LETTERS

Efficacy of nebulised colomycin in patients with non-cystic fibrosis bronchiectasis colonised with *Pseudomonas aeruginosa*

Colonisation with *Pseudomonas aeruginosa* is a feature of bronchiectasis and is associated with more severe disease and lower quality of life.[1] Nebulised colomycin, a polymixin, bactericidal antibiotic with potent activity against most Gram-negative organisms, including *P aeruginosa*, is frequently employed in these patients, but evidence is lacking for this approach.

We have retrospectively assessed the efficacy of nebulised colomycin in *P aeruginosa*-colonised patients receiving a minimum of 6 months treatment. Patients who received concomitant prophylactic macrolide treatment for >4 weeks were excluded. Bronchiectasis was confirmed in all patients by standard high-resolution CT criteria, and all received nebulised colomycin 1–2 megaunits twice daily through a standard jet nebuliser and compressor. The annualised frequency of hospital admissions, infective exacerbations requiring rescue antibiotics and sputum positivity for *P aeruginosa* per patient, prior to, and while on, colomycin treatment was compared. Forced expiratory volume in 1 s (FEV_1) and self-reported sputum volume within 6 months of starting treatment and most recent on treatment were also compared. The data were analysed by non-parametric Wilcoxon test (table 1).

Nineteen patients (9 males/10 females), mean age 66 years, were assessed between January 2000 and April 2007. Four patients had idiopathic disease and 15 disease of known cause. The mean length of data collection before commencing colomycin was 23.6 months and the mean duration on treatment was 21.2 months (range 6–39 months) with 17 patients still on treatment at assessment (two patients discontinued treatment, one erroneously and one due to lack of efficacy).

Only one previous study has interrogated the role of inhaled colomycin in this patient group. Steinfort *et al*[2] retrospectively reviewed the efficacy of nebulised colomycin in 18 patients with chronic bronchial sepsis (14 with bronchiectasis) of whom 14 were colonised with *P aeruginosa*. There was a significant attenuation in decline in FEV_1 and forced vital capacity (FVC), and an improvement in quality of life. *P aeruginosa* was eradicated in three patients but admission and exacerbation frequency were not assessed. Inhaled colomycin is frequently employed in patients with cystic fibrosis chronically colonised with *P aeruginosa*, but there is little evidence to support this. One study, involving 115 patients comparing inhaled colomycin with tobramycin over 4 weeks, demonstrated a significant decrease in the sputum *P aeruginosa* density with both treatments but a significant improvement in FEV_1 in the tobramycin group only.[3]

Table 1 Clinical outcomes pre- and postnebulised colomycin

n=19	Precolomycin Mean (SD)	Postcolomycin Mean (SD)
Exacerbation frequency/year	7.8 (3.0)	2.7 (2.1)*
Admission frequency/year	3.0 (2.7)	0.95 (1.1)†
P aeruginosa sputum positivity/year	4.2 (2.5)	0.5 (0.5)* (eradication n=3‡)
Sputum volume (ml)	31 (20.8)	14 (13.5)*
FEV_1 (litres)	1.13 (0.6)	1.14 (0.6)

*p<0.001; †p=0.002.
‡Eradication=≥2 negative *P aeruginosa* sputum cultures postcolomycin.
FEV1, forced expiratory volume in 1 s.

Several studies have assessed the efficacy of nebulised tobramycin in *P aeruginosa*-colonised patients with non-cystic fibrosis bronchiectasis.[4 5] Tobramycin consistently reduced sputum *P aeruginosa* density with eradication rates of 13–35%, but resistance emerged in 7–11% of patients. The only study addressing exacerbations demonstrated a significant reduction in admission, but not exacerbation, frequency.[5] Both studies showed a non-significant decrease in FEV_1 on treatment, probably due to increased bronchospasm, with drug intolerance in up to 10% mainly due to airway symptoms.

In conclusion, nebulised colomycin appeared to reduce exacerbation frequency, hospitalisation, sputum *P aeruginosa* positivity and sputum volume in *P aeruginosa*-colonised patients with bronchiectasis and was well tolerated. These results are encouraging in this severely affected patient group and strengthen the need for a prospective, adequately powered, randomised controlled trial to investigate the efficacy of this treatment further.

