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Adjunctive Daptomycin Attenuates Brain Damage and Hearing Loss More Efficiently than Rifampin in Infant Rat Pneumococcal Meningitis

Denis Grandgirard,^a Melchior Burri,^a Philipp Agyeman,^{a,b} and Stephen L. Leib^{a,c}

Neuroinfection Laboratory, Institute for Infectious Diseases, University of Bern, Switzerland^a; Department of Pediatric Infectiology, Inselspital, University Hospital, Bern, Switzerland^b; and Biology Division, Spiez Laboratory, Federal Office for Civil Protection, Spiez, Switzerland^c

Exacerbation of cerebrospinal fluid (CSF) inflammation in response to bacteriolysis by beta-lactam antibiotics contributes to brain damage and neurological sequelae in bacterial meningitis. Daptomycin, a nonlytic antibiotic acting on Gram-positive bacteria, lessens inflammation and brain injury compared to ceftriaxone. With a view to a clinical application for pediatric bacterial meningitis, we investigated the effect of combining daptomycin or rifampin with ceftriaxone in an infant rat pneumococcal meningitis model.

Eleven-day-old Wistar rats with pneumococcal meningitis were randomized to treatment starting at 18 h after infection with (i) ceftriaxone (100 mg/kg of body weight, subcutaneously [s.c.], twice a day [b.i.d.]), (ii) daptomycin (10 mg/kg, s.c., daily) followed 15 min later by ceftriaxone, or (iii) rifampin (20 mg/kg, intraperitoneally [i.p.], b.i.d.) followed 15 min later by ceftriaxone. CSF was sampled at 6 and 22 h after the initiation of therapy and was assessed for concentrations of defined chemokines and cytokines. Brain damage was quantified by histomorphometry at 40 h after infection and hearing loss was assessed at 3 weeks after infection. Daptomycin plus ceftriaxone versus ceftriaxone significantly ($P < 0.04$) lowered CSF concentrations of monocyte chemoattractant protein 1 (MCP-1), MIP-1 α , and interleukin 6 (IL-6) at 6 h and MIP-1 α , IL-6, and IL-10 at 22 h after initiation of therapy, led to significantly ($P < 0.01$) less apoptosis, and significantly ($P < 0.01$) improved hearing capacity. While rifampin plus ceftriaxone versus ceftriaxone also led to lower CSF inflammation ($P < 0.02$ for IL-6 at 6 h), it had no significant effect on apoptosis and hearing capacity. Adjuvant daptomycin could therefore offer added benefits for the treatment of pediatric pneumococcal meningitis.

Acute bacterial meningitis in infancy and childhood is a severe illness with high mortality and morbidity. Long-term sequelae are frequent, especially in resource-poor regions of the world (17, 53), and persist for decades, as shown in an Australian study, which documented neurological sequelae (hearing loss, epilepsy, cognitive impairment, and educational problems) in survivors of childhood meningitis up to 12 years after infection (29). Nowadays, *Streptococcus pneumoniae* is the most common etiological agent of acute bacterial childhood meningitis besides *Neisseria meningitidis* and is associated with a particularly high rate of hearing loss and other neurological sequelae (16). In both autopsy studies of human victims of bacterial meningitis (48) and experimental models of pneumococcal meningitis (7, 22, 24, 28), cortical necrosis and apoptotic cell death in the dentate gyrus of the hippocampus are reproducible histopathologic correlates of neurological damage. Furthermore, injury of neuronal and hair cells in the cochlea, as documented in experimental pneumococcal meningitis in rats (14, 32), has been identified as a morphological correlate of hearing loss.

In our current understanding of the pathophysiology of pneumococcal meningitis, the host-mediated immune response, triggered by subcapsular components of bacteria, contributes substantially to brain damage (25, 34, 63). Bacterial components are recognized by membrane-bound host cell receptors, such as TLR2 and TLR4 (3, 33). Through signaling by MyD88 (1, 35), proinflammatory cytokines and chemokines are released, which in turn induces leukocyte migration into the cerebrospinal fluid (CSF) space (34). Activated leukocytes, predominantly neutrophils, release inflammatory mediators, including reactive oxygen species

(ROS) (42) and matrix metalloproteinases (43), which have both been shown to contribute to brain damage.

