The effect of antibiotics on bacterial colonisation of vascular cannulae in a novel in-vitro model

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Summary. An in-vitro model for studying semi-quantitatively the bacterial colonisation of the external and internal surfaces of peripheral intravascular cannulae is described. Using this model, we studied the effect of ciprofloxacin, teicoplanin and fusidic acid on cannula colonisation by *Staphylococcus epidermidis*. Exposure of colonised cannulae to sub-MICs of ciprofloxacin and fusidic acid reduced bacterial attachment, whereas sub-MIC levels of teicoplanin had little effect. Pre-exposure of *S. epidermidis* to sub-MICs of ciprofloxacin and fusidic acid also reduced slime production and colonisation. In comparison, pre-exposure of *S. epidermidis* to teicoplanin 1.0 mg/L did not influence colonisation, whereas at 0.1 mg/L it was reduced. The model allowed investigation of bacterial colonisation of cannulae and offers a screening system for the assessment of potential agents for the prophylaxis and treatment of these infections.

Introduction

Intravascular (i.v.) cannulae used in patient management for the administration of fluids, parenteral nutrition and drugs, may become colonised with bacteria or fungi resulting in infections ranging from localised thrombophlebitis to septicaemia. The incidence of cannula-related infections can be relatively high, with up to 12% of patients with central venous cannulae having an associated bacteraemia (Maki *et al.*, 1976). The causative organisms are well documented, a large proportion being coagulase-negative staphylococci (Eykyn, 1984). In comparison, the mechanism whereby bacteria attach and colonise cannulae is still poorly understood. Several in-vitro models have been developed to study bacterial adherence (Locci *et al.*, 1981; Sugarman, 1982; Bayston, 1984) but they have not enabled assessment of colonisation of the external and internal cannula surfaces, both of which can be implicated (Elliott *et al.*, 1984). These models have also not included bacterial growth kinetics which may influence colonisation. We have recently developed an in-vitro model to study bacterial colonisation of both internal and external surfaces of cannulae and related it to bacterial growth rate. The model simulates peripheral cannulae with a static internal volume surrounded by

a continuous flow of fluid. In this paper we describe this model and the effect of ciprofloxacin, fusidic acid and teicoplanin on *Staphylococcus epidermidis* colonisation of cannulae.

Materials and methods

Intravenous cannulae and the model

Cannulae made of fluorinated ethylene propylene copolymer (FEP-teflon) supplied by H. G. Wallace and Co., Colchester, Essex were used in these studies. The in-vitro model design is shown diagrammatically in fig. 1. It consisted of a 1-L glass fermenter (LH Fermenters Ltd.) with a stainless steel top plate. A steel tube (3 mm diameter), on to which a 5-ml syringe was attached, passed through the top plate of the fermenter and was used for sampling from the 700 ml of broth contained within the vessel. Controls for the growth conditions of the organisms within the fermenter included a magnetic stirrer with variable speed control, a temperature probe and heater, a pH electrode with appropriate automatic alkali or acid supply to maintain a constant pH, and a filtered air supply. Peripheral cannulae were detached from their hubs and a distal 3.5-cm section was supported under the surface of the broth, suspended on an 18-gauge needle attached to a stainless steel tube, 3 mm in diameter. Only the cannulae were immersed in the broth. The tube, on to which a syringe was attached, passed through the top plate of the fermenter to enable sampling through the cannulae. The model also had a facility for either continuous or batch culture.

Volumes (700 ml) of nutrient broth at 37°C in the

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Fig. 1. Schematic diagram of in-vitro colonisation model, consisting of a 1-L glass fermenter (13), containing broth, with a stainless steel top plate, and a fixed stirrer (5) driven from below by a magnet (16). The temperature of the broth was monitored by a detector (2) and held constant by a heater (3) with appropriate control systems (17). The pH of the broth was monitored by an electrode (12) and stabilised by an automatic delivery system (15) for either acid or alkali (14) via a direct feed (10). Aeration was achieved via a filter (9). The broth was sampled through a tube (4) attached to a syringe (1). Up to five cannulae (8) were suspended on stainless steel needles attached to a 3-mm stainless steel tube (6, 11) which passed through the top plate.

