

Penicillin concentrations in serum and cerebrospinal fluid after intramuscular injection of aqueous procaine penicillin 0.6 MU with and without probenecid

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SUMMARY Paired specimens of cerebrospinal fluid and serum were taken from 21 patients to estimate penicillin concentrations two to three hours after the last dose of a course of 14-21 daily intramuscular injections of procaine penicillin 0.6 MU. Of 10 patients treated with procaine penicillin alone, eight had no detectable penicillin and two had sub-treponemicidal concentrations (<0.018 mg/l) in the cerebrospinal fluid. Of 11 patients treated with procaine penicillin as above and probenecid 2 g a day by mouth, three had no detectable penicillin, two had sub-treponemicidal concentrations, and six had treponemicidal concentrations of penicillin in the cerebrospinal fluid. All 21 patients had treponemicidal concentrations of penicillin in the serum. This dose of procaine penicillin alone or with probenecid is therefore not recommended for treating neurosyphilis.

Introduction

Aqueous procaine penicillin 0.6 MU daily up to a total of 6-12 MU is a recommended treatment regimen for syphilis.¹⁻³ All studies of this regimen have shown that a treponemicidal concentration of penicillin is only rarely achieved in cerebrospinal fluid sampled early in the course of treatment,^{4,7} which is therefore not recommended for treating neurosyphilis. With the addition of oral probenecid 2 g daily, however, one study showed that treponemicidal concentrations of penicillin were achieved in two of three patients on the second to fourth day of treatment.⁵

The aim of this study was to find out whether a treponemicidal concentration of penicillin is achieved in the cerebrospinal fluid by accumulation at the end of a course of daily intramuscular procaine penicillin 0.6 MU, and whether the addition of probenecid 2 g daily would guarantee a therapeutic concentration.

Patients and methods

We treated 21 patients infected with late treponemal disease with daily intramuscular aqueous procaine penicillin 0.6 MU for 14-21 days. In alternate patients probenecid 500 mg was given by mouth every six hours for the duration of the course of injections. Ten patients (seven men and three women) received penicillin alone and 11 patients (all men) received penicillin and probenecid. Specimens of cerebrospinal fluid and serum were taken two to three hours after the last dose of penicillin was given. Penicillin concentrations in the samples of serum and cerebrospinal fluid were measured using an agar well horizontal diffusion bioassay adapted from the method described by Sutherland and Rolinson.⁸ Bioactivity was expressed as equivalents of mg/l penicillin as the pure free acid.

Assay plates were prepared for use on the same day. A 150 ml volume of DST Agar (Oxoid), held at 56°C after autoclaving, was inoculated with 1.5 ml 24 hour glucose broth culture of *Sarcina lutea* (NCTC 8340) before being poured into a 25 × 25 cm plastic bioassay dish (Nunc Intermed, Gibco, Paisley, Scotland), giving an agar layer 2.5 mm

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deep. Wells 7 mm in diameter (volume about 0.1 ml) were cut in the agar, whose surface was then dried by incubation at 37°C for 15 minutes. The wells were filled to the top with standard penicillin solutions and the test samples; prediffusion was allowed to occur for one hour at room temperature before the plates were incubated at 30°C for 18 hours. The diameters of the inhibition zones (two for each well) were measured on a Leebrook zone reader.

PENICILLIN STANDARDS

Immediately before each assay solutions containing 0.006, 0.0125, 0.025, 0.05, 0.10, 0.50, and 1.0 mg/l penicillin as the free acid were prepared from Laboratory Standard Reference potassium benzyl penicillin (assigned potency 99.8%) (Gist-Brocades, West Byfleet, Surrey) that had been dried to constant weight. These standard solutions were made up in aqueous 0.05 mol/l phosphate buffer, pH 7.0.

VALIDATION OF BIOASSAY

The lower limit of sensitivity was 0.010 mg/l. (Use of Oxoid antibiotic medium No 2 was found to reduce the sensitivity of the assay to half that obtained with DST Agar.)

The potency of the penicillin standards was confirmed by using them to determine the MIC for the Oxford staphylococcus by tube dilution: the observed result was 0.025 mg/l (the expected concentration was 0.015-0.030 mg/l). The precision of the assay was assessed by calculating the coefficient of variation for three internal controls—that is, 0.0125, 0.025, and 0.05 mg/l. Interassay variations on four different plates were 6%, 9%, and 26% respectively; the corresponding 95% confidence limits were 0.011-0.014, 0.021-0.029 and 0.025-0.075 mg/l. Intraassay variation was not assessed formally, but each clinical sample was assayed in duplicate (on the same plate) and the mean of four inhibition zone diameters was read against the standard curve.

