*Epidemiol. Infect.* (1991), **106**, 283–288
*Printed in Great Britain*



# Effect of penicillin and chloramphenicol on the growth and endotoxin release by *N. meningitidis*

M. C. MELLADO[1], R. RODRÍGUEZ-CONTRERAS*[1], A. MARISCAL[1], J. D. LUNA[2], M. DELGADO RODRÍGUEZ[1] AND R. GALVEZ-VARGAS[1]

[1] *Departments of Preventive Medicine and* [2] *Biostatistics, School of Medicine, University of Granada, Spain*

(*Accepted 4 October 1990*)

## SUMMARY

The action of two antibiotics, penicillin and chloramphenicol, on bacterial growth and endotoxin liberation was studied in 18 strains of *Neissevia meningitidis* isolated from blood and CSF of patients with meningococcal infections. The antibiotics were administered both separately and in combined form in doses equivalent to 1 MIC and 100 MIC. Penicillin was found to produce a faster and more intense bactericidal effect than chloramphenicol during the first hour, whereas at 12 h these differences were not significant. This could explain the initial worsening observed in some infected patients when large doses of penicillin are administered. An increased liberation of endotoxin after adding penicillin was observed in six of the strains studied, whereas the remaining 12 did not show significant increases. The six strains (belonging to serogroup B) were known to have an enhanced capacity for spontaneous endotoxin liberation.

## INTRODUCTION

Several authors [1–3] have shown that the administration of antimicrobials with a rapid bactericidal action in cases of Gram-negative bacterial septicaemia can cause massive bacterial lysis and provoke endotoxic shock. In the case of meningococcal infections, *in vitro* studies have shown an increase in endotoxin liberation after the administration of large doses of penicillin [4–6]. In addition, it has been noted that high doses of penicillin have caused an initial apparent worsening of the clinical condition of some patients, more frequently in those with septicaemia that in those with meningitis due to massive *in vivo* lysis of microorganisms [1, 2, 7].

In this study 18 strains of *Neissevia meningitidis*, isolated from patients with septicaemia or meningitis, were studied in order to observe the *in vitro* effects of penicillin and chloramphenicol.

* For correspondence and requests for reprints: Prof. Rodríguez-Contreras, Dpto. de Medicina Preventiva, Facultad de Medicina, Avenida de Madrid 11, 18012 – Granada, Spain.

## MATERIAL AND METHODS

### *Bacterial strains*

Eighteen strains of *N. meningitidis*, all epidemiologically unrelated, were isolated by the Service of Microbiology of the University of Granada Hospital (Spain) from cerebrospinal fluid (CSF) and blood samples of patients admitted with meningococcal infections.

### *Technique*

The technique developed by Andersen and Solberg [7] was used. First, the minimal inhibitory concentrations (MIC) were calculated for the strains studied. Bacteria were cultured in a protein-free liquid medium (medium 199) at 37 °C. The meningococci previously cultured for 18 h on blood agar at 37 °C in an atmosphere of 5% $CO_2$ were washed three times in 0·9% saline. A bacterial inoculum was prepared at an optical density of 0·6 nm, the equivalent of $10^8$–$10^9$ colony forming units (c.f.u.)/ml. Seven hundred and fifty microlitres of the bacterial suspension were transferred to test-tubes containing 20 ml of the medium 199. These were shaken and then incubated at 37 °C for 4 h. Afterwards, penicillin and chloramphenicol were added, separately or in combined form at final concentrations equalling 1 MIC and 100 MIC. The cultures were observed for 12 h after the addition of the antibiotics. Samples were taken at 1 h and 12 h for the determination of endotoxins. These samples were filtered through 0·45 $\mu$m Millipore filters to prevent passage of bacteria, and then kept at −70 °C until the endotoxin determinations were done. The number of c.f.u. for the two time periods studied (1 and 12 h) were calculated by dilution and plate count.

### *Endotoxin assay*

The technique of Harthug and colleagues [8] was used. The details are given in the accompanying paper [9].

