The Journal of Laryngology and Otology
August 1997, Vol. **111**, pp. 760–762

Cilia from a cystic fibrosis patient react to the ciliotoxic *Pseudomonas aeruginosa* II lectin in a similar manner to normal control cilia – a case report

ELIZABETH C. ADAM*, DIETLIND U. SCHUMACHER†, UDO SCHUMACHER*

Abstract
The ciliary beat frequency measurements taken from a nasal polyp from a cystic fibrosis patient were similar to that of the control nasal polyps. The addition of a ciliotoxic lectin produced by *Pseudomonas aeruginosa* stopped the beating of the cilia as in the controls. This reaction could be blocked by the pre-incubation of the lectin with its inhibitor fucose. As in the control, the addition of fucose after the cilia had slowed resulted in a return to normal ciliary beating within 24 hours. This shows that the delta F508 CF mutation observed in this patient does not affect ciliary beating and suggests that treatment with fucose in the early stages of a *Pseudomonas aeruginosa* infection could be advantageous for cystic fibrosis patients.

Key words: Cilia; *Pseudomonas aeruginosa*; Lectins, Cystic fibrosis

Introduction

A common problem in cystic fibrosis patients is a persistent *Pseudomonas aeruginosa* infection in the respiratory tract. To date no effective method of *P. aeruginosa* eradication has been developed once it has colonized the patient's airways (Warner, 1992). *P. aeruginosa* has developed several mechanisms to avoid the body's defence mechanisms, amongst which is an alteration of mucociliary clearance by affecting the cilia. The cilia are covered with a carbohydrate-rich coat, the glycocalyx, and it has been shown that the lectin *P. aeruginosa* (PAII) binds to these cilia. Lectins are carbohydrate-binding proteins and on binding of PAII to the glycocalyx of cilia of normal human airway epithelium, these cilia stop beating, thus affecting the mucociliary clearance. It has also been shown that it is possible to block this effect by pre-incubating the PAII with fucose, the carbohydrate for which PAII is specific, thus preventing the lectin from binding to the cilia. Furthermore it has been demonstrated that the early effect caused by PAII is reversible after fucose addition and that the ciliary activity can be returned to normal within 24 hours, demonstrating the potential of new avenues of treatment for *P. aeruginosa* airway infections (Adam *et al.*, 1997).

However, no study has been undertaken to investigate whether this effect of PAII also applies to the cilia of cystic fibrosis patients, who are the most likely to succumb to *P. aeruginosa* infections. This study carries out a comparison of the PAII effect on cilia from control and CF patients.

Case report

A 12-year-old girl with cystic fibrosis (homozygous for delta F508) was referred with nasal blockage to the ENT Department at the Royal South Hants Hospital, Southampton. At the age of four she was first diagnosed as suffering from nasal polyps and subsequently underwent eight separate nasal polypectomies for recurrent nasal polyps. Both her upper and lower respiratory tract were free from *P. aeruginosa* infection although *Staphylococcus aureus* colonization was observed in both. On nasendoscopy a recurrence of nasal polyps was diagnosed, filling both middle meatus and extending into the nasal cavities. A coronal computed tomography (CT) scan of her sinuses showed diffuse polypoid rhinosinopathy. The maxillary sinuses were completely opacified due to retained secretions. She underwent endoscopic removal of the nasal polyps and clearing of the ethmoid sinuses of polypoid musoca and her sphenoid was opened. As is common in cystic fibrosis patients the sphenoids were rudimentary. Wide middle meatus antrostomies were fashioned and the maxillary sinuses were cleared of viscous secretions. At the time of operation a swab was taken from the middle meatus area for culture and sensitivity which showed *Staph. aureus* but no *P. aeruginosa* infection.

As controls we used nasal polyps from 10 patients who suffered from severe nasal polyposis. Specimens were retrieved during endoscopic sinus surgery. There was no history of CF in these patients and their mean age of 43 (range 22–62) makes cystic fibrosis as the underlying condition highly unlikely.

Immediately after removal the polyps were prepared for culture. The surface epithelium was carefully cut into small pieces, secured in small Petri dishes (for all methodological details see Adam *et al.*, 1997) and maintained at 37 °C in a humidified atmosphere. The following day the ciliary beat frequency (CBF) of an untreated explant was determined using special purpose software and was shown to be well within the normal range between 11 and 14 Hz. Having determined the frequency, addition of PAII was made to the culture medium at a final concentration of 1 μM. After

From the Department of Human Morphology*, University of Southampton, and the ENT Department†, Royal South Hants Hospital, Southampton, UK.