fermenter were inoculated with 0.5 ml of an overnight broth culture and incubated at 37°C for variable times. Five cannulae were introduced into the fermenter and broth culture was immediately drawn up into only their distal 3 cm. The phase of growth of the cultures was determined by viable counts before and after addition of the lines. At intervals the cannulae were removed from the fermenter and a semi-quantitative estimation made of the numbers of bacteria colonising their surfaces. To distinguish loosely attached from colonising bacteria, a cannula was initially washed by immersion with gentle agitation in five changes of 10 ml of fresh broth preheated to 37°C; 1–2 ml of broth was then flushed through the cannula over 2 min, by means of an attached syringe. This was repeated four times. Viable counts of bacteria in the various washings were determined to evaluate the efficiency of these procedures. To estimate the number of firmly attached bacteria on the internal surface of a cannula, 5 ml of broth at 37°C was flushed through within 20 s and then drawn back up. This procedure was repeated four times and again viable counts were made of bacteria present in these washings. To determine semi-quantitatively the colonisation of bacteria on the outside of the devices, the roll-plate method of Maki *et al.* (1977) was adopted with 10% nutrient agar plates which were

found to give the highest counts. After bacteriological sampling of the inner and outer surfaces, some of the cannula tips were placed in 5 ml of broth and sonicated for 30 s with a Branson Sonicator (Dawes Instruments Ltd, London). Counts of bacteria in this broth were subsequently made.

The internal and external surfaces of some of the cannulae were also examined by scanning electronmicroscopy (SEM) before and after washing to determine bacterial colonisation. To facilitate SEM examination of the internal surfaces, the cannulae were cut longitudinally as previously described (Cheesbrough *et al.*, 1985). The numbers of bacteria present on 1-cm lengths of both surfaces of the cannulae were then determined directly. Enumeration of colonising bacteria was not attempted by SEM when the cultures were exposed to antibiotics, because some of the organisms appeared disrupted and their viability was, therefore, uncertain.

Media

Nutrient Broth No. 2 (Oxoid) was used for growing the bacteria and nutrient agar, prepared by addition of agar 12 g/L for viable count determinations.

Organisms

Two clinical isolates of *S. epidermidis* (strains 1 and 2) were used; they were slime producers, as demonstrated by the method described by Christensen *et al.* (1982). The organisms were originally isolated from both blood cultures and the distal tips of central venous cannulae obtained from two patients with clinically proven cannula-associated infections.

Viable counts

Samples were obtained from the broth cultures and cannulae for viable count estimations. The bacteria were washed three times in fresh broth and tenfold dilutions were made in nutrient broth. Duplicate 0.1-ml portions were plated on to nutrient agar plates and the number of colonies counted after incubation at 37°C for up to 48 h.

Scanning electronmicroscopy

Cannulae were added to glutaraldehyde 3% in 0.2 M sodium cacodylate buffer (pH 7.2) for at least 1 h at 20°C. This was then replaced with fresh sodium cacodylate buffer, left for a further 10 min and the procedure was repeated. The cannulae were sequentially dehydrated for 10 min in 25%, 50%, 75%, 95% and absolute ethanol with 0.2 M sodium cacodylate buffer, cut longitudinally and dried in a Polaron critical point drier (Polaron Equipment Ltd, Watford, Herts). The samples were mounted on to stubs, coated with gold in a Polaron E5100 sputter coater, and examined in an optimised Cambridge S600 scanning electronmicroscope at an accelerating voltage of 1.5 kV. Washed and unwashed cannulae were examined.

Antibacterial agents

Ciprofloxacin was provided by Bayer (UK) Ltd, fusidic acid by Leo Laboratories Ltd, UK, and teicoplanin by Merrell-Dow Pharmaceuticals Ltd, UK. Appropriate concentrations were prepared by dissolving weighed powder in nutrient broth.

Antibiotic titrations

Serial twofold dilutions of the antibacterial agents were dispensed in 1-ml volumes of nutrient broth. Equal volumes of bacterial broth cultures, in the logarithmic phase of growth, were added to achieve an inoculum of *c.* 10^5 cfu/ml. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined as previously described (Elliott *et al.*, 1987).

Effect of antibiotics on colonisation

Some broth cultures in the model were exposed to the antibiotics before addition of the cannulae. To ensure that antibiotic-containing broth reached the lumen of the

Fig. 2. Growth kinetics of *S. epidermidis* strain 2 in nutrient broth in the model. The antibiotics were added at a point indicated by the arrow to achieve final concentrations of either 0.1 or 10 mg/L. A concentration of 1.0 mg/L was also tested and gave viable-count readings intermediate between those at 0.1 and 10 mg/L.

cannulae, it was drawn up and down the 3 cm distal part several times with the attached syringe.

