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Effect of macrolide antibiotics on ciliary motility in rabbit airway epithelium in-vitro

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Abstract—We have studied ciliary beat frequency (CBF) of rabbit cultured tracheal epithelium by a photoelectric method in-vitro. Addition of erythromycin and roxithromycin increased CBF in a dose-dependent fashion, whereas clarithromycin was without effect. The rank order potency of macrolide was roxithromycin > erythromycin ≫ clarithromycin. The roxithromycin-induced increase in CBF was not altered by propranolol, AA-861, or verapamil, but partially attenuated by indomethacin. Roxithromycin increased intracellular cAMP concentrations. These results suggest that certain macrolides can stimulate airway ciliary motility probably via prostaglandin- and cAMP-dependent regulatory pathways, which may affect mucociliary transport function in the respiratory tract.

The macrolide antibiotics are widely used as an alternative to β-lactams for the treatment and prevention of airway infections caused mainly by Gram-positive organisms and non-bacterial species such as *Mycoplasma pneumoniae*. Macrolide antibiotics also modulate the actions of a variety of inflammatory cells including polymorphonuclear leucocytes (Roche et al 1986), lymphocyte and macrophages (Anderson 1989), and directly inhibit airway secretion of mucus glycoprotein (Goswami et al 1990) and water (Tamaoki et al 1992). Thus, the clinical effects of these drugs may depend on their anti-inflammatory and anti-secretory actions as well as their anti-microbial properties.

Mucociliary transport plays a principal role in the lung defence mechanism by cleaning bacteria and cellular debris from the peripheral to central airways. This function depends on the interaction between ciliary epithelium and airway mucus, where the ciliary length, the pattern of beating, the beat frequency of cilia and their co-ordination appear to be important factors (Wanner 1977). Therefore, stimulation of ciliary motility might prevent the retention of inhaled microorganisms, thereby inhibiting bacterial colonization on the airway mucosa.

In the present study, to determine whether erythromycin and newly developed macrolide antibiotics, roxithromycin (Jones et al 1983) and clarithromycin (Kohno et al 1989), can affect airway ciliary motility, we measured ciliary beat frequency (CBF) of rabbit tracheal epithelium by a photoelectric technique in-vitro.

Materials and methods

Tissue preparation. Japanese White rabbits (1·8–2·4 kg) were anaesthetized with intravenous sodium pentobarbitone (35 mg kg^{-1}). The trachea was removed and its mucosa was dissected free from the underlying connective tissue, cut into small pieces (1–2 mm^3), rinsed several times with Dulbecco's phosphate-buffered saline, and placed on a microscope coverglass in a petri dish. Tissues were then incubated in Medium 199 containing 10% foetal calf serum with 100 units mL^{-1} each of penicillin and streptomycin at 37°C in a CO_2 incubator (95% air–5% CO_2). On the seventh day of incubation, tissues were mounted in a Rose chamber to measure CBF.

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Photoelectric technique. The photoelectric technique to measure CBF has been described in detail previously (Tamaoki et al 1989a). Briefly, we used a microscope equipped with a phase-contrast condenser and an on-base type of halogen illuminator (Nikon, Optiphoto-XF, Tokyo, Japan), to the head of which the photometer (Hamamatsu Photonics, NFX-II, Hamamatsu, Japan) with a built-in periplanatic eyepiece, a limiting aperture, and a lateral focusing telescope were attached. The beating action of cilia interfered with the passage of light through the preparation, varying the voltage output of the photometer which was amplified and continuously recorded on a pen recorder (VP 6213; Panasonic, Tokyo, Japan). In addition to CBF, we assessed ciliary co-ordination by the image of beating recorded on a video camera capable of freeze-frame replay (Sony, VO-5800, Tokyo, Japan) (Fig. 1) and ciliary dis-co-ordination was defined as the loss of metachronal wave on the free border of the cell clump (Wanner et al 1983).

