasone (Fortecortin; Merck, Mollet del Vallés, Barcelona, Spain) of 0.25 mg/24 h divided every 12 hours was given intravenously. Ten minutes later, antibiotic therapy was administered. CSF samples were taken after 2 hours, 6 hours, 24 hours, and 26 hours of therapy. Hydration was ensured throughout the experiment. Mortality was assessed at 26 hours. Surviving animals were euthanized using a lethal dose of thiopental sodium at the end of each experiment.

Therapeutic groups

Antibiotic iv therapy was then administered for 26 hours using one of the following therapy schedules: ceftriaxone ($n = 8$ rabbits) at 100 mg/kg once daily, vancomycin at 15 mg/kg every 12 hours plus ceftriaxone ($n = 9$ rabbits), daptomycin ($n = 8$ rabbits) at 15 mg/kg given once daily, daptomycin ($n = 9$ rabbits) 15 mg/kg given once daily plus dexamethasone at 0.125 mg/kg every 12 hours, daptomycin ($n = 9$ rabbits) at 25 mg/kg given once daily, and daptomycin ($n = 10$ rabbits) 25 mg/kg given once daily plus dexamethasone at 0.125 mg/kg every 12 hours. Untreated controls received saline. The pharmacokinetics and pharmacodynamics studies and the dosing regimens for antibiotics and dexamethasone have been previously described.[10,11]

Determination of TNF-α levels in CSF

TNF-α levels were determined by enzyme-linked immunosorbent assay, according to the instructions of the manufacturer eBioscience, an Affymetrix company (Campus Vienna Biocenter 2, Vienna, Austria).

Statistical analysis

All TNF-α counts are presented as picograms per milliliter [mean ± standard deviation (SD)]. Differences in TNF counts between therapeutic groups were evaluated for statistical significance using analysis of variance. An unpaired Student *t* test with Bonferroni correction and analysis of variance was used to determine statistical significance. For all tests, differences were considered to be statistically significant when $p < 0.05$.

Results

The CSF TNF-α levels measured in pg/mL in rabbits infected with a penicillin- and cephalosporin-resistant strain of *S. pneumoniae* and treated with different antibiotic therapies are presented in Table 1.

All the therapeutic groups showed good efficacy in terms of the decrease of bacterial counts. At 6 hours of treatment, CSF samples from all antibiotic groups were below 3 log from the initial inocula.

There were no significant differences in mortality at 26 hours in the therapeutic groups.

The control group did not present any significant variation in TNF-α levels during the experiment; at 24 hours there was a slight decrease.

The ceftriaxone group had the highest levels of TNF-α and presented an inflammatory peak after the administration of the antibiotic at 2 hours and 26 hours (2 hours after the 2nd dose). Levels at 2 hours were significantly higher than at baseline ($p = 0.02$) and at 26 hours the differences compared with 24 hours almost reached significance ($p = 0.07$).

The ceftriaxone plus vancomycin combination group showed a moderate increase in inflammation after antibiotic administration, but lower than the ceftriaxone alone group.

However, daptomycin 15 mg/kg showed a constant release of TNF-α levels without any significant increase in inflammation at any point during treatment. Daptomycin 25 mg/kg showed a slight decrease after the second antibiotic administration, and was the antibiotic group with the lowest proinflammatory levels at 26 hours. These CSF TNF-α levels did not present an inflammatory peak after antibiotic administration, and so no statistical differences were found at either 2 hours or 26 hours.

CSF TNF-α levels were significantly higher in rabbits treated with ceftriaxone than in rabbits treated with daptomycin (both doses) at 2 hours and at 26 hours ($p = 0.03$). The TNF-α levels obtained from rabbits treated with daptomycin at the doses of 15 mg/kg and 25 mg/kg alone and with dexamethasone at 0.25 mg/kg are presented in Figure 1.

Daptomycin 15 mg/kg plus dexamethasone reached the same CSF inflammatory levels as daptomycin 15 alone; the differences between the therapeutic groups were not significant.

Concomitant administration of dexamethasone with high-dose daptomycin increased the reduction in CSF TNF-α levels and the difference after the first antibiotic administration was significantly lower than at baseline ($p = 0.02$). The inflammatory response measured in the daptomycin 25 mg/kg plus dexamethasone group was significantly lower than daptomycin 25 alone throughout the treatment ($p < 0.00$).

Discussion

Pneumococcal meningitis is firmly associated with a high prevalence of brain injury and neurological sequelae due to the synthesis and release of the main proinflammatory mediators such as TNF-α into the central nervous system.

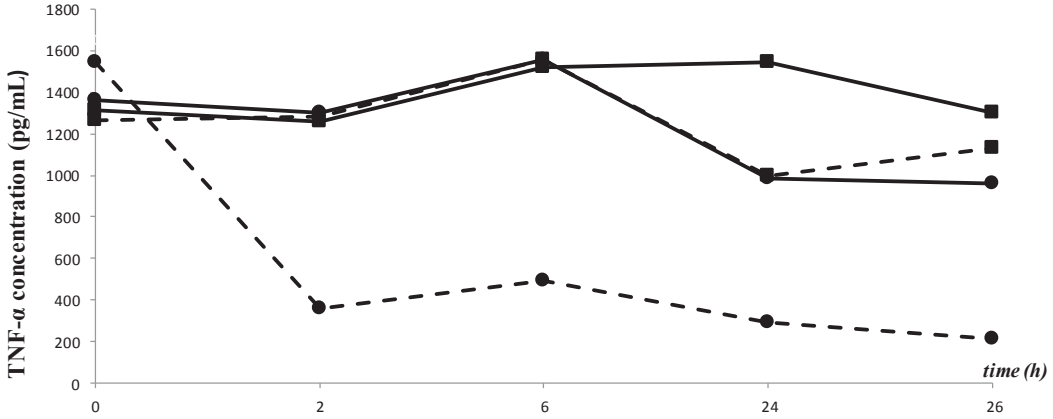
It has already been demonstrated in an experimental infected rabbit model that TNF-α plays a fundamental role in the pathogenesis of bacterial meningitis and its reduction could notably improve therapeutic treatment.[12]

