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RESEARCH ARTICLE

An experimental study of a rat model of emphysema induced by cigarette smoke exposure and the effect of Survanta therapy

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ABSTRACT

The present study was performed to test the therapeutic effects of Survanta (an exogenous surfactant) on a Wistar rat model of emphysema. Thirty-five adult male Wistar rats were divided randomly into the following groups; control subgroups Ia&b (n = 14); emphysematous model subgroups IIa,b&c (n = 21) exposed to cigarette smoke (CS), received phosphate buffer solution (PBS) and Survanta respectively. The levels of serum myeloperoxidase (MPO), lung tissue lactate dehydrogenase (LDH), alkaline phosphatase (ALP) as well as antioxidants: catalase (CAT), superoxide dismutase (SOD) and oxidative stress: malondialdehyde (MDA) markers were measured. Immunohistochemical staining of the lung was applied with anti-P53, anti- tumor necrosis factor (TNFα) and anti-proliferating cell nuclear antigen (PCNA) to reveal the changes of the lung structure. The mean linear intercepts (MLI) of alveoli were measured to assess alveolar size. In emphysematous rats, the serum level of MPO and tissue LDH, ALP & MDA were significantly increased while; CAT and SOD were significantly decreased. Pictures analysis for all immunostains was clearly increased. In Survanta treated group, a significant improvement in all previously mentioned findings while; no improvement in alveolar diameter was detected. These results conclusively demonstrate that Survanta administration improves the inflammatory biochemical and histochemical parameters of the emphysematous lung.

1. Introduction

Pulmonary emphysema is a major public health problem. It is characterized by alveolar wall destruction, distal airspace enlargement and reduction in the capillary exchange area. Consequently, the patients suffer from dyspnea and inadequate oxygenation (Vestbo et al., 2013). Although many factors are encountered in its development, cigarette smoking (Dijkstra et al., 2013) and deficiency in secretory immunoglobulin A are still the major risk factors (Richmond et al., 2016). Undoubtedly, the growing phenomenon of cigarette smoking has become one of the most expensive and pervasive health problems in the developed world (World Health Organization, 2008).

Therapeutic strategies in the treatment of emphysema depend mainly on improvement in lung function and capacity. Some investigators have adopted drugs, such as beta-agonists and anticholinergics (Chapman et al., 2011) and corticosteroids (Magnussen et al., 2014). Surgical trials using one-way endobronchial valves have been tried by others (Klooster et al., 2015). However, these treatments are symptomatic, slow disease progress and improve pulmonary function. Most of these treatments are considered chronic and palliative (Naunheim et al., 2006). Recently, Seimetz et al. (2015) tried to prevent emphysema via phosphodiesterase 4 and 5 administration during chronic cigarette smoking.

The pulmonary surfactant is a complex mixture of phospholipids and proteins. It is secreted mainly by pneumocyte type II and Clara cells. Up to now, about six types of surfactant associated proteins have been recognized; SP-A, SP-B, SP-C, SP-D, surfactant-associated protein 2 (SFTA2/SP-G) and surfactant-associated protein 3 (SFTA3/SP-H). Both SP-A & SP-D are hydrophilic and involved in defense and control of lung inflammation (van Eijk et al., 2000). In contrast, SP-B & SP-C are hydrophobic and involved in the reduction of the surface tension of alveoli. Using a 3D model, the SP-G structure was first discovered by Rausch et al. (2012). In the lung, the SP-G is localized in the bronchiolar epithelium. Its physicochemical properties are similar to SP-B and SP-C. However, the SP-H or SFTA3 is distributed in most of the lung tissue (Schicht et al., 2014). It is responsible for the enhancement of alveolar macrophage phagocytosis (Diler et al., 2014). Extrapulmonary localization of different types of SPs has been documented (Bräuer et al., 2007; Rausch et al., 2012; Sheats et al., 2016). Surprisingly, *Staphylococcus aureus* and *Pseudomonas aeruginosa* express

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and secrete four types of human surfactant proteins (Bräuer et al., 2013).

Several investigators have considered exogenous surfactants as a promising treatment for many airway diseases. In premature infants, exogenous surfactant therapy is a standard life-saving intervention for the prevention and treatment of neonatal respiratory distress syndrome (NRDS) (Hohlfeld, 2002; Babu et al., 2003; Engle, 2008). The use of exogenous surfactant is documented in adult patients with lung injury associated respiratory failure as described in pneumonia (Gan et al., 2001), and pulmonary contusion (Raghavendran et al., 2011). Survanta is an exogenous surfactant containing SP-B and SP-C. Its phospholipid fraction is obtained by mincing cow lungs. It maintains the openness of alveoli and terminal conducting airways, promotes normal blood gasses and lowers airway resistance (Polin et al., 2014).

Most of the animal-derived surfactants contain variable amounts of SP-B. The SP-B has antioxidant capacities (Kerecman et al., 2008) and anti-inflammatory properties (Seger and Soll, 2009). In addition, its potent inhibitory effect on human neutrophil elastase (HNE) after combination with a small molecule of HNE inhibitor has been documented (Guarnieri et al., 2010). Moreover, SP-B plays a role in activation of alveolar macrophages and demonstrates an antimicrobial activity (Yang et al., 2010).

