CLINICAL RESEARCH

Spectinomycin resistant gonococci

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Abstract

A study was conducted examining the properties of 10 clinical isolates of spectinomycin resistant gonococci from patients attending clinics at St Mary's and St Thomas's Hospitals, London. All of the isolates produced β -lactamase and contained plasmids of 2.6, 4.4, and 24.5 megadaltons and required proline for growth. None produced aminoglycoside modifying enzymes. Resistance to spectinomycin was transferred from some of the isolates by transformation but at a much lower frequency than resistance to streptomycin. The isolates from St Mary's Hospital were detected after therapy with spectinomycin, whereas those from St Thomas's Hospital were not. Four recent non- β -lactamase producing gonococci isolated at St Mary's Hospital and two isolated at St Thomas's Hospital also were not related to use of spectinomycin.

Introduction

Spectinomycin is one of the antibiotics recommended for uncomplicated anogenital infection caused by penicillinase producing strains of *Neisseria gonorrhoeae* (PPNG). In 1981 Ashford *et al* reported spectinomycin resistance in a PPNG isolated from a man previously treated with spectinomycin.¹ Shortly afterwards Easmon *et al* reported a similar case.² Twenty two such strains have now been isolated from patients attending the sexually transmitted disease clinics at St Mary's

and St Thomas's Hospitals, London. We report our observations on the genetic control and mechanism of resistance to spectinomycin in the first 10 strains.

Materials and methods

At St Mary's Hospital gonococci were isolated on GC base agar (Difco) supplemented with IsoVitalX (1%) and with vancomycin, colistin, amphotericin B, and trimethoprim. At St Thomas's Hospital gonococci were isolated on Columbia Agar (Oxoid CM331) supplemented with 10% saponin lysed horse blood and with vancomycin, colistin, nystatin, and trimethoprim. Gonococci were identified by their Gram stain, oxidase reaction, and either by their ability to utilise glucose but not maltose, sucrose, or lactose or by a fluorescent antibody method. Auxotyping was done by Catlin's method³ and β -lactamase was detected by the chromogenic cephalosporin (nitrocefin) test.⁴ Organisms were screened for susceptibility to spectinomycin by an agar diffusion test with a 100 μ g spectinomycin disc: isolates that gave an inhibition zone of more than 20 mm diameter were regarded as sensitive. None of the resistant isolates gave a zone of inhibition. Minimum inhibitory concentrations of antibiotics were determined by an agar dilution technique on Oxoid Diagnostic Sensitivity Test Agar (CM261) supplemented with 5% saponin lysed horse blood (lysed blood agar); in addition, IsoVitalX (1%) was added to the medium at St Mary's Hospital. The inoculum size was about 10^3 colony forming units. Plasmids were analysed by a rapid screening technique, the samples being electrophoresed in 0.6% agarose and stained with ethidium bromide.⁵

For transformation experiments crude deoxyribonucleic acid was extracted from the donor strains with sodium dodecyl sulphate and heat. Donor DNA was diluted 1/10 before mixing with recipient, which was maintained in a colony type 2 (p^{++}). The rapid method of Janik *et al* was used⁶ and transformants selected singly on GC agar base containing IsoVitalX (1%) and one of the following antibiotics: penicillin (1 mg/l), streptomycin (100 mg/l), or spectinomycin (100 mg/l). Transformation frequencies were determined after three to five days of incubation at 36°C in 7% carbon dioxide. Cotransformation was determined for streptomycin resistant and spectinomycin resistant transformants on GC agar base containing IsoVitalX (1%) and the appropriate antibiotic.

For attempts to detect spectinomycin modifying enzymes the cellulose phosphate paper binding method was used as described,⁷ except that suspensions of gonococci were made from overnight growth on a lysed blood agar plate. Controls consisted of tubes containing no antibiotic and a set of tubes in which the cell extract was from a strain of *Escherichia coli* that produces AAD(3'')⁽⁹⁾ which adenylates both streptomycin and spectinomycin.

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For attempts to detect inactivation of streptomycin or spectinomycin by phosphorylation we used the same reaction mixture as for adenylation but with non-radioactive adenosine triphosphate and with the volumes doubled. After incubation the residual antibiotic concentration was measured by bioassay in diagnostic sensitivity test agar with *E coli* NCTC 10418 as the indicator organism.

Results

Table I lists the numbers of spectinomycin resistant PPNG compared with the numbers of PPNG and the total numbers of gonococci isolated at St Mary's and St Thomas's Hospitals between January 1981 and the end of August 1983. In addition, six spectinomycin resistant non-PPNG were isolated during 1983 (four at St Mary's, two at St Thomas's); such strains may have been overlooked in earlier years because not all gonococci were then screened for resistance to spectinomycin.

