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Effect of erythromycin on *Haemophilus influenzae* endotoxin-induced release of IL-6, IL-8 and sICAM-1 by cultured human bronchial epithelial cells

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ABSTRACT: Although several studies have demonstrated that low-dose, long-term erythromycin treatment is effective in the management of patients with chronic lower respiratory tract infections, such as chronic bronchitis, bronchiolitis and bronchiectasis, the mechanisms underlying the action of erythromycin are not clear.

We have cultured human bronchial epithelial cells (HBEC) as explant cultures from surgical tissue, and have investigated the effect of erythromycin on *H. influenzae* endotoxin (HIE)-induced release of inflammatory mediators in these cultures. Confluent epithelial cell cultures were incubated with 100 μg·mL^{-1} HIE ±0.1–10 μg·mL^{-1} erythromycin and were investigated for interleukin-6 (IL-6), interleukin-8 (IL-8) and soluble intercellular adhesion molecule-1 (sICAM-1) released into the culture medium after 24 h.

HIE significantly increased the release of IL-6 from 3.9±1.5 pg·μg^{-1} cellular protein (in control untreated cultures) to 12.1±1.5 pg·μg^{-1} cellular protein, and IL-8 from 83.7±8.2 pg·μg^{-1} cellular protein (in control cultures) to 225.7±44.8 pg·μg^{-1} cellular protein. Similarly, HIE led to a significantly greater release of sICAM-1 from 0.04±0.01 ng·μg^{-1} cellular protein, in control cultures, to 3.8±0.9 ng·μg^{-1} cellular protein. Incubation of the epithelial cultures in the presence of 0.1–10 μg·mL^{-1} erythromycin significantly blocked the HIE-induced release of IL-6, IL-8, and sICAM-1, at all concentrations of erythromycin investigated. Erythromycin also attenuated neutrophil chemotaxis and adhesion to human endothelial cells, mediated by incubation with conditioned medium obtained from HIE-exposed epithelial cell cultures, *in vitro*.

These results suggest that *H. influenzae*-induced release of inflammatory mediators from airway epithelial cells could contribute to chronic airway inflammation, and that this effect may be modulated by treatment with erythromycin.
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Infections of the respiratory tract with Gram-negative bacteria have been a leading cause of death from chest infections in the hospital [1]. Studies of *Haemophilus influenzae*, a commonly occurring pathogen in infectious conditions of the lower respiratory tract, have demonstrated that this micro-organism and its products can lead to airway epithelial cell damage and death. Studies by WILSON and COLE [2] have demonstrated that factors produced by *H. influenzae* in culture filtrates can lead to immediate ciliary slowing and dyskinesia, epithelial disruption, cellular extrusion and cell death *in vitro*. Recent studies in our laboratory have demonstrated that *H. influenzae* endotoxin (HIE) significantly increased the expression and release, from cultured human bronchial epithelial cells (HBEC), of proinflammatory cytokines [3]. These studies suggest that bronchial epithelial cells may play an important role in the pathogenesis of chronic inflammatory airway disease, since epithelial cell-derived cytokines can influence the activity of inflammatory cells, including neutrophils, the chief effector cells in lower respiratory tract infections, such as chronic bronchitis, bronchiectasis and diffuse panbronchiolitis [4].

Although erythromycin, a macrolide antibiotic, is widely used as an anti-infective agent in bacterial exacerbations of chronic bronchitis and community-acquired pneumonia, it may also have an anti-inflammatory role. In Japan, erythromycin at a dose of 250–500 mg daily for 3–6 months has been shown to be effective in the management of patients with diffuse panbronchiolitis [5]. The precise mechanisms underlying the anti-inflammatory action, however, are not clearly understood. Studies by UMEKI [6] have suggested that erythromycin may exert an anti-inflammatory role by inhibiting superoxide production by neutrophils. Similarly, ICHIKAWA *et al*. [7] have suggested that erythromycin may act by reducing neutrophils and neutrophil-derived elastolytic-like activity in the lower respiratory tract of patients with bronchiolitis.

In the present study, we have hypothesized that erythromycin may exert its anti-inflammatory effect by modulating either the expression and/or release of pro-inflammatory cytokines, from human airway epithelial cells, which affect neutrophil activity. To test this hypothesis, we have investigated the effect of erythromycin on HIE-induced release of interleukin-6 (IL-6), interleukin-8 (IL-8) and soluble intercellular adhesion molecule-1 (sICAM-1), in HBEC *in vitro*. Additionally, we have studied the effect of this agent on neutrophil chemotaxis and adhesion to human endothelial cell cultures *in vitro*.

