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Histopathological changes and mRNA expression in lungs of horses after inhalation anaesthesia with different ventilation strategies

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ABSTRACT

Inappropriate mechanical ventilation can lead to ventilator-induced lung injury (VILI). Aim of this study was to evaluate the effects of inhalation anaesthesia and ventilation with and without recruitment (RM) and PEEP titration on alveolar integrity in horses.
Twenty-three horses were divided into 4 groups (group OLC ventilated with OLC, group IPPV ventilated with intermittent positive pressure ventilation, group NV non-ventilated, and group C non-anaesthetized control group).
After sedation with xylazine and induction with diazepam and ketamine anaesthetized horses were under isoflurane anaesthesia for 5.5 h. The horses were euthanized and tissue samples of the dependent and non-dependent lung areas were collected. Histopathological examinations of the lung tissue as well as relative quantification of mRNA of IL-1β, IL-6, iNOS, MMP1 and MMP9 by PCR were performed.
Horses of group OLC had significantly less alveolar congestion and atelectasis but greater alveolar overdistension compared to groups NV and IPPV. In groups OLC and group IPPV an increase in IL-1β/6 and MMP1/9 was detected compared to groups NV and C.
In conclusion, in breathing spontaneously or IPPV-ventilated horses a higher degree of atelectasis was detected, whereas in OLC-ventilated horses a higher degree of overdistention was present. Elevated levels in IL and MMP might be early signs of VILI in ventilated horses.

1. Introduction

During general anaesthesia and dorsal recumbency horses develop a large alveolo- arterial oxygen partial pressure gradient and become regularly hypoxaemic even when lungs are ventilated with oxygen-rich gas (Hubbell, 1991). Poor oxygenation in horses is the result of ventilation perfusion mismatch and the formation of atelectasis in dependent lung areas causing increased right to left shunting of the blood (Nyman and Hedenstierna, 1988).

Lachmann (1992) suggested a strategy to open atelectatic lungs by sufficient inspiratory pressures und keeping them open by sufficient positive end-expiratory pressure (PEEP). He proposed that a treatment combining an initial pressure increase beyond the opening pressures of collapsed alveoli with enough PEEP to stabilize the opened units would reduce atelectasis and improve lung function (Lachmann, 1992). This recruitment maneuver with a stepwise increase in inflation pressures in combination with the titration of PEEP was able to open the lung and keep it open in ponies and horses (Levionnois et al., 2006; Wettstein et al., 2006; Schürmann et al., 2008; Ambrósio et al., 2013; Hopster et al., 2016). However, the safety of mechanical ventilation with respect to cardiovascular changes as well as morphological alterations of the lung has been discussed controversially. Several early experimental and clinical studies in different species suggest that "inadequate" mechanical ventilation may adversely affect the lungs (Greenfield et al., 1964; Kolobow et al., 1987; Carlton et al., 1990; Parker et al., 1990; Tsuno et al., 1990). Pulmonary inflammation induced by mechanical ventilation is the result of mechanical trauma and biotrauma (Pinhu et al., 2003). Regarding lung injury one distinguishes between mechanical trauma produced by atelectasis, high tidal volume or ventilation pressure, and pulmonary biotrauma induced by local or systemic inflammation caused by mediators released from the ventilated lung.

In rats, ventilator-induced lung injury and edema occurs when a certain degree of lung overinflation due to high pressures is reached (Dreyfuss and Saumon, 1993). But when PEEP was added by holding the end-inspiratory pressure constant, the development of edema is delayed and the severity of tissue injury diminished (Dreyfuss et al., 1988; Dreyfuss and Saumon, 1993).

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Aim of this study was to evaluate the effects of inhalation anaesthesia and mechanical ventilation with and without recruitment (RM) and PEEP-titration on alveolar integrity in horses.

2. Materials and methods

2.1. Animals

The study was approved by the Ethical Committee for Animal Experiments of Lower Saxony, Germany, number 33.14-42502-04-11/0572. Twenty-three systemically healthy experimental horses were used for this terminal study. Eighteen animals were randomized to three different groups, "open-lung-concept"-ventilation (OLC), intermittent positive pressure ventilation (IPPV) or non-ventilated (NV), prior to induction of anaesthesia. Horses of groups OLC, IPPV and SV were part of an experimental abdominal surgery trial and were euthanized for tissue collection and undergraduate student teaching in anatomy. Five horses were used as controls (C) and were not anaesthetized and not ventilated. These control horses were part of an experimental orthopaedic trial.

2.2. Anaesthesia

Premedication (0.8–1.1 mg kg^{-1} xylazine (Xylapan®, Vetoquinol GmbH, Ravensburg, Germany) IV) and induction of anaesthesia (0.05 mg kg^{-1} diazepam (DiazepamAbZ® 10 mg, AbZ Pharma GmbH, Blaubeuren, Germany) and 2.2 mg kg^{-1} ketamine (Narketan®, Vetoquinol GmbH, Ravensburg, Germany) IV) were identical in all groups. Anaesthesia was maintained with isoflurane (Isofluran® CP, CP-Pharma, Burgdorf, Germany) in oxygen balanced with a constant rate infusion (CRI) of xylazine of 0.5 mg kg^{-1} h^{-1}. The isoflurane concentration was adjusted to ensure adequate depth of anaesthesia. Intravenous lactated Ringers solution (Ringer-Laktat-Lösung®, B. Braun, Melsungen AG, Germany) was administered at a rate of 5 mL kg^{-1} h^{-1}. Dobutamine (Dobutamin-ratiopharm® 250 mg, ratiopharm GmbH, Ulm, Germany) was given to effect to maintain a mean arterial blood pressure above 60 mm Hg during anaesthesia.