All the patients from whom spectinomycin resistant PPNG were isolated at St Mary's Hospital, but none of those from St Thomas's Hospital, had been treated with spectinomycin. All the strains carried plasmids of 2.6, 4.4, and 24.5 megadaltons and all required proline for growth (table II). Two of the patients seen at St Mary's Hospital were sexual contacts but resistance to spectinomycin apparently developed after treatment as two independent events. There was no obvious epidemiological link between any of the other patients seen at St Mary's. Two patients seen at St Thomas's Hospital were sexual contacts.

TABLE I—Frequency of isolation of spectinomycin resistant penicillinase producing strains of *Neisseria gonorrhoeae* (PPNG)

	St Mary's Hospital			St Thomas's Hospital		
	No of gonococci	No of PPNG	No of spectinomycin resistant PPNG	No of gonococci	No of PPNG	No of spectinomycin resistant PPNG
1981	2992	57	1	2095	61	0
1982	3403	212	6	2319	102	2
1983*	2084	174	12	1685	65	1

*January to August inclusive.

TABLE II—Properties of spectinomycin resistant gonococci

Strain	Plasmids	Auxotype	Minimum inhibitory concentration of spectinomycin (mg/l)
<i>St Mary's Hospital isolates</i>			
E77603	2.6, 4.4, 24.5	pro ⁻	1024 (NA)*
H93470	2.6, 4.4, 24.5	pro ⁻	512 (16)
H91732	2.6, 4.4, 24.5	pro ⁻	1024 (NA)
E83900	2.6, 4.4, 24.5	pro ⁻	512 (16)
H94985	2.6, 4.4, 24.5	pro ⁻	R (NA)
H91550	2.6, 4.4, 24.5	pro ⁻	256 (NA)
E84162	2.6, 4.4, 24.5	pro ⁻	512 (16)
E84799	2.6, 4.4, 24.5	pro ⁻	512 (16)
<i>St Thomas's Hospital isolates</i>			
858259	2.6, 4.4, 24.5	pro ⁻	1024
943948	2.6, 4.4, 24.5	pro ⁻	1024

*Results in parentheses show susceptibility to spectinomycin for pretreatment isolates. (NA = Strain not available for testing.)
R = Resistant to spectinomycin by disc test.

TABLE III—Transformation of resistance to spectinomycin, streptomycin, and penicillin

Recipient	Donor	Total viable count	No of transformants resistant to:			Frequency of transformation to streptomycin resistance (%)	No of streptomycin resistant transformants that were spectinomycin resistant/No tested	Frequency of transformation to spectinomycin resistance (%)	No of spectinomycin resistant transformants that were streptomycin resistant/No tested
			Streptomycin	Spectinomycin	Penicillin				
HI	E77603	2.1 × 10 ⁷	6.6 × 10 ³	< 1	< 1	0.03	3/50		
	H93470	2.2 × 10 ⁷	1.3 × 10 ³	< 1	< 1	0.50	0/50		
	H91732	4.0 × 10 ⁹	1.0 × 10 ⁵	0.2 × 10	< 1	0.003	0/50	5 × 10 ⁻⁸	1/8
	E83900	3.2 × 10 ⁹	2.8 × 10 ⁶	0.5 × 10	< 1	0.09	1/50	2 × 10 ⁻⁷	1/4
	H94985	4.8 × 10 ⁸	4.2 × 10 ⁵	4.0 × 10	< 1	0.09	0/50	8 × 10 ⁻⁶	1/30
	H91550	2.7 × 10 ⁹	1.2 × 10 ⁶	5.0 × 10	< 1	0.04	0/49	2 × 10 ⁻⁶	1/40
	E84162	9.3 × 10 ⁸	8.5 × 10 ⁵	< 1	< 1	0.09	0/50		
	E84799	1.1 × 10 ⁸	3.3 × 10 ³	< 1	< 1	0.03	0/50		
140	E77603	2.1 × 10 ⁸	3.9 × 10 ⁵	< 1	< 1	0.19	2/96		
	E77603	2.4 × 10 ⁸	3.4 × 10 ⁶	< 1	< 1	0.14	4/96		

The minimum inhibitory concentrations of spectinomycin were in the range 256 to 1024 mg/l for the resistant isolates (table II) and were 16 mg/l for the pretreatment isolates. Isolates before and after treatment did not differ in their sensitivity to other agents: minimum inhibitory concentrations were > 1024 mg/l for streptomycin, 4 mg/l for gentamicin, 2 mg/l for chloramphenicol, 1 mg/l for tetracycline, 0.5-1.0 mg/l for fusidic acid, 0.12-0.25 mg/l for erythromycin, 0.03-0.12 mg/l for rifampicin, and 0.25-0.50 mg/l for ceftioxin.

DNA from spectinomycin resistant PPNG transformed resistance to spectinomycin to a sensitive recipient at a frequency of less than one transformant per 10⁸ colony forming units of the recipient. A similar, low transformation rate was seen for penicillin, and this contrasted with the much higher rate seen for streptomycin—one transformant per 10³ colony forming units of the recipient (table III).