Materials and methods

All chemicals and reagents were of tissue culture grade and, unless otherwise stated, were obtained from the Sigma Chemical Co. (Poole, UK).

Isolation and culture of bronchial epithelial cells (HBEC)

HBEC were cultured as explant cultures from tissue of 10 individuals who had presented for lung surgery at St. Bartholomew's Hospital, according to a technique described previously [8].

Preparation of H. influenzae *endotoxin (HIE)*

HIE was prepared from *H. influenzae* (strain NCTC 8143; Reference Laboratory, London, UK) as described previously [3]. Briefly, a suspension of 10 g lyophilized bacteria in 175 mL water, was sonicated and mixed with 90% phenol (w/v). Following vortexing and centrifugation at 3,000×g for 30 min at 0°C, the aqueous phase was aspirated and dialysed against running water for 48 h. The dialysed suspension was centrifuged at 100,000×g for 4 h at 4°C, and the supernatant containing the crude HIE was treated with 100 $\mu g \cdot mL^{-1}$ ribonuclease A and 100 $\mu g \cdot mL^{-1}$ amylase, for 4 h at 37°C, to release the HIE associated with ribonucleic acid (RNA) and glycogen present in the suspension. At the end of incubation, proteinase-K was added to inactivate the ribonuclease and amylase, and the suspension was subjected to a further cycle of phenol extraction, dialysis and ultracentrifugation, as above, to obtain the "purified" HIE.

The biological activity of the HIE was assessed by the Limulus amoebocyte lysate (LAL), as described previously [3].

Effect of erythromycin on HIE-induced release of IL-6, IL-8 and sICAM-1

Fully confluent HBEC were washed with culture medium and incubated with a single concentration of 100 $\mu g \cdot mL^{-1}$ HIE, which had previously been demonstrated to be optimal in inducing the release of inflammatory cytokines from bronchial epithelial cells [3]. Sets of at least six separate cultures each were then either treated with 0.1–10 $\mu g \cdot mL^{-1}$ erythromycin lactobionate (Abbot laboratories, Queenborough, Kent, UK) or untreated, and incubated at 37°C in a 5% CO_2 in air atmosphere for 24 h. The effect of each treatment regimen was investigated on the same day. At the end of incubation, the medium from each culture was stored at -70°C until analysis for IL-6, IL-8 and sICAM-1, using commercially available enzyme-linked immunosorbent assay (ELISA) kits (British Biotechnology Ltd, Abingdon, UK). The cells in each culture were collected for protein analysis [9], and the results for the mediators released by HBEC were expressed as $pg \cdot \mu g^{-1}$ cellular protein.

In order to determine whether or not release of cytokines in HIE-treated HBEC was specifically due to the endotoxin, separate sets of HBEC were incubated with 100 $\mu g \cdot mL^{-1}$ HIE ±10–100 $\mu g \cdot mL^{-1}$ polymyxin B for 24 h. At the end of incubation, the medium and the cells were collected and analysed for IL-8 and total cellular protein, as described above. In a separate set of experiments, HBEC were also incubated for 24 h with 50–5,000 $pg \cdot mL^{-1}$ IL-1β ±1 $\mu g \cdot mL^{-1}$ erythromycin, a concentration found to be optimal in reducing cytokine release. At the end of incubation, the medium and the cells were collected and analysed for IL-8, as described above.

Interaction of erythromycin with HIE, IL-6, IL-8 and sICAM-1

The effect of erythromycin on HIE activity was assessed by incubating 100 $\mu g \cdot mL^{-1}$ HIE with 0.1–10 $\mu g \cdot mL^{-1}$ erythromycin in Medium 199 for 24 h, at 37°C in 5% CO_2 in air. At the end of incubation, the medium was assayed for endotoxin activity by the LAL test, as described previously [3].

Similarly, the effect of erythromycin on detectability and concentration of recombinant human IL-6, IL-8 and sICAM-1 was investigated by incubating different concentrations of these mediators with 0.1–10 $\mu g \cdot mL^{-1}$ erythromycin in Medium 199, for 24 h at 37°C in 5% CO_2. The concentration of each mediator present in the medium at the end of this incubation was assessed by ELISA.