It was thought that the surfactant-associated proteins (SP-A, SP-D) alone are responsible for lung immunity. However, the study of Kerecman et al. (2008) unexpected reported anti-inflammatory and antioxidant effects of surfactant phospholipid (dipalmitoyl phosphatidylcholine) without SP-A and SP-D on stimulated rat macrophages line NR8383 induced by lipopolysaccharide. Also, Kerecman et al. (2008) demonstrated the anti-inflammatory and antioxidant activities of beractant (an exogenous surfactant). They suggested that these exogenous surfactant products may depend upon their modified specific preparation or their dose in the treatment of some pulmonary diseases. In addition, the phosphatidylglycerol and palmitoyl-oleoyl-phosphatidylglycerol (anionic phospholipids of lung surfactant) have been implicated as major pulmonary regulators of innate immunity. Moreover, palmitoyl-oleoyl-phosphatidylglycerol blocks viral infection (Kurt-Jones et al., 2000) and suppresses proinflammatory activation during bacterial infection (Kuronuma et al., 2009).

Based on this, therapy with exogenous surfactants is on the horizon. However, the therapeutic safety and efficacy of surfactant in emphysema lack experimental confirmation. The correlation between the SP-C mutation and development of lung diseases (Guillot et al., 2009) and the elastolytic effect of SP-B after combination with human elastase inhibitor (Guarnieri et al., 2010; Antunes and Rocco, 2011) encourage us to use the exogenous surfactant containing SP-B and SP-C such as Survanta in an emphysematous rat model induced by cigarette smoke. The emphysematous changes in the lung will be associated with destruction and decreased synthesis of the surfactant layer lining the alveoli. Thus, we hypothesized that replacement of the internally damaged layer of surfactant by exogenous surfactant (Survanta) might alleviate the lung injury caused by emphysema.

2. Materials and methods

2.1. Study population

The study was performed in the Histology & Cell Biology and Physiology departments, Faculty of Medicine, Zagazig University, Egypt. A total 35 adult male Wistar albino rats (8–10 weeks, 100–120 gm) were purchased from the animal house, Faculty of Medicine, Zagazig University, Egypt. Rats were kept in ventilated stainless steel cages in a clean room at a controlled temperature (20–25 °C) and illumination (12 h light/dark). They were fed a standard pellet diet and water ad-libitum. This diet was designed by the National Research Institute for Nutrition, Giza, according to the National Nutrition Database for Standard Reference. The animals were left for one week for acclimatization. The experimental protocol was conducted as stipulated in the Guide for Care and Use of Laboratory Animals Guidelines of the National Institutes of Health (NIH) and approved by the local authorities of Zagazig University, Egypt.

2.2. Animal treatments

Cleopatra cigarettes (king size, Eastern Company, Egypt) were obtained from Hyper One Mall (the 10th of Ramadan, El- Sharqia, Egypt). Each cigarette contains 1 mg of nicotine and 15 mg of tar.

The Survanta (8 mg/ml suspension; Abbott Laboratories, Columbus, OH, USA) was purchased from El-Eman pharmacy (Zagazig, El-Sharqia, Egypt). It is a natural bovine lung extract containing phospholipids, neutral lipids, fatty acids, and surfactant-associated proteins to which colfosceril palmitate (dipalmitoylphosphatidylcholine), palmitic acid, and tripalmitin are added. Its protein content consists of two hydrophobic, low molecular weight, surfactant-associated proteins, SP-B and SP-C. Each ml contains 25 mg/ml phospholipids, 0.5–1.75 mg/ml triglycerides, 1.4–3.5 mg/ml free fatty acids and 0.1–1.0 mg/ml protein. It was stored at 2–8 °C and protected from light. Before administration, it was warmed by hand for 8 min and visually inspected for discoloration.

2.3. Emphysematous model

Our smoking inhalation system device was based on the work of Cendon et al. (1997) and Kozma et al. (2014). The rats were exposed to the smoke of 10 cigarettes/three times/day for 30 weeks. Each exposure period was one hour (from 9:00 a.m. to 10:00 a.m.; from 12:00 p.m. to 1:00 p.m.; from 7:00 p.m. to 8:00 p.m.). During each exposure period, the animals were exposed to smoke for 10 min, followed by 5 min of exposure to fresh room air to avoid hypoxia.

2.4. Experimental design

The rats were randomly divided into the following groups; control group I (n = 14), which was further divided equally into; subgroup Ia exposed to room air and subgroup Ib received 1 ml sterile phosphate-buffered saline (PBS). The 21 emphysematous animals (group II) were further divided equally into three subgroups; subgroup IIa exposed to room air, subgroup IIb received 1 ml sterile PBS and subgroup IIc received 4 doses of Survanta (6.5 mg in 1 ml PBS/rat) at an interval of five days in between. All medications were introduced via thin intratracheal catheters after tracheostomy under light anesthesia with an intraperitoneal injection of 0.6 ml of 1% sodium methohexital (Wang et al., 2006).

2.5. Body weight

Initial weights of all rats were recorded on the first day of the experiment and at the end of the experiment.

2.6. Tissue preparation for histological lung evaluation and homogenate

The rats were euthanized with an intraperitoneal injection of 0.2 ml of 260 mg/ml sodium pentobarbital after 4 weeks post-last treatment. Then, instillation of fixative (10% neutral buffered formalin, pH 7.4) was carried out under a constant pressure of 20 cmH_2O. The caudal lobes of right lungs were dissected, immersed in fixative and processed for paraffin block. Then, 5 μm