THERMAL STRESS INDUCTION OF CILIARY BEAT FREQUENCY OF CULTURED BRONCHIAL EPITHELIAL CELLS. B.R. Prouse, D.J. Romberger, R.A. Robbins, S.I. Rennard, J.H. Sisson. University of Nebraska Medical Center and Department of Veterans Affairs Medical Center, Omaha, NE.

Thermal stress (TS) via induction of heat shock proteins (HSP) confers protective effects in lung injury. Mucociliary clearance plays a protective role in airways. Thus, we hypothesized that TS may enhance mucociliary clearance by upregulating ciliary beat frequency (CBF) of bronchial epithelial cells (BECs). We determined the CBF of bovine BECs *in vitro* after exposure to TS. BEC cultures were exposed to TS of 43°C for 60 min and returned to 37°C for the remainder of the experiment. Non-TS cells kept at 37°C for the entire time were used as controls. CBF was measured using phase contrast microscopy, videotape analysis, and computerized frequency spectrum analysis at timepoints from 0-24 hours. BECs exposed to TS demonstrated increased CBF in comparison to non-TS cells. Maximal increase of CBF was observed at approximately 3 hrs after TS (TS, 17.1 ± 0.8 Hz; non-TS, 9.9 ± 0.9 Hz) and the increase was sustained at 24 hrs (TS, 13.1 ± 0.4 Hz; non-TS, 10.9 ± 0.7 Hz). The increase in CBF at 3 hrs after TS parallels the increase in expression of HSP70 in TS BECs by Western blot. Thus, TS, perhaps through induction of HSP, increases CBF of cultured BECs. Upregulation of CBF by TS may confer a protective property to injured airway epithelium.

LUNG MUCUS CLEARANCE IN ACUTE INFECTIVE EXACERBATIONS. A. Hasani, S. Rotondetto*, J.E. Agnew, D. Pavia, S.W. Clarke, M. Spiteri*. Department of Thoracic Medicine, Royal Free Hospital, London and Department of Respiratory Medicine*, City General Hospital, Stoke-on-Trent, England (UK).

Acute exacerbations of chronic obstructive airways disease challenge conducting airways clearance by dint of profound changes in mucus volume and properties. We have assessed tracheobronchial mucus clearance over a 6-hour observation period in 12 patients (9M/3F with mean±SD age of 63±7 years) at the start of acute exacerbation and one week later after oral antibiotic therapy (6 patients on amoxycillin, 500mg tds; 6 patients on ciprofloxacin, 500mg bd). Mean % predicted forced expiratory volume in 1 second (FEV1) and forced expiratory flow at 50% of vital capacity (FEF50) were 51% (SD:13) and 21% (SD:13) and after treatment 48% (SD:12) and 21% (SD:13) respectively. Clearance was assessed with a non-invasive radioaerosol technique. Radioactive particles (polystyrene spheres 5μm in diameter labelled with ^{99m}Tc) were inhaled under strictly controlled conditions and monitored with two axially opposed collimated scintillation detectors (positioned anteroposteriorly to the chest). After allowance for initial particle distribution, clearance was moderately subnormal (approximately 50% tracheobronchial clearance in 5 hours). Clearance (approximately 50% clearance in 4 hours) did not significantly improve after one week of antibiotic treatment. Mean cough frequency did however decrease significantly ($p<0.02$ for all 12 patients; $p<0.05$ for ciprofloxacin sub-group) as did the number of sputum samples produced over the 6-hour observation period ($p<0.01$ for all 12 patients). If a reduced cough frequency implies a reduced role for cough-aided clearance within the airways it is possible that mucus transport by true mucociliary clearance may have slightly improved over the 7-day period. Reducing cough may possibly also have lessened the risk of cough-mediated damage to the airways epithelium.

THE EFFECTS OF SODIUM TAURODIHYDROFUSIDATE ON MUCOCILIARY TRANSPORT RATE AND MUCUS GLYCOPROTEIN SECRETION USING TWO *EX-VIVO* ANIMAL MODELS.
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Sodium taurodihydrofusidate (STDHF) has been clinically shown to enhance the intranasal absorption of peptide and protein drugs (Hedin et al. (1993) J. Clin. Endocrin. Metab. 76(4): 962-7). However, it is important that none of the components included in nasal preparations should affect the mucociliary clearance mechanism to a significant extent. The aim of our study was to examine the effects of STDHF and STDHF in combination with egg phosphatidylcholine (PC) on the mucociliary transport rate (MTR) of the frog palate and to evaluate the effect of STDHF on mucus glycoprotein secretion by excised rat tracheae. The excised palates of frogs (*Rana pipiens*) were incubated in a humidity chamber at 25°C. The mean MTR of graphite particles applied to the palate after the addition of 1% STDHF in HEPES buffer was compared to the mean basal MTR in HEPES alone. To assess the production of mucus glycoprotein, excised rat tracheae were incubated in cell growth medium (pH 7.4) to which radiolabelled mucin precursors and 1% STDHF had been added. Samples of the medium were taken over a 30 h time period and proteins present in the samples were assessed for radioactivity. The results of both experiments were tested for significance using the two-tailed Mann-Whitney U-test. The results showed that 1% (15.5 mM) STDHF significantly decreased MTR by 90% ($n=6$, $P<0.05$) and that this effect could be mitigated by the presence of 5 mM PC. STDHF (1%) also caused a significant increase of mucus glycoprotein secretion by the tracheae compared to the control ($n=4$, $P<0.05$). The effect of STDHF on the mucociliary apparatus could alter the time that nasal formulations remain in contact with the absorbing mucosa whereas inclusion of PC may modify this effect.