Isolation and purification of human neutrophils

Neutrophils were isolated from human blood according to the method described previously [3]. Neutrophils were isolated and purified from human blood by dextran sedimentation and centrifugation on discontinuous density Percoll gradients. Neutrophil numbers and purity were determined in an improved Neubauer chamber, after staining with Kimura's stain [10]. Neutrophil cell viability was assessed by trypan blue exclusion and only preparations of >95% purity and >95% viability were used in further investigations.

Assay for neutrophil chemotaxis

Neutrophil chemotaxis was studied using the modified Boyden chamber technique [11]. Neutrophil migration was assessed over 90 min at 37°C in response to

0.5 mL conditioned medium from HIE-, HIE+erythromycin-treated HBEC, and medium 199 + 1.0 μg·mL^{-1} erythromycin + 100 μg·mL^{-1} HIE. At the end of incubation, the membrane was removed and, after fixation in absolute alcohol for 5 min, was washed and stained in Harris' haematoxylin stain for 1 min (BDH Laboratory Supplies, Lutterworth, UK). The stained membrane was cleared in CNP 30 reagent (BDH Laboratory Supplies, Lutterworth, UK) and after mounting in Styrolite ™ mounting medium, was immediately examined microscopically for neutrophils coming through to the other side of the membrane. Neutrophils were counted in 10 random high power fields (HPF) and the chemotactic activity was expressed as the mean number of cells·HPF^{-1}. All slides were read by two independent observers blinded to the experimental conditions.

Assay for neutrophil adherence

Neutrophil adherence was investigated by estimating the number of neutrophils adhering to human endothelial cell (HEC) cultures established from cell line ECV 304 (European Collection of Animal Cell Cultures, Porton Down, UK), as described previously [3]. Briefly, confluent HEC were incubated in: 1) conditioned medium from HIE-, HIE + erythromycin-treated, and untreated HBEC; and 2) Medium 199 + 1.0 μg·mL^{-1} erythromycin + 100 μg·mL^{-1} HIE for 6 h at 37°C and washed three times with medium 199. Neutrophils, 0.5×10^6, were added to each culture and, following incubation at 37°C for 30 min, the nonadherent neutrophils were washed off. The HEC were then incubated with 0.5 mL tetramethylbenzidine (TMB) solution (2 mM TBM + 0.1% (w:v) cetyltrimethylammonium bromide (CTAB) in 0.1 M sodium acetate buffer, pH 4.2), and 0.7 mM hydrogen peroxide, and the reaction colour developed was estimated by measuring the absorbance at 620 nm. The number of neutrophils adhering to the HEC was calculated from a calibration curve prepared for the reaction colour developed from cell suspensions containing known numbers of neutrophils.

Statistical analysis

All data were tested for normality prior to further evaluation. All results were expressed as mean±SEM, and differences in means were compared using Student's t-test. All values of p less than 0.05 were considered to be significant.

Results

Analysis of IL-8 released by HBEC into the culture medium, demonstrated that this was significantly increased by treatment of the cells with HIE, confirming our previous findings [3]. Release of IL-8 was significantly increased from 83.7±8.2 pg·μg^{-1} cellular protein, in control untreated cultures, to 225.7±44.8 pg·μg^{-1} cellular protein ($p<0.05$), in cultures treated with HIE (fig. 1a).