In other experiments the effect of the antibiotics on cannulae already colonised was investigated. The colonised cannulae were removed from the model, washed as previously described and then immersed in 100 ml of fresh broth in a 250-ml flask which was shaken slowly. Antibiotics were added to the broth which was again introduced into the cannula lumen. These cannulae were sampled without further washing. All experiments were performed in duplicate and for comparative studies only cannulae which were colonised in the same broth were used. For control experiments no antibiotics were added.

Results

Bacterial attachment to cannulae

The growth kinetics of the two isolates of *S. epidermidis* were similar. The bacteria reached logarithmic phase of growth after incubation for

Table I. Attachment of *S. epidermidis* strain 2 to the inner and outer surfaces of peripheral intravenous cannulae immersed in a broth culture of *S. epidermidis* in the logarithmic phase of growth

Incubation time* of cannulae in broth culture (h)	Number of <i>S. epidermidis</i> † on			
	inner surface		outer surface	
	viable counts (cfu)	SEM counts	viable counts (cfu)	SEM counts
0.5	129 ± 44	84 ± 64	48 ± 36	49 ± 27
1	364 ± 86	180 ± 52	142 ± 66	110 ± 58
2	780 ± 220	ND	294 ± 112	ND

ND = not done.

* At each time interval four cannulae were sampled, two for viable counts (3-cm distal lengths) and two for SEM (1-cm distal lengths).

† Mean of eight experiments ± SD.

2 h (fig. 2) and the cannulae were then added. After 30 min, bacteria had attached on to the cannulae (table I). Some of the cannulae had surface defects and bacteria appeared to attach initially to these areas without slime production (fig. 3). Bacterial multiplication on the cannulae was evident 1 h after immersion in the broth cultures with the appearance of new division sites, the formation of microcolonies, and a rise in viable counts. The number of colonising bacteria on the cannulae increased during the following 3 h (table I). Colonisation predominantly occurred at the distal tips of the cannulae with more bacteria attaching to the internal surfaces.

Slime production by the attached bacteria was first detected by SEM (fig. 4) after immersion for 3 h in broth cultures in the logarithmic phase of

growth. In comparison, slime production was detected within 2 h of colonisation by bacteria in the stationary phase of growth. In all experiments, the slime gradually accumulated, embedding the colonising bacteria. Similar results were obtained for both strains of *S. epidermidis* studied. In some experiments, despite being immersed in bacterial cultures for up to 16 h, some of the cannula surfaces remained uncolonised. These areas were predominantly smooth, with no surface irregularities.

The washing procedures removed most of the loosely attached bacteria from the cannulae. This was demonstrated by a rapid reduction in bacteria present in the sequential washings. The final washings always contained < 20 cfu/ml. SEM studies on the cannulae also confirmed the efficacy of these procedures. This was particularly evident 3 h

Fig. 3. Scanning electronmicrograph showing early stages of colonisation with bacteria adhering to ridges on the cannula surface in the absence of slime. Bar = 4 µm.

Fig. 4. Scanning electronmicrograph of cannula surface after immersion for 3 h in broth culture of *S. epidermidis* strain 2. Bacteria, surrounded by slime, are shown attached on to cannula surface defects. Bar = 4 µm.

Table II. Susceptibilities of two strains of *S. epidermidis* to ciprofloxacin, fusidic acid and teicoplanin

Antibiotic	Strain 1		Strain 2	
	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)
Ciprofloxacin	0.5	0.5	0.25	0.25
Fusidic acid	2.0	8.0	0.125	0.25
Teicoplanin	2.0	8.0	0.25	0.25

after colonisation when only bacteria associated with slime remained attached on to the cannulae after washing. A further control on these sampling methods was by sonicating the cannulae after washing and subsequent sampling. This resulted in further bacteria being isolated from the cannulae. However, in no instance did the number of organisms derived by this method account for greater than 10% of the total recorded by the original sampling technique.

Antibiotic titrations

All three antimicrobial agents were uniformly active against *S. epidermidis* strain 2 and all were less active against *S. epidermidis* strain 1 (table II).