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REFERENCES

1. **Wilson CB,** Jones PW, O'Leary CJ, *et al*. Effect of sputum bacteriology on the quality of life of patients with bronchiectasis. *Eur Respir J* 1997;**10**:1754–60.
2. **Steinfort DP,** Steinfort C. Effect of long-term nebulized colistin on lung function and quality of life in patients with chronic bronchial sepsis. *Intern Med J* 2007;**37**:495–8.
3. **Hodson ME,** Gallagher CG, Govan JRW. A randomised clinical trial of nebulised tobramycin or colistin in cystic fibrosis. *Eur Respir J* 2002;**20**:658–64.
4. **Barker AF,** Couch L, Fiel SB, *et al*. Tobramycin solution for inhalation reduces sputum *Pseudomonas aeruginosa* density in bronchiectasis. *Am J Respir Crit Care Med* 2000;**162**:481–5.
5. **Drobnic ME,** Sune P, Montoro JB, *et al*. Inhaled tobramycin in non-cystic fibrosis patients with bronchiectasis and chronic bronchial infection with *Pseudomonas aeruginosa*. *Ann Pharmacother* 2005;**39**:39–44.

Maternal smoking during pregnancy decreases Wnt signalling in neonatal mice

Epidemiological studies have shown that maternal smoking during pregnancy is a risk factor for the development of asthma. However, the mechanisms underlying the increased risk of developing asthma are largely unknown. We have shown that maternal smoking during pregnancy increases smooth muscle layer and collagen III deposition around the airways in mouse offspring in association with increased airway hyper-responsiveness.[1] Other factors also appear to contribute to the development of increased airway hyper-responsiveness. We hypothesise that lung development is such a factor. Since genes in the Wnt-β-catenin pathway are essential for lung development and epithelial stem cell differentiation/expansion, we investigated the effects of maternal smoking during pregnancy on Wnt gene expression in lung tissue from neonatal offspring.

Balb/c mice were exposed to fresh air or cigarette smoke from 3 weeks before conception until delivery. Offspring (n = 16 from non-smoking mothers, n = 18 from smoking mothers) were killed 1 day after birth. RNA was isolated from total lung tissue and qRT-PCR was performed using microfluidic card assay.

We found that maternal smoking during pregnancy decreased the expression of *Foxa2*, *Fzd-7*, *Egf*, *Ctnnb1*, *Fn1* and *Pdgfra* (encoding forkhead box a2, frizzled receptor 7, epidermal growth factor, β-catenin, fibronectin and platelet-derived growth factor receptor a, respectively) in lung tissue from neonatal offspring (figure 1). Maternal

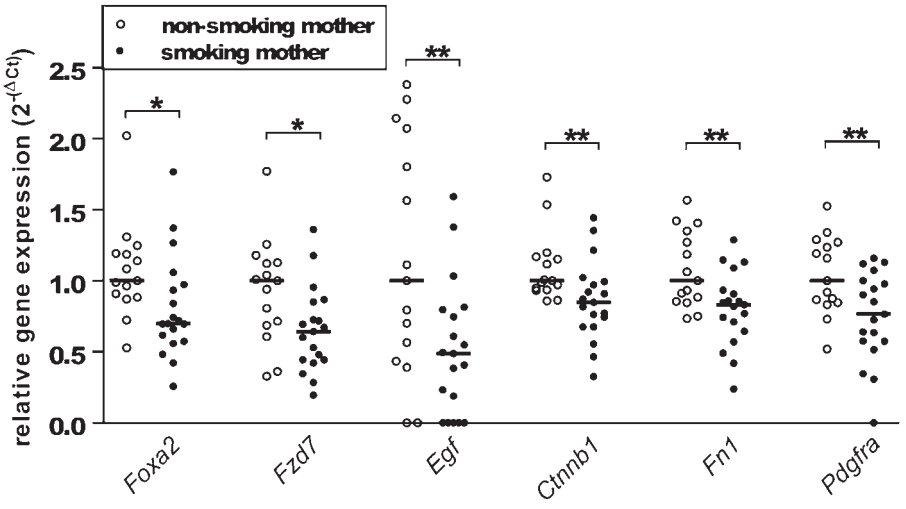

Figure 1 Relative gene expression in lung tissue of offspring from non-smoking mothers (open symbols) and smoking mothers (closed symbols). Data were normalised to GAPDH in order to correct for differences in input using the formula $\Delta Ct = Ct(GAPDH) - Ct(gene\ of\ interest)$. The relative expression levels were calculated by $2^{-(\Delta Ct)}$ and levels are given relative to the non-smoking mother group = 1. *p<0.05, **p<0.01.

smoking had no effect on the expression levels of *Wnt-5* and *-7, Fzd-4* and *-10*, and *Pdgfa* (encoding wingless proteins 5 and 7, frizzled receptors 4 and 10 and platelet-derived growth factor, respectively) and expression of *Dkk1* (encoding dikkopf) was not detected. For an overview of the effects of maternal smoking during pregnancy on the Wnt pathway in the lung tissue of offspring, see figure 2 in the online supplement.