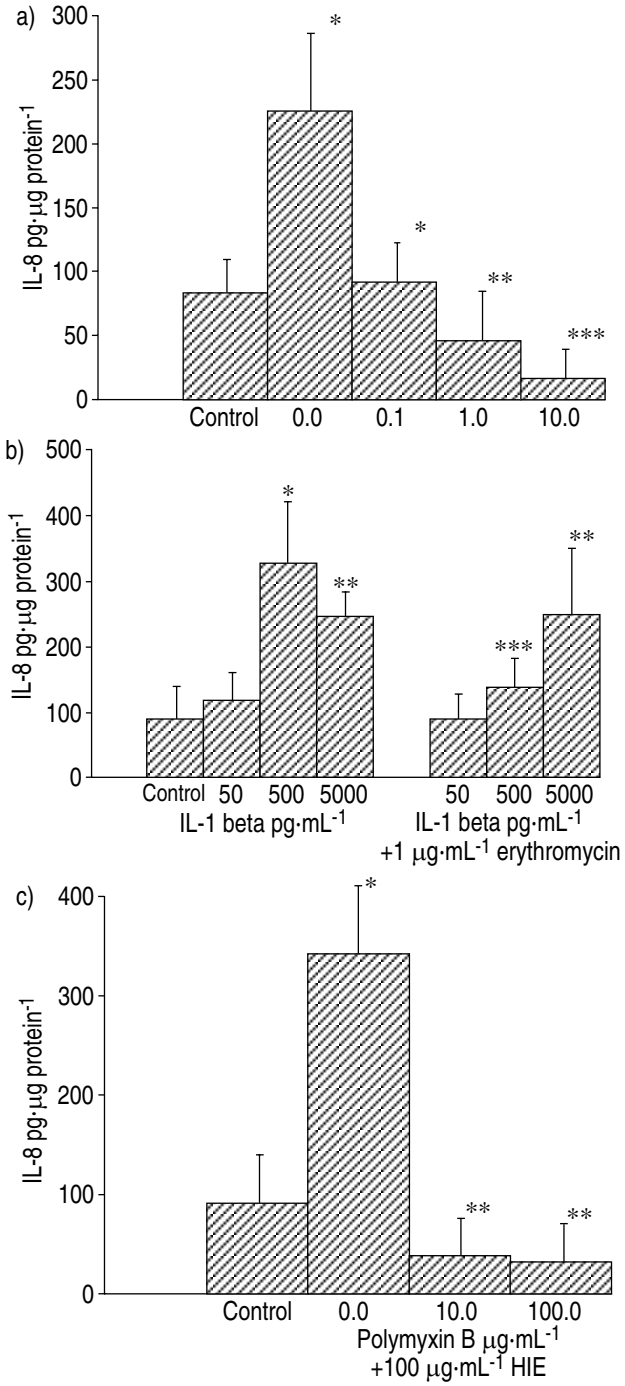

Fig. 1. – Release of interleukin-8 (IL-8) from human bronchial epithelial cell cultures (HBEC) incubated for 24 h with a) 0–10 μg·mL^{-1} erythromycin + 100 μg·mL^{-1} H. influenzae endotoxin (HIE), b) 1.0 μg·mL^{-1} erythromycin + 50–5000 pg·mL^{-1} interleukin-1β (IL-1β), and c) 0–100 μg·mL^{-1} Polymyxin B + 100 μg·mL^{-1} HIE. Untreated cultures were used as controls. Results are expressed as mean±SEM (n=6 for each treatment group). (Figure 1a: *: $p<0.05$ for HIE-treated HBEC *vs* control cultures and HBEC incubated with HIE + 0.1 μg·mL^{-1} erythromycin, **: $p=0.005$ for HIE-treated HBEC *vs* HIE +1.0 μg·mL^{-1} erythromycin-treated HBEC, ***: $p<0.001$ for HIE-treated HBEC *vs* control cultures and HIE +10.0 μg·mL^{-1} erythromycin-treated HBEC; Figure 1b: *: $p=0.005$ for HBEC treated with 500 pg·mL^{-1} IL-1β ±1.0 μg·mL^{-1} erythromycin *vs* control cultures, ***: $p<0.04$ for HBEC treated with 500 pg·mL^{-1} IL-1β ± 1.0 μg·mL^{-1} erythromycin *vs* control cultures; Figure 1c: *: $p<0.001$ for HIE-treated HBEC *vs* control cultures, **: $p<0.001$ for HIE-treated HBEC *vs* HIE +10 and 100 μg·mL^{-1} Polymyxin B-treated HBEC).

Whilst incubation of the cultures in the presence of 0.1 μg·mL^{-1} erythromycin completely blocked the HIE-induced IL-8 release (91.6±13.5 pg·μg^{-1}; p<0.05), incubation in the presence of 1.0 and 10 μg·mL^{-1} erythromycin significantly reduced the concentration of IL-8 released by the epithelial cultures (45.0±22.0 (p=0.005) and 15.5±4.5 pg·μg^{-1} cellular protein (p<0.001), respectively) (fig. 1a).

Incubation of HBEC in the presence of 50–5,000 pg·μg^{-1} IL-1β, demonstrated that this cytokine increased the release of IL-8 in a dose-dependent manner, and was maximally active at a concentration of 500 pg·μg^{-1}. Release of IL-8 was increased from 90.7±20.7 pg·μg^{-1} cellular protein in control cultures, to 117.5±16.3, 325±73.9 (p=0.005), and 244.5±21.1 (p<0.001) pg·μg^{-1} cellular protein, in cultures incubated with 50, 500 and 5,000 pg·mL^{-1} IL-1β, respectively. Incubation of HBEC in the presence of 1.0 μg·mL^{-1} erythromycin significantly attenuated the 500 pg·mL^{-1} IL-1β-induced release of IL-8 (fig. 1b).

