HEPARIN INHIBITS *PSEUDOMONAS* ADHERENCE TO SOFT CONTACT LENSES

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SUMMARY

Adherence of bacteria to the surface of contact lenses may play an important role in contact lens intolerance and corneal infections. To decrease the capability of bacteria to adhere to contact lenses we incubated two types of soft contact lenses with two strains of *Pseudomonas aeruginosa* (serotypes 0:11 and 0:8) at a concentration of 5×10^7 c.f.u./ml for 12 hours. When heparin was added to the medium at a concentration of 1000 IU/ml the numbers of bacteria adhering to the contact lenses were significantly fewer than in the controls ($p<0.005$). Our results suggest that heparin, either included in contact lens solutions or bonded to the surface of the contact lens, may decrease contact-lens-related morbidity.

Infectious keratitis is the most serious complication related to contact lens use. Frequently the agent responsible is *Pseudomonas aeruginosa*,[1–4] and extended wear has been demonstrated to increase this risk.[5,6]

P. aeruginosa has a high capability of adhering to the surface of different types of unused contact lenses,[7,8] and deposits may facilitate such adherence.[9] The role of adherent bacteria in contact-lens-related pathology is unknown, but there is evidence of the presence of bacteria and biofilm in the contact lenses of patients with corneal ulcers.[10]

Decreasing the ability of bacteria to adhere to contact lenses may influence the incidence of contact-lens-related infection. Such a procedure would have to: (1) be safe for the eye, (2) produce no contact lens spoilage, (3) be effective for different bacteria and contact lenses, (4) induce no bacterial changes, and (5) be durable. In this study we investigated the effect of heparin on the adherence of *P. aeruginosa* to hydrogel contact lenses.

MATERIALS AND METHODS

Bacteria

Two strains of *P. aeruginosa* were isolated from a contact-lens-related corneal ulcer (PA1) and from a pulmonary infection (PA2). PA1 was serotype 0:11 and PA2 was serotype 0:8. Isolates were obtained after incubation with tryptic soy agar (TSA), and aliquots maintained at 80 °C in tryptic soy broth (TSB) supplemented with 10% glycerol.

For each experiment bacteria were allowed to grow overnight in blood-agar at 37 °C. One colony was introduced into Müller–Hinton broth with 5 μCi/ml D–[6-^{3}H] glucose (Amersham, Amersham, UK) and maintained at 37 °C for 20 hours. The bacterial suspension was centrifuged three times in phosphate-buffered saline (PBS) at 9000 rpm and the concentration adjusted to 10^8 c.f.u./ml by colorimetry (Api, ATB 1550). One millilitre of the final suspension was introduced into scintillating liquid (Ready Protein +, Beckman, Fullerton, CA) in order to establish isotope uptake.

Contact Lenses

Two types of hydrogel contact lenses were used, the choice being based on surface, water content and composition characteristics: (1) Soft-Mate (Barnes Hind, Sunnyvale, CA), ionic bufilcon-A with 45% water; and (2) Hydron (Allergan Medical Optics, Irvine, CA), non-ionic polymacon with 38% water. In order to avoid variability in thickness all contact lenses ranged from +0.50 to –0.50 dioptres.

Experiments

Therapeutic sodium heparin (Leo, Madrid, Spain) was diluted in PBS to a concentration of 200 or 2000 IU/ml. One millilitre of each was introduced into a plastic well (Cluster 24, Coster, Cambridge, MA) together with 1 ml bacterial suspension and one of the contact lenses. Note that the final concentration of bacteria in this medium was 5×10^7 c.f.u./ml and of heparin 100 or 1000 IU/ml. Wells were maintained at room temperature in an orbital stirrer for 12 hours. For each experiment one contact lens was introduced into 1 ml bacterial suspension and 1 ml PBS as control.

Contact lenses were separated with sterile forceps,

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Table I. Soft-Mate contact lens: number of bacteria × 10^3 per lens

	PA1 (0:11)	PA2 (0:8)
Control	11 545±5445	5441±1686
Heparin 100	8179±5372 (NS)	4119±1039 (NS)
Heparin 1000	3362±1946*	1246± 329*

*Statistically significant ($p<0.005$); NS, not significant.

