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Oral amoxycillin, an alternative treatment for neurosyphilis

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SUMMARY A serum amoxycillin concentration of 0·11 g/l was established as being treponemicidal in a rabbit model with orchitis. Seventeen patients treated with amoxycillin 2 g by mouth three times a day plus 500 mg probenecid twice a day attained treponemicidal CSF amoxycillin concentrations. Thus amoxycillin by mouth offers an effective alternative method for treating patients with neurosyphilis.

Introduction

Penicillin G has been the treatment of choice for syphilis for over four decades.[1] However, because spirochaetaemia may ensue during the earliest stages of the infection and treponemes can invade the central nervous system before any clinical signs or symptoms develop,[2-4] debate continues about the preparation of penicillin necessary to cure neurosyphilis reliably. Conventional treatment has been shown to give inadequate concentrations of penicillin to eliminate *Treponema pallidum* from the cerebrospinal fluid (CSF),[5-9] and *T pallidum* has been isolated from the CSF of patients treated with benzathine penicillin.[5] Some clinicians faced with the prospect of their patients developing neurosyphilis 10 to 25 years later have therefore recommended admitting the patients to hospital and administering high doses of penicillin intravenously to ensure that adequate amounts cross the blood brain barrier.[10]

Clearly, an oral therapeutic antibiotic regimen would be a welcome alternative. We here describe studies establishing treponemicidal concentrations of amoxycillin, and show that concentrations attained in the CSF of 17 patients treated with 6 g amoxycillin plus 2 g probenecid were adequate to cure neurosyphilis.

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Patients, materials, and methods

ANIMALS

Because *T pallidum* cannot be grown in vitro, in vivo studies in animals have been used to establish the minimum concentrations of antibiotics necessary to kill the organism (minimum bactericidal concentrations).[11] We used virgin male New Zealand albino rabbits (weighing 3-5 kg) and only used rabbits that gave negative results to the rapid plasma reagin (RPR) test (Macro Vue Card; Hynson, Westcott and Dunning, Division of Becton Dickinson, Baltimore, Maryland, USA) and the fluorescent treponemal antibody absorbed (FTA-ABS) test using fluorescein labelled goat anti-rabbit IgG (Sylvania, Grand Island, New York, USA). The rabbits were housed in separate cages and the ambient temperature was maintained at 62-70°F. In conducting the research described in this report, we followed the *Guide for laboratory animal facilities and care* promulgated by the committee of the guide for laboratory animal facilities and care of the Institute of Laboratory Animal Resources, National Academy of Sciences, the National Research Council.

RABBIT MODEL TO ESTIMATE MINIMUM BACTERICIDAL CONCENTRATION

The rabbits, which were not pretreated with corticosteroids, were infected by testicular inoculation.[5] The treponemes were either freshly harvested from testicular extracts of infected rabbits, suspended in basal reducing medium consisting of phosphate buffered saline (pH 7·1) with 5% bovine plasma, sodium thioglycolate, cysteine, glutathine, and sodium pyruvate, or else thawed after being stored in liquid nitrogen (in reducing medium plus 20% dimethyl sulphoxide). The rabbits were injected intratesticularly with 10^6 to 10^7 motile treponemes. Clinical orchitis developed in infected rabbits in 11 to 14 days, and infection with syphilis was confirmed by seroconversion in both the RPR and FTA-ABS tests.

Amoxycillin 0·03 mg/kg, 0·1 mg/kg, 0·5 mg/kg, 1·0 mg/kg or 3·0 mg/kg (Beecham Laboratories, Bristol, Tennessee, USA) or penicillin 25 mg/kg (Pfizer Laboratory, New York, USA) were injected intravenously over three minutes into the marginal ear every eight hours for 10 days. Four control rabbits were injected with normal saline sodium chloride (0·9%). The rabbits were examined daily for 24 days, then killed by intravenous injections of pentobarbital, and their testes were removed aseptically. Any surviving spirochaetes were extracted by mincing the testes in 50 ml basal media. The testicular extract was examined by dark field microscopy within 40 minutes of killing the rabbit, and 1 ml of the supernatant was then injected into each testicle of a second rabbit (a test of cure rabbit). The second (test of cure) rabbits were held for 40 days and killed in the same way. Serum was obtained for RPR and FTA-ABS tests before each rabbit was injected and when it was killed.

