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Concentrations of Doxycycline and Penicillin G in Sera and Cerebrospinal Fluid of Patients Treated for Neuroborreliosis

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Concentrations of doxycycline and penicillin G in serum and cerebrospinal fluid (CSF) were analyzed in 46 patients during treatment for neuroborreliosis. Twenty patients were treated intravenously with penicillin G at 3 g every 6 h (q6h), and 26 patients were treated orally with doxycycline at 200 mg q24h. All samples were collected on day 13 of treatment. The median concentrations of penicillin G in serum were 0.5, 37, and 5.6 μg/ml before and 1 and 3 h after drug administration, and that in CSF was 0.5 (range, 0.3 to 1.6) μg/ml after 2 to 3 h. The median concentrations of doxycycline in serum were 2.1, 6.1, and 4.7 μg/ml before and 2 and 6 h after drug administration, and that in CSF was 0.6 (range, 0.4 to 2.5) μg/ml after 4 h. All patients had concentrations of penicillin G or doxycycline in CSF above the lowest reported MICs of penicillin G (0.003 μg/ml) and doxycycline (0.12 μg/ml) for *Borrelia burgdorferi*. However, no patients had a drug concentration in CSF above the highest reported MIC of penicillin G (8 μg/ml), and only one had a drug concentration in CSF above the highest reported MIC of doxycycline (2 μg/ml), despite good clinical response to treatment. No treatment failure or relapse was observed during a 1-year follow-up, although one patient treated with penicillin G and one treated with doxycycline were retreated because of residual pain. The chosen dosages of penicillin G and doxycycline seem to give sufficient concentrations in serum and CSF for the treatment of neuroborreliosis.

Lyme borreliosis is a tick-borne spirochetosis with a varying clinical picture. The etiological agent, *Borrelia burgdorferi*, may spread locally in the skin or disseminate through the blood to other organs, including the nervous system (33). Several studies (24–26, 32, 35) have shown the efficacy of parenteral penicillin G (PcG) and cephalosporins in the treatment of neuroborreliosis, and other studies (3, 4, 13, 15) have shown that intravenously or orally administered doxycycline is an adequate alternative. The reported MIC of PcG for *B. burgdorferi* varies from 0.003 to 8 μg/ml (2, 12, 20, 28–30), and the MBC varies from 0.05 to >50 μg/ml (1, 2, 7, 10, 11, 20). The MIC of doxycycline varies from 0.12 to 2 μg/ml (12, 28), and the MBC varies from 1.6 to 6.4 μg/ml (1, 7, 11). In the present study, we analyzed the concentrations of PcG and doxycycline in serum and cerebrospinal fluid (CSF) from patients during treatment for neuroborreliosis and correlated the results with the clinical outcome of treatment. The observed concentrations were also compared with the previously reported MICs and MBCs of these antibiotics for *B. burgdorferi.*

MATERIALS AND METHODS

Patients. Forty-six patients with neuroborreliosis, aged 16 to 88 years (mean age, 48 years), were studied. The patients were diagnosed and treated at the Departments of Infectious Diseases, Danderyd Hospital and Roslagstull Hospital (now Huddinge Hospital), Stockholm, Sweden, from 1987 to 1990 and participated in a prospective, open, and randomized study comparing PcG and doxycycline for the treatment of neuroborreliosis (13). All patients gave informed consent. Neuroborreliosis was defined as clinical signs and symptoms of meningoradiculitis, encephalomyelitis, or chronic meningitis (36), with pleocytosis and elevated anti-*B. burgdorferi* antibody titers in serum (87%), CSF (84%), or both (74%). Seventy-four percent of the patients had intrathecal antibody production. CSF analysis showed between 6×10^6 and $1{,}190 \times 10^6$ (median, 106×10^6) leukocytes per liter. Spirochetes were cultured from the CSF of two patients. No antibodies were detected in their CSF, and one was seronegative. Clinical symptoms included headache, neck pain, malaise, vomiting, low-grade fever, neuromuscular pain, disturbed sensibility, ataxia, or paresis. Fifty percent of patients had preceding erythema migrans. For 14 days, 20 patients were treated intravenously with PcG at 3 g every 6 h (q6h), and 26 received doxycycline orally at 200 mg q24h. PcG was administrated as an approximately 5-min intravenous injection to 18 patients and as a 30-min infusion to 2 patients. All patients improved during treatment, and there were no significant differences between the two treatment groups in patient scoring of symptoms, CSF analysis, or serological follow-up for 1 year. There were no treatment failures, although one patient in each treatment group was retreated because of residual symptoms (13).