Table II. Hydron contact lens: number of bacteria × 10^3 per lens

	PA1 (0:11)	PA2 (0:8)
Control	10 150±3640	7174±1686
Heparin 100	6552±2875*	5389± 882*
Heparin 1000	2466± 633**	1125± 263**

Statistically significant: *$p<0.005$; **$p<0.05$.

washed in a vortex with 2 ml PBS in order to separate non-attached bacteria, introduced into vials with scintillating liquid and radioactivity counted in a beta counter (LS 3801, Beckman, Palo Alto, CA) for a minimum of 15 minutes.

Media were cultured on blood-agar for the assessment of bacterial growth and final contamination. In all cases *P. aeruginosa* grew; one experiment was rejected because of contamination with *Proteus* sp.

Each of the experiments included: (1) 1 ml bacterial suspension for the radioactivity control, (2) PA1 or PA2 with heparin 100 mIU/ml, (3) PA1 or PA2 with heparin 1000 IU/ml and (4) a contact lens control. All experiments were repeated at least 8 times. Results were expressed as the number of bacteria per contact lens. Statistical analysis of the results included the non-parametric Mann–Whitney test and Kruskal–Wallis test.

RESULTS

After 12 hours of incubation the numbers of *P. aeruginosa* adhering to the Soft-Mate contact lens (control) were 11 545±5445 × 10^3 for PA1 and 5441±1686 × 10^3 for PA2 (Table I). In the presence of 100 IU/ml heparin the results were 8179±5372 × 10^3 and 4119±1039 × 10^3 respectively (not significant). In the presence of 1000 IU/ml heparin the results were 3362±1946 × 10^3 and 1246±329 × 10^3 respectively, both being significantly lower compared with the controls ($p<0.005$).

For the Hydron contact lens the numbers of bacteria (control) adhering after 12 hours were 10 150±3640 × 10^3. for PA1 and 7174±1686 × 10^3 for PA2 (Table II). When 100 IU/ml heparin were present these numbers changed to 6552±2875 × 10^3 and 5389±882 × 10^3 respectively, statistically lower than the controls ($p<0.05$). In the presence of 1000 IU/ml heparin the results were 2466±633 × 10^3 and 1125±263 × 10^3 (highly significant, $p<0.005$).

DISCUSSION

P. aeruginosa is a bacterium frequently isolated from corneal ulcers related to contact lens use, and serotype 0:11 has been reported to be the most frequent isolate.[11,12] In our study adherence of this serotype was higher than that of serotype 0:8, being even more marked on one of the contact lens types. Moreover, Mayo *et al.* found that strains isolated from contact lens solutions are more likely to have a high number of plasmids.[12] Several studies have shown the capability of *P. aeruginosa* to adhere to soft contact lenses[5,6,13] and how adherence could be influenced by different factors such as the presence of mucin or other tear compounds.[14–16] Also, *P. aeruginosa* adherent to contact lenses can induce a bacterial keratitis under specific circumstances.[17,18]

The results of our study show clearly that heparin can inhibit the numbers of *P. aeruginosa* attached to the surface of two types of soft contact lens. The anti-adherent bacterial effect of heparin was first documented by Hanno *et al.* in 1978 and Parsons *et al.* in 1979, in studies on experimental infections of the bladder.[19,20] Other authors found similar results.[21–23] Ruggieri *et al.* compared its anti-adherent effect on five bacterial species; it was least effective for *P. aeruginosa*.[22,23] The mechanism by which heparin inhibits bacterial attachment is not fully understood, but it is not based on its anticoagulant effect.[24] Heparin is a glycosaminoglycan and when exposed to a substrate forms a molecular layer of water that prevents attachment of bacteria to the surface via adhesins.[19] Thus, heparin does not seem to have an antibacterial effect.

The use of heparin for the prevention of contact-lens-related corneal infections could be approached in two different ways: (1) by adding it to contact lens solutions or (2) by coating the contact lens with it. The first possibility comes directly from our method. From the biological characteristics of heparin no local side effects or changes in the bacteria are expected since it does not have a direct effect on the bacteria. Further, it could diminish the amount of deposits of bacterial origin with its antigenic charge[25,26] and thus reduce the risk of conjunctival inflammation.[27]

The other potential approach, of incorporating a layer of heparin on the surface of the contact lens, has already been done with intraocular lenses to reduce bacterial deposits (Pharmacia, Uppsala, Sweden) and in urethral catheters to decrease the risk of infection (heparin-bonded polyurethane (HBP), Bard Co., Lombard, IL). Since contact lenses are subject to manipulation and abrasion, which could remove the heparin coating, a disposable soft heparin-coated contact lens could be useful in cases where the risk of infection is high (e.g. therapeutic lenses) or where there is a history of contact lens intolerance.

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