PATIENTS

Seventeen patients, 15 men and two women, with latent syphilis (positive FTA-ABS test results but no signs or symptoms of primary or secondary syphilis) were treated for 14 days with amoxycillin 2 g by mouth three times a day plus probenecid 500 mg by mouth twice a day. Before administering the third or fourth dose we obtained samples of CSF and serum to measure amoxycillin concentrations.

STRAIN OF *T PALLIDUM*

The Nichol's strain of *T pallidum* was used as the test organism.[12] It had been maintained for over 35 years in liquid nitrogen and by innoculation of rabbits in the laboratory of the department of immunology, Walter Reed Army Institute of Research.

SERUM AND CSF CONCENTRATIONS OF AMOXYCILLIN

Rabbit serum was obtained by cardiac puncture 30 minutes after the fourth dose of amoxycillin had been injected. We pipetted 20 µl of the serum or CSF and a known concentration of amoxycyillin diluted in normal rabbit serum on to 5 mm discs (Schleicher and Schwell, 74051 Keene, New Hampshire, USA). The discs were placed on antibiotic medium No 5 (Difco Laboratories, Detroit, Michigan, USA) in which spores of *Bacillus subtilis* (ATCC6633) were suspended. (Test plates were kept at 40°C for no longer than seven days.) The zones of inhibition were measured. The amoxycillin concentrations were estimated from a graphic plot (linear regression analysis, $\log_{10}$) of the known antibiotic concentrations.[13]

Serum and CSF amoxycillin concentrations in patients were estimated in the same way.

Results

STUDIES OF RABBITS

All the rabbits treated with $\geqslant 0{\cdot}1$ mg/kg amoxycillin were cured (table I). Orchitis resolved clinically, no spirochaetes were seen on examination of the testicular extract by dark field microscopy, and the second group of rabbits (the test of cure group) injected with a testicular extract from the first group failed to develop any antibodies (seroconvert). Only two of four rabbits treated with 0·03 mg/kg amoxycillin were cured. Thus, a serum concentration of $\geqslant 0{\cdot}11$ mg/l amoxycillin was found to be curative.

STUDIES IN MAN

All 17 patients with normal cell numbers and concentrations of protein and glucose in their CSF before treatment achieved CSF amoxycillin concentrations greater than the minimum treponemicidal concentrations after receiving the third or fourth dose (table II). One patient developed mild transient diarrhoea but was able to complete the 14 day course. Amoxycillin was discontinued in another patient after seven days because of the development of a generalised morbilliform skin rash.

Discussion

Because *T pallidum* cannot be cultivated in vitro, animal models have traditionally been used to estimate the MIC of antibiotics against this organism. Eagle *et al* used a rabbit testicular syphiloma model and, by examining saline extracts for motile treponemes, estimated that a serum penicillin concentration of 0·005-0·01 mg/l was necessary to cure syphilis.[11] We used a similar assay to measure the MIC of amoxycillin for *T pallidum*.

TABLE I *Results of serological tests and dark field examinations of testicular extracts from 16 syphilitic rabbits treated with amoxycillin and an equal number of rabbits (test of cure rabbits) injected with testicular extracts from the treated rabbits*

			Treated rabbits				*Test of cure rabbits*		
Rabbit No	*Antibiotic*	*Dose (mg/kg)*	*Mean serum Amoxycillin concentration (mg/l)**	*RPR*	*FTA-ABS*	*Dark field†*	*RPR*	*FTA-ABS*	*Dark field†*
1	Amoxycillin	3·0	1·90	+	+	−	−	−	−
2	Amoxycillin	3·0		+	+	−	−	−	−
3	Amoxycillin	1·0	0·71	+	+	−	−	−	−
4	Amoxycillin	0·5	0·42	+	+	−	−	−	−
5	Amoxycillin	0·1	0·11	+	+	−	−	−	−
6	Amoxycillin	0·1		+	+	−	−	−	−
7	Amoxycillin	0·1		+	+	−	−	−	−
8	Amoxycillin	0·03	0·03	+	+	−	−	−	−
9	Amoxycillin	0·03		+	+	−	−	−	−
10	Amoxycillin	0·03		+	+	−	+	+	−
11	Amoxycillin	0·03		+	+	−	+	+	−
12	Penicillin	25		+	+	−	−	−	−
13	Penicillin	25		+	+	−	−	−	−
14	0·9% Sodium chloride			+	+	+	+	+	+
15	0·9% Sodium chloride			+	+	+	+	+	+
16	0·9% Sodium chloride			+	+	+	+	+	+