Studies investigating the effect of polymyxin B on HIE-induced release of IL-8 from the HBEC demonstrated that this compound significantly reduced the concentration of IL-8 from 341.2±41.1 pg·μg^{-1} cellular protein, released in HIE-treated cells, to 36.8±9.6 (p<0.001) and 30.6±11.2 (p<0.001) pg·μg^{-1} cellular protein, released in HIE +10 or +100 μg·mL^{-1} polymyxin B treated cells, (fig. 1c).

Studies of IL-6 and sICAM-1 demonstrated that the release of these mediators from HBEC was also significantly increased by treatment of the cells with HIE. Analysis of IL-6 demonstrated that release of this cytokine was significantly increased from 3.9±1.5 pg·μg^{-1} cellular protein, in control untreated cultures, to 12.1±1.5 pg·μg^{-1} cellular protein (p<0.005), in HIE-treated cultures. Incubation of the cultures in the presence of 0.1 and 1.0 μg·mL^{-1} erythromycin significantly reduced the HIE-induced release of IL-6 from 12.1±1.5 pg·μg^{-1} cellular protein to 4.1±0.6 (p<0.001) and 1.4±0.5 (p<0.001) pg·μg^{-1} cellular protein, respectively. Although 10 μg·mL^{-1} erythromycin also reduced the concentration of IL-6 down to 5.5±2.9 pg·μg^{-1} cellular protein, this was not significant, possibly demonstrating a high-dose inhibition effect (fig. 2).

Similarly, analysis of sICAM-1 demonstrated that the release of this mediator was significantly increased from 0.04±0.01 ng·μg^{-1} cellular protein, in control cultures, to 3.8±0.9 ng·μg^{-1} cellular protein, in HIE-treated HBEC (p<0.001) (fig. 3). Erythromycin, 0.1, 1.0 and 10.0 μg·mL^{-1}, significantly decreased the HIE-induced release of sICAM-1 to 0.05±0.006, 0.03±0.007 and 0.04±0.004 ng·μg^{-1} cellular protein (p<0.005), respectively.

Analysis of the effect of erythromycin at all concentrations studied, demonstrated that this did not alter cell viability nor was it cytotoxic, as indicated by trypan blue exclusion test. Additionally, erythromycin did not have any direct inactivating effect on HIE, as indicated by the LAL test for endotoxin activity, at any concentration. Similarly, incubation of recombinant human IL-6, IL-8 and sICAM-1 in the presence of the different concentrations of erythromycin did not adversely affect the detectability or concentration of these cytokines by ELISA.

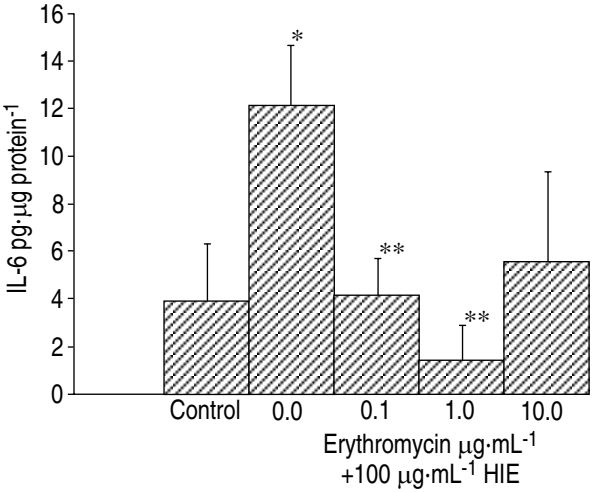

Fig. 2. – Effect of erythromycin on *H. influenzae* endotoxin (HIE)-induced release of interleukin-6 (IL-6) by human bronchial epithelial cells (HBEC) after 24 h. Six cell cultures were used for each set of experiments. All cell cultures, other than control, were exposed to 100 μg·mL^{-1} HIE ±erythromycin. Results are expressed as mean±SEM. *: p<0.005 for HIE-treated HBEC *vs* control cultures; **: p<0.001 for HIE-treated HBEC *vs* HIE + 0.1–1.0 μg·mL^{-1} erythromycin-treated HBEC.

