Single-dose benzathine penicillin in infants at risk of congenital syphilis — results of a randomised study

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Objective. To determine the efficacy of single-dose benzathine penicillin G in infants at high risk of congenital syphilis.

Design. Randomised study comparing benzathine penicillin with no therapy.

Setting. Peninsula Maternal and Neonatal Service, Cape Town.

Subjects. Asymptomatic infants born to mothers with untreated syphilis whose VDRL titre was 32 or more.

Outcome measures. The number of cases of congenital syphilis was determined by results of IgM Western blots and follow-up VDRL titres.

Results and conclusions. Of 8 patients followed up in the non-treatment group, 4 had congenital syphilis while 0/11 had the disease ($P = 0.035$) in the group receiving benzathine penicillin. Although the exact failure rate is unknown, benzathine penicillin is effective in preventing symptomatic congenital syphilis when administered to high-risk newborns.

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The management of the asymptomatic newborn whose mother has untreated or incompletely treated syphilis in pregnancy remains an important paediatric problem. In South Africa, 7 - 12% of women attending antenatal clinics have positive serological tests for syphilis[1-3] and therapy during pregnancy is frequently inadequate or non-existent.[3] Consequently large numbers of infants require evaluation and management for potential congenital syphilis.

The optimal therapy for these apparently healthy infants, however, is still being debated. In 1988, the Centers for Disease Control (CDC) modified existing guidelines so that asymptomatic patients would receive a minimum of 10 days of treatment with either procaine penicillin or aqueous penicillin G.[4] Until recently it was our policy at Groote Schuur Hospital to follow the World Health Organisation recommendations for patients without evidence of neurosyphilis, i.e. well infants with normal cerebrospinal fluid levels. Management included serial follow-up and monitoring of serological tests or, when that was not possible, a single dose of benzathine penicillin.[5,6]

Although benzathine penicillin has been widely used in asymptomatic infants of mothers with syphilis, there have been few definitive reports of its efficacy and, indeed, isolated cases of treatment failure have been noted.[7]

We carried out a study in which infants at risk for congenital syphilis were randomised to receive either a single dose of benzathine penicillin or no therapy. We determined outcome by means of clinical assessment and serological testing (Venereal Disease Research Laboratory (VDRL) and IgM Western blotting) at birth and follow-up.

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Methods

Background

At the time of commencing the study, the hospital policy was similar to that proposed by the WHO;[6] i.e. patients with clinical signs compatible with congenital syphilis were treated, while apparently healthy infants were monitored and serial VDRL titres were determined. Those with a fourfold increase in titre or whose serum did not revert to negative by 6 months were regarded as infected and treated with procaine penicillin 50 000 U/kg/day for 10 days.

It has not been our policy to perform routine lumbar punctures on clinically healthy infants. Several studies have indicated that the investigation is of little value in this group of patients.[8,9]

We did not screen patients or mothers for HIV infection. When the study was carried out, the prevalence of the disease was low among antenatal clinic attenders at the Peninsula Maternal and Neonatal Service in the Western Cape — approximately 1:1 000 (A Keen — personal communication).

Patient selection

We studied asymptomatic infants at high risk of congenital syphilis. We regarded infants in this category as those born to mothers with untreated syphilis, i.e. positive VDRL and treponema pallidum haemagglutination (TPHA) tests, with a VDRL titre of 32 or more.[10] Infants were regarded as asymptomatic at birth if: (*i*) there were no clinical signs of congenital syphilis; (*ii*) the radiographs of the long bones were normal; and (*iii*) the rheumatoid factor latex test was negative at birth.[11]

Mothers were interviewed soon after birth; only infants who could be followed up were included in the study, which commenced within 72 hours of birth. Infants were randomised to receive either no treatment or a single injection of benzathine penicillin 50 000 U/kg.

