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Beneficial effect of antibiotics on ciliary beat frequency of human nasal epithelial cells exposed to bacterial toxins

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Abstract

In the present study, we explored whether the cilio-inhibitory effect induced by toxins derived from bacterial infections could be compensated for by a cilio-stimulatory effect of antibiotics. Human nasal epithelial cells (HNEC) expressing beating cilia were grown as monolayers. Ciliary beat frequency (CBF) was determined using an inverted microscope coupled with a high-speed digital camera. Clarithromycin and neomycin did not influence ciliary activity. Bacitracin, clindamycin, gramicidin and roxithromycin increased CBF significantly: by 50 ± 12%, 54 ± 16%, 31 ± 16% and 31 ± 18%, respectively. A 30 min exposure to *Staphylococcus aureus* enterotoxin B (SEB) and *Pseudomonas aeruginosa* lipopolysaccharide (PAL) decreased CBF significantly, by 37 ± 16 and 28 ± 12%, respectively. In contrast with exposure to the toxin alone, co-incubation of the nasal monolayer cells with PAL and bacitracin or clindamycin did not result in a decrease in CBF after 30 and 60 min. The effect of SEB could be compensated for by bacitracin but not by clindamycin. After a 12 h pre-incubation period with SEB, co-incubation with either bacitracin or clindamycin resulted in the complete recovery of CBF. This study suggests that topical antibiotic treatment of nasal infections could result in a dual positive effect, namely treatment of the bacterial infection and recovery of ciliary activity.

Introduction

Mucociliary clearance is one of the most important local defence mechanisms of the respiratory tract (Jorissen 1998). Chronic or recurrent respiratory tract infections are associated with disturbance of mucociliary clearance (Scadding et al 1995). It is therefore important not to impair this defence mechanism. One of the basic functional parameters that determines mucociliary clearance is the ciliary beat frequency (CBF) (Jorissen et al 2000).

The microbiology of acute bacterial rhinosinusitis consists mainly of *Haemophilus influenzae* and *Streptococcus pneumoniae* (Scheid & Hamm 2004), whilst the main agents in chronic sinusitis are *Staphylococcus aureus*, coagulase-negative staphylococci, *Pseudomonas aeruginosa* and anaerobic bacteria (Bachert et al 2003). Several authors have reported that products derived from these organisms can affect CBF. Particular efforts have been made to characterize the interaction of *H. influenzae* with respiratory epithelium and to identify the compounds responsible for the decrease in CBF after exposure to a culture filtrate of *H. influenzae* (Ferguson et al 1988; Read et al 1992). *S. pneumoniae* appears to have an effect on nasopharyngeal integrity (Lagrou et al 2003) but no direct effect on CBF was observed (Ferguson et al 1988). *P. aeruginosa* has been shown to release factors that cause slowing of human nasal cilia in-vitro (Wilson et al 1985), and attempts have been made to identify these factors (Hingley et al 1986). The most important factors are pyocyanin and 1-hydroxyphenazine, both of which slow CBF and disrupt the nasal epithelium (Wilson et al 1988; Kanthakumar et al 1993). Amitani and colleagues have shown that proteinases produced by *P. aeruginosa* also decrease CBF in association with epithelial disruption (Amitani et al 1991). Recently, several authors have examined the effect of different toxins produced by *S. aureus* on ciliary activity (Yun et al 1999; Kim et al 2000; Min et al 2006). All of the toxins examined in these studies (α-toxin, β-toxin and enterotoxin α, respectively)

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decreased CBF. Furthermore, exposure to toxins derived from *S. aureus* has been shown to induce allergic rhinitis (Okano et al 2005).