PARTICLES AND THE RESPIRATORY BRONCHIOLE: PATTERNS OF DEPOSITION AND CLEARANCE. KE Pinkerton, J Peake, CG Plopper, DM Hyde, BK Tarkington. School of Veterinary Medicine, University of California, Davis, CA

Respiratory bronchioles (RB) form the transitional zone between the conducting airways and alveoli in the primate lung. These anatomical units are defined as airways with occasional alveolar outpocketings along their walls. The interdigitation of bronchiolar epithelial cell types with alveolar epithelial cells in the RB represents a unique microenvironment for cell and tissue responses to particles and oxidant pollutants. The purpose of this study was to determine the deposition patterns of particles in RBs of Rhesus monkeys. Animals were exposed for a 3 hour period to aerosolized fluorescent yellow-green microspheres 1μ in diameter. Within two hours of the end of exposure the lungs were fixed by intravascular perfusion of glutaraldehyde-paraformaldehyde solution. Immediately following exposure, numerous single microspheres were scattered in a patchy distribution along the conducting airways, RBs, and alveolar ducts with the highest concentrations found on the bifurcation ridges. In RBs, more microspheres were found on the alveolated side of the RB wall compared with the non-alveolated side. Microspheres within the RB were scattered on the surfaces of tissue crests forming the mouth openings of the alveoli and into the alveoli. By 24 hours, most microspheres had cleared from the conducting airways. In striking contrast, many microspheres remained in RBs and alveoli. The distribution of microspheres at 24 hours in RBs had shifted primarily to small clusters rather than single microspheres. Microspheres on the alveolated side of the RB continued to be the dominant location of spheres in the RB with little clearance from this level in the lungs. We conclude that particles depositing in respiratory bronchioles are preferentially localized to the alveolated portions of the wall, undergo active redistribution following deposition, but unlike particles in the conducting airways, are retained at sites of deposition in RBs for at least 24 hours following inhalation.

A REGIONAL MODEL OF PARTICLE DEPOSITION IN FIVE SPECIES OF LABORATORY ANIMALS. MG Ménache[1], FJ Miller[2] and OG Raabe[3]. [1]Duke University, Durham, NC, [2]CIIT, RTP,NC, [3]University of California, Davis, CA, USA.

Interpretation of toxicological responses in animals can be improved by information on dose delivered to the target site. Knowledge of dose at the target site is needed to extrapolate from an observed experimental result to other species (generally humans) or to other exposure regimens in the same species. An empirical model of regional deposition in the extrathoracic (ET), tracheobronchial (TB) and pulmonary (P) regions has been developed from published data on particle deposition in animals. The model describes regional deposition of particles in the aerodynamic diameter range ($d_{ae}>0.5$ μm) for a mean inspiratory flow rate (Q) and is analogous to a series of filters or compartments removing particles on inhalation and exhalation. Data on regional deposition were used to estimate regional efficiencies (deposition in a compartment normalized to mass entering the compartment). The expected values of efficiency (η) were fit using logistic functions with d_{ae} or d_{ae}^2Q as the independent variables:

$$E(\eta)=\frac{1}{1+e^{\alpha+0.4343\beta \ln x}}$$

	ET		TB		P	
indep var (X)	d_{ae}^2Q		d_{ae}		d_{ae}	
parameter	α	β	α	β	α	β
mouse	0.7	-2.2	1.6	-2.9	1.1	-3.2
hamster	2.0	-3.5	1.9	-2.9	1.1	-7.2
rat	6.6	-5.5	1.9	-2.1	2.2	-9.5
guinea pig	2.3	-1.3	2.5	-0.9	0.8	0.6
rabbit	4.3	-1.6	2.8	-2.3	2.6	-2.0

(This abstract does not reflect US EPA Policy.)

Species Differences in Tracheal Mucus Rheology and Clearance Rate
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Considerable differences between species in airway size, epithelial potential difference (PD) and mucociliary clearance rates have been derived from previous studies. Mucus rheology is one of the major contributing factors to mucociliary clearance. In this study we compared mucus rheological properties and transportability of samples collected from six mammalian species and related the data to mucociliary clearance and tracheal diameter. The samples were collected by cytology brush or endotracheal tube technique; no mucokinetic treatment was applied. Rheologic analysis was performed by magnetic microrheometry (King 1988) and reported as viscoelasticity, log G* at 1 rad/s. Mucociliary clearability (MCI) was assessed directly by the frog palate assay, except in rat and horse, where it was computed from rheological properties. Tracheal mucociliary velocity (TMV) was measured by bronchoscopic or radiologic observations of tracer particle clearance. The data are reported as means ± SD.

	Trach diam (mm)	TMV (mm/min)	log G* (1 rad/s)	MCI (normalized)
Rat	3.4	1.9±0.7	2.63±.25	*(0.71±.10)*
Rabbit	5.	3.2±1.1	2.83±.28	.54±.13
Ferret	4.	10.7±3.7	2.57±.49	.58±.25
Dog	16.	12.7±1.3	2.25±.38	.71±.24
Man	20.	15.5±1.3	2.19±.39	.93±.41
Horse	45.	10.0±1.5	1.75±.15	*(1.03±.04)*

Different experimental conditions and methods of collection may account for some of the variation seen, but the overall trend is very clear - viscoelasticity decreases with increasing size of the animal. These variations in mucus rheology, which could reflect interspecies differences in PD, may assist in mimmizing differences in tracheobronchial residence times of material to be cleared.

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