Serum and CSF samples. All samples were collected on day 13 of treatment. Serum samples were drawn before and 1, 3, and 5 h after the end of intravenous administration of PcG and before and 2, 4, and 6 h after oral administration of doxycycline. CSF samples were drawn 2 to 3 h after administration of PcG and 4 h after administration of doxycycline. The samples were stored at −70°C until analyzed.

Antibiotic concentrations. PcG concentrations in serum and CSF were measured with an agar diffusion assay (9). Paper discs 6 mm in diameter were used as diffusion centers. The agar medium was Antibiotic Medium No. 1 (Difco), pH 6.6. The test strain was *Bacillus cereus* ATCC 11778. The concentration was calculated from standard curves made from discs with known concentrations of PcG. The standard discs (Biodisk) contained standard dilutions of PcG made up in pooled human blood plasma. To validate the assay for PcG, five independent experiments were performed. The standard curves in the interval between 0.25 and 8 μg/ml gave a coefficient of correlation of 0.995 to 1.0 in all experiments. Spiked samples containing 0.5 μg of PcG per ml gave a mean value of 0.52 with a 95% confidence interval of 0.47 to 0.57 μg/ml. A spiked sample containing 4 μg/ml gave a mean of 4.5 μg/ml with a 95% confidence interval 3.8 to 5.2 μg/ml. The minimum measurable concentration was 0.3 μg/ml. Concentrations of doxycycline in serum and CSF were determined with a high-performance liquid chromatographic assay (6). Serum proteins were precipitated with an equal volume of 20% (wt/wt) trichloroacetic acid. CSF samples were passaged without pretreatment. The injection volumes were 100 μl for serum and 25 μl for CSF samples. The interassay coefficients of variation were 7.1% for a sample con-

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TABLE 1. Antibiotic concentrations in serum

Drug	Median (range) of concn in serum (μg/ml) at postadministration time (h) of:						
	0	1	2	3	4	5	6
PcG	0.5 (0.3–2.3)	37 (5–114)		5.6 (0.7–17)		0.9 (0.3–9.7)	
Doxycycline	2.1 (0.5–9.1)		6.1 (2.3–12.3)		5.5 (2.2–13.8)		4.7 (1.8–11.7)

taining doxycycline at 1.0 μg/ml and 3.8% for a sample containing doxycycline at 5.0 μg/ml. The lower limit of detection was 0.2 μg/ml.

Blood-brain barrier (BBB) function. Serum and CSF samples were analyzed for albumin content, and the BBB function was calculated as the CSF/serum albumin ratio (normal value, <0.008).

RESULTS

Antibiotic concentrations in serum and CSF. Concentrations of PcG and doxycycline in serum are shown in Table 1 and Fig. 1, and concentrations in CSF are shown in Fig. 2. The median concentration of PcG in CSF was 0.5 (mean, 0.7; range, 0.3 to 1.6) μg/ml after 2 to 3 h, corresponding to 2 to 10% (mean, 7%) of the simultaneous concentration in serum. Patients with a peak PcG concentration below the median of 37 μg/ml of serum had a mean concentration in CSF of 0.50 (range, 0.3 to 0.9) μg/ml compared with 0.80 (range, 0.4 to 1.6) μg/ml for patients with higher peak values in serum. The coefficient of correlation between the area under the concentration-time curve from 0 to 5 h for PcG in serum and the concentration of PcG in CSF was 0.577 ($P = 0.015$). The median concentration of doxycycline in CSF was 0.6 (mean, 0.8; range, 0.4 to 2.5) μg/ml after 4 h, corresponding to 3 to 36% (mean, 15%) of the simultaneous concentration in serum. Patients with a peak doxycycline concentration below the median of 6.1 μg/ml of serum had a mean concentration in CSF of 0.62 (range, 0.5 to 1.0) μg/ml compared with 0.97 (range, 0.4 to 2.5) μg/ml for patients with a higher concentration in serum. There was no significant correlation between the concentrations of doxycycline in serum and CSF.