While bacterial components are continuously released during the natural course of pneumococcal meningitis, antimicrobial therapy with expanded-spectrum cephalosporins (i.e., ceftriaxone [CRO] and cefotaxime) results in rapid bacterial cell lysis and a sudden release of large amounts of microbial components (46, 60). Thus, an overshooting host-mediated inflammatory reaction, which contributes to brain damage, is elicited at the beginning of the antimicrobial therapy.

Most currently investigated strategies to improve the outcome in experimental meningitis models involve the inhibition of different steps of the host-driven inflammatory cascade. Matrix metalloproteinase inhibition, reduction of reactive oxygen species by antioxidants, and inhibiting mediators of apoptosis are possible candidates for inhibiting host immune reaction in bacterial meningitis (25). In clinical practice, only adjuvant use of the anti-inflammatory corticosteroid dexamethasone has been shown to be beneficial in adults with bacterial meningitis (4, 11) and in childhood meningitis caused by *Haemophilus influenzae* type b (38, 49), a pathogen that has virtually been eradicated in global

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Address correspondence to Stephen L. Leib, stephen.leib@ifik.unibe.ch.

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regions that have implemented the corresponding vaccination. Efficacy of dexamethasone therapy in children with pneumococcal meningitis is disputed (11, 51). Also, the benefit of adjunctive dexamethasone treatment in adults with bacterial meningitis has been questioned by a meta-analysis of 2,029 individual patient data (64) and recent clinical studies (52).

Furthermore, in experimental models of meningitis, dexamethasone has been shown to induce apoptosis in the dentate gyrus (8, 58) and to increase learning impairments (41).

A promising approach to reduce inflammation early in the cascade of events leading to the pathophysiology of bacterial meningitis is the “soft killing” of bacteria (56), i.e., to minimize the release of inflammatory bacterial compounds by using nonlytic antibiotics. The two most extensively studied nonbacteriolytic antibiotics in experimental meningitis are daptomycin (DAP), a cyclic lipopeptide, which disrupts cell membrane function and cell wall metabolism (6), and rifampin (RIF), which inhibits protein synthesis by inhibition of the DNA-dependent RNA polymerase (65). Both antibiotics reduce the release of bacterial cell components or toxins *in vitro* (15, 59) and *in vivo* (59, 60). Several experimental animal studies exploring the use of these nonlytic antibiotics in pneumococcal bacterial meningitis have demonstrated their beneficial effects. Daptomycin has been shown to reduce the inflammatory reaction and brain damage compared to ceftriaxone in an experimental pneumococcal meningitis model in rats (26, 27). Meanwhile, rifampin and clindamycin reduced the release of lipoteichoic acid and had a neuroprotective effect in an experimental rabbit model of pneumococcal meningitis (9, 10). Furthermore, pretreatment with rifampin 1 h prior to ceftriaxone therapy limited the extent of brain injury (59), and the combination of daptomycin with ceftriaxone was shown to be more active than vancomycin plus ceftriaxone (18) in experimental pneumococcal meningitis in rabbits.

The primary aim of this study was to assess the potential benefits of sequential use of nonlytic before lytic antimicrobial therapy in experimental pneumococcal meningitis in an experimental setting that more closely mimics the clinical situation with regard to the interval between adjuvant and antibiotic therapy. In the present study, daptomycin and rifampin, two well-described nonlytic antibiotics given 15 min before ceftriaxone, were compared to ceftriaxone monotherapy. We challenged the hypothesis that by acting before ceftriaxone-induced lysis of bacteria, nonlytic antibiotics would limit the release of bacterial components and positively impact on the inflammatory reaction and outcome of infected animals.

