PATHOLOGIC CHANGES IN THE RESPIRATORY TRACT OF PIGS INDUCED BY EXPOSURE TO FEED DUST WITH OR WITHOUT ADDED ENDOTOXIN

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Abstract

We hypothesize that exposure to airborne dust and endotoxin induces an upregulation of inflammatory cell function and associated pathologic changes in the respiratory system of pigs. A continuous flow exposure chamber was build to investigate the effect of airborne dust and endotoxin in a more controlled environment. So far, pigs have been exposed for 5 weeks to chamber background (controls), dust (11.3 mg/m^3) and dust (10 mg/m^3) and ET (2 µg/m^3). From the BAL analysis, we concluded that dust and dust+ET exposed pigs tend to respond by day 17 of the experiment.

Introduction

Respiratory diseases in swine cause important problems for the swine industry all over the world. It is widely accepted that respiratory diseases in swine are a multifactorial problem, which is implied by the name Porcine Respiratory Disease Complex (1,2). However, various infectious agents (virus, bacteria, mycoplasma) are still considered as the primary etiological agents. We believe that, similar to the respiratory problems in swine producers, non-infectious airborne contaminants in swine buildings might be critically important contributing causes. Surprisingly, the effect of such contaminants on the respiratory system of pigs, which often are continuously exposed to dust throughout their life, has never been investigated. Therefore, we hypothesize that chronic exposure to airborne contaminants (i.e. dust and endotoxin) induces inflammatory changes in the respiratory tract of pigs, leading to impaired disease resistance. To investigate this hypothesis, we exposed pigs to different combinations of dust and endotoxin. This paper describes the results of exposure of pigs to the chamber background (control),feed dust only and feed dust with added endotoxin.

Material and Methods

Continuous Flow Exposure Chamber

A continuous flow exposure chamber was built in a semi-isolation room at Charmany Animal Resource Center of the UW- Madison, School of Veterinary Medicine and has been described in more detail elsewhere (3).

Preparation of exposure material

A pelleted 18% corn-soybean meal, without added antibiotics or fat and phenol extracted endotoxin from *Escherichia coli* (O55:B5; Sigma Chemical Company, MO) were used as exposure material. For the dust exposed group, 1 kg of feed pellets were placed daily in the rotating drum to produce a target dose of 10 mg/m^3 dust. For the dust with endotoxin group, 200 mg of endotoxin in 50 ml of pyrogen free water was added to 1 kg of feed pellets. This mixture was prepared one day prior to exposure, was dried overnight at room temperature and was considered as one daily exposure dose.

Environmental sampling

Total dust in the exposure chamber was measured 40 cm above the floor. Dust was collected with an IOM inhalable dust sampler (SKC, Eighty Four, PA) connected to a vacuum pump (flow= 2 L/min), located outside of the exposure chamber. Dust was measured by gravimetric analysis of the filters before and after sampling. The filters were extracted in appropriate buffers and supernatants were analyzed for 1) endotoxin with the endotoxin specific Limulus ES-II test; 2) β-1,3-glucan with the β-1,3-glucan specific BG-Star; 3) peptidoglycan and β-1,3-glucan with the non-specific Silk Larvae Plasma(SLP) reagent. All toxin measurements were done by Wako Pure Chemical Ltd., Japan.

Experimental Design

Ten early weaned pigs (10-12 days old) were purchased from a high health herd for each experiment. Upon arrival at Charmany Animal Resource center, pigs were kept in a semi-isolation room and raised to 4 weeks with a commercial phase feed. At the age of 4.5 weeks, 6 pigs were moved into the room with the continuous flow exposure chamber and were fed an 18% pelleted corn-soybean meal, similar to the exposure material. From 5 weeks of age on, pigs were placed in the continuous flow exposure chamber for 5 hours/day during 25 days out of a 35 day study period. Pigs were exposed to the background environment in the chamber, to dust or dust+endotoxin (ET). Each pig was weighed and bronchoalveolar lavages (BAL) were performed on Days 0, 17, and 36 of the study period. Blood samples were collected on Days 1, 18 and 35. On Day 36, pigs were anesthetized, exsanguinated and necropsied. For each experimental group, 4 control pigs were housed in a standard semi-isolation room. These pigs were treated the same as the experimental pigs, although no BAL was collected on Days 0 and 17.