Antibiotic concentrations in CSF in relation to BBB function. Eleven (55%) of 20 patients treated with PcG had a declining but still measurable BBB dysfunction at day 13 of treatment, with a mean CSF/serum albumin ratio of 0.015 (range, 0.009 to 0.023; upper normal value, 0.008). The mean concentration of PcG in CSF was 0.85 (range, 0.4 to 1.6) μg/ml for patients with BBB dysfunction compared with 0.40 (range, 0.3 to 0.6) μg/ml for patients without BBB dysfunction. The coefficient of correlation between the albumin ratio and the concentration of PcG in CSF was 0.492 ($P = 0.028$). Fourteen (54%) of 26 patients treated with doxycycline had a BBB dysfunction at day 13, with a mean CSF/serum albumin ratio of 0.016 (range, 0.009 to 0.035). The mean concentrations of doxycycline in CSF were similar in patients with BBB dysfunction (mean, 0.77 [range, 0.4 to 2.5] μg/ml) and those without BBB dysfunction (mean, 0.83 [range, 0.4 to 1.6] μg/ml). There was no significant correlation between the albumin ratio and the concentration of doxycycline in CSF.

Antibiotic concentrations and clinical outcome of treatment. Forty-three of 46 patients were followed up for 1 year, two PcG-treated patients were followed up for 2 weeks and 3 months, respectively, and one doxycycline-treated patient was followed up for 7 months. All patients improved during anti-

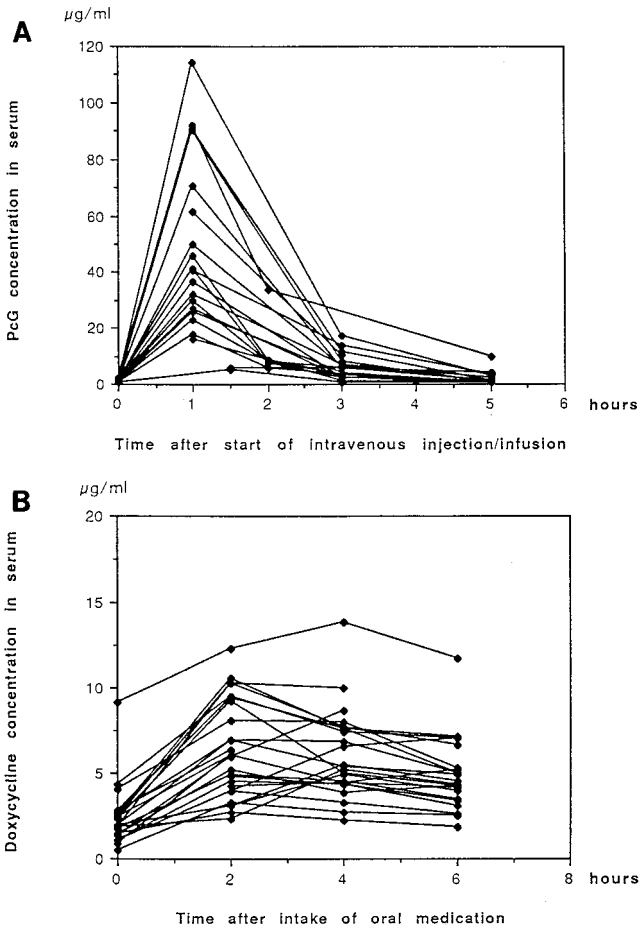

FIG. 1. (A) Concentration of PcG in serum after intravenous injection ($n = 18$) or infusion ($n = 2$) at day 13 of treatment. (B) Concentration of doxycycline in serum after oral intake ($n = 26$) at day 13 of treatment.