MATERIALS AND METHODS

Animal model of meningitis. All animal studies were approved by the Animal Care and Experimentation Committee of the Canton Bern (license no. 26/07) and followed the Swiss national guidelines for the performance of animal experiments. A well-characterized model of infant rat pneumococcal meningitis was used (27, 44). Eleven-day-old Wistar rats ($n = 112$; Charles River, Germany) were injected intracisternally (i.c.) with 10 μ l of saline containing $\log_{10} 6.173 \pm 0.149$ CFU/ml of *Streptococcus pneumoniae* (clinical isolate of a serotype 3 strain). Eighteen hours later, CSF samples were obtained by puncture of the cisterna magna and were cultured quantitatively on sheep blood agar plates to document meningitis. The number of bacteria in CSF was determined by plating serial dilutions of 10 μ l of CSF on blood agar plates. Animals were randomized to receive ceftriaxone ($n = 32$; Rocephin, Roche Pharma; 100 mg/kg of body weight, subcutaneously [s.c.], twice a day [b.i.d.]), daptomycin ($n = 42$; Cubicin [kindly provided by Cubist Pharmaceuticals], 10 mg/kg, s.c., daily), or rifampin ($n = 38$; Rimactan, Sandoz Pharmaceuticals AG; 20 mg/kg, intraperitoneally [i.p.], b.i.d.). Fifteen minutes after daptomycin or rifampin treatment, animals were treated with ceftriaxone. The dosages of daptomycin, rifampin, and ceftriaxone used in this study were within the range of those used in previously published work (26, 27, 55, 59). CSF was sampled 6 h (24 h postinfection [hpi]) or 22 h (40 hpi) after treatment. A subset of the animals were sacrificed at 40 hpi by an overdose of pentobarbital (100 mg/kg, i.p.) and subsequently perfused with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS), and brains were removed and fixed in PFA for subsequent histology. Treatments were repeated for a total of 3 days in surviving animals. Three weeks after infection, animals were tested for hearing.

Myeloperoxidase assay. Myeloperoxidase (MPO) is an enzyme specific for azurophil granules and represents 5% of the total cellular protein of neutrophils (2). Here MPO was measured in CSF samples as an index of leukocyte influx. Five microliters of uncentrifuged CSF were resuspended in 200 μ l HETAB solution (0.5% hexadecyltrimethylammonium bromide in 100 mM potassium phosphate buffer [pH 6.0]) and repeatedly submitted to 3 cycles of freeze-thawing, sonication, and centrifugation for 5 min at $10,000 \times g$ at 4°C. Supernatants were stored at -80°C until use. Assays were performed in triplicate by mixing 155 μ l of HETAB buffer with 10 μ l of samples and 10 μ l of *o*-dianisidine (20 mg/ml in water) in a 96-well plate. The reaction was initiated by the addition of 25 μ l of 2 mM hydrogen peroxide in water. Absorbance was measured at 450 nm every 30 s for 10 min at 37°C using the ThermoMax microplate reader (Molecular Devices Inc., Sunnyvale, CA). The linear domain of the curve was used to determine the increase in absorbance. MPO activity was expressed in relative units corresponding to V_{max} as determined using SOFTmax PRO software version 3.1.2 (Molecular Devices Inc., Sunnyvale, CA).

Cytokine expression in the CSF. Microsphere-based multiplex assays (Lincplex; Millipore Corporation, Billerica, MA) were used to assess the CSF concentrations of the following cytokines: interleukin 1 β (IL-1 β), IL-6, IL-10, IL-18, tumor necrosis factor alpha (TNF- α), MIP-1 α , and monocyte chemoattractant protein 1 (MCP-1). The choice of cytokines is based on the results of previous studies with the experimental model used. The present study was not meant to explore an extensive panel of cytokine/chemokine levels but to judge from the effect of the described therapeutic modalities on the host inflammatory reaction known to occur in the model. Depending on the amount of harvested material, CSF was diluted 5- to 9-fold to a final volume of 25 μ l. A minimum of 100 beads per analyte was measured using a Bio-Plex 200 station (Bio-Rad Laboratories, Hercules, CA). Calibration curves from recombinant standards were calculated with Bio-Plex Manager software version 4.1.1 using a five-parameter logistic curve fitting. When cytokine concentration was below the detection limit, an arbitrary value corresponding to the detection limit provided by the manufacturer multiplied by the dilution factor of the samples was used for statistical analysis. The lower detection limits (in pg/ml) provided by the manufacturer were as follows: IL-1 β , 2.32; IL-6, 9.8; IL-10, 5.41; IL-18, 4.78; TNF- α , 4.44; MCP-1, 3.81; and MIP-1 α , 1.94.