In this study, we wanted to explore whether the detrimental effects of bacterial toxins could be reversed. In the first part, we assessed the effect of selected bacterial toxins on CBF: *S. aureus* enterotoxin B (SEB) and *P. aeruginosa* lipopolysaccharide (PAL). SEB is a single-chain protein with a low molecular weight and is rich in polar amino acid residues; it contains only one disulphide bond and is produced in high amounts by the bacteria (200–500 μg mL^{-1} cell culture) (Chu 1968). Lipopolysaccharides from other bacteria have been shown to decrease CBF (Ueda et al 2001). PAL is composed of three components: a lipid A tail, a core oligosaccharide and an O-antigen which consists of 15–20 repeating monomers of a 3–5-sugar unit (Al-Tahhan et al 2000).

Acute and chronic bacterial rhinosinusitis can be treated with antibiotics, among other agents. Various antibiotics have been shown to have an effect on ciliary beating. For instance, exposure of rabbit tracheal epithelium to erythromycin resulted in a rapid increase in CBF, followed by a gradual decline (Tamaoki et al 1992). These results were confirmed for erythromycin and roxithromycin. Clarithromycin, on the other hand, has no effect on ciliary activity (Takeyama et al 1993). Dirithromycin appears to have no effect on CBF (Rutman et al 1998), while exposure to ofloxacin elicited a decrease in CBF (Gosepath et al 2002). Long-term antibiotic therapy has been reported to be associated with an increase in ciliary activity (Scadding et al 1995). In that study, 10 patients with chronic rhinosinusitis were treated for 3 months with amoxicillin, flucloxacillin, cefadril or co-trimoxazole, the antibiotic for each patient being selected on the basis of previous sinonasal cultures. CBF was determined on nasal brushings before and after the 3 months' treatment. CBF increased in all patients; the mean increased from 9.3 ± 2.3 Hz to 13.7 ± 1.6 Hz.

In the current study we have explored the effect of antibiotics of four different classes that are commonly prescribed for respiratory infections and can be administered nasally: bacitracin (MW 1423) and gramicidin (MW 1884) are polypeptide antibiotics; clarithromycin (MW 748) and roxithromycin (MW 837) are macrolides; clindamycin (MW 425) is a lincomycin; and neomycin (MW 615) is an aminoglycoside. Considering the CBF-decreasing effect of toxins and the cilio-stimulatory effect of some antibiotics, we were interested to see whether a decrease in CBF induced by bacterial toxins could be compensated for by the antibiotics.

Materials and Methods

Chemicals and materials

Protease type XIV and penicillin–streptomycin solution (10 000 IU mL^{-1} and 10 000 μg mL^{-1}, respectively) were purchased from Sigma Chemical Co. (St Louis, MO, USA). DMEM-Ham's F12 1:1 medium, Ultroser G and NU-serum were obtained from Life Technologies Ltd (Paisley, Scotland, UK). Saline (0.9% NaCl) was obtained from Fisher Scientific (Loughborough, UK). Bacitracin, neomycin sulfate (denoted neomycin) and clindamycin hydrochloride (denoted clindamycin) were purchased from Certa (Braine-l'Alleud, Belgium). Gramicidin and both toxins, namely SEB and PAL, were obtained from Sigma. Roxithromycin was provided by Roussel Uclaf (Paris, France) and clarithromycin by Abbott (Abbott Park, IL, USA).

Cell isolation, cell culture and incubation

Human nasal epithelial cells (HNEC) were isolated from nasal biopsies according to the procedure described by Jorissen et al (1989). Use of the nasal specimens was approved by the Committee of Medical Ethics of the University Hospitals Leuven. The nasal specimens were obtained from patients with nasal polyps.