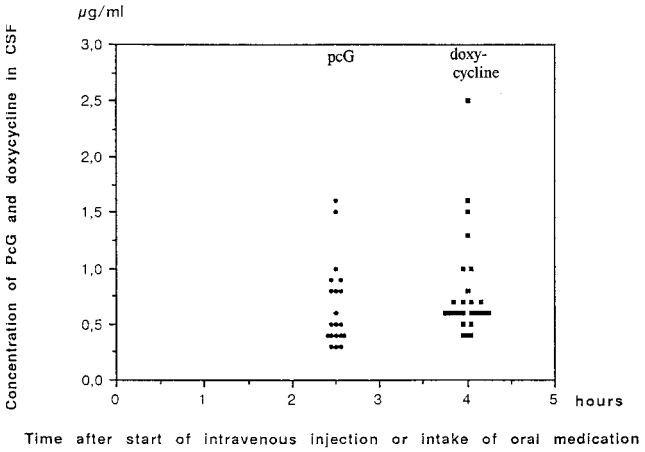

FIG. 2. Concentrations of PcG ($n = 20$) and doxycycline ($n = 26$) in CSF at day 13 of treatment.

biotic treatment and showed no symptoms of relapse during the follow-up. One patient treated with PcG and one treated with doxycycline were retreated with antibiotics because of residual pain after 4 months and 2 weeks, respectively. CSF analysis was normalized in the first patient and showed declining signs of inflammation in the second patient before retreatment, and no additional symptoms had appeared.

The number of patients with some residual symptoms at the end of treatment and during follow-up at 3, 6, 9, and 12 months did not differ significantly between patients with concentrations of PcG or doxycycline above and below the median concentrations in serum and CSF (Fisher exact test; $P > 0.05$ [not significant]). The patient that was retreated after initial treatment with PcG had concentrations of PcG in serum of 41 and 3.3 μg/ml 1 and 5 h after drug administration, respectively, and a concentration in CSF of 1.0 μg/ml after 2 h. The other retreated patient, initially treated with doxycycline, had concentrations of doxycycline in serum of 1.3 and 8.7 μg/ml before and 4 h after drug administration, respectively, and a concentration in CSF of 0.6 μg/ml after 4 h.

DISCUSSION

Concentrations of PcG and doxycycline in serum and CSF in the present study were similar to those reported previously (3, 4, 18, 19, 21, 22, 31, 37). BBB dysfunction correlated with a higher concentration of PcG, but not of doxycycline, in CSF. This may reflect a higher dependency on BBB damage for the penetration of PcG into CSF and the central nervous system compared with doxycycline, which is more lipophilic. It may also reflect an accumulation of doxycycline after 13 days of treatment. The concentrations of PcG and doxycycline in serum varied considerably between individuals (Fig. 1). The variation may, to some extent, be explained by the use of standard dosages of both drugs, regardless of patient age and weight. The concentration of doxycycline in serum is also influenced by differences in absorption after oral medication. It is possible that doxycycline carragenate, which is less dependent on gastric pH for its absorption than is the monohydrate used in this study, may give less variation in the concentration in serum (6).

Clinical improvement after antibiotic treatment did not correlate with peak levels of antibiotics in serum or concentrations in CSF. A slow improvement of paresis and sensory impairment after treatment was seen in a few patients with either high or low concentrations of PcG or doxycycline in serum and CSF, and the duration of residual symptoms seemed to depend on the severity of symptoms before treatment.