Histology and morphometry. PFA-fixed brains were cryopreserved in 18% sucrose in PBS at 4°C overnight. Forty-five-micrometer-thick coronal brain sections were used to evaluate neuronal injury of the cortex and hippocampus as described in detail previously (20). The sections were stained for Nissl substance with cresyl violet and used for all determinations. Quantification of apoptotic nuclei in the hippocampal dentate gyrus was performed as described previously (7). In brief, cells exhibiting characteristic histomorphologic features of apoptosis were counted in 4 different slices spanning the hippocampus. Using a $\times 400$ magnification, three visual fields in each of the two blades (upper and lower) of the dentate gyrus were inspected for the presence of cells showing morphological signs indicative of apoptosis (condensed, fragmented dark nuclei, apoptotic bodies). The number of apoptotic cells in each visual field was determined, and a mean value per animal was calculated from all inspected fields.

For the measurement of cortical brain damage, the entire cortex was evaluated for damage by using a systematic uniform random sampling procedure, with a random start and a sampling frequency of 15 slices throughout the brain. Stained sections were scanned, and the total and damaged cortical surfaces identified by decreased density of neurons were quantified by the software ImageJ. The volume of total cortex and the volume of the damaged tissue were calculated using the Cavalieri method (30), and cortical neuronal injury was expressed as the percentage of affected cortex. Histopathological evaluations were done by investigators who were unaware of the clinical, microbiologic, and treatment data for the respective animal.

Audiometry. Auditory brainstem responses (ABR) were recorded in response to click stimulations (presentation rate, 20 clicks/second) on both ears of animals anesthetized with a mixture of medetomidine hydrochloride (Dormitor, Orion Pharma; 1 mg/kg, i.p.) and ketamine hydrochloride (Ketalar, Pfizer; 50 mg/kg, i.p.). Subdermal needle electrodes were placed in the mastoid of the tested ear (active), at the vertex (reference), and in the cervical neck muscles (ground). The sound stimulus consisted of 100- μ s square wave impulses synthesized digitally using Sig-Gen software (Tucker-Davis Technologies System II), fed into a programmable attenuator and, after digital-to-analog (D/A) conversion, transduced by a speaker (JBL model 2405H) located 4 cm from the pinna. ABR were amplified (10^3), band-pass filtered between 100 Hz and 3 kHz (Ithaco model 1201), and averaged ($n = 500$) during a 12-ms window using the data acquisition software BioSig (Tucker-Davis Technologies System II). The intensity of the click stimulus was reduced from 110 to 0 dB sound pressure level (SPL) in 5 dB steps. The hearing threshold was defined as the lowest intensity that induced the appearance of a visually detectable first peak and an average from both ears calculated per animal.

RESULTS

Clinical parameters. No significant difference in survival was observed between the different experimental groups. Three weeks after infection, the survival proportions of rifampin-, daptomycin-, and ceftriaxone-treated animals were 85%, 83%, and 87%, respectively. As an index of disease severity, gain or loss in weight was determined for the different experimental groups. Figure 1A depicts weight changes recorded between the time of infection and 40 hpi. Interestingly, while no difference in weight change could be observed between groups for the interval between infection and treatment, statistically less weight loss was observed for the DAP group when recorded between initiation of treatment and 40 hpi. A decrease in CSF MPO activity at 24 h after infection was observed in DAP-CRO-treated animals (35.8 ± 30.1 relative units) and RIF-CRO-treated animals (30.4 ± 29.1) compared to CRO-treated animals (49.8 ± 37.0) without reaching statistical significance, as determined by the Kruskal-Wallis test ($P = 0.1$). At 40 h after infection, no difference between the groups could be observed.

