JAC

MICs of rifampicin and chloramphenicol for mucoid *Pseudomonas aeruginosa* strains are lower when human lactoferrin is present

Catherine E. Fowler[a], James S. Soothill[a]* and Lynne Oakes[b]

[a]*Department of Pathological Sciences, University of Manchester, Clinical Sciences Building, Manchester Royal Infirmary, Oxford Road, Manchester M13 9WL;* [b]*Department of Pathology, Trafford General Hospital, Moorside Road, Davyhulme, Manchester M41 5SL, UK*

MICs of rifampicin and chloramphenicol for mucoid strains of *Pseudomonas aeruginosa* were lower in the presence of human lactoferrin (0.9 mg/mL, the concentration found in cystic fibrosis sputum) than in its absence. MICs for some strains were lowered to clinically achievable levels of the antibiotics, which is compatible with impressions of greater clinical efficacy in pseudomonas infections than would be predicted by standard sensitivity tests. The routine addition of lactoferrin to sensitivity media for testing of cystic fibrosis isolates may give more useful results than conventional tests as in-vivo conditions are more closely simulated.

Introduction

In *Pseudomonas aeruginosa* infections in patients with cystic fibrosis, antibiotics that kill the bacteria *in vitro* fail to eradicate them in the patient, while treatment with antibiotics to which the bacteria are apparently resistant *in vitro* can be associated with clinical improvement. In *P. aeruginosa* infections in other patients, both rifampicin and chloramphenicol, to which the organisms were insensitive *in vitro*, appeared to confer benefit when each was given in combination with other drugs (Korvick *et al.*[1] and Woodhead, M., personal communication).

Standard sensitivity test media contain iron but sputum contains lactoferrin which limits iron availability to bacteria. Lactoferrin also has a direct effect, damaging the Gram-negative outer membrane and thus increasing the susceptibility of *Escherichia coli* to rifampicin[2,3] and of *Salmonella typhimurium* to rifampicin, ampicillin, ciprofloxacin, chloramphenicol and erythromycin.[4] We have therefore investigated the effect of lactoferrin at the very high concentration found in sputum from patients with cystic fibrosis[5] (0.9 mg/mL) on the sensitivity of *P. aeruginosa* to ceftazidime and gentamicin, antibiotics routinely used in pseudomonas infections, and to rifampicin and chloramphenicol which have little activity *in vitro* as measured by MIC but appear to benefit patients with respiratory infections caused by *P. aeruginosa*.

Materials and methods

The *P. aeruginosa* control strain was NCTC 10662. Five mucoid clinical *P. aeruginosa* isolates (993, 581, 580, 515, 642) were obtained from different cystic fibrosis patients at Booth Hall Children's Hospital, Manchester. They will be referred to as strains although they were not characterized in detail. All were identified as *P. aeruginosa* by the API 20NE system.

Rifampicin and chloramphenicol (Mast Laboratories Ltd, Bootle, UK) were 95% potent and 99.7% potent respectively. Gentamicin as gentamicin sulphate (Sigma Ltd, Poole, UK) was 64.7% potent. Ceftazidime, as ceftazidime pentahydrate (GlaxoWellcome Pharmaceuticals, Uxbridge, Middlesex, UK) was 96% potent. Human lactoferrin from *Aspergillus awamori* was provided by Agennix Inc., Houston, TX, USA. Isosensitest broth was used throughout, and was from Oxoid (Unipath Ltd, Basingstoke, UK) containing 7.0 μmol/L iron and 1.4 mmol/L bicarbonate (enough to allow all iron present to be bound by added lactoferrin, since one lactoferrin molecule binds two iron molecules).[6]

Bacteria were cultured in broth for 5 h at 35–37°C and diluted to contain approximately 10^5 cfu/mL. MICs were established for the clinical and control strains with and without 0.9 mg/mL lactoferrin by the broth microdilution method[7] modified in that an equal volume of organism sus-

*Corresponding author. Tel: +44-161-2768830; Fax: +44-161-2768826.

pension was added to each working dilution of antibiotic. Antibiotic-free controls with and without lactoferrin were included. The antibiotic concentration at which, on subculture, there was seen to be a 99.9% kill was taken as the MBC.

Rifampicin sensitivity tests were done with six replicates for strains 10662, 993 and 581 but with only three replicates for strains 580, 515 and 642. Chloramphenicol sensitivity tests were done for strains 10662, 993, 642 and 580, using nine replicates for each strain. Ceftazidime and gentamicin tests were done for strains 10662, 993, 642 and 581 with six replicates for each strain.

Statistical analysis was by the Mann–Whitney *U*-test (two-tailed). Means quoted are geometric means.

Results

In the absence of lactoferrin the antibiotics had the expected MICs for NCTC 10662.

Rifampicin MICs (Table I) against the control and strains 993 and 581 were significantly lower with lactoferrin than without ($P < 0.05$). The remaining strains were tested only three times, so significance could not be established, but the trend for decrease in MIC was maintained. For strain 515 there was a two- to four-fold decrease in MIC, and for strains 580 and 642 there was a two-fold decrease. The effect of lactoferrin on the MBCs of rifampicin for the mucoid strains of *P. aeruginosa* showed no consistent trend across the five strains. For two mucoid strains, 993 and 581, the MBCs increased significantly in the presence of lactoferrin ($P < 0.05$). For strain 581 the MBC without lactoferrin was 32 mg/L in all replicates, whereas with lactoferrin for five replicates the MBC was 128 mg/L, that for the remaining replicate being >128 mg/L. For NCTC 10662 there was no significant difference in MBCs of rifampicin with and without lactoferrin but for this strain and strains 515, 580 and 642 there were non-significant trends for reduction of MBC in the presence of lactoferrin.