A comparison of the observed concentrations of PcG and doxycycline and the MIC-MBC for *B. burgdorferi* showed that several patients with excellent response to treatment had concentrations in CSF far below some of the reported MICs and MBCs, especially regarding PcG. Berger et al. (2) reported PcG MICs of 0.005 to 0.08 U/ml (0.003 to 0.05 μg/ml), Luft et al. (20) reported 0.1 to 1.0 μg/ml, Johnson et al. (12) reported 0.25 to 2.0 μg/ml, and Preac-Mursic et al. (30) reported 0.5 to 8 μg/ml. Correspondingly, the MBC of PcG varied between 0.08 and 2.5 U/ml (0.05 to 1.5 μg/ml) (2), 0.5 and 4.0 μg/ml (7), 0.05 and 6.4 μg/ml (10), 12.8 and 25.6 μg/ml (1), and 1 and >50 μg/ml (20). Johnson et al. (11) observed PcG MBCs of 0.05 μg/ml after 1 week and 6.4 μg/ml from 4 to 6 weeks of incubation. The poor in vitro performance of PcG may be explained by the instability of the compound in the culture medium (27) during incubation for 8 days (30) or even 6 weeks (10), caused by the slow growth of *B. burgdorferi*. Also, different investigators have used different definitions of MIC (2, 12, 29, 30), and there is no standardization of laboratory conditions for susceptibility testing of *B. burgdorferi*. Consequently, the clinical relevance of reported MICs and MBCs of PcG seems doubtful. Berger et al. (1) observed a good clinical response with penicillin V in two patients with erythema migrans, despite very high penicillin MBCs for *B. burgdorferi* isolated from the patients' own skin (12 and 26 μg/ml), concentrations that probably were not reached with the dosage given. Pfister et al. (25) reported concentrations of PcG in CSF below the MIC for *B. burgdorferi* in 10 patients successfully treated for neuroborreliosis. In the present study, all patients treated with PcG had concentrations in serum and CSF above the lowest reported MIC (0.003 μg/ml) of PcG. Eighteen (90%) of 20 patients had a concentration in serum above the highest reported MIC (8 μg/ml), but no patient reached this concentration in CSF. All patients had concentrations in serum and CSF above the lowest reported MBC of PcG (0.05 μg/ml), while only 6 (30%) of 20 and (0%) of 20 reached concentrations of >50 μg/ml (the highest reported MBC) in serum and CSF, respectively. It is possible that concentrations of PcG in CSF were higher for some patients at the beginning of treatment because of more pronounced BBB dysfunction at that time. Also, the strains of *B. burgdorferi* that caused infection in the present study may be different from the approximately 50 isolates previously used to measure antibiotic susceptibility (1, 2, 7, 10–12, 20, 28–30). Finally, although antibiotic treatment reduces the duration of disease and the risk for residual symptoms, it has been shown that neuroborreliosis, in most cases, heals without antibiotic treatment (16). Consequently, host factors are probably important in clearing the infection.

The reported MIC of doxycycline shows less variation (12, 28) than that of PcG, and all patients treated with doxycycline in the present study had a concentration in serum above the lowest and highest reported MICs of doxycycline (0.12 and 2.0 μg/ml). All patients had a concentration in CSF above 0.12 μg/ml, but only one patient (4%) had a concentration in CSF above 2.0 μg/ml. All patients and 13 (50%) of 26 patients reached the lowest and highest reported MBCs (1.6 to 6.4 μg/ml) of doxycycline in serum, respectively, and 23 (88%) of 26 and (0%) of 26 patients reached these concentrations in CSF, respectively. Laboratory experiments must be interpreted with caution, as evidenced by studies of erythromycin, which has very low MICs and MBCs in vitro (1, 2, 10, 11, 20, 28–30) but is less effective clinically and in animal models (10, 30, 34), and by studies of roxithromycin, which has low MICs and MBCs and was effective in a gerbil model but less effective in humans (7). Animal studies with PcG and doxycycline show divergent and sometimes contradictory results (8, 10, 11, 23, 30). The most important criterion when evaluating the clinical relevance of the MIC and MBC for *B. burgdorferi* is probably to verify the elimination of spirochetes by culture or PCR. However, both of these methods have a low sensitivity when CSF from patients with neuroborreliosis is analyzed (14, 17). The present study indicates that intravenous administration of PcG at 3 g q6h or oral administration of doxycycline at 200 mg q24h for 2 weeks gives sufficient concentrations in serum and CSF for the treatment of neuroborreliosis.

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