To our knowledge, this is the first report showing that maternal smoking during pregnancy decreases the expression of several genes involved in Wnt signalling in offspring. We propose that this relates to lung development as follows. Foxa2 decreases alveolarisation and increases goblet cell hyperplasia when conditionally deleted from epithelial cells.[2] Interestingly, we have previously shown increased house dust mite-induced goblet cell hyperplasia in offspring from smoking mothers,[1] which could have been caused by decreased *Foxa2* expression. Furthermore, epidermal growth factor, β-catenin and the downstream Wnt target gene Fn1 were also shown to be involved in branching morphogenesis of the fetal lung.[3–5] In addition, Fzd-7 is important in neovessel formation,[6] while Pdgfra is involved in both neovessel formation and alveolarisation.[7]

Together, these findings provide a plausible argument for the effects of maternal smoking during pregnancy on Wnt-β-catenin signalling in the lungs of neonatal offspring. This may contribute to impaired lung development and an increased risk of developing asthma later in life. Moreover, Wnt signalling is involved in many other developmental processes that may thus be affected by maternal smoking during pregnancy. The relationship between defects in Wnt signalling and morphological/functional outcomes in lung tissue from offspring should therefore be investigated in more detail.

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► Figure 2 is published online only. To view these files please visit the journal online at (http://thorax.bmj.com).

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REFERENCES

1. **Blacquiere MJ,** Timens W, Melgert BN, *et al*. Maternal smoking during pregnancy induces airway remodeling in mice offspring. *Eur Respir J* 2009;**33**:1133–40.
2. **Wan HJ,** Kaestner KH, Ang SL, *et al*. Foxa2 regulates alveolarization and goblet cell hyperplasia. *Development* 2004;**131**:953–64.
3. **Chinoy MR,** Zgleszewski SE, Cilley RE, *et al*. Influence of epidermal growth factor and transforming growth factor beta-1 on patterns of fetal mouse lung branching morphogenesis in organ culture. *Pediatr Pulmonol* 1998;**25**:244–56.
4. **Maeda Y,** Dave V, Whitsett JA. Transcriptional control of lung morphogenesis. *Physiol Rev* 2007;**87**:219–44.
5. **Sakai T,** Larsen M, Yamada KM. Fibronectin requirement in branching morphogenesis. *Nature* 2003;**423**:876–81.
6. **Dufourcq P,** Leroux L, Ezan J, *et al*. Regulation of endothelial cell cytoskeletal reorganization by a secreted frizzled-related protein-1 and frizzled 4- and frizzled 7-dependent pathway. *Am J Pathol* 2008;**172**:37–49.
7. **Bostrom H,** Gritli-Linde A, Betsholtz C. PDGF-A/PDGF alpha-receptor signaling is required for lung growth and the formation of alveoli but not for early lung branching morphogenesis. *Dev Dyn* 2002;**223**:155–62.

Circulating endothelial stem cells are not decreased in pulmonary emphysema or COPD

Previous studies have suggested a role for an increased apoptosis of the endothelial cells in the pulmonary capillaries of the alveolar septa in the pathogenesis of human pulmonary emphysema.[1] In animal models, circulating endothelial stem cells, characterised by the concomitant expression of CD34+, CD133 and vascular endothelial growth factor receptor 2 (VEGF-R2), may contribute to the repair of lung damage.[2] However, it is unknown if a decrease in the blood of these stem cells contributes to the pathogenesis of pulmonary emphysema in humans. The aim of our study was to investigate by flow cytometry the number of total (CD34+) and endothelial stem (triple positive for CD34+/CD133/VEGF-R2) cells in the peripheral venous blood of current and former smokers of similar age, with or without pulmonary emphysema.

All the recruited subjects were free from concomitant diseases or drugs able to interfere with the number of circulating stem cells. Venous blood samples were obtained from 37 subjects (mean (SD) age 66.8 (1.4) years, 25M/12F, mean (SD) 33.11 (3.2) pack-years, 12 current and 25 ex-smokers). All former smokers had stopped smoking for more than 1 year. Twenty-two subjects (59.5%) had chronic obstructive pulmonary disease (COPD) according to the criteria of the Global Initiative for Chronic Obstructive Lung Disease guidelines[3] (mean post-bronchodilator forced expiratory volume in