### *Statistical analyses*

The analysis of variance using a crossed design of four factors was used. The comparison by pairs was done using Tukey's procedure.

## RESULTS

### *Antibiotic action on bacterial growth*

The mean c.f.u. values for all the strains after the application of antibiotics are shown in Table 1. At 1 h there are significant differences in the colony counts between those preparations treated with penicillin alone and with chloramphenicol alone, as well as between chloramphenicol and the combination of penicillin and chloramphenicol, both at 1 MIC and 100 MIC. At 12 h these differences were found only for the 1 MIC dose. This suggests that penicillin and the combination of penicillin and chloramphenicol reduce the number of viable microorganisms more sharply than chloramphenicol does alone at minimal as well as maximal concentrations.

Table 1. *Effect of antibiotics upon bacterial growth of* N. meningitidis

| Time | Dose | Antibiotic | No. c.f.u. $\times 10^6$ | Comparison | Texp* | *P* |
|---|---|---|---|---|---|---|
| 1 h | 1 MIC | P | 9·01 | P vs. C | 10·52 | < 0·001 |
| | | C | 37·43 | P vs. P+C | 0·03 | ns |
| | | P+C | 8·71 | C vs. P+C | 10·49 | < 0·001 |
| | 100 MIC | P | 0·36 | P vs. C | 4·16 | < 0·001 |
| | | C | 3·77 | P vs. P+C | 1·15 | ns |
| | | P+C | 0·05 | C vs. P+C | 5·35 | < 0·001 |
| 12 h | 1 MIC | P | 0·19 | P vs. C | 4·55 | < 0·001 |
| | | C | 3·60 | P vs. P+C | 0·42 | ns |
| | | P+C | 0·39 | C vs. P+C | 4·13 | < 0·001 |
| | 100 MIC | P | 0·03 | P vs. C | 1·71 | < 0·001 |
| | | C | 0·54 | P vs. P+C | 0·31 | ns |
| | | P+C | 0·01 | C vs. P+C | 2·03 | < 0·001 |

P, penicillin; C, chloramphenicol.
At time 0 the mean number of c.f.u. was $1·96 \times 10^8$.
* Texp, Value of the Student-*t*.

Table 2. *Endotoxin liberation of* N. meningitidis *under the influence of antibiotics*

| Time | Dose | Antibiotic | Endotoxin (ng/ml) | Comparison | *P* |
|---|---|---|---|---|---|
| 1 h | 1 MIC | P | 0·455 | P vs. C | < 0·01 |
| | | C | 0·426 | P vs. P+C | ns |
| | | P+C | 0·448 | C vs. P+C | ns |
| | 100 MIC | P | 0·510 | P vs. C | < 0·01 |
| | | C | 0·458 | P vs. P+C | ns |
| | | P+C | 0·483 | C vs. P+C | ns |
| 12 h | 1 MIC | P | 0·423 | P vs. C | ns |
| | | C | 0·428 | P vs. P+C | ns |
| | | P+C | 0·446 | C vs. P+C | ns |
| | 100 MIC | P | 0·406 | P vs. C | ns |
| | | C | 0·427 | P vs. P+C | ns |
| | | P+C | 0·470 | C vs. P+C | ns |

P, penicillin; C, chloramphenicol.
The mean spontaneous endotoxin liberation was 0·408 ng/ml.

*Antibiotic effect on endotoxin liberation*

The mean values of the amount of endotoxin liberated by each strain, measured at 1 h and 12 h, are presented in Table 2. Penicillin, and penicillin and chloramphenicol produce a greater liberation than chloramphenicol alone ($P < 0·01$) 1 h after its application at both 1 MIC and 100 MIC. Despite these significant differences, the increase in endotoxin liberation induced by those antibiotics is only 10–15% higher. At 12 h no significant differences exist between the action of antibiotics either separate or combined.

