Resistance to a polyquaternium-1 lens care solution and isoelectric points of Pseudomonas aeruginosa strains

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Objectives: The aim of this study was to correlate the cell surface hydrophobicity and charge of various strains of Pseudomonas aeruginosa with their resistance to a polyquaternium-1 lens care solution.

Methods: The 11 P. aeruginosa strains included were isolated from eyes, contact lenses, lens cases and lens care solutions. Cell surface hydrophobicities were determined from water contact angle measurements and surface charges were measured as a function of pH using particulate micro-electrophoresis.

Results: Strains resistant to polyquaternium-1 had an isoelectric point (IEP; pH where the bacterial zeta potential is zero) ranging from 4.0 to 5.5, whereas susceptible strains were more negatively charged than resistant strains and had an IEP between 1.3 and 1.9. Water contact angles ranged from hydrophilic (34°) to hydrophobic (124°), without showing a relation with antimicrobial resistance.

Conclusions: Results suggest that electrostatic repulsion between cationic molecules on the cell surface and quaternary ammonium compounds impedes the antimicrobial entering the cell.

Keywords: cationic molecules, quaternary ammonium compounds, hydrophobicity, surface charge

Introduction

Worldwide, 85 million people use contact lenses (CLs) as a successful form of vision correction and as an alternative to spectacles. Several complications may occur as a result of wearing CLs, of which microbial keratitis is the most serious complication as it may result in permanent visual loss.¹ One of the most commonly isolated bacterial strains is Pseudomonas aeruginosa, which can gain access to the eye via contamination of the lens, lens case or lens care solutions.²

The development of bacterial resistance to antibiotics is well recognized in medical microbiology.³ Disinfectants, such as triclosan (commonly used in soaps, toothpaste etc.), also have the potential to promote resistance by selecting for mutants that are equipped to survive in the presence of the disinfectant.³ The majority of CLs used for daily wear have to be stored and cleaned with lens care solutions. These solutions are complex mixtures, but the main components of many different commercial solutions are essentially the same. Repeated usage of these solutions may increase the probability of bacteria becoming resistant to the disinfectants used.

Strains of Serratia marcescens have been shown to become adapted to certain chlorhexidine and benzalkonium chloride based disinfecting solutions, recommended for the care of rigid gas permeable CLs.³ Lakkis and Fleiszig⁵ investigated bacterial resistance to hydrogel disinfecting solutions and concluded that some P. aeruginosa strains were resistant to a polyquaternium-1 preserved lens care solution. Resistance appeared to be correlated with cytotoxicity of the strains, but they suggested that a better understanding of the mechanisms of resistance was necessary in order to design improved CL solutions and potentially reduce CL related microbial keratitis.

An antimicrobial works at the level of the cell surface or needs to enter the cell in order to become effective. This suggests that cell surface properties may play an essential role in determining antimicrobial resistance or susceptibility to a given disinfectant. Tomeczek et al.⁶ described that lactobacilli with relatively...
hydrophilic surfaces were resistant to non-ionic 25% nonoxynol-9 and vancomycin, whereas only hydrophobic strains were susceptible. They also suggested that resistance of bacteria to ionic antimicrobials, such as polyquaternium-1, probably involves electrostatic forces rather than hydrophobic interactions.

Therefore the aim of this study was to relate the cell surface hydrophobicities and charges of 11 strains of P. aeruginosa with their known susceptibility to a polyquaternium-1 preserved lens care solution.

Materials and methods

Bacterial strains, growth conditions and harvesting

Eleven P. aeruginosa strains were collected from eyes, CLs, lens cases and lens care solutions in the United States and Australia. The strains were classified into two groups (see Table 1) based upon their resistance or adaptation to a polyquaternium-1 preserved lens care solution [solution B: sodium citrate, sodium chloride, disodium edetate (0.5%), polyquaternium-1 (0.001%)]. The strains were pre-cultured from a frozen stock in 10 mL of Tryptone soya broth (TSB; Oxoid, Basingstoke, UK) for 24 h at 37°C in ambient air. These cultures were used to inoculate 200 mL of TSB and were grown for 18 h. All strains were harvested by centrifugation for 5 min at 9600 g, washed twice and resuspended in ultra-pure water for determination of their cell surface hydrophobicity and zeta potentials.

