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Immunological and Biochemical Correlates of Adjunctive Dexamethasone in Vietnamese Adults with Bacterial Meningitis

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Abstract

Adjunctive treatment to improve outcome from bacterial meningitis has centered on dexamethasone. Among Vietnamese patients with bacterial meningitis, cerebrospinal fluid (CSF) opening pressure and CSF:plasma glucose ratios were significantly improved and levels of CSF cytokines interleukin (IL)–6, IL-8, and IL-10 and were all statistically significantly lower after treatment in patients who were randomized to dexamethasone, compared with levels in patients who received placebo.

The mortality rate associated with bacterial meningitis and the frequency of neurologic sequelae among those who survive the diseases remains high [1, 2]. Adjunctive treatment approaches to bacterial meningitis have focused on mitigating the inflammatory response in the subarachnoid space with agents such as corticosteroids. In animal models of bacterial meningitis, adjunctive dexamethasone with antimicrobial treatment ameliorated markers of subarachnoid space inflammation [3-5]. In randomized, controlled clinical trials, dexamethasone has been associated with improved cerebrospinal fluid (CSF) inflammatory parameters, but only in children with *Haemophilus influenzae* type b infection [6, 7]. In a second study involving children, dexamethasone was associated with improved CSF concentrations of tumor necrosis factor (TNF) α and platelet activating factor but was not associated with a reduction in mortality [8]. Finally, a third study failed to demonstrate any modulation of CSF inflammatory indices in children or adults with bacterial meningitis, despite an improvement in associated mortality among patients who received dexamethasone [9]. More-recent randomized controlled trials have provided conflicting

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results regarding the clinical value of dexamethasone as adjunctive treatment in bacterial meningitis for both adults and children [10-12] In this study, we examined biochemical and immunological markers of the subarachnoid space inflammatory response among adult Vietnamese patients with bacterial meningitis who were randomized to receive either dexamethasone or placebo.

Patients and methods

The study participants were from a randomized, double-blind, placebo-controlled trial of dexamethasone involving 435 patients >14 years of age who had suspected bacterial meningitis. The study was conducted from November 1996 through May 2005 at the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam [13]. A total of 217 patients were assigned to the dexamethasone group, and 218 were assigned to the placebo group. Lumbar puncture was performed and CSF opening pressure was measured at hospital admission and then repeated after initiation of treatment as the standard of care. Aliquots of CSF were sent for routine investigations, and when there was sufficient CSF collected, an aliquot was stored at −70°C for cytokine measurements. Bacterial meningitis was confirmed in 300 patients (69.0%) using conventional diagnostic approaches [13], and an additional 41 patients (11.8%) had a diagnosis that was confirmed by molecular methods (polymerase chain reaction [PCR]). Probable meningitis was diagnosed in 82 patients (19%), and an alternative diagnosis was made in 12 patients (3%).

A diagnosis of bacteriologically confirmed meningitis was made if bacteria were detected in CSF by (1) Gram or acridine orange stain, (2) latex agglutination test, (3) PCR, or (4) culture from CSF or blood samples. Details on the routine methods of pathogen detection have been described elsewhere [13]. Real-time PCR for detection of *H. influenzae* type B, *Streptococcus pneumoniae, Neisseria meningitidis* and *Streptococcus suis* was performed on stored samples as described elsewhere [14]. CSF cytokine levels were measured only in patients with confirmed bacterial meningitis for whom there were paired CSF samples obtained at the time of randomization (baseline) and again 1–4 days after initiation of treatment.

CSF concentrations of interleukin (IL)–6, IL-8, IL-10, IL-12p70, TNF-α, and IL-1β were determined using a multiplex bead array assay (CBA assay; BD Biosciences), and analysis was performed according to the manufacturer's instructions. The limit of detection was 10 pg/mL for each cytokine. Analysis was performed by a technician blinded to the treatment assignment of each patient, and paired CSF samples were analyzed simultaneously.