Another experimental study evaluated the inflammatory response in rabbits treated with ceftriaxone, finding a substantial increase in TNF-α levels 2 hours after the initiation of the therapy using this bacteriolytic antibiotic in comparison with untreated animals.[13]

Table 1 Tumor necrosis factor-alpha levels (pg/mL) in cerebrospinal fluid of rabbits with pneumococcal meningitis treated with various antibiotic schedules (mg/L).

Therapy group (dose in mg/kg/d)	CSF-TNF-α levels (pg/mL)				
	0 h	2 h	6 h	24 h	26 h
Control	1527 ± 153.86	1360 ± 496.34*	1481.18 ± 1089.6	711.40 ± 147.55	645 ± 26.340*
CRO	1263.46 ± 519.85	2125.05 ± 927.71**	1450.54 ± 522.84	1390.08 ± 729.50	2073.38 ± 634.38
CRO + VAN	1448.06 ± 571.04	1986.76 ± 119.54	1634.49 ± 1003.42	1951.90 ± 787.68	1627.47 ± 725.65
DAP 15	1313.88 ± 469.44	1260.40 ± 463.19*	1519.89 ± 653.54	1543.04 ± 541.58	1302.13 ± 108.83*
DAP 25	1364.11 ± 142.36	1304.48 ± 942.26*	1557.84 ± 1164.30	983.03 ± 91.84	959.86 ± 88.72*

Data are expressed as mean ± standard deviation.
* $p \leq 0.05$ versus ceftriaxone group.
** $p \leq 0.05$ intraceftriaxone group 2 hours versus 0 hours.
CRO = ceftriaxone; DAP = daptomycin; VAN = vancomycin.



	0 h	2 h	6 h	24 h	26 h
DAP 15	1313.88 ± 469.44	1260.40 ± 463.19	1519.89 ± 653.54	1543.04 ± 541.58	1302.13 ± 108.83
DAP 25	1364.11 ± 142.36	1304.48 ± 942.26	1557.84 ± 164.30	983.03 ± 91.84	959.86 ± 88.72
DAP 15 + DEX	1262.95 ± 598.29	1682.73 ± 529.48	1755.57 ± 362.80	1000.00 ± 457.19	1131.58 ± 441.95
DAP 25 + DEX	1544 ± 906.20	359 ± 209.14*	494.50 ± 463.68*	292.33 ± 174.88*	215.47 ± 131.54*

Data are expressed as mean ± standard deviation.

*$p \leq 0.05$ versus daptomycin 25 group.

DAP = daptomycin; DEX = dexamethasone.

Figure 1. Cerebrospinal fluid tumor necrosis-alpha levels in rabbits treated with daptomycin at 15 mg/kg and 25 mg/kg and in combination with dexamethasone at 0.25 mg/kg.

Our results corroborated those by Mahmoud et al, who found a similar response in terms of the release of TNF-α when ceftriaxone is used.

For their part, animals treated with daptomycin presented with lower CSF TNF-α levels than those treated with ceftriaxone alone or in combination with vancomycin. High-dose daptomycin was the therapy group with the strongest reduction in the inflammatory response after antibiotic administration. Our results confirm and expand on earlier experimental studies that compared the inflammatory response of ceftriaxone-treated animals with daptomycin-treated animals.[7,8,14] Those data support our findings, because it seems reasonable to attribute the effect to daptomycin's nonbacteriolytic mechanism. Nevertheless, an extensive experimental study with Wistar rats by Barichello et al[15] found no significant differences in cytokine presence either in brain tissue or in CSF levels when ceftriaxone and daptomycin therapies were compared. There is no clear explanation for the differences between their results and ours. Further research is needed to entirely clarify the point. The CSF TNF-α levels with concomitant administration of daptomycin and dexamethasone remained constant in the case of low-dose daptomycin. In high-dose daptomycin, dexamethasone modulated TNF-α release, leading to a drastic decrease of proinflammatory cytokine levels. The adjuvant therapy with dexamethasone in combination with ceftriaxone has been extensively studied. In an experimental rabbit model, Paris et al[16] demonstrated a significant reduction in TNF-α in ceftriaxone-treated animals when dexamethasone was administered. Another clinical report demonstrated a beneficial effect with the use of dexamethasone compared with placebo alone.[17] Even though additional research is needed, our results may suggest that daptomycin could present some advantages over β-lactam antibiotics with regard to preventing inflammation and thus avoiding neurological sequelae.

In conclusion, CSF TNF-α levels were significantly lower in rabbits treated with daptomycin than in rabbits treated with ceftriaxone, and did not present the peak after administration found in ceftriaxone-treated rabbits. The use of dexamethasone plus daptomycin resulted in a significantly larger reduction of CSF TNF-α levels.

Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Acknowledgments

C.C. and M.V. are members of the Spanish Network for the Research in Infectious Diseases (REIPI RD12/0015), Instituto de Salud Carlos III, Madrid, Spain. Financial support was provided by a research grant from Instituto de Salut Carlos III FIS 09/518.

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