Zeta potential measurements and their influence on polyquaternium-1 antimicrobial activity

The pH dependence of the zeta potentials was measured for all P. aeruginosa strains at 25°C at pH 2–9 in 10 mM potassium phosphate solutions. Zeta potentials were measured with a Lazer Zee Meter 501 (PenKem Inc., Bedford Hills, NY, USA). Briefly, the micro-electrophoresis chamber was filled with a bacterial suspension and a voltage difference of 150 V was applied over the chamber. The apparent zeta potentials were calculated using the Helmholtz–Smoluchowski equation.7

Table 1. Pseudomonas aeruginosa strains included in this study and their susceptibility (–) or resistance (+) to a polyquaternium-1 preserved lens care solution, together with their measured isoelectric points (IEPs) and water contact angles (θw, in degrees)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Isolation source</th>
<th>Resistant</th>
<th>IEP</th>
<th>θw (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GW</td>
<td>CL case</td>
<td>–</td>
<td>1.4 ± 0.2</td>
<td>105</td>
</tr>
<tr>
<td>AJ</td>
<td>CL solution (Boston)</td>
<td>–</td>
<td>1.8 ± 0.1</td>
<td>60</td>
</tr>
<tr>
<td>6487</td>
<td>cornea keratitis</td>
<td>–</td>
<td>1.7 ± 0.1</td>
<td>34</td>
</tr>
<tr>
<td>6294</td>
<td>cornea keratitis</td>
<td>–</td>
<td>1.9 ± 0.1</td>
<td>47</td>
</tr>
<tr>
<td>CL79</td>
<td>CL case (AOSep)</td>
<td>–</td>
<td>1.3 ± 0.7</td>
<td>124</td>
</tr>
<tr>
<td>K555</td>
<td>CL blepharitis</td>
<td>–</td>
<td>1.4 ± 0.0</td>
<td>84</td>
</tr>
<tr>
<td>B1</td>
<td>keratitis CL wearer</td>
<td>–</td>
<td>1.7 ± 0.0</td>
<td>106</td>
</tr>
<tr>
<td>K648</td>
<td>CL keratitis</td>
<td>+</td>
<td>5.2 ± 0.7</td>
<td>45</td>
</tr>
<tr>
<td>6206</td>
<td>cornea keratitis</td>
<td>+</td>
<td>5.5 ± 0.5</td>
<td>117</td>
</tr>
<tr>
<td>6073</td>
<td>cornea keratitis</td>
<td>+</td>
<td>4.8 ± 0.5</td>
<td>105</td>
</tr>
<tr>
<td>PA103</td>
<td>NA</td>
<td>+</td>
<td>4.0 ± 0.5</td>
<td>60</td>
</tr>
</tbody>
</table>

CL, contact lens; AOSep, CIBA Vision AOSep disinfection system.
The average SD over three experiments with separately cultured bacteria is ±0.3 for IEP and ±5° for contact angles.

In order to test the influence of the electrostatic interactions on the killing effect of polyquaternium-1, 1 × 10⁶ cfu of the susceptible P. aeruginosa GW was suspended in a polyquaternium-1 lens care solution with 5% TSB added and ionic strength adjusted to 10 or 200 mM potassium phosphate for 2 h at room temperature. The zeta potentials were measured and diluted plate counting was performed.

Contact angle measurements

The hydrophobicities of the bacterial cell surfaces were determined by the measurement of sessile water droplets on bacterial lawns deposited on a cellulose acetate membrane filter.8 So-called ‘plateau’, advancing type water contact angles were measured after 30–60 min of drying using a home-made contour monitor.

Statistical analysis

Differences in IEPs and water contact angles between polyquaternium-1-resistant and -susceptible strains were compared using the Mann–Whitney U-test. A P value of less than 0.05 was considered to be statistically significant.