Laboratory measurements, except for the CSF opening pressure and the ratio of CSF glucose to plasma glucose, were log-transformed before analysis. Several of the cytokine measurements were below the lower limit of detection, and therefore, methods for left-censored data were used [15]. We compared follow-up laboratory measurements between the 2 arms using the Wilcoxon rank-sum test for uncensored data or the Peto and Peto modification of the Gehan-Wilcoxon test for cytokines. We also performed an adjusted analysis that modeled the follow-up measurement as depending linearly on the baseline value of the respective laboratory measurement and the sampling day in addition to the

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study arm. In a sensitivity analysis, the analysis was adjusted by the addition of sex plus the variables most strongly associated with survival [13]: age, Glascow coma score (GCS), the presence of paresis, and meningitis caused by a bacterium other than *Streptococcus suis*. For the adjusted analyses, we used standard linear regression for uncensored data; for cytokines, we used maximum likelihood estimation accounting for left-censored data and assuming a normal distribution of the log-transformed measurements. No imputation of missing laboratory markers and no adjustment for multiplicity was performed, and all reported confidence intervals (CIs) are 95% CIs. Analyses were performed with the statistical software R, version 2.8.1 [16], and the contributed R package Nondetects and Data Analysis.

Results

From November 1996 through June 2005, 435 patients with suspected bacterial meningitis were randomly assigned to receive either a placebo or dexamethasone. Overall, 341 (78%) of 435 patients had confirmed bacterial meningitis. The number of patients with confirmed cases is higher than previously reported (300 patients) [13], because PCR identified an additional 41 confirmed patients. Paired CSF cytology or biochemistry data, collected at enrolment and 1–6 days later, was available from almost all of the confirmed patients (dexamethasone group, 154 [94%] of 164; placebo group, 164 [93%] of 177). In 146 (42%) of 341 patients, there was insufficient CSF to analyze cytokine concentrations, or the second CSF sample was collected >4 days after randomization and was therefore not analyzed. Thus, cytokine levels in paired CSF samples were measured for a total of 195 (58%) of 341 patients. Baseline characteristics of all patients with confirmed bacterial meningitis are shown in Table 1. The baseline features of patients for whom CSF cytokine levels were measured were similar to those for the overall group of patients with confirmed bacterial meningitis (Table 1).

The distribution of sampling times for the follow-up samples that were used for cytological or biochemical analysis was not statistically significantly different between the placebo and dexamethasone treatment arms ($P = .61$, by Fishers exact test). CSF glucose level ($P < .001$) and the ratio of CSF glucose to plasma glucose ($P = .02$) at follow-up were statistically significantly higher for patients who received dexamethasone than they were for patients who received placebo (Table 2), and the findings were consistent when the analysis was adjusted for additional baseline covariates that had been previously identified as being associated with outcome in this clinical trial (GCS, paresis, age, sex, and *Streptococcus suis* infection) ($P < .001$ for CSF glucose; $P = .003$ for ratio CSF glucose to plasma glucose. The CSF opening pressure was lower at follow-up among patients who received dexamethasone ($P = .04$) and was borderline significant after adjustment for additional covariates ($P = .07$). There were no statistically significant differences in follow-up measurements between the 2 arms with respect to CSF protein levels, CSF lactate levels, or CSF leukocyte count.

Baseline and follow-up CSF cytokine concentrations are shown in Table 3. The distribution of sampling times for followup samples used in cytokine analysis was not statistically significantly different between the placebo and dexamethasone treatment arms ($P = .21$, by Fisher’s exact test). CSF concentrations of IL-6, IL-8, and IL-10 were all statistically

significantly lower in follow-up samples from patients who received dexamethasone than they were in samples from patients who received placebo (Table 3). For all 3 cytokines, the results remained statistically significant ($P < .01$ for all) after adjustment for additional covariates (GCS, paresis, age, sex, and *Streptococcus suis* infection). CSF concentrations of IL-12p70, IL-1β, and TNF-α were either not measurable or were low at baseline. At follow-up, these cytokines were seldom detected in CSF, and there was no statistically significant difference in the distribution of follow-up measurements between the 2 groups or in the rate of patients with detectable values (Table 3).

