PostScript

LETTERS

Effect of azithromycin on primary bronchial epithelial cells derived from stable lung allografts

Obliterative bronchiolitis (OB), the main cause of lung allograft failure, is characterised by airway inflammation, neutrophilia, remodelling and fibrosis. Recent studies of the macrolide antibiotic azithromycin in OB, including one from our centre, have reported improved lung function with rescue therapy.[1] The mechanism for this improvement remains unclear, but a recent study suggests that neutrophilic inflammation may be an important predictor of clinical response.[2]

Macrolides have anti-inflammatory properties in several pulmonary conditions. We hypothesised that the clinical benefit observed with azithromycin in patients undergoing lung transplantation may be related to the inhibition of factors key to airway neutrophilic inflammation, remodelling and fibrosis. The bronchial epithelium is in a pivotal position as a target and orchestrator of airway inflammation and remodelling. We tested our hypothesis using a unique primary bronchial epithelial cell (PBEC) model.[3] The local research ethics committee approved the study.

Following pre-bronchoscopic assessment, surveillance bronchoscopy and bronchoalveolar lavage microbiology were performed to rule out infection. Bronchial brushings were obtained from subsegmental bronchi for PBEC cultures. Transbronchial specimens were examined to exclude acute vascular rejection. PBECs were established using endobronchial brushings from 10 clinically stable transplant recipients.

Azithromycin (Pfizer Ltd, Sandwich, UK) 20, 10 and 5 ng/ml was added to PBECs for 48 h before removal of cell supernatant and protein analysis with multiplex kits (R&D Systems Europe Ltd, Abingdon, UK) and a Luminex analyser. A methylene blue assay was used to correct data for PBEC cell number and the data were analysed using the Wilcoxon signed rank test.

Azithromycin caused a significant decrease in matrix metalloproteinase (MMP)-2, interleukin (IL)-8, granulocyte-macrophage colony stimulating factor (GM-CSF) and MMP-9 levels, with a trend towards decreased IL-6 production (table 1). Basal MMP-9 (2/10) and MMP-2 and GM-CSF levels (3/10) were below detection limits in some PBEC cultures. These factors are critical in orchestrating neutrophil influx, influencing antigen presenting cells and potentiating airway remodelling, which are relevant to the pathophysiology of OB. IL-8 is a neutrophil chemoattractant with additional roles in angiogenesis and remodelling, while IL-6 plays a part in B cell differentiation, monocyte proliferation, neutrophil recruitment, activation and degranulation, which may have pathophysiological roles in OB. GM-CSF regulates accumulation and activity of neutrophils and is increased in lung disease characterised by neutrophilic infiltration and in the post-transplant airway.[4] It is also associated with an increased number and activity of antigen presenting cells, including activated dendritic cells.

It is assumed that fibroblasts are responsible for the production of scar collagen in chronic transplant rejection. We have shown potential for injured airway epithelial cells from allografts to become fibroblasts through epithelial mesenchymal transition (EMT) and have demonstrated a key role for MMPs in facilitating this phenotypic change.[5] The ability of azithromycin to modulate the epithelial release of these factors is therefore consistent with a potential to influence alloimmunity, neutrophilic inflammation, EMT, remodelling and fibrosis. We therefore suggest that this may underlie the clinical benefit of azithromycin seen in certain patients, but further animal model and in vivo studies including randomised trials are required.

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Table 1 Effect of azithromycin at concentrations of 5, 10 and 20 ng/ml on IL-8, GM-CSF, IL-6, MMP-2 and MMP-9 levels

Azithromycin concentration (ng/ml)	IL-8 ng/10^6 cells (n = 10)	GM-CSF ng/10^6 cells (n = 7)	MMP-9 ng/10^6 cells (n = 8)	MMP-2 ng/10^6 cells (n = 7)	IL-6 pg/10^6 cells (n = 3)
0	4.4 (0.6–13.0)	1.1 (0.1–5.5)	34.2 (5.5–65.6)	2.2 (0.4–8.7)	54.6 (36.4–238.0)
5	3.4 (0.5–14.5)	0.6 (0.1–4.3)	20.4 (3.9–66.4)*	1.0 (0.4–4.1)	37.0 (24.7–91.6)
10	2.2 (0.4–16.9)	0.4 (0.1–3.3)	20.5 (3.6–93.9)	1.2 (0.4–6.9)*	39.3 (36.8–71.0)
20	1.8 (0.2–7.3)**	0.5 (0.1–2.9)*	14.6 (1.4–35.1)**	0.6 (0.4–5.0)*	35.0 (21.6–46.4)

IL, interleukin; GM-CSF, granulocyte-macrophage colony stimulating factor; MMP, matrix metalloproteinase.
Data shown are median (range) values.
Experiments were analysed for n = 10 for IL-8, n = 8 for MMP-9, and n = 7 for both MMP-2 and GM-CSF. Where azithromycin decreased analyte levels below the assay detection limit, a value halfway between the lowest level of the detection range and zero was assigned. The Wilcoxon signed rank test was used to test for statistical significance with a two-sided p value <0.05 deemed significant. *$p<0.05$; **$p<0.01$. IL-6 data (n = 3) were not statistically analysed.

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Critical care as part of respiratory training in the UK

Experience in critical care medicine is mandatory for all respiratory trainees in the UK with a need for 60 days (3 months) minimum placement in an intensive care unit (ICU). The Respiratory Critical Care Group of the British Thoracic Society[1] recently reported a survey in which there was widespread agreement with this requirement, although it was inadequately provided by a number of programmes. In addition, a proportion of trainees indicated the intention to subspecialise in intensive care medicine and were concerned that their ICU experience was diluted by having responsibilities such as acute general medical takes during their attachment. We recently carried out an email survey of anaesthetic and respiratory trainees and directors of intensive care which provides additional useful information.

The majority of ICUs in the UK operate an admission policy that depends on making initial contact with the duty anaesthetic registrar. One aspect of our survey concerned the interaction between the referring physician and the critical care ''gate keeping'' specialist. Although it might be optimal to involve the appropriate medical, emergency or surgical consultant directly in making a referral to the critical care consultant, it is often specialist registrars who refer patients, especially out of hours.

Our survey investigated whether the specialty of the referring specialist registrar affected the outcome. A total of 108 doctors (97 specialist registrars and 9 ICU lead consultants) from South Thames, West Midlands and Oxford regions were surveyed in 2006. Of the respiratory specialist registrars, 27% indicated they ''commonly'' or ''always'' had difficulty gaining admission for medical patients. No anaesthetic trainee reported difficulty ''commonly'' or ''always'' while 47% reported that