The primary cell culture used in this system has been specifically developed to exclude the remaining effect on CBF of any therapeutic compounds that could have been used in the treatment of the patients. Briefly, after rinsing the tissues three times in saline, the human nasal epithelial tissues were enzymatically dissociated using 0.1% protease solution in DMEM–Ham's F12 1:1 medium, supplemented with 50 IU mL^{-1} penicillin and 50 μg mL^{-1} streptomycin for a period of 16–24 h at 4°C. At the end of the protease incubation, large pieces of tissue were removed and the protease activity was inhibited by adding 10% NU-serum. The cells were washed three times in cell culture medium (DMEM–Ham's F12 1:1 medium supplemented with 50 IU mL^{-1} penicillin, 50 μg mL^{-1} streptomycin and 2% Ultroser G) by centrifugation (800 rev min^{-1} for 5 min at 4°C). After the last centrifugation, the cell pellets were resuspended in 10 mL of the medium and incubated for 1 h in a 25 cm^2 plastic tissue culture flask in a CO_2 incubator (5% CO_2 95% air) at 37°C to allow selective attachment of the contaminating fibroblasts and macrophages to plastic. The cell number was determined using a Bürker chamber. The cells were plated into six-well plates pre-coated with 0.2% rat tail collagen at a density of 5×10^5 cells per well, in a final volume of 3 mL medium. The medium was changed 24 h after plating and then every other day. HNEC cultures formed microscopically confluent layers, consisting of ciliated and non-ciliated cells, 5 days after plating. Experiments with CBF measurements were performed on days 8–10 after plating. All experiments were performed in an air-conditioned room at a constant temperature of 22°C. Cell culture plates were removed from the incubator 1 h before the experiment, in order to allow the medium to adapt to the environmental temperature.

Different solvent systems were used for the different experiments. The initial studies exploring the effect of antibiotics were performed in isotonic saline, which has been shown not to affect CBF (Mallants et al 2007). However, cell viability was not maintained with isotonic saline for the long-term exposure experiments with toxins. We therefore used cell culture medium as the solvent system for experiments with bacterial toxins.

In previous experiments we have explored the effect of pH on CBF, showing that CBF was not influenced in the pH range 6.5–8.0 (Agu et al 1999). The shift in pH induced by including the antibiotics in the medium was within this pH range; also, no significant alkalization was observed during

the time period of the experiments when the cell culture plates were taken out of the incubator (pH at time 0 and 120 min was 7.3 and 7.4, respectively).

Data acquisition

An inverted microscope (Olympus IX70, Olympus Corp., Tokyo, Japan) was used at a magnification of ×600. A MotionScope high-speed digital camera and PCI application software, running with Windows 2000 (Redlake MASD Inc., San Diego, CA) were used for image acquisition. The images were captured at a frame rate of 512 frames per s, with a sampling interval of 2 ms. Sequences of 1024 images were recorded for each area. Each sequence of frame-by-frame images was stored in a file folder containing 1024 TIF format files for later analysis.

Calculation of ciliary beat frequency

CBF was calculated as described previously (Dimova et al 2005; Mallants et al 2007). Briefly, a CBF value is computed locally for each pixel separately by spectral analysis of the variation of the pixel intensity over time. First, a region of interest (ROI) is selected, which is defined as all pixels for which the standard deviation of the intensity variation over time exceeds a threshold value of 5 dB. For each pixel in the ROI, the influence of noise on CBF computation is reduced by spatial averaging of the intensity signal at each time point within a 3×3 pixel region centred around that pixel. Fast Fourier transformation (FFT) analysis is then applied to the smoothed intensity signal. The CBF is then calculated as the frequency corresponding to the maximal FFT amplitude value in the range 0–20 Hz. The analysis method was performed using Matlab (The Mathworks, Inc., Natick, MA, USA) and a graphical user interface was developed for CBF measurement and histogram analysis, which allowed overall statistics (mean, s.d., median) for the CBF of all beating cilia in the image to be derived.