Discussion

A role for adjunctive dexamethasone therapy in bacterial meningitis has been considered for >30 years, but there is still no consensus on the value of this intervention [17, 18]. We recently demonstrated that dexamethasone was associated with a significant reduction in mortality among human immunodeficiency virus (HIV)–negative Vietnamese adult patients with confirmed bacterial meningitis [13]. The key finding from the present study was that dexamethasone was associated with a statistically significant (albeit small) reduction, in absolute terms, in CSF biochemical and immune markers of inflammation in Vietnamese adult patients with confirmed bacterial meningitis

Receipt of dexamethasone, compared with receipt of placebo, was associated with a statistically significantly greater improvement in the CSF glucose to plasma glucose ratio. This may reflect rapid antimicrobial-mediated killing of bacteria in the central nervous system and a dexamethasone-augmented reduction in inflammatory metabolic activity, but it could also reflect increased transport of glucose into the CSF. At the time of study enrolment, the concentration of IL-6, IL-8, and IL-10 in CSF samples from patients with bacterial meningitis was very high and decreased with therapy. CSF concentrations of TNF-α, IL-1β, and IL-12 were lower or were not measurable at presentation, and this may reflect a different quantitative balance of production, consumption, degradation, and binding to inhibitory receptors in the CSF for these molecules, compared with IL-6, IL-8, and IL-10. IL-6 and IL-8 are classical proinflammatory cytokines that are produced by diverse cell types, with IL-8 in a potent neutrophil chemoattractant and likely a mediator of the neutrophil infiltrate in the CSF of patients with bacterial meningitis [19].

The strength of the current study was the large number of patients observed and the breadth of pathogens studied and inflammatory parameters measured. A weakness of the study was the possible bias associated with not measuring cytokine responses in all patients who were randomized to receive placebo or dexamethasone. Nevertheless, the results provide a rational, although not conclusive, explanation of the clinical benefit provided by adjunctive dexamethasone in HIV-negative Vietnamese adolescent and adult patients with confirmed bacterial meningitis [13].