Results

Figure 1 shows the bacterial zeta potentials as a function of pH. Zeta potentials varied widely among the different strains and were as negative as –34 mV at pH 9 but became positive at acidic pH values. The four strains classified as resistant to polyquaternium-1 lens care solution were less negatively charged than the susceptible strains. Extrapolation and intrapolation of the pH dependence of the zeta potentials yields the pH value where the zeta potential is zero, the IEP, as presented in Table 1. The four strains resistant to polyquaternium-1 had significantly (P < 0.001) higher IEPs in the range of 4.0–5.5, whereas the susceptible strains had lower IEPs between 1.3 and 1.9.

The water contact angles measured on the bacterial cell surfaces are also presented in Table 1 and ranged from very hydrophilic (34°) to very hydrophobic (124°), showing no relation (P > 0.05) with the susceptibility of the strains to polyquaternium-1 preserved solution. No relationship was found between the water contact angles and IEPs.
The isolation source, i.e. eyes, CLs, lens case or lens care solution, did not show any correlation with resistance, zeta potentials, IEPs or cell surface hydrophobicity. 

P. aeruginosa GW showed zeta potentials of $-32$ mV in the low ionic strength solution and $-5$ mV in the high ionic strength solution. In the high ionic strength solution no cfu were found in contrast to the low ionic strength solution where $2 \times 10^7$ cfu of P. aeruginosa were isolated.

Discussion

Quaternary ammonium compounds, such as polyquaternium-1, are membrane active, cationic antimicrobial agents\(^1\) that act predominantly at the level of the inner cytoplasmic membrane with phospholipid components, thereby causing disruption in cell metabolism and cell death. McDonnell and Russell\(^9\) ascribe the relative resistance of P. aeruginosa to the lipopolysaccharide composition and cation content of its outer membrane.\(^3\)

This study shows that the resistance of the tested P. aeruginosa strains against a quaternary ammonium compound (polyquaternium-1) depends on the charge properties of the cell surfaces, most notably their IEPs. Cell surface hydrophobicity was not found to be a factor in polyquaternium-1 resistance of the P. aeruginosa strains. In this respect it is interesting to note that resistance to disinfection appears to be an inherent rather than acquired trait, since some resistant strains have been isolated prior to the introduction of disinfectants and some susceptible P. aeruginosa strains could not be made more resistant by repeated disinfectant exposure.\(^2\) Characterization of the ExsA-regulated protein profiles by western blotting of different Pseudomonas isolates showed\(^2\) exoU was involved in resistance, and an exoU mutant grew in extremely low numbers and only after long exposure as compared with the wild-type strain PA103, that was also involved in this study and found in the high IEP group (see Table 1).

IEP and cell surface charge are a reflection of the chemical composition of the cell surface. A high IEP or less negative zeta potential corresponds with a larger number of positively charged cations in the cell surface putatively due to exoU regulated proteins that exceed the number of anions below IEP. Consequently, other cations, like quaternary ammonium chlorides will experience a stronger electrostatic repulsion upon approach of the strains with a higher IEP than upon approach of strains with a lower IEP. This repulsion subsequently impedes the antimicrobial from entering the cell and becoming active. If this mechanism is indeed operative, it should be possible to manipulate the electrostatic interactions between the antimicrobial and cell surface components by adjusting, for instance, the ionic strength of the solution in which microbial killing is attempted. This hypothesis was tested on P. aeruginosa GW and no survival of the strain in the high ionic strength solution was found, suggesting that the antimicrobial experienced no electrostatic repulsion from the uncharged cell surface upon entering the cell. In the low ionic strength solution the strain survived, suggesting that electrostatic attraction between negatively charged cell surface components and the antimicrobial impeded entry of the antimicrobial into the cell.

In conclusion, the surface charge of P. aeruginosa is likely to play an important role in the susceptibility and resistance of clinical strains to polyquaternium-1 lens care solutions. Since bacterial cell surface charge can be manipulated by changing the ionic strength of the solutions, these results offer a pathway for the development of more potent solutions, especially against strains that have become resistant under currently applied conditions.

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Transparency declarations

None to declare.

References