Neuronal damage. Neuronal damage was examined on brain sections of a subset of animals which were sacrificed 40 h after infection. In the present study, only a moderate level of cortical damage was noted across all treatment arms. Cortical damage was present in 3 out of 12 CRO-treated animals, in 1 out of 15 DAP-CRO-treated animals, and in none of 13 RIF-CRO-treated animals analyzed by histomorphometry. In affected animals, the extent of cortical damage was restricted to small focal areas, not exceeding 2% of the total cortical volume. When comparing the proportions of animals which develop cortical damage, significant differences ($P < 0.0001$, chi-square test) were found between CRO (25%), DAP (7%), and RIF (0%).

A significant difference ($P < 0.01$, Kruskal-Wallis) in the ex-

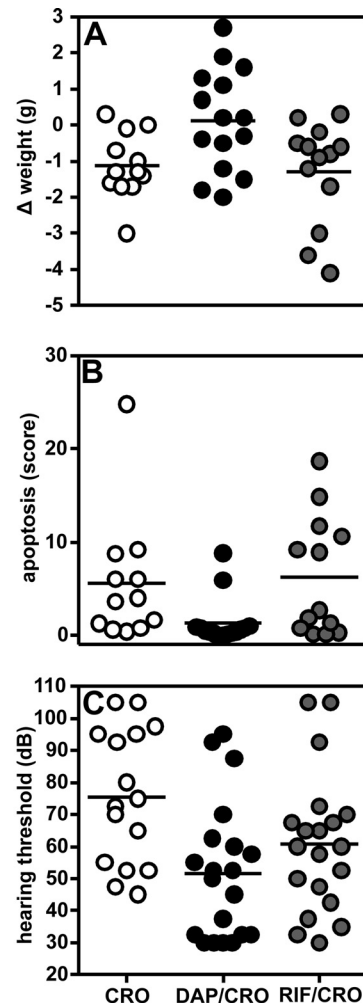


FIG 1 Weight loss (A), hippocampal apoptosis (B), and hearing loss (C) under the 3 treatment regimens. (A) Statistically significant differences ($P < 0.04$, Kruskal-Wallis) in weight loss were observed at 22 h after initiation of antibiotic therapy between groups. Pairwise comparison (Mann-Whitney test) revealed significant differences between DAP-CRO versus CRO ($P < 0.03$) and DAP versus RIF-CRO ($P < 0.04$). (B) Significantly less hippocampal injury was observed in animals treated with DAP-CRO compared to both RIF-CRO-treated ($P < 0.03$) and CRO-treated ($P < 0.01$) animals. No difference was observed between RIF-CRO and CRO. (C) Hearing loss was significantly attenuated by DAP-CRO ($P < 0.01$) in comparison to CRO, while RIF-CRO versus CRO did not differ significantly ($P = \text{NS}$).

tent of apoptosis in the dentate gyrus of the hippocampus was found between the different experimental groups. Apoptosis was significantly reduced in DAP-CRO-treated animals (1.4 ± 2.5 cells/visual field, $n = 15$) compared either to CRO-treated animals (5.6 ± 6.8 cells/visual field, $n = 12$; $P < 0.01$, Mann-Whitney test) or to RIF-CRO-treated animals (6.3 ± 6.4 cells/visual field, $n = 13$; $P < 0.03$). In contrast, no such difference was observed between RIF-CRO-treated and CRO-treated animals (Fig. 1B).

Hearing loss 3 weeks after infection. The loss of hearing capacity consecutive to pneumococcal meningitis was determined by measuring the increase in the threshold of the stimulus necessary to induce a detectable auditory brainstem response (ABR). In the present experimental setting, the hearing threshold of healthy animals was 30 to 35 dB SPL (data not shown). In infected CRO-