Bronchoalveolar lavage fluid was analyzed for cellular components, alveolar macrophage activation, procoagulant activity (PCA) and lactate dehydrogenase activity (LDH). Total cell count, differential cell count, PCA and macrophage activity were considered as markers for inflammation. LDH in BALF was measured as a non-specific biochemical marker for lung injury. On Day 0 and 17, BAL was performed on anaesthetized pigs by wedging

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a Pentax bronchoscope in the diaphragmatic lung lobe. Sixty ml of sterile phosphate buffered saline (pH 7.4) at body temperature was slowly infused and aspirated.

Plasma was analyzed for antibodies against a panel of infectious respiratory diseases (Pharmacia and UpJohn, Veterinary Services and Diagnostic Center, Worthington, MN).

At necropsy, the respiratory system was examined and scored for macroscopic lesions. The left lung lobe was immersed in 10% neutral buffered formalin and 4 different sections were collected for histopathological examination (in progress). BAL was performed by infusing 80 ml PBS in the right diaphragmatic lung lobe.

Results and Discussion

The environment in the continuous flow exposure chamber is summarized in Table 1. The dust in the background group originated from the feeder and the pigs themselves. In the dust and dust+ET group, pigs were exposed to an average of 11.3 and 10 mg/m^3 of dust, respectively, with some daily variations. Endotoxin concentrations in the background and dust group were within the range of others measured in swine confinement (4). Preliminary data indicate that about 2 µg/m^3 ET was introduced in the chamber for the dust+ET group. Silk larvae plasma results, which included both peptidoglycan and ß-1,3-glucan, were higher than those previously reported from swine confinement (4). The differences in ß-1,3-glucan concentrations between background and dust group cannot be explained at this point. Although, it is possible that there was more water insoluble ß-1,3-glucan present in the dust, which precipitated after extraction. The dust and toxin levels measured in this study were the inspirable fraction. This included particles up to 100 µm in size, which are effectively trapped in mouth and nose. Only particles smaller or equal to 5 µm are respirable and reach the alveoli. Unfortunately, we have not been able to collect measurable amounts of such small particles. Ammonia and carbon dioxide concentrations were measured at different time points and averaged 2.4 ppm and 0.12 vol%, respectively.

Pigs exposed to dust had a better growth performance and consumed more feed than background and dust+ET exposed pigs (Table 2, $p=0.07$), but the dust exposed pigs were also 1 kg heavier ($p<0.05$) at the start of the experiment. Dust+ET exposed pigs had a lower ADG than background pigs.

At necropsy, one of the dust and 2 of the dust+ET exposed pigs had minor microscopic lung lesions (Table 2). The histopathology results of 1 of these pigs indicated a foreign body granulomatous pneumonia. Since the histopathology examinations of the other dust and dust+ET exposed pigs were in progress at the time this paper was written, we could not conclude if the lesion was due to the dust exposure or the bronchoscopy procedure. Both background and dust exposed pigs had minor atrophic rhinitis lesions (Table 2). One control pig for the dust exposed group seroconverted for *M. hyopneumoniae* at Day 35 of the study period.

The BAL cell differentials are summarized in Table 3. Alveolar macrophages were the major cell in the BALF of all experimental groups. The percentage of alveolar macrophages decreased over time while the percentage of lymphocytes and neutrophils increased. The main difference between the 3 experimental groups was an earlier increase (Day 17) of percent neutrophils and lymphocytes in the dust+ET exposed pigs.