Determination of the effect of antibiotics and bacterial toxins on CBF

The concentrations of antibiotics used were selected on the basis of either their concentration in registered pharmaceutical products (bacitracin, 250 IU mL^{-1}; neomycin, 3000 IU mL^{-1}; gramicidin, 50 mg L^{-1}) or the concentration used or reported in other studies (clarithromycin 10 mg L^{-1}; clindamycin 10 mg L^{-1}; roxithromycin 10 mg L^{-1}). A nasal cream containing 250 IU mL^{-1} bacitracin and 3000 IU mL^{-1} neomycin is registered in the USA and in Europe; gramicidin is commercially available at 50 mg L^{-1}; the concentration of clarithromycin in nasal mucosa has been reported to be almost 10 mg L^{-1} (Rodvold et al 1997; Jain & Danziger 2004). In lungs, the intracellular concentration of roxithromycin amounted to 21±10 mg L^{-1}, while the concentration in epithelial lining fluids appeared to be 2.0±1.7 mg L^{-1} (Chastre et al 1987; Jain & Danziger 2004). For clindamycin, a dose of 10 mg L^{-1} in solutions for nasal irrigation maintains levels significantly above the minimal inhibitory concentration for the treatment of *S. aureus* and *P. aeruginosa* (Elliott & Stringer 2006).

In order to explore the effect of the selected antibiotics, CBF values were determined 15 min after exposure of the ciliated nasal cells. Various concentrations of each antibiotic were tested to explore the concentration-dependent effect on CBF. An exposure period of 15 min was selected as this is the normal residence time in the nasal cavity (Merkus et al 1998). All antibiotic solutions were prepared in isotonic saline adjusted to pH 7.4. Although the effect of isotonic saline on the ciliary activity remains controversial, experiments performed in our laboratory did not reveal any influence on CBF compared with cell culture medium (Mallants et al 2007).

To determine the effect of SEB and PAL on CBF, the HNEC monolayer was incubated with a solution containing 30 ng mL^{-1} SEB or 1 mg mL^{-1} PAL. CBF was measured at 15, 30, 60 and 120 min after exposure of the monolayer to the toxins, in order to explore a time-dependent effect on the ciliary activity. All solutions of bacterial toxins were made in cell culture medium.

Simultaneous exposure to toxins and antibiotics

To determine the combined effect of toxins and antibiotics, the HNEC monolayer was exposed to a solution containing 30 ng mL^{-1} SEB or 1 mg L^{-1} PAL and either bacitracin (250 IU mL^{-1}) or clindamycin (10 mg L^{-1}). CBF was measured at 0, 15, 30, 60 and 120 min after exposure, in order to determine the time-dependent effect of the co-incubation on CBF.

We also explored the long-term effect of SEB on the ciliary activity by pre-incubating the nasal monolayer with a solution containing 30 ng mL^{-1} SEB for 12 h. After the pre-incubation period, the cells were exposed to either 30 ng mL^{-1} SEB or to a solution containing both 30 ng mL^{-1} SEB and the desired concentration of the antibiotic. CBF was measured after 0, 60 and 120 min' incubation. In this way, we mimicked the in-vivo situation in which a nasal infection is treated with topical antibiotic administration. All solutions were prepared using cell culture medium.

Data presentation and statistical analysis

Each set of experiments consisted of one control condition and five different test conditions. The data for each test condition were calculated as a percentage of the average CBF value for the corresponding control condition. Six CBF measurements were made for each condition. All experiments were performed in duplicate, using cells from two different patients, resulting in a total of 12 CBF determinations for each condition (presented as mean ± s.d.).

Data were analysed using one-way analysis of variance followed by Dunnett's multiple comparison test. Conditions were considered to be significantly different from the control condition when $P < 0.05$. Absolute CBF values for the control condition ranged from 5 to 8 Hz.

Results and Discussion

Effect of antibiotics on CBF

Bacitracin (250 IU mL^{-1}), clindamycin (10 mg L^{-1}), gramicidin (50 mg L^{-1}) and roxithromycin (10 mg L^{-1}) appeared to have a stimulatory effect on CBF after 15 min' exposure,

while neomycin (3000 IU mL^{-1}) and clarithromycin (10 mg mL^{-1}) did not affect CBF at these concentrations (Table 1). As, in-vivo, the solutions would be diluted by the presence of mucus, we also studied more dilute concentrations of the antibiotics for which a cilio-stimulatory effect was observed. After a ten-fold dilution (which has been described as clinically relevant (Lebe et al 2004)), bacitracin, clindamycin, gramicidin and roxithromycin still exhibited a cilio-stimulatory effect. For bacitracin and clindamycin, the effect on CBF was slightly lower, while for gramicidin and roxithromycin there was no decrease in response on dilution (Table 1). Clindamycin was also tested at lower concentrations; its concentration-dependent effect is illustrated in Figure 1.