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References

1. Durand ML, Calderwood SB, Weber DJ, et al. Acute bacterial meningitis in adults: a review of 493 episodes. N Engl J Med. 1993; 328:21–8. [PubMed: 8416268]
2. Bohr V, Hansen B, Jessen O, et al. Eight hundred and seventy-five cases of bacterial meningitis. Part I of a three-part series: clinical data, prognosis, and the role of specialised hospital departments. J Infect. 1983; 7:21–30. [PubMed: 6631026]
3. Mustafa MM, Ramilo O, Mertsola J, et al. Modulation of inflammation and cachectin activity in relation to treatment of experimental Hemophilus influenzae type b meningitis. J Infect Dis. 1989; 160:818–25. [PubMed: 2809257]
4. Syrogiannopoulos GA, Olsen KD, Reisch JS, McCracken GH Jr. Dexamethasone in the treatment of experimental *Haemophilus influenzae* type b meningitis. J Infect Dis. 1987; 155:213–9. [PubMed: 3492570]
5. Tauber MG, Khayam-Bashi H, Sande MA. Effects of ampicillin and corticosteroids on brain water content, cerebrospinal fluid pressure, and cerebrospinal fluid lactate levels in experimental pneumococcal meningitis. J Infect Dis. 1985; 151:528–34. [PubMed: 3973406]
6. Lebel MH, Freij BJ, Syrogiannopoulos GA, et al. Dexamethasone therapy for bacterial meningitis: results of two double-blind, placebo-controlled trials. N Engl J Med. 1988; 319:964–71. [PubMed: 3047581]
7. Mustafa MM, Lebel MH, Ramilo O, et al. Correlation of interleukin-1 β and cachectin concentrations in cerebrospinal fluid and outcome from bacterial meningitis. J Pediatr. 1989; 115:208–13. [PubMed: 2787856]
8. Odio CM, Faingezicht I, Paris M, et al. The beneficial effects of early dexamethasone administration in infants and children with bacterial meningitis. N Engl J Med. 1991; 324:1525–31. [PubMed: 2027357]
9. Girgis NI, Farid Z, Mikhail IA, Farrag I, Sultan Y, Kilpatrick ME. Dexamethasone treatment for bacterial meningitis in children and adults. Pediatr Infect Dis J. 1989; 8:848–51. [PubMed: 2626285]
10. Scarborough M, Gordon SB, Whitty CJ, et al. Corticosteroids for bacterial meningitis in adults in sub-Saharan Africa. N Engl J Med. 2007; 357:2441–50. [PubMed: 18077809]
11. Molyneux EM, Walsh AL, Forsyth H, et al. Dexamethasone treatment in childhood bacterial meningitis in Malawi: a randomised controlled trial. Lancet. 2002; 360:211–8. [PubMed: 12133656]
12. Peltola H, Roine I, Fernandez J, et al. Adjuvant glycerol and/or dexamethasone to improve the outcomes of childhood bacterial meningitis: a prospective, randomized, double-blind, placebo-controlled trial. Clin Infect Dis. 2007; 45:1277–86. [PubMed: 17968821]
13. Nguyen TH, Tran TH, Thwaites G, et al. Dexamethasone in Vietnamese adolescents and adults with bacterial meningitis. N Engl J Med. 2007; 357:2431–40. [PubMed: 18077808]
14. Mai NT, Nga TV, Linh le D, et al. *Streptococcus suis* meningitis in adults in Vietnam. Clin Infect Dis. 2008; 46:659–67. [PubMed: 19413493]
15. Helsel DR. More than obvious: better methods for interpreting non-detect data. Environ Sci Technol. 2005; 39:419A–23A.
16. R Development Core Team: a language and environment for statistical computing. R Foundation for Statistical Computing; Available at: http://www.R-project.org. [Accessed 13 January 2009]
17. Coyle PK. Glucocorticoids in central nervous system bacterial infection. Arch Neurol. 1999; 56:796–801. [PubMed: 10404980]
18. Greenwood BM. Corticosteroids for acute bacterial meningitis. N Engl J Med. 2007; 357:2507–9. [PubMed: 18077815]
19. Kobayashi Y. Neutrophil infiltration and chemokines. Crit Rev Immunol. 2006; 26:307–16. [PubMed: 17073556]

Table 1
Baseline Characteristics and Outcome for Patients with Confirmed Bacterial Meningitis