Follow-up was arranged at 6-weekly intervals after birth. At these times the patients were examined clinically by one of the authors (MR) and the VDRL test was repeated. If the latter was negative at 3 months, the infant was discharged; those with positive tests and declining titres were followed up until the test became negative or features of congenital

syphilis developed. Patients whose VDRL titre increased fourfold or whose VDRL was still positive at 6 months were regarded as having congenital syphilis and treated with procaine penicillin 50 000 U/kg for 10 days.

Permission for the study was obtained from the Ethics and Research Committee of the University of Cape Town and informed consent was obtained from the mothers.

Western blotting

Serum collected at birth and follow-up visits was aliquoted, frozen and stored at –80°C. We performed batched testing and the results were used to assist the diagnosis of congenital syphilis. Whole serum was tested at birth, but IgM fractions were used for the follow-up samples. The latter procedure was followed because we noted a 10% false-positive rate when whole serum was tested in older infants outside the neonatal period.[12] The false positives were eliminated by testing of IgM fractions and were presumably brought about by the presence of rheumatoid factors (detected in 8.5% of healthy infants aged 1 - 4 months).[13]

The immunoblotting method has been described elsewhere.[12] A suspension of *Treponema pallidum* antigens was subjected to sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE) with a 12% separating gel. Electrophoretic transfer of low molecular weight standards (Biorad) and treponemal antigens to nitrocellulose paper was then carried out. Strips were reacted with test serum at a dilution of 1:100 followed by a mu chain-specific anti-IgM monoclonal antibody. The blots were developed with a biotinylated rabbit anti-mouse antibody (Dako, Glostrup, Denmark) and streptavidin-biotin horseradish peroxidase complex (Dako). Substrate was diaminobenzene with cobalt chloride and hydrogen peroxide.

IgM serum fractions were prepared using an ion exchange column (Quick Sep, System II; Isolab). The IgM concentration in the eluate was determined and the blots carried out at the same IgM level as was present in the original sample.

We regarded IgM reactivity to treponemal antigens with any of the following molecular weights as a positive blot: 72, 47, 45, 37, 30, 17 and 15 kDa.[12]

Outcome and statistics

The diagnosis of congenital syphilis was based on a fourfold rise in VDRL titre (and positive TPHA) during follow-up or a persistently positive VDRL and TPHA after 6 months. In addition, patients with a positive Western blot for IgM antibody to *T. pallidum* were regarded as having congenital syphilis.[12]

Patients who defaulted on follow-up in the non-treatment group were assumed to be uninfected; this would have the effect of reducing the apparent efficacy of treatment.

The initial study design was to enter 30 infants per group. However, the presence of 4 congenitally infected infants in the non-treatment group prompted an analysis of the data after 22 patients had been enrolled; a significant difference was found and the study terminated.

Results

Altogether, 22 infants were studied. There were no statistically significant differences between the two groups with regard to birth weight (combined mean 2 941 g), gestational age (combined mean 39.8 weeks), maternal VDRL titre at delivery (median 128 in both groups) and infant VDRL titre at birth (median 8 in both groups).

Non-treatment group

Of 10 patients enrolled, there were 4 with congenital syphilis. In 3 cases the diagnosis was based on a fourfold (or greater) rise in VDRL titre at follow-up. In addition, these 3 patients developed physical signs suggestive of congenital syphilis at follow-up, having been completely asymptomatic at birth (Table I). Western blots from these 3 patients had IgM reactivity to the 47dDa antigen (Fig. 1). Two had reactive blots at birth (Fig. 1, lanes c and d) before any other evidence of disease, either clinically or serologically. The other patient demonstrated IgM reactivity (on both whole serum and IgM fractions) by 10 weeks (Fig. 1, lanes e and f). The fourth patient with congenital syphilis had a strongly positive Western blot for IgM at birth in the absence of physical signs (Fig. 1, lane g).