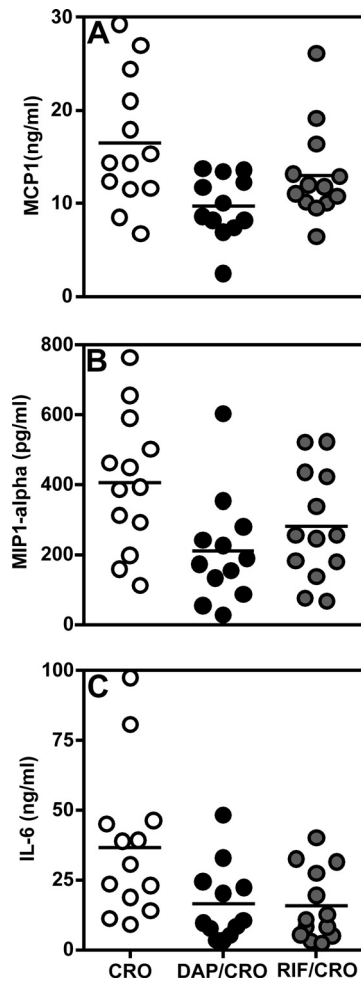


FIG 2 CSF concentrations of cytokines and chemokines in the 3 treatments of experimental pneumococcal meningitis at 6 h after initiation of therapy. Overall comparison between groups revealed statistically significant differences for MCP-1 ($P = 0.028$), MIP-1 α ($P = 0.028$), and IL-6 ($P = 0.014$), as determined by the Kruskal-Wallis test. Pairwise comparison between groups was significant for DAP-CRO versus CRO for all cases and RIF-CRO versus CRO only for IL-6 at 6 h after therapy.

TABLE 1 CSF cytokine/chemokine concentrations at 24 and 42 hpi (6 and 24 h after initiation of antibiotic therapy)^b

Time postinfection	Cytokine/chemokine	Concn (pg/ml) ^a			Kruskal-Wallis <i>P</i> value
		CRO	DAP-CRO	RIF-CRO	
24 hpi	IL-1 β	174.7 (11.6–848.4)	67.75 (11.6–451.6)	80.25 (11.6–811.9)	0.32
	IL-6	30,605 (9,127–97,523)	10,017 (3,369–48,270)	10,950 (1,727–40,143)	0.017
	IL-10	878.7 (27.1–4,511)	27.1 (27.1–1,849)	418 (27.1–3,396)	0.22
	IL-18	218.2 (23.9–511.6)	135.8 (23.9–3,170)	123 (23.3–1,035)	0.63
	TNF- α	141.6 (22.2–236.8)	91.1 (22.2–367.7)	150.1 (22.2–316.5)	0.72
	MCP-1	14,366 (6,758–29,248)	9,337 (2,455–13,723)	11,801 (6,415–26,139)	0.028
	MIP-1α	393.1 (113.1–765.4)	181.6 (27.3–604.1)	256.8 (67.7–523.2)	0.028
	42 hpi	IL-1 β	70.46 (11.6–282.3)	11.6 (11.6–159.8)	48 (11.6–368.4)
IL-6		68.6 (49–415.5)	49 (49–88.2)	49 (49–88.2)	0.04
IL-10		37.9 (27.1–3,934)	27.1 (27.1–48.7)	27.1 (27.1–118.1)	0.005
IL-18		473.2 (33.5–2,600)	277.7 (47.1–3,425)	605.8 (23.9–2,193)	0.4
TNF- α		30.6 (2.9–54.1)	19.7 (3.6–37.8)	22.2 (2–54.9)	0.45
MCP-1		1,088 (19.1–7,814)	1,213 (19.1–2,686)	1,238 (19.1–6,011)	0.94
MIP-1α		37.7 (9.7–153.4)	9.7 (9.7–32.7)	11.1 (9.7–288.8)	0.012

^a Concentrations expressed as median (minimum-maximum).

^b Text is in bold when overall comparison between groups revealed statistically significant differences as determined by the Kruskal-Wallis test ($P < 0.05$).

treated animals, this threshold was increased to 75.3 ± 21 dB SPL ($n = 16$). Significant differences in thresholds were found ($P < 0.01$, Kruskal-Wallis) by comparing the three experimental groups. Hearing loss assessed by an increase in the hearing threshold observed in CRO-treated animals was significantly attenuated by DAP-CRO treatment (51.8 ± 21.3 dB SPL, $n = 20$; $P < 0.01$, Mann-Whitney). A trend for attenuation was also observed by RIF-CRO versus CRO treatment (60.8 ± 21.7 dB SPL, $n = 20$; $P = 0.054$) (Fig. 1C).