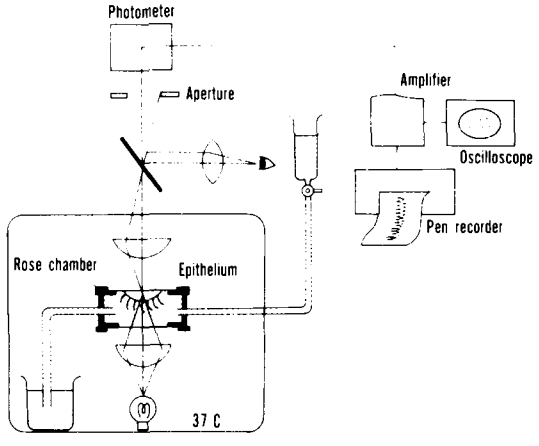

FIG. 1. Schematic diagram of a photoelectric method, an analytical device for the measurement of airway ciliary motility.

Effects of macrolides on CBF. The preparation was allowed to equilibrate for 30 min in Krebs–Henseleit solution. To assess the effects of macrolides on CBF, we first determined the baseline CBF, and medium was drained off the chamber. Perfusate was replaced with Krebs–Henseleit solution containing each of erythromycin (10^{-5} M, Sigma, St Louis, MO, USA), roxithromycin (10^{-5} M, Roussel Uclaf, Paris, France), clarithromycin (10^{-5} M, Taisho Pharmaceutical Co., Tokyo, Japan) or an equivalent volume of the solvent alone (dimethylsulphoxide), and 5 min later, CBF was measured again. To examine a dose-response relationship, each drug at a concentration ranging from 10^{-9} to 10^{-3} M was cumulatively added to the chamber and the highest recorded value in response to each concentration was determined. Because the ciliary stimulating effect was most pronounced with roxithromycin, this macrolide was used in subsequent experiments. To assess the time course of the effect of roxithromycin on CBF, roxithromycin (10^{-5} M) was added to the chamber and CBF was continuously recorded for 30 min.

Effects of pharmacologic blocking agents. To assess possible contributions of β-adrenoceptors, arachidonic acid metabolites and Ca^{2+} influx to the action of roxithromycin, we compared roxithromycin (10^{-5} M)-induced increases in CBF in the absence and presence of the following pharmacologic blocking agents: propranolol (10^{-5} M, Sigma), a β-adrenoceptor antagonist; indomethacin (3×10^{-6} M, Sigma), a cyclo-oxygenase inhibitor; AA-861 (2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadignyl)-1,4-benzoquinone, 10^{-6} M, Takeda Chemicals, Osaka, Japan), a lipoxygenase inhibitor (Tamaoki et al 1991); and verapamil (10^{-5} M), a Ca^{2+}-entry blocker.

Measurement of cyclic AMP. To assess whether the alteration of cyclic (c) AMP levels was involved, we measured concentrations of cAMP in epithelial cells (Tamaoki et al 1989b). Fifteen minutes after the addition of roxithromycin (10^{-5} M) to the chamber in the presence of 3-isobutyl-1-methylxanthine (10^{-3} M), tissues were placed in ice-cold 10% trichloroacetic acid and sonicated. In control tissues, solvent only (dimethylsulphoxide) was applied. After the extraction of trichloroacetate with ether, the residue was dissolved in acetate buffer, and cAMP levels were determined in duplicate by ^{125}I-radioimmunoassay (New England Nuclear, Boston, MA, USA), normalized for protein content of the tissues as determined by the method of Lowry et al (1951).

Statistics. All values are expressed as means ± s.e. Statistical analysis was performed by analysis of variance or Newman-Keuls multiple comparison test; $P < 0.05$ was considered significant.

Results

Effects of macrolides on CBF. The baseline CBF values obtained before drug addition were not different between groups (11·8 ± 0·4 Hz for clarithromycin, 12·2 ± 0·5 Hz for erythromycin, 11·5 ± 0·3 Hz for roxithromycin). Addition of roxithromycin (10^{-5} M) to the chamber increased CBF of rabbit tracheal epithelium by 34·3 ± 5·5% (Table 1, $P < 0.001$, n = 8). Erythromycin also elicited an increase in CBF, but this effect was smaller than that of roxithromycin. No significant effect was observed on the addition of clarithromycin. Thus, the rank order potency of the effect of macrolides on ciliary stimulation was roxithromycin > erythromycin ≫ clarithromycin.