	Patients with confirmed bacterial meningitis		Patients with CSF cytokine measurements	
Variable	**Dexamethasone group (*n* = 164)**	**Placebo group (*n* = 177)**	**Dexamethasone group (*n* = 88)**	**Placebo group (*n* = 107)**
Age, median years (IQR)	43 (32—58)	40 (28—51)	45.5 (33.5—59)	41 (29—52)
Male sex	115 (70.1)	136 (76.8)	64 (72.7)	81 (75.7)
Duration of illness, median day (IQR)	3 (2–6)	3 (2–5)	3 (2–5)	3 (2–5)
Findings at hospital admission				
Glascow coma score, median value (IQR)	13 (9–15)	13 (10–15)	12 (9–15)	13 (10–15)
Papilloedema	8 (4.9)	4 (2.3)	1 (1.1)	2 (1.9)
Cranial nerve palsy	16 (9.8)	19 (10.7)	7 (8.0)	9 (8.4)
Paresia	29 (17.7)	25 (14.1)	13 (14.8)	11 (10.3)
Positive test results				
CSF gram stain	106 (64.6)	116 (65.5)	59 (67.0)	71 (66.4)
CSF acridine orange stain stain	11 (6.7)	13 (7.3)	4 (4.5)	7 (6.5)
CSF culture	108 (65.9)	113 (63.8)	59 (67.0)	74 (69.2)
Blood culture	65 (39.6)	62 (35.0)	36 (40.9)	37 (34.6)
Latex antigen	23 (14.0)	27 (15.3)	13 (14.8)	15 (14.0)
PCR	128 (78.0)	134 (75.7)	74 (84.1)	81 (75.7)
Index of CSF inflammation, median value (IQR)				
White blood cell count, ×1000 cells/μL	3785 (1055–8150)	2808 (1125–7605)	3290 (1155–8580)	3285 (1510–8600)
Glucose level, mg/dL	20 (10–36)	23 (10–38)	19 (10–39)	20 (10–35)
Lactate level, mmol/L	9.1 (6.1–14.7)	10.1 (6.1–14.3)	11.6 (7.6–16.7)	11.0 (6.3–16.0)
Protein level, mg/L	253 (153–420)	244 (159–421)	290 (142–434)	249 (170–445)
Causative organism				
Streptococcus suis	72 (43.9)	65 (36.7)	42 (47.7)	41 (38.3)
Streptococcus pneumoniae	36 (22.0)	42 (23.7)	22 (25.0)	25 (23.4)
Neiseria meningitidis	14 (8.5)	14 (7.9)	8 (9.1)	7 (6.5)
Klebsiella pneumoniae	7 (4.3)	3 (1.7)	2 (2.3)	1 (0.9)
Escherichia.coli	6 (3.7)	3 (1.7)	4 (4.6)	1 (0.9)
Staphylococcus aureus	3 (1.8)	6 (3.4)	1 (1.1)	3 (2.8)
Hemophilus influenzea	2 (1.2)	6 (3.4)	0	4 (3.7)
Death	10 (6.1)	22 (12.5)	3 (3.4)	9 (8.4)

NOTE. Data are no. (%) of patients, unless otherwise indicated. CSF, cerebrospinal fluid; IQR, interquartile range; PCR, polymerase chain reaction.

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Table 2

Cerebrospinal Fluid (CSF) Opening Pressure, Leukocyte Count, and Biochemical Analysis Results for 341 Patients with Confirmed Bacterial Meningitis

	Dexamethasone group (*n* = 164)		Placebo group (*n* = 177)		*P* for unadjusted comparison of follow-up values	Adjusted comparison of follow-up values	
CSF parameter	**At baseline**	**At follow-up[a]**	**At baseline**	**At follow-up[b]**		**Estimate of dexamethasone effect (95% CI)**	***P***
Opening pressure							
No. of patients	128	135	144	143			
Median cm (IQR)	20 (14–34)	13.5 (9–18)	20 (15–28)	14 (11–20)	.04	−1.97 (−3.84 to −0.09)	.04
Leukocyte count							
No. of patients	164	153	176	164			
Median leukocytes per mm^3 (IQR)	3785 (1065–8135)	825 (260–2630)	2808 (1138–7433)	865 (400–1943)	.40	0.95 (0.70–1,28)[b]	.74
Glucose level							
No. of patients	164	154	177	164			
Median mg/dL (IQR)	20 (10–36)	63 (45–80)	23 (10–38)	44 (27–55)	<.001	1.61 (1.41–1,84)[b]	<.001
Ratio of CSF glucose to plasma glucose							
No. of patients	162	154	177	162			
Median % (IQR)	15 (8–31)	40 (32–48)	17 (9–30)	37 (24–49)	.02	4.36 (0.84–7.89)	.02
Lactate level							
No. of patients	145	134	156	148			
Median mmol/L (IQR)	11.60 (7.07–17.10)	4.20 (3.33–6.28)	10.80 (6.82–15.72)	4.65 (3.23–6.13)	.73	0.99 (0.88–1.10)[b]	.79
Protein level							
No. of patients	162	148	172	159			
Median mg/L (IQR)	253 (155–419)	108 (69–176)	244 (159–421)	108 (66–165)	.84	1.03 (0.88–1,20)[b]	.70

NOTE. CI, confidence interval; IQR, interquartile range.