As the highest effect on CBF was obtained with bacitracin and clindamycin (increases of 50 ± 12% and 54 ± 16%, respectively), these compounds were used in further studies.

Penicillin and streptomycin, which are included in the cell culture medium, had no effect on CBF (data not shown).

Table 1 Ciliary beat frequency (CBF) of human nasal epithelial cell (HNEC) monolayers after exposure to different concentrations of selected antibiotics. CBF values were determined after 15 min exposure and are expressed as a percentage of the CBF of the corresponding control condition

Antibiotic	Concentration	CBF (% control)	*P* value
Bacitracin	250 IU mL^{-1}	150 ± 12	< 0.001
	25 IU mL^{-1}	137 ± 28	< 0.001
Clarithromycin	10 mg L^{-1}	122 ± 20	n.s.
Clindamycin	10 mg L^{-1}	154 ± 16	< 0.001
	1 mg L^{-1}	134 ± 19	< 0.001
Gramicidin	50 mg L^{-1}	131 ± 16	< 0.01
	5 mg L^{-1}	138 ± 30	< 0.001
Neomycin	3000 IU mL^{-1}	99 ± 15	n.s.
Roxithromycin	10 mg L^{-1}	131 ± 18	< 0.001
	1 mg L^{-1}	132 ± 12	< 0.001

Values are means ± s.d. (n = 12).
n.s., not significant.

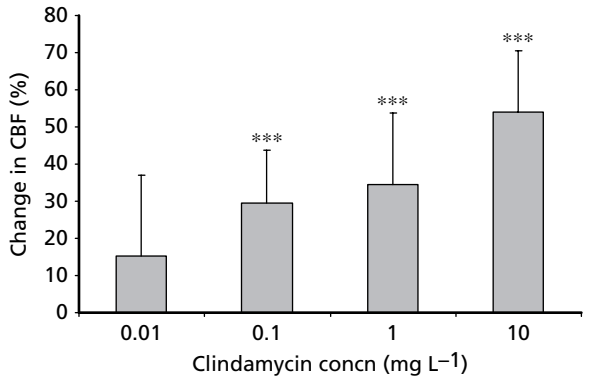

Figure 1 Concentration-dependent effect of clindamycin on ciliary beat frequency (CBF) in human nasal epithelial monolayers. Data show the change in CBF after 15 min exposure to different concentrations of clindamycin (mean ± s.d.; n = 12). ***$P < 0.001$ for test condition vs corresponding control condition.

Effect of bacterial toxins on CBF

The time-dependent effect of 30 ng mL^{-1} (SEB) and 1 mg mL^{-1} PAL on CBF is shown in Figure 2. The concentration of SEB was chosen on the basis of a study in which 30 ng mL^{-1} staphylococcal enterotoxin A produced a statistically significant decrease in CBF when using New Zealand White rabbits (Min et al 2006). SEB (30 ng ml^{-1}) showed an immediate effect on CBF, resulting in a significant decrease within 15 min of exposing the HNEC to the toxin (30 ± 19%). As bacterial toxins are expected to reside in the nasal cavity for a longer period of time, their effect was studied for 120 min. No time-dependent effect of SEB on CBF was observed (Figure 2): CBF decreased by 37 ± 16%, 38 ± 11% and 26 ± 11% after 30, 60 and 120 min' exposure to SEB, respectively. In contrast to the immediate decrease in CBF observed in this study, Min and colleagues (2006) reported a significant effect only after 4 h exposure to staphylococcal enterotoxin A (30 ng mL^{-1}).