Inflammatory reaction. Modulation of the inflammatory reaction in response to antimicrobial therapy was analyzed by measuring the CSF concentration of cytokines and chemokines at different time points following the initiation of antimicrobial treatment. The DAP-CRO combination therapy resulted in lower CSF concentration of cytokines/chemokines. Statistical significance (Kruskal-Wallis) was reached for MIP-1 α , MCP-1, and IL-6 at 6 h after therapy (Fig. 2) and MIP-1 α , IL-6, and IL-10 at 22 h after therapy (Table 1). For those cytokines/chemokines with significant differences in CSF concentration, pairwise analysis (Mann-Whitney) revealed a statistically significant difference for the comparison of DAP-CRO versus CRO but not for RIF-CRO versus CRO (except IL-6 at 6 h after therapy) or DAP-CRO versus RIF-CRO.

DISCUSSION

Effective antimicrobial treatment of bacterial meningitis depends on antibiotics that have a fast bactericidal effect on the meningitis pathogen. For both childhood meningitis and pneumococcal meningitis in adults, it was shown that a delay in CSF sterilization leads to an adverse outcome; i.e., a higher incidence of neurologic abnormalities, including moderate to profound hearing impairments at the time of hospital discharge and follow-up (39) or increased mortality (5).

The emergence of resistant bacterial strains, i.e., penicillin-resistant pneumococci, is therefore of concern, as failure to cover for these strains may lead to delayed sterilization of CSF. Although most studies have not shown differences in outcome between penicillin-resistant and -susceptible strains (12, 19), failure related to

this resistance has been observed and may be linked to a worse clinical outcome (57) or increased mortality (5).

Modern meningitis therapy with an expanded-spectrum cephalosporin, including vancomycin in regions where penicillin-resistant pneumococci pose a threat, fulfills these requirements well. But on the downside, bacteriolytic action of these cell wall-active substances additionally supports the host inflammatory reaction and may contribute to the worsening of the patient's outcome (45, 47, 61). Therefore, strategies combining adequate antimicrobial therapy with adjuvant therapies preventing the excessive host inflammatory reaction have been proposed to reduce neurological damage and ameliorate the outcome in terms of mortality and morbidity (46). It has been shown that the nonlytic antimicrobials rifampin and daptomycin are promising candidates for the reduction of inflammation and brain damage in experimental pneumococcal meningitis. For rifampin, it has been shown that it attenuates inflammation and brain damage if given 1 or 6 h (versus 15 min in this study) before ceftriaxone (21, 59). Similarly, daptomycin has been shown to be noninferior to ceftriaxone in experimental pneumococcal meningitis (18) and to reduce inflammation and brain damage in this experimental setting (26, 27). Our work adds to these studies by a head-to-head comparison of the two nonlytic antimicrobial compounds in a study with a clinically acceptable interval of 15 min (an interval that is accepted for the adjuvant administration of dexamethasone) between administration of the nonlytic antimicrobials and ceftriaxone. In addition, this is the first study to report on the clinically relevant outcome of hearing loss.

Animals in the daptomycin-ceftriaxone treatment arm had significant lower CSF levels of the chemokines MCP-1, MIP-1 α , and IL-6 compared to ceftriaxone monotherapy, a trend also observed for the rifampin-ceftriaxone treatment arm, although only reaching statistical significance for IL-6 at 6 h after the initiation of therapy. This measurable reduction of the inflammatory reaction in the daptomycin-ceftriaxone treatment arm also translated into less cortical damage, less hippocampal apoptosis, and less hearing loss compared to the ceftriaxone treatment arm. For animals in the daptomycin-ceftriaxone treatment arm, a significant reduction in weight loss was noted compared to the other two treatment arms. This is likely due to a decrease in the release of cytokines and chemokines and subsequent reduction in systemic inflammation (27, 60). Taken together, the present results are in accord with our previous findings that daptomycin monotherapy leads to a reduction of proinflammatory cytokines and attenuation of neuronal damage compared to ceftriaxone monotherapy in experimental pneumococcal meningitis (26). Contrary to what has previously been shown in a rabbit model of pneumococcal meningitis (59), rifampin-ceftriaxone treatment was not effective in protecting against hippocampal apoptosis. Differences in time-kill kinetics between daptomycin and rifampin may explain the observed difference in neuroprotection since a beneficial effect by pretreatment with rifampin was observed only with an interval of 1 and 6 h before ceftriaxone administration (21, 59).

