Effect of Respirable and Total Endotoxin on Neutrophilic Inflammation of Lower Airways in Young Thoroughbred Racehorses in Sydney, Australia.

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Summary

Recent evidence indicates that exposure to stable environments play an important role in the pathogenesis of inflammatory airway disease (IAD) in racehorses. In order to determine the role of airborne particle-related endotoxin and (1→3)-β-D-glucan, a case-control study was performed in two to three year old Thoroughbred racehorses (n=112) housed in racetrack stables in Sydney, Australia. Respirable endotoxin concentration, but not respirable (1→3)-β-D-glucan concentration, was significantly associated with percentage of neutrophils in tracheal aspirate (TA) samples. Specifically, after adjusting for actual confounding variables, a dose-response increase in percentage of neutrophils occurred in lower airways as respirable endotoxin concentrations increased in breathing zone air beyond a threshold of ~4 to 5 ng/m^3.

Introduction

Inflammatory airway disease, characterised by increased proportions of neutrophils in TA samples, is very common in young Thoroughbred racehorses worldwide. For example, four out of ten young racehorses developed neutrophilic IAD within 2 weeks of entering racetrack stables and half of these horses still had evidence of IAD after 4 weeks housed in stables (our unpublished data). The majority of these racehorses have no infectious cause identified. In humans, strong evidence indicates that airborne particle-associated endotoxin, a major pro-inflammatory glycolipid component of Gram-negative bacterial cell walls, contributes to acute neutrophilic airway inflammation when inhaled[1, 2]. In addition, research suggests that (1→3)-β-D-glucan, also found in organic particles, has synergistic or modulatory effects when combined with endotoxin[3,4]. Therefore, a case-control study was designed to investigate the association between exposure to organic particles containing endotoxin and/or (1→3)-β-D-glucan normally present in stable environments, other risk factors, and presence of neutrophils in lower airways of racehorses.

Materials and Methods

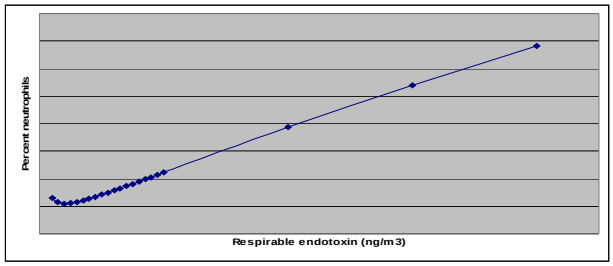
Only two to three year old healthy racehorses entering three racetrack stable complexes in the Sydney metropolitan region for race training were included. All were free of airway inflammation on arrival. The same information was obtained on Day 0, when horses arrived at stables and were exposed to different environments, and on Day 14 and 28. Data collected included details of horse signalment, previous and current history, endoscopic findings, details of TA sample collection, and

management, environment and stable information. Blood was collected for serological analysis for Equine Herpes Virus 1 and 4, Equine Adenovirus, Equine influenza virus 1 and 2, and Equine Rhinovirus A and B. Tracheal aspirates were collected using a standard endoscopic technique and using a guarded catheter[5]. Samples were taken from all horses within two to four hours of morning training. Carbon dioxide (CO_2) and ammonia (NH_3) concentrations in the stable also were measured. Horses were classified as having IAD (cases) if cytological examination of smears from TA samples demonstrated greater than 20% neutrophils. Cases were classified on Day 14 or 28 and only if they were free of IAD when they entered racetrack stables at Day 0. Horses were classified as controls if cytological examination of TA smears demonstrated < 20% neutrophils. At least 1 control per case was selected and control horses were selected at the same time as cases were identified. Respirable and total particle samples were collected from the breathing zone of cases and controls onto polycarbonate micro-pore filters. The endotoxin and (1→3)-β-D-glucan concentrations of particles were analysed with commercially available endotoxin (Endospecy) and glucan (Fungitec G) specific assays respectively using standard techniques[6].

For the purpose of this study, the outcome of interest was "percentage of neutrophils in lower airways" (using data from cases and controls) whereas the primary study factors were respirable and total endotoxin and (1→3)-β-D-glucan. All other variables were considered potential confounders or effect modifiers. Data were analysed using univariable and multivariable linear regression techniques (SAS v.8). The final multivariable model was determined after evaluation of biologically important interaction terms and potential confounding variables.

Results

From March 2000 to December 2001, samples of total and respirable particles were collected from 112 horses. Of these, 54 (48%) had IAD as defined whereas 58 horses (52%) did not have IAD. Total endotoxin, total and respirable (1→3)-β-D-glucan were not significantly associated with changes in percentage of neutrophils in TA in this study. In addition, bedding type, hard feed type, NH_3 concentration, exposure to viruses, indicators of box ventilation such as open windows or fans in stalls, environmental variables such as pollution index, humidity, temperature and wind, and difficulty of TA procedure and coughing during TA procedure were not associated with changes in percentage of neutrophils in lower airways.



The final multivariable model included the study variable respirable endotoxin ($F_{2,96}$ = 4.78, P = 0.01), and the confounding variables previous location, type of hay fed ($F_{2,96}$ = 2.88, P = 0.06), training progress, bacterial growth in TA, and CO_2 grade.

This model accounted for 25% of the variability in percentage of lower airway neutrophils. After controlling for confounding variables, and substituting values for endotoxin between 1 and 40 ng/m^3 into the multivariable model equation, an initial exponential then linear (or dose-response) increase in percentage of neutrophils occurred in lower airways (P=0.002) when exposed to respirable particle endotoxin. This is qualitatively demonstrated in the figure above.

Discussion

This study is the first to quantify concentrations of total and respirable endotoxin and (1→3)-β-D-glucan in breathing zone particles in racetrack stables, using a large population of horses. Furthermore, these results demonstrated that exposures to respirable and total endotoxin in "natural" stable environments could be as high as experimental airborne challenges used to induce lower airway inflammation in horses[7].

The results demonstrated that there was a significant exposure-response relationship between the average percentage of neutrophils in lower airways and exposure to >4 to 5 ng/m^3 of respirable endotoxin in breathing zone dust, after accounting for confounding variables. The response to endotoxin is due to activation of macrophages and epithelial cells in airways and subsequent release of pro-inflammatory mediators, which result in neutrophil invasion from airway walls and from circulation[8]. These results agreed with experimental evidence of the effects of inhaled lipopolysaccharide in lower airways[7]. However, this study emphasised that inhalation of endotoxin at concentrations present in airborne organic dust in many racetrack stables, which are well below "threshold doses" of respirable endotoxin used in inhalation studies, still result in increases in percentage of neutrophils in lower airways. This is most likely because the horses in our study received almost continuous exposure to the stable environment, and other agents present in stable dust such as peptidoglycans and proteases may enhance the inflammatory response to endotoxin[3, 9].

In contrast to airborne respirable endotoxin, total particle endotoxin and respirable and total (1→3)-β-D-glucan concentrations were not found to be significant risks for increases in neutrophil percentages in lower airways of racehorses. As well, no interactions between these agents or other variables were found. Finally, the results of this and other studies[7], demonstrate that endotoxin is a fundamental component of airborne particulate matter, which contributes to acute neutrophilic IAD in young racehorses housed in stables.

References

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