PAL (1 mg mL^{-1}) has been reported to decrease CBF significantly after a 30 min exposure period (Rautiainen et al 1994). This concentration was therefore selected for this study. Exposure of the HNEC to 1 mg mL^{-1} PAL resulted in an immediate decrease in CBF (18 ± 13% after 15 min). As with SEB, no further decrease in CBF with exposure time to PAL was observed: after 30, 60 and 120 min, the decrease in CBF amounted to 28 ± 12%, 21 ± 8% and 21 ± 9%, respectively (Figure 2). The data obtained for PAL are also comparable with an in-vivo study performed by Ueda and colleagues (2001) in which CBF decreased by 16% after exposure of guinea pigs to a solution containing 2.5 mg mL^{-1} lipopolysaccharides from *Escherichia coli*.

As a continuous decrease in CBF was observed in the study by Min and colleagues (2006), we also studied the effect of an incubation period of 720 min before CBF measurements. No additional decrease in CBF was observed compared with a 30 min exposure period to SEB: decreases in CBF were 34 ± 12% versus 37 ± 16% after 30 and 720 min'

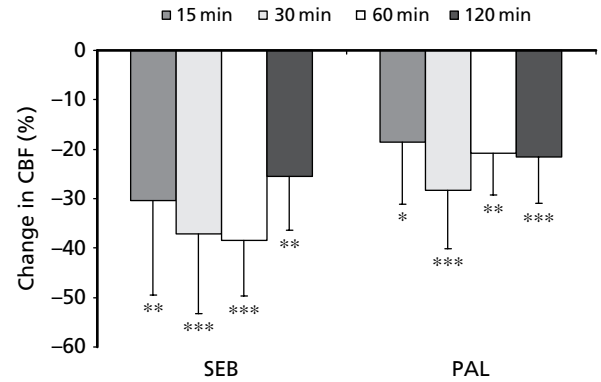

Figure 2 Ciliary beat frequency (CBF) at 15, 30, 60 and 120 min after exposure of human nasal epithelial cell monolayers to *Staphylococcus aureus* enterotoxin B (SEB; 30 ng mL^{-1}) or *Pseudomonas aeruginosa* lipopolysaccharide (PAL; 1 mg mL^{-1}). Both SEB and PAL decreased CBF significantly at all time points. Values are mean ± s.d. (n = 12). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ for test condition vs corresponding control condition.

exposure, respectively. These results confirm the results discussed above, where no additional effect on CBF was observed when the HNEC were exposed to SEB for 120 min. Apparently, SEB resulted in an immediate effect on CBF and did not elicit additional ciliotoxicity after long-term exposure.

Simultaneous exposure to bacterial toxins and antibiotics

The results of exposure of the HNEC monolayers to bacterial toxins and antibiotics simultaneously are presented in Figure 3 (SEB) and Figure 4 (PAL). As described above, CBF decreased significantly after exposure of the ciliated nasal cells to SEB (30 ng mL^{-1}). When the nasal cells were exposed to SEB (30 ng mL^{-1}) in combination with bacitracin (250 IU mL^{-1}), CBF values were similar to control condition values at 15, 30 and 60 min, suggesting that the cilio-stimulatory effect of bacitracin could compensate for the cilio-inhibitory effect of SEB. In contrast to these results, co-incubation of the nasal cell monolayer with SEB and clindamycin (10 mg mL^{-1}) resulted in decreases in CBF of 17 ± 12%, 26 ± 17% and 28 ± 13% after 15, 30 and 60 min' exposure, respectively. Although CBF was decreased significantly by SEB in the presence of clindamycin, a tendency for clindamycin to partly compensate for the CBF-decreasing effect of SEB was observed.

Co-incubation of PAL (1 mg mL^{-1}) with either bacitracin (250 IU mL^{-1}) or clindamycin (10 mg L^{-1}) did not reveal any differences in CBF compared with the control condition; the exposure of the nasal cells to PAL alone decreased CBF (Figure 4). The results obtained after 15, 30 and 60 min' exposure were comparable. The effect of PAL on CBF alone or in combination with bacitracin or clindamycin appeared to be independent of the exposure time.