Assessment of the biological relevance of erythromycin modulation of HIE-induced release of these inflammatory mediators demonstrated that neutrophil chemoattraction was significantly reduced to 35±10.0 cells·HPF^{-1} (p<0.02), for conditioned medium (CM) from HIE- and erythromycin-treated HBEC, compared to 70.5±7.4 cells·HPF^{-1} for CM from HIE-treated cultures (fig. 4). Neutrophil chemotaxis observed for CM from HIE- and erythromycin-treated cultures was not found to be significantly different from neutrophil chemotaxis (28.3±2.5 cells·HPF^{-1}) observed for CM from control untreated cultures.

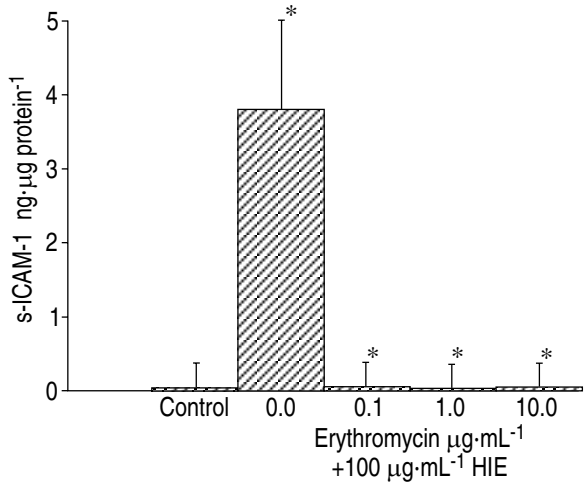

Fig. 3. – Effect of erythromycin on *H. influenzae* endotoxin (HIE)-induced release of soluble intercellular adhesion molecule (sICAM-1) by human bronchial epithelial cells (HBEC) after 24 h. Six cell cultures were used for each set of experiments. All cell cultures, other than control, were exposed to 100 μg·mL^{-1} HIE ±erythromycin. Results are expressed as mean±SEM. *: p<0.001 for HIE-treated HBEC *vs* control cultures; and HIE + 0.1–1.0 μg·mL^{-1} erythromycin-treated HBEC.

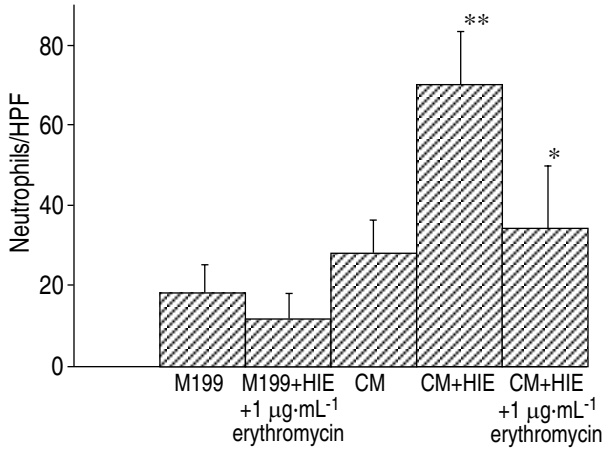

Fig. 4. – Effect of medium 199 + 100 μg·mL^{-1} *H. influenzae* endotoxin (HIE) + 1.0 μg·mL^{-1} erythromycin, conditioned medium (CM) from human bronchial epithelial cells (HBEC) for 24 h, CM from HBEC incubated with 100 μg·mL^{-1} HIE + 1.0 μg·mL^{-1} erythromycin for 24 h, on neutrophil chemotaxis *in vitro*. CM from six HBEC cultures were used for each set of experiments. Results are expressed as mean±SEM. *: p<0.002 for CM from HBEC incubated with HIE + 1.0 μg·mL^{-1} erythromycin *vs* CM from HIE-treated HBEC; **: p<0.001 for CM from HBEC incubated with HIE *vs* Medium 199 + HIE + erythromycin and CM from control cultures.

