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## Changes in Bronchoalveolar Lavage in Camels *Camelus dromedaries* with Experimentally Induced Pneumonic Pasteurellosis

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**Abstract :** To investigate possible changes in bronchoalveolar (BAL) composition in camels with bacterial infection, we inoculated camels intratracheally with *Pasteurella haemolytica*. Phospholipids in BAL were determined with the help of High - performance liquid chromatography (HPLC). There was a significant decrease in Dipalmitoyl phosphatidylcholine (DPPC) and phosphatidylglycerol (PG) with concomitant non-significant increase in the other phospholipid species. These changes may indicate damage of alveolar type II cells and /or metabolic disturbance in the pulmonary surfactant.

**Key words :** Pulmonary surfactant, camel, phospholipid composition, *Pasteurella haemolytica*

### Introduction

Bronchoalveolar lavage (BAL), a routine method of sampling lung excretion, has been used successfully in the physiological, biochemical and cytological evaluation of pulmonary diseases and pulmonary surfactant components (Honda *et al.*, 1988; Lesur *et al.*, 1993).

The pulmonary surfactant consists mainly of about 90% lipids and 5-10% surfactant - specific proteins (Van Golde *et al.*, 1988). In vertebrates phospholipids comprise the largest part of lipid fraction (Clercx *et al.*, 1989). The main phospholipids are DPPC and PG. These are synthesized in alveolar type II cells and secreted in alveolar spaces, where it prevents the collapse of the pulmonary alveoli (Clercx *et al.*, 1989).

Alteration of alveolar surfactant phospholipids is common feature of various pulmonary diseases (Lesur *et al.*, 1993). Changes in phospholipids profile have been reported in alveolar proteinosis (Onoera *et al.*, 1983), bacterial pneumonia (Baughman *et al.*, 1984) Sarcoidosis (Baughman *et al.*, 1985) and Adult Respiratory Distress Syndrome (Jackson *et al.*, 1986). Weiss *et al.* (1991) studied changes in BAL fluid components in calves, infected with *P. haemolytica* and reported changes in lavage total cell count, neutrophil count, total proteins, albumin concentration and ALP and LD activities. Pneumonic pasteurellosis is prevalent disease that often results in severe lung necrosis, lung injury and death. As part of study on the composition of the pulmonary surfactant of the camel, we present in this manuscript alteration in the phospholipids of the surfactant of camels with experimentally induced pneumonic pasteurellosis.

### Materials and Methods

Four healthy male camels, 2-4 years old were purchased and transported to an isolation facility near the experimental farm of the College. The camels were kept for two weeks before starting the experiment. The animals were positioned in sitting posture and secured with ropes. The camels were sedated with xylazine (0.5 mg/kg body weight), ten minutes before surgery. The surgical technique of Weiss *et al.* (1991) for calves was followed. Amid - cervical transtracheal incision was made. A modified catheter with inflatable cuff was introduced into the right diaphragmatic lung lobe and advanced till resistance was met, then was withdrawn 3 inches and secured in place. The catheter was modified by cutting off the tip to allow aspiration. Hundred ml sterile calcium and magnesium - free phosphate buffer saline solution (PBSS) was slowly infused. The fluid was aspirated by suction. The lavage procedure was repeated twice for each animal pre and post bacterial inoculation. The two samples were then pooled.

After sample collection the camels were euthanized,

necropsied and lungs were removed and placed in neutral buffered 10% formalin for further investigations.

**Inoculum:** The bacteriological part of the experiment was conducted in the microbiology laboratory at the department of Veterinary Medicine, College of Agriculture and Veterinary Medicine.

A field isolate of *P. haemolytica* AI strain 12296 was inoculated on brain-heart infusion agar plates. After 12 hours, the organisms were harvested and incubated in brain - heart infusion broth at 37°C for 3 h followed by centrifugation. Bacteria were then harvested to 10<sup>9</sup> organisms / mL of PBSS.

**Experimental procedure:** Lavage fluid was obtained twice from each animal before inoculation as control. Each animal was then inoculated with 5 x 10<sup>8</sup> *P. haemolytica* organisms suspended in 50 mL PBSS.

**Sample collection and analysis:** BAL was collected before inoculation and 6 hours post inoculation. The lavages were then filtered and centrifuged at 1000 rpm for 10 minutes to remove cells and debris. After removal of cells and debris from lung lavage samples, an aliquot of the lavage fluid from the two samples was centrifuged (4 °C, 1 h, 75000 x g) to collect the surfactant pellet. The pellet was resuspended in distilled water, frozen (-70 °C) and lyophilized.

**Lipid extraction of the surfactant pellet:** The method of Folch *et al.* (1957) was employed for extraction of the lipids of the surfactant pellet.