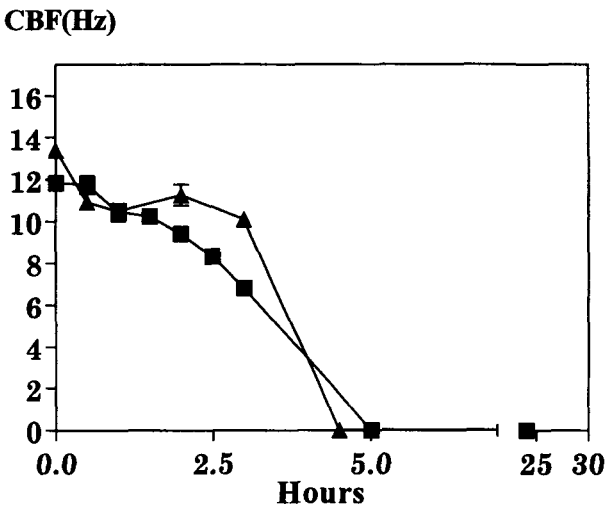

FIG. 1

Ciliary beat frequencies (CBF) of nasal polyp explants over a 24-hour period. The effect of the lectin PAII on a control culture (▲) was similar to that on a cystic fibrosis culture (■).

five hours all ciliary activity had ceased (Figure 1) and when left for 24 hours showed no signs of recovery. Another explant was treated in a similar manner, but this time the PAII was pre-incubated with 3 mM fucose (for which it is specific) for 30 minutes. Twenty-four hours later the cilia were still beating normally (Figure 2). A further explant then received an addition of PAII to the culture medium followed by an addition of fucose (12 μM final concentration) after three hours when the CBF had reduced from 12.45 Hz to 5.66 Hz. The CBF was monitored until the next day when it was shown to have returned to normal (Figure 3). These three variables were each repeated three times on different explants and in all cases showed similar responses.

Discussion

The cystic fibrosis patient in this study has never had a recognized *P. aeruginosa* infection and demonstrates a CBF comparable to that of control patients. Cystic fibrosis

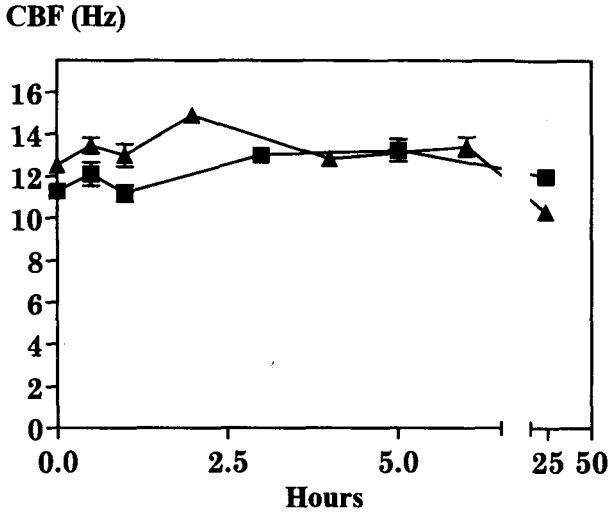

FIG. 2

Ciliary beat frequencies (CBF) of nasal polyp explants over a 24-hour period. Addition of the lectin PAII pre-incubated with its specific sugar fucose had little effect on the CBF of control explants (▲). Similar results were obtained from the cystic fibrosis patient (■).

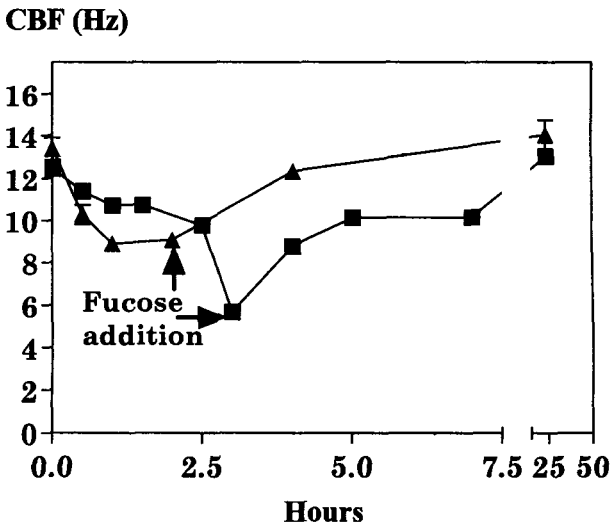

FIG. 3

Ciliary beat frequencies (CBF) of nasal polyp explants over a 24-hour period. The lectin PAII was added at time 0 and when a significant drop in CBF had occurred, a fucose addition was made. Note that in both cases a full recovery of CBF was made (▲ control culture, ■ cystic fibrosis culture).