As described above, the influence of SEB on CBF was not affected by the exposure time: there was no additional decrease in CBF after a 720 min incubation period with 30 ng mL^{-1} SEB compared with short-term exposure. When the HNEC monolayer was pre-incubated for 12 h with SEB (30 ng mL^{-1}), CBF decreased at the beginning of the experiment (Figure 5). After this pre-incubation period, the HNEC monolayer was incubated with SEB in combination with bacitracin (250 IU mL^{-1}) or clindamycin (10 mg L^{-1}). In this setup, both bacitracin and clindamycin resulted in a complete recovery of the ciliary activity after both 60 and 120 min' exposure (Figure 5). When the nasal cells were incubated

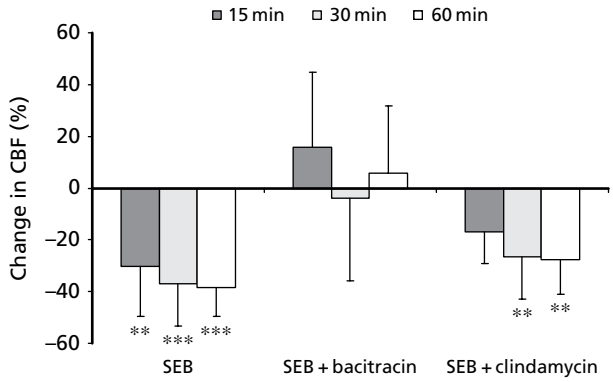

Figure 3 Ciliary beat frequency (CBF) of human nasal epithelial cell (HNEC) monolayers after 15, 30 and 60 min exposure to *Staphylococcus aureus* enterotoxin B (SEB; 30 ng mL^{-1}) alone or in combination with bacitracin (250 IU mL^{-1}) or clindamycin (10 mg L^{-1}). Simultaneous exposure of the HNEC to SEB and bacitracin abolished the cilio-inhibiting effect of the bacterial toxin. Co-incubation with SEB and clindamycin showed a tendency to decrease the cilio-inhibiting effect of SEB. Values are mean ± s.d. (n = 12). $^{**}P < 0.01$; $^{***}P < 0.001$ for test condition vs corresponding control condition.

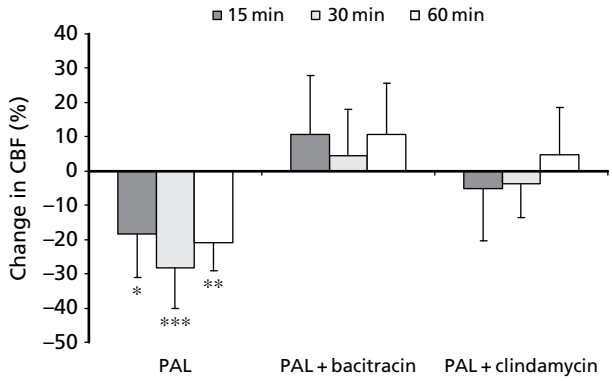

Figure 4 Ciliary beat frequency (CBF) of human nasal epithelial cell (HNEC) monolayers after 15, 30 and 60 min exposure to *Pseudomonas aeruginosa* lipopolysaccharide (PAL; 1 mg mL^{-1}), alone or in combination with bacitracin (250 IU mL^{-1}) or clindamycin (10 mg L^{-1}). Simultaneous exposure of the HNEC to PAL and bacitracin or clindamycin completely abolished the cilio-inhibiting effect of the bacterial toxin. Values are mean ± s.d. (n = 12). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ for test condition vs corresponding control condition.