Cumulative application of roxithromycin and erythromycin increased CBF in a dose-dependent fashion, the concentration required to produce a half-maximal effect (EC50) being 4×10^{-7} M for roxithromycin and 7×10^{-7} M for erythromycin. In contrast, clarithromycin had no effect at concentrations of up to 10^{-3} M (Fig. 2).

As demonstrated in Fig. 3, addition of roxithromycin (10^{-5} M) to the chamber elicited a rapid increase in CBF from the baseline value of 11·1 ± 0·3 to the maximal value of 16·5 ± 0·5 Hz ($P < 0.001$, n = 8). This response was followed by a gradual decline, but CBF after 10 min of incubation was still significantly greater than the baseline values ($P < 0.05$); the solvent dimethylsulphoxide did not alter epithelial CBF. Dis-co-ordination of ciliary beating was not observed in any of the experiments.

Table 1. Effects of clarithromycin, erythromycin and roxithromycin on ciliary beat frequency (CBF) of rabbit cultured tracheal epithelium. Each drug was added to the chamber at a concentration of 10^{-5} M and the highest recorded value was depicted. Values are expressed as percentage of the baseline CBF determined before drug addition.

Drug	Ciliary beat frequency (% baseline ± s.e. n = 8)
Clarithromycin	6·8 ± 3·8
Erythromycin	20·4 ± 3·8**
Roxithromycin	34·3 ± 5·5***

**$P < 0.01$, ***$P < 0.001$, compared with baseline values.

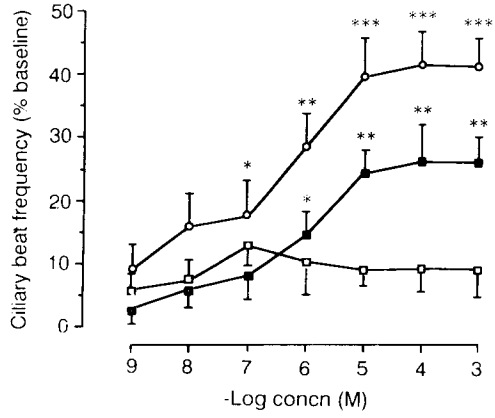

FIG. 2. Concentration-dependent effects of roxithromycin (○), erythromycin (■) and clarithromycin (□) on ciliary beat frequency (CBF). Each drug was added in a cumulative manner to the chamber. Values are expressed as percentage of the baseline CBF obtained before drug addition. Each point represents mean ± s.e.; n = 6. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with baseline values.

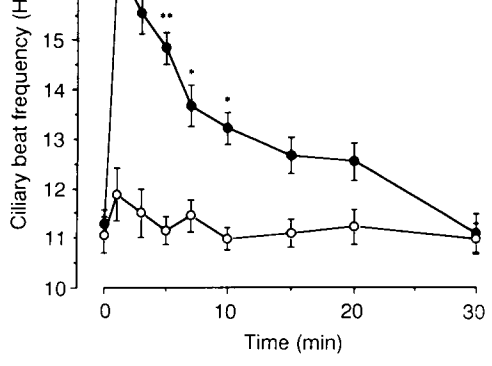

FIG. 3. Time-course of the effects of roxithromycin (●) and its vehicle dimethylsuphoxide (○) on ciliary beat frequency (CBF). After determining the baseline CBF, each agent was added at time 0 to the chamber. Each point represents mean ± s.e.; n = 8. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with responses for the solvent alone.

The increase in CBF produced by roxithromycin was not significantly altered by pretreatment of cells with propranolol, AA-861, or verapamil, but it was partially inhibited by the cyclo-oxygenase inhibitor, indomethacin (Table 2).