A significant decrease in hearing loss was observed in the daptomycin-ceftriaxone treatment arm. Hearing loss during pneumococcal meningitis may result in part from the production of highly reactive oxygen species (ROS) (31). Treatment with rifampin has been shown to decrease the level of ROS compared to ceftriaxone in an experimental meningitis model in rabbits (9). In this study, hearing loss was determined by audiometry 3 weeks

after infection. At that time, hearing loss is permanent, in contrast to the early reversible form of hearing loss observed during the acute stage of the disease in experimental models. The permanent hearing impairment best finds its morphological correlate in the reduction of spiral ganglion neuronal cells, which was not observed until 48 hpi (32) and after damage to the hippocampus has already occurred. Daptomycin-ceftriaxone may therefore protect against hearing loss by reducing the production of ROS or by influencing pathophysiological events occurring later than those involved in hippocampal injury.

Overall, our study results favor sequential therapy with daptomycin before standard meningitis therapy with ceftriaxone as an alternative to treatment with rifampin-ceftriaxone or ceftriaxone monotherapy. While daptomycin possesses potent *in vitro* bactericidal activity against most clinically relevant strains of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant enterococci (13), it lacks any activity against Gram-negative bacteria.

Although *Streptococcus pneumoniae* and *Streptococcus agalactiae* have been shown to be the predominant species causing meningitis in a recent U.S. survey, Gram-negative bacteria, i.e., *Neisseria meningitidis* and *Haemophilus influenzae*, still represent 20% of all cases (62). Furthermore, *Neisseria meningitidis* was responsible for 80% of the meningitis burden in the "meningitis belt" of sub-Saharan Africa during the 2010 epidemic (66). Therefore, for empirical meningitis therapy, daptomycin would have to be combined with a second antibiotic targeting Gram-negative bacteria, e.g., an expanded-spectrum cephalosporin.

Another main issue to be considered in the treatment of central nervous system infection is the difficulty to reach therapeutic antibiotic concentrations in the CSF due to the limited permeability of the blood-brain barrier. For daptomycin, it has been shown in a rabbit pneumococcal meningitis model that penetration into the CSF space is enhanced by the presence of inflamed meninges (5% versus 2% with noninflamed meninges) (23). The addition of dexamethasone reduced the penetration of daptomycin into CSF to 2% in the same experimental model (18). The CSF penetration of rifampin in a similar experimental model was 3 to 5% and seemed less affected by concomitant dexamethasone therapy (50). More recently, in patients with suspected or confirmed neurological infections, CSF penetrations of 0.8% (36) to 5% (54) were documented for daptomycin. It has been proposed that the differences between these studies are due to the variations in the degree of inflammation of the meninges. Furthermore, when the differences in protein binding between CSF and serum were considered, the CSF penetration of daptomycin increased to 11.5% (36). Successful treatment of meningitis caused by vancomycin-resistant enterococci (37) or methicillin-resistant *Staphylococcus aureus* (40) have been reported using daptomycin at high doses (6 to 12 mg/kg) and/or in combination with gentamicin or linezolid. These doses are higher than the doses currently approved for the treatment of complicated skin and soft tissue infections (4 mg/kg) or *S. aureus* bloodstream infections (6 mg/kg), but higher doses, such as those used for the treatment of meningitis, may still be safe and well tolerated (67) and result in higher levels in the CSF.

In conclusion, our experimental data indicate that combining the nonlytic antibiotic daptomycin with ceftriaxone in a 15-min sequence may represent a beneficial strategy for the empirical treatment of bacterial meningitis. By decreasing the host-driven inflammatory reaction and by rapidly killing bacteria in the CSF

space, including strains resistant to other antibiotics, daptomycin-ceftriaxone may contribute to improved outcome of patients with pneumococcal meningitis.

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