Table I. Mean MICs and MBCs (mg/L) of rifampicin against *P. aeruginosa* control strain and clinical isolates with (+) and without (−) 0.9 mg/mL lactoferrin

Isolate	MIC		MBC	
	−	+	−	+
10662	20	5	32	18
993	64	14	81	>128
581	32	8	32	>128
580	32	16	64	51
515	32	10	64	16
642	16	8	51	32

MICs of chloramphenicol (Table II) were lower ($P < 0.05$) in the presence of lactoferrin for NCTC 10662 and strains 993 and 580 (two-fold decrease) and strain 642 (four-fold decrease). MBCs of chloramphenicol were all >128 mg/L, beyond the range of antibiotic concentrations tested, and clinically irrelevant.

With ceftazidime no significant change in MIC was found on addition of lactoferrin for two out of three mucoid strains tested or for NCTC 10662 (Table II). However, for mucoid strain 993, both the MIC and MBC were decreased ($P < 0.05$).

With gentamicin, lactoferrin did not significantly change the MICs for any strain. MBCs of gentamicin for NCTC 10662 and mucoid strains 993 and 581 were not significantly changed by lactoferrin. The MBC for strain 642 was increased by the presence of lactoferrin ($P < 0.05$).

Discussion

For both rifampicin and chloramphenicol, the MICs for all strains of *P. aeruginosa* were lower with lactoferrin than

Table II. Mean MICs and MBCs (mg/L) of chloramphenicol, ceftazidime and gentamicin against *P. aeruginosa* control strain and clinical isolates with (+) and without (−) 0.9 mg/mL lactoferrin

Isolate	Chloramphenicol MIC		Ceftazidime MIC		MBC		Gentamicin MIC		MBC	
	−	+	−	+	−	+	−	+	−	+
10662	64	30*	1.4	0.9	1.8	1.1	1.6	1.3	2	2.5
993	13	5*	1	0.4*	2.5	0.6*	4	4	5	8
642	87	24*	16	14	91	114	8	8	16	29*
580	32	19*	ND	ND	ND	ND	ND	ND	ND	ND
581	ND	ND	2.8	2	6.3	5.0	3.6	5.3	4	8

ND, not determined.
*Significant difference between results in the presence and absence of lactoferrin.

without it. In some instances such antibiotic concentrations are attainable *in vivo,* perhaps explaining the impression of response of respiratory tract infection by *P. aeruginosa* to chloramphenicol when given to treat suspected coincidental infection (Woodhead, M., personal communication). The results are consistent with the increased susceptibility of *E. coli* to rifampicin[2,3] and of *S. typhimurium* to rifampicin and chloramphenicol in the presence of lactoferrin.[4] Previous work [2] has shown enhanced bacterial killing by antibiotics in the presence of lactoferrin. In our studies, for some antibiotics and strains the MBC increased in the presence of lactoferrin, whereas for others it fell. Iron deprivation might be expected to reduce bacterial killing by slowing growth, whereas outer membrane damage might be expected to increase killing. The interaction of these conflicting factors may have given rise to the strain-specific effects seen.

Our results suggest that sensitivity testing should be carried out under conditions as close to those *in vivo* as possible, and the addition of lactoferrin is one practical step. For specialized fields such as cystic fibrosis, media could be tailored to conditions at the site of infection. Rifampicin and possibly chloramphenicol should be tested in infections caused by *P. aeruginosa*, and antibiotics previously thought to be unsuitable in other infections should be tested in conditions closer to those *in vivo* and clinically reappraised if results are encouraging.

Acknowledgements

References

1. Korvick, J. A., Peacock, J. E., Muder, R. R., Wheeler, R. R. & Yu, V. L. (1992). Addition of rifampin to combination antibiotic therapy for *Pseudomonas aeruginosa* bacteremia: prospective trial using the zelen protocol. *Antimicrobial Agents and Chemotherapy* **36**, 620–5.

2. Ellison, R. T., Ghiel, T. J. & LaForce, F. M. (1988) Damage of the outer membrane of enteric Gram-negative bacteria by lactoferrin and transferrin. *Infection and Immunity* **56**, 2774–81.

3. Ellison, R. T., LaForce, M., Giehl, T. J., Boose, D. S. & Dunn, B. E. (1990) Lactoferrin and transferrin damage of the Gram-negative outer membrane is modulated by Ca^{2+} and Mg^{2+}. *Journal of General Microbiology* **136**, 1437–46.

4. Naidu, A. S. & Arnold, R. R. (1994). Lactoferrin interaction with *Salmonellae* potentiates antibiotic susceptibility *in vitro*. *Diagnostic Microbiology and Infectious Diseases* **20**, 69–75.

5. Jacquot, J., Tournier, J. M., Carmona, T. G., Puchelle, E., Chazalette, J. P. & Sadoul, P. (1983). Proteines des secretions bronchiques dans la muscoviscidose. Role de l'infection. *Bulletin Européen de Physiopathologie Respiratoire* **19**, 453–8.

6. Montreuil, J., Mazurier, J., Le Grand D. & Spik G. (1985). Human lacto-transferrin: structure and function. In *Proteins of Iron Storage and Transport* (Spik, G., Montreuil, J., Crighton, R. R. & Mazurier, J., Eds), pp. 25–38. Elsevier, Amsterdam.

7. Working Party of the British Society of Antimicrobial Chemotherapy. (1991). A guide to sensitivity testing. *Journal of Antimicrobial Chemotherapy* **27**, Suppl. D.