The results of the BAL analysis are summarized as mean change over baseline (Day 0 sampling) in Figures 1-3. Superoxide anion (SO) synthesis was measured as a parameter for BAL cell activity and originated from alveolar macrophages and neutrophils. Dust exposed pigs had increased SO production on Day 17 (Figure 2). On Day 36, SO production in background pigs was nearly double the increase observed in dust and dust+ET exposed pigs. A negative change of LDH concentration in BALF was found in background pigs on Day 17, while LDH increase was higher for dust and dust+ET exposed pigs on Day 36 (Figure 2).

Fibrin deposition in alveoli is present in different lung diseases and may partially be explained by an increased PCA. Procoagulant activity in BALF originates from alveolar macrophages and endothelial cells. Since PCA was measured as the abilitiy of BALF to shorten the clotting time of normal swine plasma, a lower clotting time indicated an increased PCA. From Figure 3, it was concluded that PCA decreased over time in background and dust exposed pigs and that the decrease was earlier in the dust exposed pigs (Day 17). This decrease coincides with a decrease in alveolar macrophages. An increased PCA was found at Day 36 in the dust+ET exposed pigs.

Although some of the results cannot be fully explained at this time due to incomplete analysis of data, it can be concluded that there is a trend for the dust and dust+ET exposed pigs to respond earlier in the study period (Day 17). The assumption can be made that the later response of the background pigs might be due to an additive effect of the daily dust exposure. This could indicate that longterm exposure to low dust concentrations can be as detrimental as short term exposure to high concentrations. More studies need to be done to support these assumptions.

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Table 1. Description of environment in continuous flow exposure chamber and semi-isolation room for background control, dust and dust+endotoxin exposed pigs

	Experimental Group		
Airborne Contaminant	Background	Dust	Dust+ET
Days	24	25	25
Dust (mg/m^3)	2.5 ± 0.2[1]	11.3 ± 2.0[2]	10.1 ± 0.5[2]
ET (ng/m^3)	46.8 ± 4.2	40.0 ± 4.0	ND
Glucan (ng/m^3)	207.6 ± 62.0	15.2 ± 1.5	ND
SLP (µg/m^3)[3]	10.5 + 1.7	8.3 + 0.7	ND

[1] mean ± standard error; [2] 23 samples, ND - Not Done; [3] SLP - non-specific for peptidoglycan and β-1,3-glucan

Table 2. Summary of health parameters measured in background control, dust and dust+endotoxin exposed pigs

	Experimental Group [1]		
	Background	Dust	Dust+ET
ADG (g)[2]	623 ± 42[4]	711 ± 30	605 ± 52
Lung Score	0	0.9 ± 0.8	0.8 ± 0.7
AR Score[3]	0.7 + 0.7	0.3 + 0.3	0.0

[1] 6 Pigs/Group; [2] ADG-Average Daily Gain; [3] score 0 to 9; [4] Mean ± Standard error

Table 3. Cell differential in bronchoalveolar lavage fluid of background control, dust and dust+endotoxin exposed pigs

	Experimental Group [1]		
	Background	Dust	Dust+ET
Total Cells			
Day 0	7.6 ± 1.4[2]	11.3 ± 0.5	11.8 ± 1.3
Day 17	11.2 ± 1.6	16.0 ± 1.6	11.0 ± 1.5
Day 36	13.7 + 1.7	14.2 + 2.0	15.2 + 2.5
Alv Macroph.	91.8%	95.0%	93.2%
Day 0	91.8%	91.0%	84.0%
Day 17	83.7%	88.6%	85.0%
Day 36			
Lymphocytes			
Day 0	3.5%	3.8%	2.7%
Day 17	4.2%	4.9%	8.9%
Day 36	8.8%	6.9%	8.1%
Neutrophils			
Day 0	4.8%	1.2%	4.0%
Day 17	4.0%	3.3%	6.8%
Day 36	7.1%	3.5%	6.7%