[a]The 318 follow-up samples were obtained on days 1–6 after randomization. Sampling day was day 1 for 19 (6%) of patients, day 2 for 218 (69%), day 3 for 69 (22%), day 4 for 5 (2%), day 5 for 3 (1%), and day 6 for 4 (1%).

[b]Because data was log-transformed before analysis, this corresponds to an (antilog-transformed) multiplicative effect (eg, follow-up CSF glucose level for patients who received dexamethasone is estimated to be higher than that for patients who received placebo by a factor of 1.61).

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Table 3

Cerebrospinal Fluid (CSF) Cytokine Concentrations at Baseline and Follow-Up for 195 Patients with Confirmed Bacterial Meningitis

	Dexamethasone group (*n* = 88)		Placebo group (*n* = 107)		*P* for unadjusted comparison of follow-up values	Adjusted comparison of follow-up values	
CSF parameter	**Baseline**	**Follow-up[a]**	**Baseline**	**Follow-up[a]**		**Estimate of dexamethasone effect (95% CI)**	***P***
IL-6							
No. (%) of patients with detectable values	83 (94)	83 (94)	103 (96)	105 (98)			
Median $\log_{10}$ pg/mL (IQR) for patients with detectable values	4.97 (4.38–5.37	3.23 (2.43–4.19)	4.89 (4.5–5.5)	3.65 (2.8–4.33)	.01	−0.43 (−0.73 to −0.12)	.006
IL-8							
No. (%) of patients with detectable values	86 (98)	86 (97)	106 (99)	106 (99)			
Median $\log_{10}$ pg/mL (IQR) of patients with detectable values	4.33 (3.81–4.68)	3.24 (2.66–3.69)	4.3 (3.82–4.68)	3.45 (2.94–3.89)	.03	−0.21 (−0.41 to −0.008)	.04
IL-10							
No. (%) of patients with detectable values	83 (94)	43 (49)	106 (99)	74 (69)			
Median $\log_{10}$ pg/mL (IQR) of patients with detectable values	2.58 (2.06–3.09)	1.57 (1.19–1.94)	2.53 (2.04–3.06)	1.52 (1.25–1.87)	.02	−0.24 (−0.42 to −0.06)	.01
IL-12							
No. (%) of patients with detectable values	14 (16)	5 (6)	17 (16)	5 (5)			
Median $\log_{10}$ pg/mL (IQR) of patients with detectable values	1.33 (1.18–1.64)	1.27 (1.26–1.46)	1.3 (1.22–1.69)	1.20 (1.15–1.22)	.71[b]	0.08 (–0.26 to 0.42)	.64
IL-1β							
No. (%) of patients with detectable values	72 (82)	38 (43)	90 (84)	51 (48)			
Median $\log_{10}$ pg/mL (IQR) of patients with detectable values	2.63 (2.13–3.3)	1.77 (1.42–1.92)	2.44 (2.17–3.24)	1.87 (1.51–2.02)	.27[b]	−0.17 (−0.45 to 0.10)	.22
TNF-α							
No. (%) of patients with detectable values	64 (72)	12 (14)	73 (68)	11 (10)			
Median $\log_{10}$ pg/mL (IQR) of patients with detectable values	2.28 (1.49–3.16)	1.23 (1.16–1.39)	2.07 (1.57–3.35)	1.31 (1.17–1.51)	.50[b]	0.08 (−0.23 to 0.38)	.62

NOTE. IL, interleukin; TNF tumor necrosis factor.

[a] Samples were obtained on days 1–4 after randomization. Sampling day was day 1 for 6 (3%) of the patients, day 2 for 144 (74%), day 3 for 44 (23%), and day 4 for 1 (1%).

[b] Comparisons of the rates of detectable values by Fisher's exact test were also nonsignificant. *P* values were .76 (IL-12), .57 (IL-1*β*), and .51 (TNF-*α*).