**High Performance Liquid Chromatography of the surfactant lipids :** Fractionation of phospholipids by HPLC was carried out by the method of Pison *et al.* (1986) as modified by Engen and Clark (1990) for reducing elution time, maintaining good resolution and reproducibility. Standard solutions of phosphatidic acid (PA), phosphatidylethanolamine (PE), Phosphatidylserine (PS), Dipalmitoyl Phosphatidylcholine (DPPC), Sphingomyelin (SPH), Lysophosphatidylcholine (LPC) and Lysophosphatidylinositol (PI) were obtained from Sigma Chemicals Company and prepared at concentrations between 1-15 mg/ml in chloroform : methanol : hexane mixture (5 : 4 : 1 v/v/v).

When surfactant samples were to be analysed by HPLC system, the dried samples (10 mg) were reconstituted with 0.25 ml of above mixture. The mobile phase of HPLC system consisted of acetonitrile : methanol : H<sub>2</sub>SO<sub>4</sub> (100 : 4 : 0.25, v/v/v). the injected sample volume was 20 µl of lipid extract or phospholipids standard solution. All surfactant phospholipids were separated in the following system PA, PG, PI, PE, PS,

DPPC, SPH and LPC. The relative percent of bands were automatically printed by the computing chromatography integrator. The different phospholipids were identified by comparing the retention time of each band by retention time of standard. Analysis of variance and the standard Student's (t) test were used to test differences between the two groups. Data are presented as mean  $\pm$  SD.

## Results

The phospholipids contents of BAL collected from camels before and after bacterial inoculation are shown in Table 1. DPPC was the most abundant phospholipid isolated comprising about 65% of the total phospholipids. Phosphatidylglycerol was the next most abundant at 11.26  $\pm$  2.43%. The other phospholipids were in substantially smaller quantities. Six hours post inoculation of *P. haemolytica*, the percentage of DPPC decreased significantly to about 54%. Phosphatidylglycerol also decreased significantly to 7.47% when compared to the pre-inoculation control. This significant decrease in DPPC and PG was accompanied by a relative non-significant increase in the other phospholipid species.

Table 1: Percentage of the phospholipid fractions of normal and infected camel BAL

Phospholipid %	Normal	Infected
PA	3.44 <sup>a</sup> $\pm$ 1.12	5.24 <sup>a</sup> $\pm$ 1.88
PG	11.62 <sup>a</sup> $\pm$ 2.43	7.47 <sup>b</sup> $\pm$ 1.62
PI	3.57 <sup>a</sup> $\pm$ 2.60	6.84 <sup>a</sup> $\pm$ 2.83
PE	4.35 $\pm$ 0.92	6.56 $\pm$ 1.24
PS	2.24 $\pm$ 0.63	4.83 $\pm$ 1.68
DPPC	65.82 <sup>a</sup> $\pm$ 3.21	54.42 <sup>b</sup> $\pm$ 2.46
SPH	4.84 <sup>a</sup> $\pm$ 2.76	8.74 <sup>a</sup> $\pm$ 3.14
LPC	3.91 <sup>a</sup> $\pm$ 1.82	5.86 <sup>a</sup> $\pm$ 2.24

Means on the same row having different superscripts are significantly different at  $P < 0.05$ .

## Discussion

Results obtained in this work clearly demonstrated that bacterial inoculation significantly altered the phospholipids profile of BAL in camels. The most obvious changes noticed were decrease in both DPPC and PG with concomitant non-significant increases in other phospholipids.

It is well known that DPPC, the main phospholipid in pulmonary surfactant, is synthesized in alveolar type II cells and secreted into alveolar space (Van Golde, 1988). Decrease in DPPC has been reported in many pulmonary diseases like, in Human Adult Respiratory Failure, Pneumonia and Cardiogenic Lung Oedema (Gunther *et al.*, 1996); in Idiopathic Pulmonary Fibrosis and Sarcoidosis (Honda *et al.*, 1988) and in calves exposed to bovine herpes virus -1 or Parainfluenza -3 virus (Engen and Brown, 1991).

The decrease in DPPC after *P. haemolytica* inoculation may reflect some damage to alveolar type II cells that resulted in decreased synthesis of DPPC or as a result of degradation of preformed DPPC as a result of metabolic disturbances. Van Golde (1976) reported that phospholipase A is located at the plasma membrane of the epithelial cells of alveolar lining. Damage of this plasma membrane due to inflammation or invasion of micro organisms may increase the release of phospholipase A into alveolar lumen causing degradation of DPPC.

Phosphatidylglycerol (PG) is known to be the second characteristic phospholipid in pulmonary surfactant. Appreciable amount of PG are contained in alveolar type II cells but only traces are found in other cells (Masson and Williams, 1980). For this reason, changes in PG in the surfactant will reflect more precisely the damage in alveolar type II cells than does DPPC which is present in other cells as well. The present results showed also that the other

phospholipids of BAL increased non-significantly. However, these phospholipids are considered minor components of pulmonary surfactant and their exact function is not well clarified (Honda *et al.*, 1988). It can be concluded from this study that inoculation of camels with *P. haemolytica* changed the phospholipid profile in BAL, specially decreases in DPPC and PG. This may indicate damage of alveolar type II cells and metabolic disturbance in pulmonary surfactant.

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