Effect of antimicrobial agents on *S. epidermidis* in broth cultures

After addition of ciprofloxacin or fusidic acid at concentrations above the MIC (10 mg/L) to broth cultures of *S. epidermidis* strain 2, the bacterial growth rate slowed for a short period, followed by a prolonged fall in viable counts which was more evident with the quinolone (fig. 2). In comparison, teicoplanin 10 mg/L resulted in a larger reduction in viable organisms. When the antibiotics were added at the sub-MIC of 0.1 mg/L, the fall in numbers of viable bacteria was not so marked, with fusidic acid appearing the least active. Addition of the antibiotics to a final concentration of 1.0 mg/L resulted in a response intermediate between the 0.1 and 10.0 mg/L concentrations. Similar but less pronounced responses to the antibiotics were observed with *S. epidermidis* strain 1.

Effect of antimicrobial agents on cannula colonisation

When teicoplanin at a final concentration of 0.1 mg/L was added to broth containing cannulae pre-colonised with *S. epidermidis* strain 2, a greater number of bacteria remained attached than when

Table III. Attachment of *S. epidermidis* strain 2 to the inner and outer surfaces of peripheral cannulae after being colonised for 1 h in a broth culture in the logarithmic phase of growth and then transferred to fresh broth containing ciprofloxacin, fusidic acid or teicoplanin for a further hour

Antibiotic	Concentration (mg/L)	Number of bacteria (cfu $\pm$ SD)* on	
		internal surface	external surface
Ciprofloxacin	0.1	25 $\pm$ 12	3 $\pm$ 2
	1.0	18 $\pm$ 12	14 $\pm$ 8
Fusidic acid	0.1	18 $\pm$ 8	12 $\pm$ 6
	1.0	16 $\pm$ 7	17 $\pm$ 15
Teicoplanin	0.1	680 $\pm$ 122	980 $\pm$ 370
	1.0	92 $\pm$ 32	290 $\pm$ 78
Control (no antibiotic)		640 $\pm$ 180	286 $\pm$ 102

* Mean of six experiments each with four cannulae; counts include organisms recovered after sonication of cannulae.

Table IV. Attachment of *S. epidermidis* strain 2 to cannulae after immersion for 1 h in broth cultures in the logarithmic phase of growth; the cultures were exposed to the antibiotics at 0.1 or 1.0 mg/L final concentration for 1 h before addition of the cannulae to the model

Antibiotic	Concentration (mg/L)	Number of bacteria (cfu $\pm$ SD)* on	
		internal surface	external surface
Ciprofloxacin	0.1	25 $\pm$ 11	22 $\pm$ 11
	1.0	17 $\pm$ 12	10 $\pm$ 6
Fusidic acid	0.1	48 $\pm$ 14	35 $\pm$ 12
	1.0	18 $\pm$ 12	19 $\pm$ 13
Teicoplanin	0.1	14 $\pm$ 9	15 $\pm$ 8
	1.0	192 $\pm$ 56	170 $\pm$ 84
Control (no antibiotic)		330 $\pm$ 102	158 $\pm$ 68

* Mean of four experiments, each with three cannulae.

ciprofloxacin or fusidic acid was added (table III). Ciprofloxacin and fusidic acid reduced the expected cannula colonisation, with only a few bacteria remaining attached to the cannulae. SEM studies confirmed the reduction of bacterial colonisation in the presence of ciprofloxacin and fusidic acid. The bacteria that remained attached to these cannulae were mainly associated with slime, but a few also appeared swollen or collapsed.

Broth cultures in the model were also pretreated

for 1 h with either 0.1 or 1.0 mg/L of the antibiotics before addition of the cannulae. Ciprofloxacin and fusidic acid reduced subsequent colonisation (table IV). Similarly, pretreatment with teicoplanin at 0.1 mg/L decreased colonisation whereas at 1.0 mg/L bacterial adhesion appeared largely unaffected. Production of slime by the remaining attached bacteria in the presence of sub-MICs of ciprofloxacin or fusidic acid was sparse whereas with teicoplanin it appeared no different from that by the untreated control bacteria.

The results obtained from identical experiments performed on *S. epidermidis* strain 1 showed similar colonisation trends.