*Mean concentration achieved in each treatment group.
†Testicular extracts of the treated rabbits were examined by dark field microscopy after 24 days and in the test of cure rabbits after 40 days.
+ = positive, − = negative test results.

TABLE II *Serum and CSF amoxycillin concentrations achieved in 17 patients treated with amoxycillin and probenecid by mouth*

	*Amoxycillin concentrations (mg/l)**	
Case No	*Serum*	*Cerebrospinal fluid*
1	NT	0·54
2	4·32	0·36
3	3·04	1·56
4	24·32	0·76
5	24·32	0·76
6	5·76	0·72
7	4·56	0·32
8	6·08	0·32
9	8·96	1·12
10	0·76	0·36
11	6·08	0·32
12	2·28	0·32
13	12·16	0·32
14	12·16	0·32
15	NT	0·77
16	4·48	0·32
17	24·32	0·32

*Simultaneous blood and CSF concentrations measured before the third or fourth dose (CSF amoxycillin concentration $\geq$0·11 mg/l was established as being curative.)
NT = not tested.

Because of the sampling error associated with aspirating syphilomas, we elected to reinject testicular extracts into a second cohort of rabbits as a test of cure. The testicular extract from one rabbit treated with 0·03 mg/kg amoxycillin (0·03 mg/l serum concentration) gave negative results on dark field examination, but successfully infected a second rabbit.

Serum amoxycillin concentrations greater than 0·11 g/l cured syphilitic orchitis in rabbits. Faber *et al*,[15] using an in vitro *T pallidum* immobilisation assay, found a concentration of 0·07 g/l to be treponemicidal. Thus the results from the two studies agreed.

How often neurosyphilis develops after primary syphilis is not certain. Spirochaetaemia can occur before clinical evidence of disease develops, as shown by the transfer of syphilis by the transfusion of blood obtained from asymptomatic donors (before they developed a chancre).[1 2 4] Invasion of the central nervous system can result whenever spirochaetaemia develops, and neurological involvement has been documented in 8-40% of patients with secondary syphilis.[14] *T pallidum* has been isolated from patients with fewer than 5 lymphocytes/ml and normal concentrations of protein and glucose in the CSF.[2] Thus either treated patients must be closely followed up or a treatment that reliably cures invasion of the central nervous system must be used.

Benzathine penicillin G (total dose 7·2 MIU given intramuscularly in weekly doses of 2·4 MIU for three successive weeks) has long been considered to be adequate treatment for neurosyphilis. Troubling reports of failures have, however, continuously appeared.[5-9] Aqueous procaine penicillin G[7] and aqueous intravenous penicillin have therefore been recommended by various workers.[3] A reliable oral antibiotic regimen offers obvious advantages.

All 17 of our patients developed CSF amoxycillin concentrations greater than 0·3 mg/l, which ensured

that their treatment provided adequate antibiotic concentrations. These findings agree with those of Faber *et al*[15] and Clumeck *et al.*[16] The CSF amoxycillin concentrations in the study reported here were generally lower than those described by Faber *et al*,[15] who estimated CSF concentrations after seven days of treatment of six patients with raised cell counts in the CSF.

As with any oral antibiotic regimen, patient compliance is necessary. Thus selection of patients by the treating doctor is important. In our experience discussing the potential outcome of inadequately treated neurosyphilis is often sufficient to convince most patients of the necessity to complete this course of treatment.

A third reliable alternative for treating neuro-Syphilis is procaine penicillin G 6000 000 IU to 2·4 MIU given intramuscularly once a day plus probenecid 500 mg by mouth six times a day for 14 days.[7]

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