The maximum liberation of endotoxin was 1 h after the addition of penicillin at a dose of 100 MIC. However, analysis of endotoxin liberation after the

https://doi.org/10.1017/S0950268800048421 Published online by Cambridge University Press

Table 3. *Strains in which endotoxin liberation increased after the addition of penicillin*

| Strain | Spontaneous endotoxin liberation (ng/ml) | Endotoxin liberation after penicillin mean* (ng/ml) | Increase (%) |
|---|---|---|---|
| E2 | 0·922 | 1·452 | 57 |
| E11 | 0·388 | 1·296 | 234 |
| E13 | 0·346 | 0·803 | 132 |
| E14 | 0·387 | 0·563 | 45 |
| E27 | 0·709 | 0·889 | 25 |
| E0371 | 0·610 | 0·759 | 24 |

* Mean liberation between 1 h and 12 h after adding the antibiotics.
All the strains were serogroup B.

administration of antibiotics to each of the 18 strains studied showed that the quantity of endotoxin increased substantially in only six of the strains (Table 3). The increase ranged between 24 and 234 %. These same six strains were previously shown to be substantial spontaneous liberators of endotoxin (i.e. without antibiotics), whereas the endotoxin-liberating capacity of the remaining 12 strains was not appreciably modified by the application of antibiotics (Table 4). Furthermore, with some of these latter strains, the addition of antibiotics decreased the amount of endotoxin liberated. This decrease was greater for strains belonging to serogroup A (mean −35·2 %) than for those belonging to serogroup B (−8·2 %).

All strains producing an increased endotoxin liberation after adding antibiotics belong to serogroup B. No differences in behaviour could be attributed to isolation site (CSF or blood).

## DISCUSSION

Bearing in mind the observations on the clinical effects of the administration of antibiotics in cases of meningococcal disease, we studied the effects of penicillin and chloramphenicol at final concentrations of 1 MIC and 100 MIC, the latter being the most usual dose in clinical use. Bacterial survival and endotoxic activity were measured both 1 h and 12 h after the administration of antibiotics in order to approach clinical reality, as the severity of the patient's condition usually peaks during the early hours of antimicrobial treatment.

Penicillin and the penicillin-chloramphenicol combination, in concentrations of 1 or 100 MIC, have a more intense bactericidal action than chloramphenicol alone ($P < 0·01$) during the first hour. At 12 h, these differences were observed only at the lower dose; at the higher dose the effect of the different antibiotic regimens was very similar.

In general endotoxin liberation after the addition of the antibiotics is significantly greater compared to spontaneous liberation from these strains. Time studies suggest that very large doses (100 MIC) of penicillin produce the greatest liberation of endotoxin in the first hour supporting this view that certain patients may suffer an apparent deterioration shortly after receiving high doses of this

Table 4. *Strains in which endotoxin liberation did not increase after the addition of penicillin*

| Strain | Spontaneous endotoxin liberation (ng/ml) | Endotoxin liberation after penicillin mean* (ng/ml) | % of variation |
|---|---|---|---|
| E8 | 0·109 | 0·104 | −4·6 |
| E12 | 0·264 | 0·248 | −6·1 |
| E19 | 0·116 | 0·107 | −7·8 |
| E21 | 0·117 | 0·125 | 6·8 |
| E24 | 0·132 | 0·109 | −17·4 |
| E25 | 0·182 | 0·105 | −42·3 |
| E68/69 | 0·140 | 0·139 | −0·7 |
| E0666 | 0·418 | 0·414 | −1·0 |
| E0487 | 0·230 | 0·206 | −10·4 |
| E0564 | 0·204 | 0·108 | −47·1 |
| E02027 | 0·747 | 0·502 | −32·8 |
| E01654 | 0·218 | 0·108 | −50·5 |

* Mean liberation between 1 h and 12 h after adding penicillin.

The strains E0487, E0564, E02027, and E01654 were serogroup A, and the remaining were serogroup B.

antibiotic [7]. Further it is known that laboratory mice injected with liberating strains of meningococci respond very poorly to penicillin treatment [5].