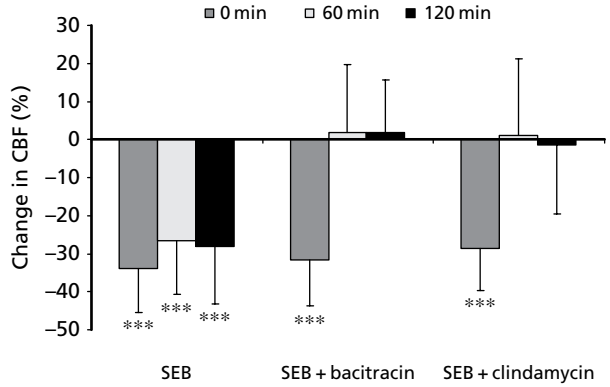

Figure 5 Ciliary beat frequency (CBF) of human nasal epithelial cell (HNEC) monolayers after 12 h pre-incubation with *Staphylococcus aureus* enterotoxin B (SEB; 30 ng mL^{-1}). CBF was measured immediately after the pre-incubation (0 min) and after 60 and 120 min incubation with SEB, alone or in combination with bacitracin (250 IU mL^{-1}) or clindamycin (10 mg L^{-1}). Exposure to the bacterial toxin alone maintained a significant decrease in ciliary activity, while inclusion of bacitracin or clindamycin resulted in a complete recovery of the CBF. Values are mean ± s.d. (n = 12). $^{***}P < 0.001$ for test condition vs corresponding control condition.

with the toxin alone, CBF values remained significantly lower at the time points measured and no recovery was apparent. These results suggest that the cilio-inhibiting effect of SEB is reversible. A discrepancy was observed for the effect of clindamycin in the short- versus long-term exposure experiments: although both studies showed that SEB had less effect in the presence of clindamycin, complete recovery of CBF was observed for the 12 h SEB pre-incubation experiment, whereas clindamycin only partly compensated for the SEB-induced decrease in CBF in the short-term exposure experiment. We can only speculate about the mechanism underlying this discrepancy. One of the possible reasons might be that a partial degradation of SEB occurred so that its effect could be more easily compensated for by clindamycin.

The underlying mechanism of the CBF-modulating effect of the antibiotics and the bacterial toxins remains unknown. The interactions could occur at different levels. Three ubiquitous second messengers have been reported to enhance ciliary beating: the intracellular calcium concentration, cAMP and cGMP (Zagoory et al 2002). Although it seems that each of these second messengers represents an independent pathway for the regulation of CBF, these pathways are closely interconnected and their combined effect is required to achieve a strong and sustained enhancement of CBF. The exact mechanisms by which these second messengers regulate ciliary beating remain uncertain. The mechanism is best understood for cAMP, which regulates ciliary beating by activation of cAMP-dependent protein kinase. This phosphorylates the outer dynein arm, which can in turn mediate a switch from the slow to the fast dynein-duty cycle and thus increase CBF. The increase in CBF induced by elevated intracellular calcium concentrations appears to be divided into two parts: an initial response which seems to be directly mediated by calcium-binding proteins, and a prolonged increase in CBF which seems to rely on additional signalling mechanisms, such as the ones activated by cAMP and cGMP. On the other hand, protein kinase C has been reported to slow ciliary beating, although the underlying mechanism is not fully understood (Salathe 2007). The observations presented in this manuscript warrant further research to explore how the toxins and the antibiotics interact with these regulatory systems.

Conclusion

We have examined the effect of antibiotics and bacteria-derived toxins on the ciliary activity of HNEC cultures. Fifteen min' exposure to bacitracin, clindamycin, gramicidin or roxithromycin resulted in a significant increase in CBF. Neomycin and clarithromycin did not influence ciliary activity. The results suggest that topical antibiotic treatment of nasal infections could result in a dual positive effect, namely treatment of the bacterial infection and recovery of ciliary activity. It would be interesting to explore the mechanisms underlying the cilio-stimulating effect of the antibiotics and the cilio-inhibiting effect of bacterial toxins, and how these mechanisms interact. In this way, a rationale could be developed that takes into account whether an antibiotic is preferentially delivered orally or nasally for the treatment of nasal bacterial infections. It would also be interesting to elucidate the extent of these effects in-vivo.

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