STORAGE AND ASSAY OF CLINICAL SAMPLES

All specimens of serum and cerebrospinal fluid were frozen to -20°C immediately they were received in the laboratory and stored at this temperature until assay. All the samples from the 21 patients were assayed together in one batch. This meant that specimens had been stored for variable periods before assay. The results, however, did not show a trend towards lower penicillin concentrations in samples that had been stored the longest. The actual concentrations obtained corresponded closely to those reported by other workers in serum and cerebrospinal fluid from patients given the same

dosage of penicillin when the assay had been completed within two or three days of collection.⁵

Samples of cerebrospinal fluid were assayed undiluted. Serum samples were assayed at 1/10, 1/30 and 1/90 dilutions in the same aqueous buffer used for preparation of the penicillin standards. (The serum penicillin concentration was taken as the mean of the three concentrations calculated from these dilutions.)

Results

The table shows that all 21 patients had treponemidal concentrations of penicillin in the serum, the

TABLE Concentrations of penicillin in serum and cerebrospinal fluid from 21 patients

Case No	Treatment regimen	Course (days)	Concentrations (mg/l) in:	
			Serum	CSF
1	Procaine penicillin 0.6 MU daily intramuscularly	14	1.08	0.010
2		21	1.29	ND
3		14	0.49	ND
4		14	0.76	ND
5		14	0.78	0.012
6		14	0.98	ND
7		14	0.99	ND
8		14	1.92	ND
9		14	0.76	ND
10		14	1.31	ND
11	Procaine penicillin 0.6 MU daily intramuscularly plus probenecid 2 g daily by mouth	14	0.8	ND
12		14	1.56	0.025
13		21	2.48	0.039
14		14	1.23	ND
15		14	1.20	ND
16		14	1.39	0.010
17		14	1.55	0.024
18		14	1.36	0.014
19		14	1.60	0.024
20		14	3.68	0.027
21		14	1.70	0.026

CSF = cerebrospinal fluid; ND = not detectable.

lowest concentration being 0.49 mg/l. Of the 10 patients who were given procaine penicillin alone, eight had no detectable penicillin in the cerebrospinal fluid and the two others had concentrations still below the minimal treponemidal level of 0.018 mg/l. Of the 11 patients treated with procaine penicillin and probenecid, three had no detectable penicillin in the cerebrospinal fluid, two had sub-treponemidal concentrations, and six had treponemidal concentrations. None of the specimens of cerebrospinal fluid had abnormal cell counts, protein, or positive results to the rapid plasma reagin (RPR) and *Treponema pallidum* haemagglutination (TPH) tests for syphilis. There was no correlation between the patients' body weights and the concentrations of penicillin in the serum or cerebrospinal fluid. The figure shows the

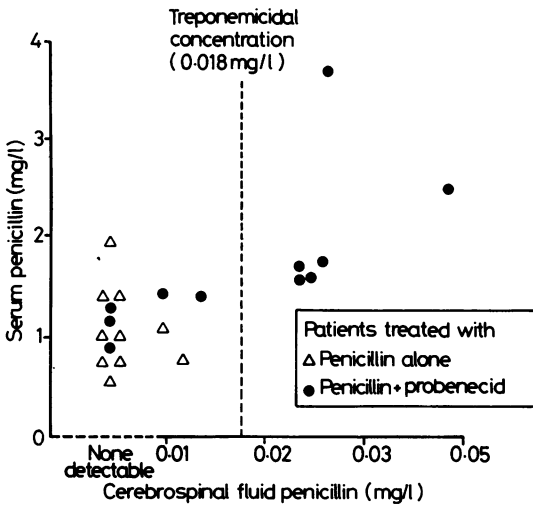


FIGURE Relation of concentrations of penicillin in cerebrospinal fluid and serum from 21 patients.

relation of the concentrations in the cerebrospinal fluid to those in the serum. In patients in whom penicillin was found in the cerebrospinal fluid the mean ratio of cerebrospinal fluid to serum was 1.25%.

Discussion

The study reported here shows that daily intramuscular aqueous procaine penicillin 0.6 MU alone does not achieve a treponemicidal concentration of penicillin in the cerebrospinal fluid⁴⁻⁷ even after 21 days' treatment, and the addition of oral probenecid 2 g daily cannot be relied on to produce treponemicidal concentrations in the cerebrospinal fluid by

the end of a 14 day course of treatment.⁵ Although two patients who received a 21 day course of procaine penicillin and probenecid did achieve treponemicidal concentrations of penicillin in the cerebrospinal fluid, the number was too small for any conclusion to be made.

In the treatment of neurosyphilis it is logical to choose a regimen which would consistently achieve a treponemicidal concentration of penicillin in the cerebrospinal fluid, such as intramuscular procaine penicillin 1.8 MU with oral probenecid 2 g daily, which can be given to outpatients,⁹ or intravenous benzyl penicillin 10 MU every six hours, a procedure which requires admission to hospital.⁶

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