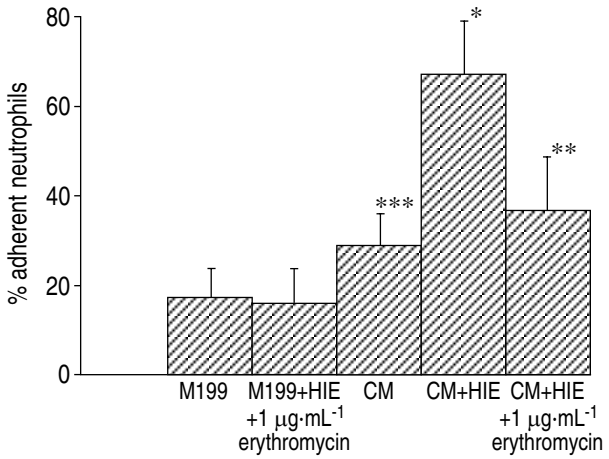

Fig. 5. – Effect of medium 199 + 100 μg·mL^{-1} *H. influenzae* endotoxin (HIE) + 1.0 μg·mL^{-1} erythromycin, conditioned medium (CM) from human bronchial epithelial cells (HBEC) for 24 h, CM from HBEC incubated with 100 μg·mL^{-1} HIE + 1.0 μg·mL^{-1} erythromycin for 24 h, on adherence of neutrophils to cultured human endothelial cells *in vitro*. CM from six HBEC cultures were used for each set of experiments. Results are expressed as mean±SEM. *: p<0.001 for CM from HIE-treated HBEC *vs* CM from control cultures and Medium 199 + HIE + erythromycin; **: p<0.002 for CM from HIE- and erythromycin-treated HBEC *vs* CM from HIE-treated HBEC; ***: p<0.01 for CM from control cultures *vs* Medium 199 + HIE + erythromycin.

Analysis of the direct effect of solutions of HIE or erythromycin in Medium 199 demonstrated that neither compound significantly altered neutrophil chemotaxis, when tested as suspensions in Medium 199 (fig. 4).

Similarly, assessment of the effect of CM from HIE- and erythromycin-treated cultures on neutrophil adherence to endothelial cell cultures demonstrated that HIE significantly increased neutrophil adherence from 28.4± 2.5 % (for CM from untreated HBEC) to 66.7±7.1% (p<0.001) (fig. 5). Treatment of cultures with CM from HIE- and erythromycin-treated cultures significantly reduced adherence of the neutrophils to 36.1±7.5% (p<0.001) (fig. 5). Medium 199 + HIE or erythromycin did not significantly alter neutrophil adhesion to HEC, when compared to adhesion with Medium 199 alone.

Discussion

Our studies have demonstrated that both polymyxin B and erythromycin attenuated HIE-induced release of inflammatory mediators, including IL-8, IL-6 and sICAM-1, from HBEC and that erythromycin also attenuated IL-1β induced release of IL-8. Additionally, these studies have demonstrated that erythromycin also reduces HIE-induced neutrophil chemotaxis and adhesion to human endothelial cells *in vitro*. These results suggest that the effect of HIE on mediator release from epithelial cells is a specific effect, and that erythromycin is likely to interfere with this effect. Whilst it is possible that erythromycin may act either to inhibit the expression or the release of these inflammatory mediators from human bronchial epithelial cells, it is not possible to determine which of the two processes is affected from the present studies. In order to investigate further the specific mechanism through which erythromycin may operate, we are currently investigating the effect of erythromycin on both HIE- and IL-1β-induced changes in the concentrations of specific messenger ribonucleic acid (mRNA) transcribed for the mediators investigated in the present study.

In this study, a single concentration of 100 μg·mL^{-1} HIE was selected on the basis of our previous studies, which demonstrated that maximal cytokine release from epithelial cell cultures occurred when cells were incubated with 100 μg·mL^{-1} HIE, and that this was comparable with concentrations of commercially available endotoxin preparations from *Klebsiella pneumoniae* and *Escherichia coli*, required to induce similar quantities of cytokines in our model system [3]. Indeed, studies by JOHNSON and INZANA [12] demonstrated that concentrations of up to 40 μg·mL^{-1} HIE did not have any significant effect on the ciliary activity of rat tracheal organ cultures for up to 5 days.

Studies by MARTIN *et al*. [13] and HUEMANN *et al*. [14], however, demonstrated that the activity of lipopolysaccharide (LPS)-induced release of tumour necrosis factor-α (TNF-α) from macrophages and monocytes, respectively, is increased by a 1,000 fold, when lipopolysaccharide binding protein (LBP) is added to the cells. These authors additionally demonstrated that the activity of LPS-LBP complex is dependent on the presence of the CD14 receptor complex on these cells. Preliminary studies in our laboratory have demonstrated that the bronchial epithelial cells do not express CD14, and it is possible that this may be the cause of the lack of epithelial cell response to low concentrations of HIE. It is also possible that the HIE used in the present studies may not be as potent as that derived from more virulent strains, such as encapsulated *H. influenzae* type b.