Discussion

Several models developed to study the attachment and colonisation of bacteria on to plastic cannulae have been described previously (Locci *et al.*, 1981; Sugarman, 1982; Peters *et al.*, 1982; Bayston, 1984). However, these models have not incorporated bacterial growth kinetics nor have they differentiated between bacterial colonisation of the internal and external cannula surfaces, which can occur *in vivo* (Cheesbrough *et al.*, 1985). The design of the present model allowed the colonisation of bacteria, in predetermined phases of growth, on both cannula surfaces to be studied. This approach was of particular importance in the subsequent studies on changes in cannula colonisation following antibiotic treatment.

The present findings confirm that bacterial colonisation occurs on both cannula surfaces, with more organisms attaching to the inner surface. A possible explanation for this observation was the predominantly granular appearance of the inner surface, compared with the relatively smoother outer-cannula surface. These defects, to which bacteria can adhere initially (Locci *et al.*, 1981; Elliott *et al.*, 1984), may have enabled a larger number of organisms to colonise the internal surface. It was also noted that slime production by bacteria was evident earlier in the stationary phase of growth than with rapidly dividing organisms. Bacteria within the cannula lumen may have reached this stationary phase of growth before those surrounding the cannulae, due to limitation of nutrients. The organisms in the inner surface may, therefore, have subsequently produced more slime, resulting in increased adhesion and colonisation. Christensen *et al.* (1982), using a static model to examine colonisation, similarly reported that slime production depended on the culture medium, which can in turn influence growth rate.

Following development of the model, it was used to test the effects of various antibiotics on bacterial colonisation of cannulae. In recent years it has become increasingly recognised that antibiotics, particularly at low concentrations, interfere with bacterial adhesion (Shibl, 1985), offering a potential approach to the management of these cannula-associated infections. Sub-MICs of antibiotics were, therefore, assessed in the model. Pretreatment of *S. epidermidis* with ciprofloxacin or fusidic acid, 0.1 or 1.0 mg/L or teicoplanin 0.1 mg/L reduced subsequent cannula colonisation, whereas colonisation was largely unaffected by teicoplanin 1.0 mg/L. In comparison, exposure of previously colonised cannulae to the same concentrations of ciprofloxacin or fusidic acid reduced colonisation whereas teicoplanin 0.1 mg/L resulted in enhanced colonisation. Proctor *et al.* (1982) have similarly demonstrated that sub-MICs of β -lactam antibiotics can increase binding of staphylococci to fibronectin whereas antimicrobials acting at other sites decreased this interaction. Shibl (1985) also described enhanced adherence of *S. aureus* in the presence of penicillin. Sheth *et al.* (1985) have likewise demonstrated that slime-producing coagulase-negative staphylococci, when attached to polyvinyl chloride, are able to survive exposure to apparently lethal concentrations of the cell wall acting antibiotic nafcillin. Proctor *et al.* (1982) suggested that the β -lactam drugs produce cell wall gaps due to interference with transpeptidation resulting in uncovering of binding sites or release of increased amounts of binding substances. A similar mechanism may occur in the presence of certain concentrations of teicoplanin, which inhibits either transglycosylation or transpeptidation, resulting in increased bacterial adherence. However, as it is not yet clear which bacterial structures are directly associated with adhesion on to solid substrates (Hogt *et al.*, 1985, 1986, 1987), the mechanism for the action of these β -lactam antibiotics at sub-MICs needs to be elucidated further.

The effect of ciprofloxacin, which inhibits DNA gyrase resulting in cell-wall lesions (Elliott *et al.*, 1987), or fusidic acid, which acts on the ribosome, further corresponds to the previous reports of clindamycin and lincomycin decreasing adherence of *S. aureus* (Shibl, 1985). The difference in response between these antibiotics and those acting primarily on the cell wall may reflect not only mode of action as suggested by Proctor *et al.* (1982), but also the ability to penetrate the slime layer produced by the bacteria, which in turn may offer protection from therapy (Shibl, 1985).

The continued survival and presence of bacteria

on cannula surfaces despite exposure to any of the agents tested, but particularly teicoplanin, confirms the difficulty in attempting to treat this type of infection *in vivo*, which usually requires removal of the device (Eykyn, 1984). The present results suggest that cell wall-acting antibiotics may, at certain concentrations, actively encourage cannula colonisation, thus compounding the problem. In comparison, antimicrobial agents such as ciproflox-

acin or fusidic acid, acting at other sites, may be potentially more efficacious, particularly if used for the prevention rather than treatment of cannula infections. The present studies with this model demonstrate its value as a relatively rapid screening method to assess potential agents for the prevention or eradication of cannula infections.

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