2.3. Ventilation strategy

Following induction of anaesthesia and tracheal intubation, the horses were positioned in dorsal recumbency. Horses of group NV ($n = 6$) were allowed to breathe spontaneously. Horses of group IPPV ($n = 6$) were ventilated by intermittent positive pressure ventilation. In group OLC ($n = 6$) horses were ventilated by an "open-lung-concept". For ventilation a pressure limited and pressure cycled large animal ventilator (Vet.-Tec. Model JAVC 2000 J.D. Medical Distributing Company Phoenix, USA) equipped with a custom made PEEP-valve (Dräger Medical Deutschland GmbH, Lübeck, Germany) was used.

Mechanical ventilation in groups IPPV and OLC started with a positive inspiratory pressure (PIP) of 20 cm H_2O and a PEEP of 0 cm H_2O. In group OLC, after 40 min of IPPV, an incremental PEEP titration with steps of 5 cm H_2O every 10 min up to a PEEP of 20 cm H_2O was performed maintaining a constant airway pressure difference (ΔP) of 20 cm H_2O. The actual lung recruitment (RM) consisted of a stepwise increase in ΔP by 25, 30, 35 and 40 cm H_2O obtained by increasing peak inspiratory pressure (PIP) to 45, 50, 55 and 60 cm H_2O, while maintaining PEEP at 20 cm H_2O (Fig. S1a) followed by decremental PEEP titration until PIP and PEEP reached pressures of 20 cm H_2O and 0 cm H_2O, respectively. A second incremental and decremental PEEP-titration to a PEEP of 30 cm H_2O with constant ΔP but without RM was conducted (Fig. S1b).

2.4. Respiratory mechanics

Respiratory gas flows, volumes and pressures and dynamic thoracic compliance (C_{dyn}) were measured and calculated by a pitot type spirometric system. A horse-lite sensor, was located between the endotracheal tube and the Y-piece and was calibrated by three liter calibration syringe (Hans Rudolph Meena Medical, Bedford). In horses of group OLC arterial blood samples were taken and blood gases were measured every 10 min during recruitment.

2.5. Bronchoalveolar lavage

Bronchoalveolar lavages (BAL) were carried out 5 days before and at the end of anaesthesia. In horses of group C, BAL was performed before euthanasia. Bronchoalveolar lavage was performed with a fiberoptic endoscope (Olympus Optical CO Europe GmbH, Hamburg) which was passed through the nose or the endotracheal tube (groups OLC, IPPV and NV at end of anaesthesia) into the lung until it wedged in the most distal bronchus, which was usually the fourth or fifth generation subsegment of the caudal lobe. A lavage of dorsal and of ventral lung areas was performed. Bronchoalveolar lavage was performed with 500 mL of warm (37 °C) sterile physiologic saline. The fluid was instilled through a solution administration set plugged into a saline bottle on the proximal end into the channel of the BAL tube and pushed into the airway by pressure exerted using a pressure bulb. The solution was collected through a side port using gentle suction applied with a suction pump. Two representative samples of 200 and 500 μL were placed on an object slide, dried and stained using Pappenheim and examined using light microscopy (cell count: macrophages, lymphocytes, neutrophils, mast cells, eosinophils, erythrocytes, epithelial cells).

2.6. Histopathology

Following euthanasia a right thoracotomy was performed to collect tissue samples of dorsal (= dependent lung) and ventral (= non-dependent) lung areas.

Complete removal of the lungs was not possible because horses were used for studentical education (anatomic situs). For histopathologic examination by a board certified pathologist (BJ) (blinded to the protocol), lung tissue samples were fixed in 10% neutral buffered formalin, processed by routine methods, embedded in paraffin wax, cut into at 5 μm thick sections, and stained with hematoxylin and eosin, as well as with periodic acid-Schiff (PAS) reaction to confirm the integrity of the basal lamina and elastica-van Gieson stain to show eventually breaks within elastic fibers. For electron microscopy, lung tissues were fixed in 5% glutaraldehyde with 0.1 M cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon®. Ultrathin sections were stained with lead citrate and uranyl acetate. Incidence and amount of alveolar edema, interstitial edema, alveolar damage, hyaline membranes, alveolar hemorrhages, neutrophilic infiltration, alveolar overdistension, alveolar macrophages, congestion and atelectasis were scored with a score from 0 to 5 (Appendix) described by Dreyfuss (Dreyfuss and Saumon, 1998).

2.7. Real time PCR

Two biopsies were collected from dorsal and ventral lung areas and fixed in an aqueous, nontoxic tissue storage reagent that rapidly permeates tissues to stabilize and protect cellular RNA (RNA-later). The biopsies for PCR were stored at −80 °C until analysis. Relative quantification of equine cytokine mRNA expression was performed by quantitative real-time PCR (RT-PCR). Biopsies were pulverized under liquid nitrogen and RNA was extracted using trizol extraction followed by purification with the PureLink RNA Mini Kit (Ambion/Invitrogen) according manufacturers' instructions. RNA was reverse transcribed to cDNA using reverse transcription reagents from Applied Biosystems (Switzerland).