Studies investigating the effect of erythromycin in the treatment of infective lung conditions have demonstrated that in Japan, in particular, this drug is often used empirically in the treatment of diffuse panbronchiolitis [5, 15–17], a disease entity that is characterized by chronic inflammation of the respiratory bronchioles and infiltration by chronic inflammatory cells [18]. This condition progresses insidiously, and finally results in respiratory failure due to repeated episodes of respiratory tract infections. Studies from Europe, however, have demonstrated that this agent is effectively used in the treatment of acute exacerbations of chronic bronchitis and community-acquired pneumonia, diseases characterized by frequent isolation of *H. influenzae*, a pathogen which is specifically adapted to colonize and damage the lower respiratory tract [19].

Although the clinical effectiveness of erythromycin in the management of these conditions has been suitably established, studies of the mechanisms underlying the effect of erythromycin are not clear and have produced inconsistent data with respect to the predominant mode of action of this drug. Whilst some studies have suggested that this drug acts primarily as an antibacterial agent, others have suggested that it may have anti-inflammatory effects.

BRISSON-NOEL *et al*. [20] reviewed the mechanisms underlying the antibacterial role of erythromycin and other macrolide antibiotics have demonstrated that these agents exhibit their antimicrobial activity by interfering with protein synthesis in the microorganism. These authors suggested that the macrolide antibiotics act primarily by binding reversibly to the 50 S ribosomal subunits of sensitive micro-organisms, and consequently stimulate the dissociation of the peptidyl-transfer ribonucleic acid (tRNA), from the ribosomes during translocation to the mRNA, rather than preventing the formation of the peptide bond [20]. Studies by DOWLING *et al*. have suggested that resistance of variants of *Legionella* spp. to erythromycin may occur partly as a result of modification of the target sites on the ribosome [21].

Our findings of an anti-inflammatory role of erythromycin in the present study, however, are in accordance with the findings of others, who have also demonstrated similar effects of erythromycin in different model systems. Recently, IINO *et al*. [22] investigated the effect of LPS-induced release of TNF-α from human monocytes, and demonstrated that this was significantly reduced by treatment with erythromycin. Incubation of LPS-stimulated monocytes with the nonmacrolide drugs, minocycline hydrochloride, ofloxacin or penicillin G, did not have any effect on TNF-α release [22]. More recently, KATODA *et al*. [23] investigated the effect of 4 weeks of treatment with oral erythromycin, on neutrophil chemotactic activity (NCA) and neutrophil accumulation in bronchoalveolar lavage (BAL) fluid of patients with diffuse panbronchiolitis, and demonstrated that these were significantly reduced. Similarly, ICHIKAWA *et al*. [4] have reported that erythromycin may reduce neutrophils and neutrophil-derived elastolytic-like activity in BAL obtained from patients with bronchiolitis. ANDERSON [24] has suggested that erythromycin may exhibit anti-inflammatory effects by inhibiting the generation of superoxide by activated neutrophils.

In summary, our studies also suggest that erythromycin exhibits anti-inflammatory effects. It is possible that erythromycin could exert its anti-inflammatory effects by directly inhibiting the activity of inflammatory cells *in vivo*. Our studies, however, suggest that this agent is more likely to act indirectly by modulating the synthesis and/or release of the proinflammatory mediators, such as IL-8 and sICAM-1, which affect the activity of neutrophils, the key effector cell in the pathogenesis of intermittently exacerbated bacterial infections, such as bronchiectasis, chronic bronchitis and cystic fibrosis. These findings may be of particular significance in the management of such conditions, since the concentrations of erythromycin found to be effective in the present study are similar to the peak plasma concentrations of 1.5–10 $\mu g \cdot mL^{-1}$ obtained in clinical practice after administration of single oral or intravenous doses of 250–1,000 mg erythromycin [25]. MARLIN *et al*. [26] have measured the concentrations of erythromycin present both in plasma and sputum of 10 chronic bronchitics treated with 500 mg *t.d.s* erythromycin for 8 days, and demonstrated that the sputum concentrations were approximately 10% of the plasma levels and reached a maximum of 2.02 $\mu g \cdot mL^{-1}$. Moreover, the sputum erythromycin concentrations achieved were in a similar range to the minimal inhibitory concentrations for common respiratory pathogens. Indeed, our studies have suggested that erythromycin may be active at even lower concentrations, since a concentration of 0.1 $\mu g \cdot mL^{-1}$ significantly inhibited HIE-induced release of IL-6, IL-8 and sICAM-1, from the epithelial cells.

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