Title: Validation of a hypertussive model of cough in the rabbit

Body: Background Potential anti-tussive agents are usually evaluated in naïve guinea-pigs, however, the clinical translation is poor. Animal models which demonstrate hypertussive responses have not been exploited in drug development programmes. Aim To establish the effect of ozone on the citric acid provoked cough response in the rabbit and to evaluated whether the anti-cholinergic drug, tiotropium bromide is anti-tussive in this model. Method Thirty, juvenile male New Zealand Rabbits were studied using whole body plethysmography and a microphone. Rabbits were screened on day 1 and then assessed in a individually controlled 2x2 randomised protocol each 7 days. 3 hours before each experiment rabbits were pre-treated with either nebulised tiotropium bromide (250µM) or vehicle (saline) at 3L.min-1 for 10 mins. One hour before each experiment the rabbits were exposed to either air or ozone (2ppm) at 3L.min-1. During the experiment, rabbits acclimatized for 5mins before being exposed to 10 minutes of nebulised citric acid at either 0.4M or 0.8M. Results Acute ozone exposure sensitised rabbits into a hypertussive state in a dose dependent manner from $1.38 \pm 1.02$ to $6.26 \pm 1.59$ in the 0.4M citric acid group (control vs ozone, n = 8) and $0.13 \pm 0.13$ to $8.63 \pm 1.71$ in the 0.8M citric acid group (control vs ozone, n = 8). Tiotropium bromide did not attenuate the cough response in the ozone sensitised rabbit ($9.63 \pm 3.39$, n = 8) when compared to the control group ($0.51 \pm 0.26$, n = 8). Conclusion We have demonstrated that rabbits are relatively refractory to citric acid, however, cough number can be significantly increased following exposure to ozone. Under these conditions we found no evidence of an anti-tussive effect by tiotropium bromide.