Our findings are in keeping with those of Shenep and colleagues [10] who have shown that chloramphenicol does not induce endotoxin liberation in rabbits infected with an *Eschevichia coli* strain, whereas gentamicin and moxalactam do. Their conclusion that endotoxin liberation *in vivo* during therapy for sepsis caused by Gram-negative bacteria depends on the type of antimicrobial given might also be applied to our *in vitro* results. Goto and Nakamura [11] in their *in vitro* study analysed a strain of *E. coli* and its endotoxin liberation under the influence of bactericidal antibiotics (aminobenzylpenicillin and streptomycin sulphate) and the bacteriostatic antibiotic tetracycline hydrochloride. Tetracycline produced less endotoxin liberation than bactericidal antibiotics, probably due to less destructive action. Bactericidal antibiotics increased endotoxin liberation three-fold after 6 h incubation, but do not induce endotoxin liberation after more prolonged times of incubation [11].

Analysis of endotoxin liberation from each of the 18 strains separately revealed that the increased production was confined to six strains only (Table 3). These six strains also released endotoxin spontaneously in quantity. The remaining 12 strains made little response to treatment, suggesting that antibiotic induced endotoxin liberation is associated with a greater spontaneous liberating capacity.

Our results agree with the *in vitro* findings of Andersen and Solberg [6] who studied three strains of *N. meningitidis* and who, however, reported much higher endotoxin liberation in induction while most of our methods were taken from theirs, the endotoxin quantification methods used by them differed from ours.

We suggest that bactericidal antibiotics can induce endotoxin liberation, especially in strains which also exhibit similar spontaneous activity and this phenomenon may be linked to clinical features in some cases.

## REFERENCES

1. Elin RJ, Wolff SM. Biology and endotoxin. Ann Rev Med Selected Trop Clin Sci 1977; **27**: 127–41.
2. Hopkin DAB. Top-rapid destruction of gram-negative organism. Lancet 1977; ii: 603–4.
3. Hopkin DAB. Frapper fort ou frapper doucement: a gram-negative dilemma. Lancet 1978; ii: 1193–4.
4. Andersen BM, Solberg O. The endotoxin liberating effects of antibiotics on meningococci in vitro. Acta Pathol Microbiol Scand (B) 1980; **88**: 231–6.
5. Andersen BM, Solberg O. The virulence of endotoxin liberating and non-liberating variants of *N. meningitidis* in mice. Effect of benzylpenicillin treatment. In: Proceedings of the VIIIth International Congress of Infectious and Parasitic Diseases, Stockholm (Sweden), 1982: 7–11.
6. Andersen BM, Solberg O. Effect of benzylpenicillin in mice infected with endotoxin-liberating and non-liberating variant strains of *N. meningitidis*. Scand J Infect Dis 1984; **16**: 256–66.
7. Andersen BM, Solberg O. Endotoxin liberation and invasivity of *N. meningitidis*. Scand J Infect Dis 1984; **16**: 247–52.
8. Harthug S, Bjorkatn B, Osterud B. Quantitation of endotoxin in blood from patients with meningococcal disease using a limulus lysate in combination with chromogenic substrate. Infection 1983; **11**: 192–5.
9. Mellado MC, Rodríguez-Contreras R, Fernandez-Crehuet M, Lopez-Gigosos R, Delgado Rodriguez M, Galvez-Vargas R. Endotoxin liberation by strains of *N. meningitidis* isolated from patients and healthy carriers. Epidemiol Infect 1991; **106**: 289–295.
10. Shenep JL, Barton RP, Mogan KA. Role of antibiotic class in the rate of liberation of endotoxin during therapy for experimental gram-negative bacterial sepsis. J Infect Dis 1985; **151**: 1012–18.
11. Goto H, Nakamura S. Liberation of endotoxin from *Escherichia coli* by the addition of antibiotics. Japan J Exp Med 1980; **50**: 35–43.