When streptomycin resistant transformants were tested for spectinomycin resistance cotransformation was found to be between 0% and 6% (table III). Only a small number of spectinomycin resistant transformants were available for study, and the frequency of cotransformation of streptomycin resistance was between 2.5% and 25% (table III). Although these rates were lower than would be expected for closely linked antibiotics, the rate was probably still higher than would occur spontaneously in the gonococcus.

No enzymes that adenylated, phosphorylated, or acetylated spectinomycin or streptomycin were detected in any of the gonococci.

Discussion

The mechanism of the resistance to spectinomycin and streptomycin in these gonococci was not clear. It seemed unlikely to have been caused by enzymatic inactivation of the compounds, since we failed to detect such enzymes. Furthermore, the only enzyme that modifies spectinomycin so far detected in Gram negative organisms, AAD(3'')(9), also modifies streptomycin to which the pretreatment spectinomycin sensitive isolates were resistant. We believe that the resistance was unlikely to be the result of poor uptake of aminocyclitols, since the degree of resistance was high and sensitivity to gentamicin, another aminocyclitol, was unaffected. Thus we conclude that the resistance was due to a change in the ribosome. Even here, however, there are problems: firstly, our strains were not quite as resistant as previously reported ribosomally resistant gonococci^{8,9}; and, secondly, the hypersensitivity to fusidic acid which has been reported for spectinomycin resistant mutants (Sox; quoted by Sparling *et al*¹⁰) was not apparent in our strains.

The spectinomycin resistance did not seem to be plasmid determined, at least directly, since, apart from the cryptic small plasmid, no plasmids other than those associated with penicillin resistance and its transfer were detected. This finding supports our conclusion that aminoglycoside modifying enzymes were not implicated, since these enzymes are mostly plasmid determined.

Apart from the two pairs of sexual partners there were no clear epidemiological links between the strains, though all required proline and had the same 2.6, 4.4, plus 24.5 megadalton plasmid content and the erythromycin and tetracycline sensitivity pattern characteristic of β -lactamase producers from Asia.¹¹ An interesting phenomenon, which we cannot explain,

was that the spectinomycin resistant PPNG strains from St Mary's Hospital were all isolated after treatment with spectinomycin, whereas those from St Thomas's Hospital were not.

We do not know whether these strains first acquired the ability to produce β -lactamase and then mutated to spectinomycin resistance or whether they represented gonococci with an abnormally high propensity to mutate to spectinomycin resistance that have acquired the 4.4 plus 24.5 megadalton plasmids for β -lactamase production and transfer. Wider screening of gonococci for resistance to spectinomycin may help to answer this question. Our experience so far is that of over 3000 non- β -lactamase producing gonococci tested by the disc diffusion method only six (four from St Mary's Hospital and two from St Thomas's Hospital) were resistant to spectinomycin even though spectinomycin was introduced as first line treatment for gonorrhoea in heterosexuals at St Mary's Hospital in January 1983. In contrast to the situation often seen with spectinomycin resistant PPNG, all six of these spectinomycin resistant non-PPNG were isolated before spectinomycin treatment.

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Role of spleen in immune response to polyvalent pneumococcal vaccine

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Abstract

The immune response of lymphocytes to subcutaneously administered pneumococcal vaccine was studied in five patients without spleens and in five healthy subjects. Seven days after immunisation circulating B cells synthesising IgG antipneumococcal capsular polysaccharides (anti-PCP) appeared in both groups. Twenty one days after vaccination this B cell population had disappeared and a B cell subset which secreted IgM and IgG anti-PCP in the presence of pokeweed mitogen was detected in the normal but not in the splenectomised subjects.

In the splenectomised group polyclonal IgM synthesis induced by pokeweed mitogen was defective.

It was concluded that the early events of the immune response to PCP may be mediated by lymph nodes but that, later, the spleen acquires a central role in producing lymphocyte subsets capable of synthesising specific antibodies and that this might explain the increased sensitivity of splenectomised subjects to pneumococcal infection.

Introduction

Despite the use of antibiotics pneumococcal infections continue to cause illness and death throughout the world.^{1 2} Patients who do not have spleens are particularly at risk³ and many clinical trials have assessed the efficacy of polyvalent pneumococcal vaccines in these patients. Some studies have shown the effectiveness of vaccination in affording protective concentrations of specific antibodies,⁴⁻⁷ while others have shown a limited serological response.^{8 9}

Experimentally the cellular immune events that occur after immunisation with pneumococcal capsular polysaccharide (PCP) depend on the spleen for the response to the T cell independent antigen.^{10 11} We have investigated at different intervals after vaccination in vitro IgM and IgG anti-PCP synthesis by peripheral blood lymphocytes in five splenectomised and five normal subjects to see if the same applies in man.

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