[1] 6 Pigs/Group; [2] Mean ± Standard error, x 10^6

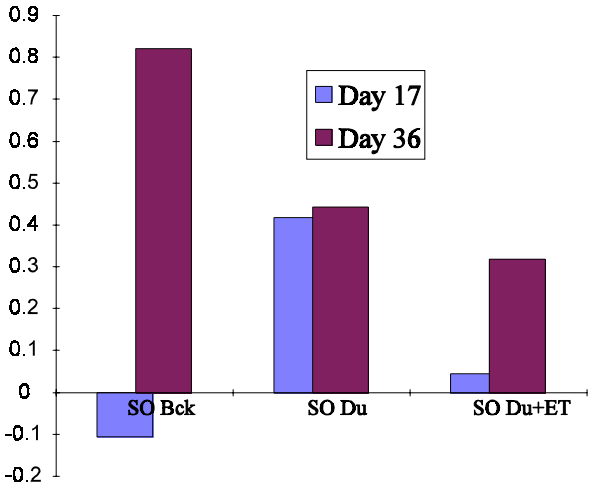

Figure 1. Effects of exposure to chamber background (Bck), feed dust (Du) or feed dust+endotoxin (Du+ET) on superoxide activity (SO, nmol/min/10^6) in bronchoalveolar lavage fluid of pigs. Results are presented as mean change over baseline (Day 0 sampling).

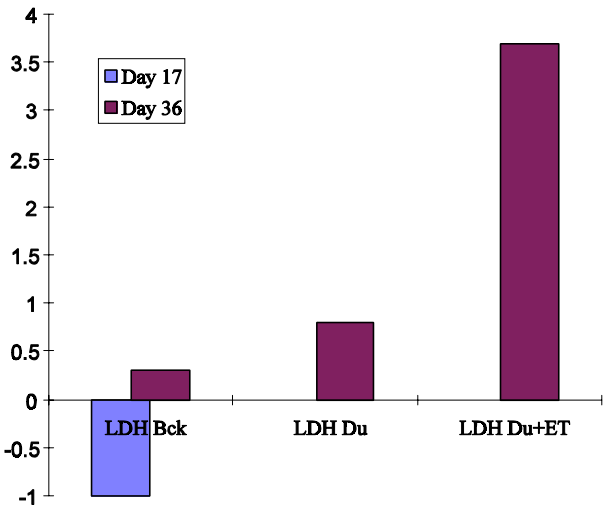

Figure 2. Effects of exposure to chamber background (Bck), feed dust (Du) or feed dust+endotoxin (Du+ET) on lactate dehydrogenase activity (LDH, U/L) in bronchoalveolar lavage fluid of pigs. Results are presented as mean change over baseline (Day 0 sampling).

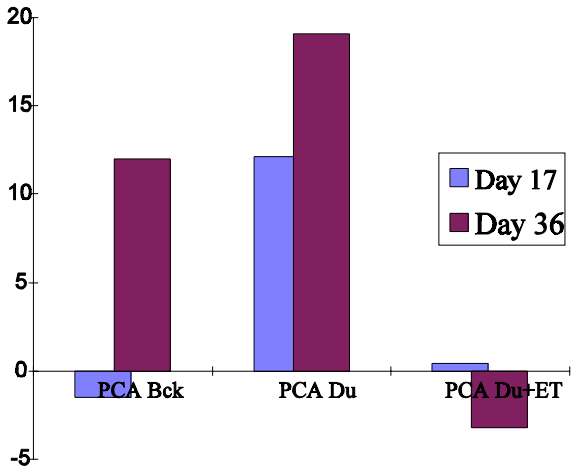

Figure 3 Effects of exposure to chamber background (Bck), feed dust (Du) or feed dust+endotoxin (Du+ET) on procoagulant activity (PCA, sec) in bronchoalveolar lavage fluid of pigs. Results are presented as mean change over baseline (Day 0 sampling).