Table I. Details of infants diagnosed with congenital syphilis on the basis of a 4-fold rise in VDRL titre at follow-up or a positive IgM Western blot

	Infant data						
		Serial VDRL titre					
Maternal VDRL titre	Infant treated*	Birth	6 wks	12 wks	24 wks	IgM Western blot	Clinical features at follow-up
32	N	16	4	1 024		+ve (at 12 wks)	Periosteal reaction (at 12 wks)
64	N	2	1	> 2 048		+ve (birth)	Skin rash (at 12 wks)
> 2 048	N	4	128			+ve (birth)	Periosteal reaction (at 6 wks)
1 024	N	8	ND			+ve (birth)	ND
> 2 048	Y	512	8	1	–ve	+ve (birth)	Nil (at 24 wks)
512	Y	32	2	–ve		+ve (birth)	Nil (at 12 wks)

* Benzathine penicillin 50 000 U/kg at birth.
Y = yes; N = no; ND = not done.

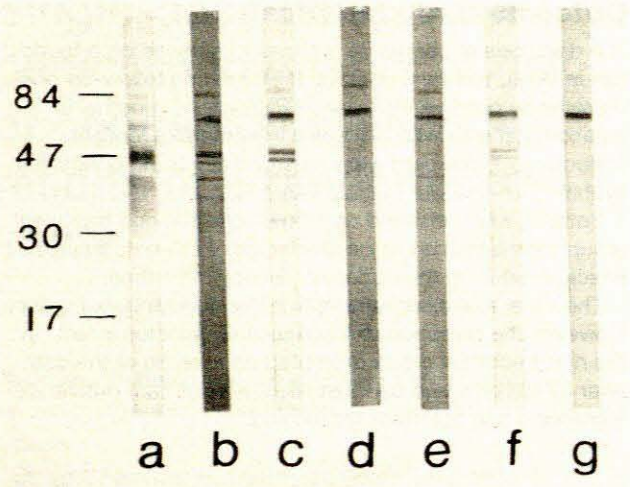

Fig. 1. Reaction profiles in Western blots. Lane a — profile with pooled IgG from *T. pallidum*-infected rabbit; lane b — IgM reactivity in serum from control infant with symptomatic congenital syphilis; lanes c and d — IgM profile at birth in 2 untreated, infected infants; lanes e and f — IgM reactivity at birth and 3 months in an untreated, infected infant; lane g — IgM pattern at birth in an untreated infant lost to follow-up.

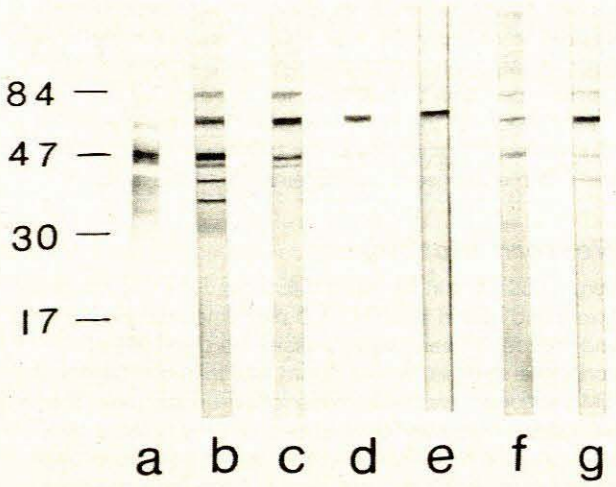

Fig. 2. Reactivity in Western blots. Lane a — pooled IgG from *T. pallidum*-infected rabbit; lane b — IgM profile with pooled serum from adults with recent syphilis; lanes c, d and e — IgM reactivity in sera from treated infant at birth, 3 months and 18 months; lanes f and g — IgM pattern obtained with sera from a treated infant at birth and 3 months.

There were 4 infants who were not infected. Their VDRL titres had declined to zero by 4 months and they remained healthy during this time. These infants had negative immunoblots at birth and, in addition, showed no IgM reactivity to the relevant treponemal antigens when column fractions from the follow-up sera were tested.