patients normally become infected with *P. aeruginosa* by the age of 10–14 years and after this time almost all cystic fibrosis patients exhibit *P. aeruginosa* colonization (de Bentzmann *et al.*, 1996). *P. aeruginosa* is resistant to most commonly used antibiotics and as the *P. aeruginosa* infections continue, the bacteria produce ciliotoxins (Hingley *et al.*, 1986) and proteases which cause tissue damage (Fick *et al.*, 1985), resulting in reduced muco-ciliary transport. It is hence fortunate that this patient had no history of *P. aeruginosa* infection thus excluding any potential influence on CBF that this infection might have.

On adding PAII to the cultures, they displayed identical behaviour to the control cultures in that all ciliary activity had ceased within five hours (Figure 1). This effect on the CBF of the cystic fibrosis cultures could be completely inhibited by pre-incubation of the lectin with fucose resulting in blockage of the fucose binding sites (Figure 2), again identical to normal cultures. In addition, the cystic fibrosis airway epithelium cultures behaved exactly as the control airway cultures in that the addition of fucose reversed the decline (Figure 3). These results are in comparison to earlier work (Adam *et al.*, 1997), however, it remains to be established the latest possible stage at which fucose can be added to these cultures and still allow the cilia to return to a normal beat pattern.

Our results therefore show that the interaction between PAII and the cilia of CF patients is the same as that in the normal controls and therefore the delta F508 CF mutation as seen in this patient does not have any effect on this lectin-sugar interaction.

The fact that the action of PAII is inhibitable by fucose and the potential that the early stages of infection might be reversible add weight to the suggestion that therapeutic treatment with fucose could be of great benefit to CF patients. Although the primary pathology of CF patients lies in viscous mucus which results in poor muco-ciliary clearance and in turn allows adherent bacteria to remain in the respiratory tract for longer than normal, it has also been shown that *P. aeruginosa* will multiply profusely when transplanted onto tracheal cells from CF patients (Ko *et al.*, 1997). Therefore any form of prevention of

https://doi.org/10.1017/S0022215100138551 Published online by Cambridge University Press

attachment of this bacterium to the respiratory tract would seem to be advantageous for cystic fibrosis patients, especially in the early stages of infection.

References

Adam, E. C., Mitchell, B. S., Schumacher, D. U., Grant, G., Schumacher, U. (1997) *Pseudomonas aeruginosa* II lectin stops human ciliary beating: therapeutic implications of fucose. *American Journal of Respiratory and Critical Care Medicine* **155:** 2102–2104.

De Bentzmann, S., Roger, P., Puchelle, E. (1996) *Pseudomonas aeruginosa* adherence to remodelling respiratory epithelium. *European Respiratory Journal* **9:** 2145–2150.

Fick, R. B., Baltimore, R. S., Squier, S. V., Reynolds, S. Y. (1985) IgG proteolytic activity of *Pseudomonas aeruginosa* in cystic fibrosis. *Journal of Infectious Diseases* **151:** 589–598.

Hingley, S. T., Hastie, A. T., Kueppers, F., Higgins, M. L., Weinbaum, G., Shryock, T. (1986) Effect of ciliostatic factors from *Pseudomonas aeruginosa* on rabbit respiratory cilia. *Infection and Immunity* **51:** 254–262.

Ko, Y. H., Delannoy, M., Pederson, P. L. (1997) Cystic fibrosis, lung infections, and a human tracheal antimicrobial peptide (hTAP). *FEBS Letters* **405:** 200–208.

Warner, J. O. (1992) Immunology of cystic fibrosis. *British Medical Bulletin* **48:** 893–911.

Address for correspondence:
E. C. Adam,
Human Morphology,
University of Southampton,
Bassett Crescent East,
Southampton SO16 7PX.

Fax: +44 (0)1703 594433
email: ECA@soton.ac.uk