Intracellular cAMP levels. Incubation of epithelial cells with roxithromycin (10^{-5} M) increased intracellular cAMP levels from 42·4 ± 7·9 to 72·3 ± 12·7 pmol (mg protein)$^{-1}$ ($P < 0.05$, n = 8), whereas dimethylsulphoxide alone was without effect (Table 3).

Discussion

Our in-vitro studies demonstrate that the macrolide antibiotics roxithromycin and erythromycin increase CBF in rabbit tracheal epithelium without alterations in co-ordinated pattern

Table 2. Effects of pretreatment of cells with propranolol, verapamil, indomethacin or AA-861 on the response of ciliary beat frequency to roxithromycin (3×10^{-7} M). Responses are expressed as means ± s.e.; n = 8.

Drug pretreatment	Ciliary beat frequency (% baseline ± s.e.)
None	116 ± 2
Propranolol 10^{-5} M	118 ± 2
Verapamil 10^{-5} M	114 ± 3
Indomethacin 3×10^{-6} M	105 ± 2*
AA-861 10^{-5} M	115 ± 3

*$P < 0.05$ compared with roxithromycin alone.

Table 3. Effects of roxithromycin and dimethylsulphoxide vehicle on cyclic AMP levels in rabbit cultured tracheal epithlium. Data are means ± s.e.; n = 8.

Treatment	Cyclic AMP (pmol (mg protein)$^{-1}$)
Control	42·8 ± 6·7
Vehicle	39·5 ± 6·7
Roxithromycin	74·3 ± 12·3*

*$P < 0.05$ compared with control values.

of beating action. In view of the theoretical model of mucociliary pumping (Ross & Corrison 1974) and Reynold's number (Sleigh 1989), our results suggest that these macrolides may play a beneficial role in airway mucociliary clearance by stimulating epithelial ciliary motility to transport mucus, cellular debris and inhaled bacteria in the respiratory tract (Wanner 1977). In contrast to the effects of roxithromycin and erythromycin, clarithromycin did not alter CBF. Anderson et al (1984) showed that serum concentrations of erythromycin of $1{\cdot}6 \times 10^{-6}$ M were obtained following the ingestion of 500 mg. Thus, a therapeutic dose of the macrolides appears to be sufficient to increase ciliary motility. However, further studies may be required to confirm whether the observed effects of macrolides directly reflect the improvement of mucociliary clearance. Erythromycin possesses immunomodulatory actions including stimulation of leucocytes motility and phagocytosis (Anderson 1989), and roxithromycin shows an antioxidant-like effect. Goswami et al (1990) reported that erythromycin can decrease glycoprotein secretion from human airways in-vitro. The findings from our laboratory have shown that this macrolide selectively inhibits Cl^- secretion across airway mucosa, which may lead to the decreased water secretion toward the lumen (Tamaoki et al 1992). Taken together, our present results could implicate clinical improvement in mucus mobilization observed after macrolide treatment.

The regulatory pathway of airway ciliary motility includes a variety of factors, including β-adrenoceptor function, arachidonic acid metabolism, Ca^{2+} influx and the presence of protein kinase A (Widdicombe 1991). The increase in CBF produced by roxithromycin was not affected by pretreatment of cells with the β-adrenoceptor antagonist propranolol, the lipoxygenase inhibitor AA-861 (Tamaoki et al 1991), or the Ca^{2+}-entry blocker verapamil, but it was partially attenuated by the cyclo-oxygenase inhibitor indomethacin. Therefore, the ciliary stimulatory effect of roxithromycin is not associated with β-adrenoceptor, lipoxygenase products, or Ca^{2+} entry, but may be attributable, at least in part, to cyclo-oxygenase products. Airway epithelial cells synthesize and release various prostaglandins, among which prostaglandin E_2 is the major product (Widdicombe et al 1989) and can stimulate cAMP accumulation (Smith et al 1982). Intracellular cAMP levels were increased by roxithromycin at a concentration that stimulated ciliary motility. Thus, the mechanism of action of macrolides may be related to prostaglandin synthesis and the subsequent production of the ciliary stimulating substance cAMP (Tamaoki et al 1989b).

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