In 2 cases follow-up was incomplete; in 1 case the VDRL titre had declined to 1 by 4 months and the infant (who remained asymptomatic throughout) could not be traced for repeat VDRL. The other patient died of diarrhoeal disease at the age of 2 months; follow-up serology was not obtained. Immunoblots from these 2 infants were negative. As discussed above, for the purposes of analysis these 2 cases were regarded as not infected.

Treatment group

Twelve patients were enrolled in this group. Eleven were followed up and the VDRL titres declined to zero in the absence of any signs of disease. The remaining infant died of a congenital heart defect (hypoplastic left heart syndrome) aged 2 weeks; there was no evidence of congenital infection at postmortem. This case was excluded from the statistical analysis.

Two of the 12 treated infants had an IgM response to the 47 kDa antigen at birth (Fig. 2, lanes c and f). Following treatment with a single dose of benzathine penicillin, the IgM was no longer demonstrable in either patient by 3 months of age (Fig. 2, lanes d, e and g). None of the remaining 10 patients had IgM reactivity with the relevant treponemal antigens at birth or follow-up.

When the two groups were compared in respect of outcome, there were significantly fewer cases of congenital syphilis in the treatment group (0/11 compared with 4/10; $P = 0.035$ (Fisher's exact test)). As a result of this, it was decided to terminate the study.

Discussion

Overall, the use of benzathine penicillin resulted in a significant decline in the number of cases of congenital syphilis in the high-risk group studied. There were no treatment failures although the number of patients who were indisputably infected was small. (Only 2 patients in the treatment group had positive Western blots.) Once benzathine penicillin had been demonstrated to have a therapeutic effect the study was discontinued.

Of the 3 untreated infants who developed disease at follow-up, 2 had positive Western blots at birth; this sensitivity of 66% at birth was similar to that of 75% reported in a previous study among infants with normal long-bone radiographs.[12] It should be noted that in the present study all cases were IgM RF latex test negative at birth.

We regarded the 2 newborns in the treatment group with reactive blots as infected; we have not observed false-positive blots at birth, either in the untreated infants in this study who were followed up or among 21 neonates of seropositive mothers described in a previous publication.[12] A single dose of benzathine penicillin appeared to have been effective in these 2 patients as there was no clinical development of disease and the Western blots had reverted to negative by 3 months.

A recently published study compared the efficacy of benzathine penicillin with procaine penicillin in 152 newborns whose mothers had untreated or incompletely treated syphilis during pregnancy.[14] There were no treatment failures reported with either regimen. However, relatively few infants were included whose mothers were untreated (8 in the benzathine group and 11 recipients of procaine penicillin). Altogether, 3 infants had positive FTA-ABS IgM tests at birth and were therefore probably infected.[15]

It is clear that reported experience with benzathine penicillin in high-risk asymptomatic patients with congenital

syphilis is scarce. However, there have been few reports of treatment failure over the years in spite of widespread use of benzathine penicillin. The occurrence of disease following therapy has been noted after use of both benzathine and crystalline penicillin.[7,16,17] It is likely that treponemes in environments such as the cerebrospinal fluid and aqueous humour are able to persist because of poor penetration of the antibiotic into these body compartments.[18,19] The fact that unsuccessful treatment with benzathine penicillin appears to be rare may be related to the observation that cerebrospinal fluid involvement in asymptomatic newborns is uncommon.[8,9]

To determine the risk of late-onset disease after benzathine penicillin treatment accurately requires the evaluation of a relatively large cohort of high-risk patients. This is important because the failure rate appears to be low (possibly of the same order as that observed in adults with primary or secondary syphilis, i.e. 3 - 5%).[20] These data are essential to determine whether the longer duration of therapy, i.e. the 10 - 14 days proposed by the CDC (and now recommended by the South African Department of Health) is necessary.

In conclusion, the present study has confirmed that benzathine penicillin G is effective in preventing symptomatic congenital syphilis when administered to high-risk newborns. The risk of treatment failure, however, requires further evaluation.

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