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Physiological and Pathological Evaluation of Mechanically Ventilated Anesthetized Pigs

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ABSTRACT

Prolonged assisted ventilation during anesthesia and critical care is associated with increased morbidity and mortality in both humans and animals. However, no study of mechanically ventilated patients has examined the mortality-related physiology and pathology. Our study investigated physiological, hematological and pathological changes in pigs that died during anesthesia. Six pigs were placed under ventilation-assisted anesthesia for up to 48h. The heart rate (HR), respiratory rate (RR), mean arterial blood (MAP), end-tidal carbon dioxide ($ETCO_2$), and oxygen saturation (SpO_2) were monitored every 15min until the pigs dies. Blood glucose, serum lactate, pH, and serum bicarbonate (HCO_3^-) were measured every 12h until death. Tissues were harvested from lung, liver, heart, and kidney of pigs immediately after death during anesthesia. All pigs died during anesthesia. Times of death varied. One pig died at approximately 18h (Group I), two pigs at 24h (Group II), one pig at 36h (Group III), and two pigs at 48h (Group IV). Decreased MAP was reported in all groups throughout anesthesia. Each group showed different changes before death; Group I and III showed decreased blood glucose (45mg/dL) and (17mg/dL), respectively; Group III showed elevated serum lactate (9.76 mmol/l) and reduced pH (7.13) that suggested metabolic acidosis; Group IV showed increased $ETCO_2$ (64.35±5.79 mmHg). Pigs that survived longer showed a higher level of cellular injury to the liver and respiratory system. Blood glucose, MAP, and $ETCO_2$ should be carefully monitored during anesthesia for better patient outcomes and reduced mortality.

Key words: Anesthesia, Long-Term, Ventilation, Porcine, Physiology, Pathology.

INTRODUCTION

The prolonged use of mechanically assisted ventilation in operating rooms or intensive care units poses a major concern due to the associated mortality (Chelluri et al. 2004; Fernandez-Zamora et al. 2018). In the 2000s, a study was made of cases of in-hospital mechanical ventilation lasting from 24h to 96h (Douglas et al. 2002). The study showed that 47.4% of the patients died in the hospital, 32.6% of discharged patients died within a year and that the mortality rate increased with time spent on the ventilator. Another study of mechanically ventilated patients reported a mortality rate 43.9% in the hospital but found that only 9% of discharged patients died within 6 months (Douglas et al. 1997). However, neither of the reports showed significant physiological or pathological data. Therefore, the pathogenesis of mortality during long-term anesthesia with mechanical ventilation is still unclear.

The cardiovascular system of the pig is similar to that of the human. A pig of approximately 30 to 40 kg has a heart of a similar size to the heart of a human child and the lung physiology of the pig also mimics that of humans (Hannon et al. 1990; Pehböck et al. 2015; Gabriel et al.

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2021). A study of mechanically ventilated pigs was made during anesthesia maintained for 96h with ketamine and pentobarbital (Goldmann et al. 1999). The study showed non-significant changes in blood cell profiles, blood chemistry and intestinal pathology. And yet, the physiology and pathology of mechanically ventilated human patients is still unclear despite the associated high mortality. We propose that the physiological, blood and pathological changes in pigs that died at the early experimental endpoint may reflect both mortality and co-mortality factors in humans.

MATERIALS AND METHODS

Animals

The investigation was approved by Chulalongkorn University Laboratory Animal Center (CULAC), Chulalongkorn University, Thailand No. 1973017. Six mixed breed male pigs weighing between 20 and 30 kg were divided into four groups according to the time of death during anesthesia: at 18h (Group I), 24h (Group II), 36h (Group III), and 48h (Group IV).

Anesthetic Procedures

Eight hours after food was withdrawn, the pigs were sedated with intramuscular injections of 0.5mg/kg of xylazine (X-LAZINE, L.B.S. Laboratory Ltd. Part., Thailand) and 3mg/kg of Tiletamine-zolazepam (Zoletil 100, Virbac Laboratories, France). Anesthesia was then induced in the sternal recumbent position using 4mg/kg of propofol (Pofol, Dongkook Pharm, Korea) administered intravenously, and maintained via inhalation of 1.5-2.0% isoflurane (Aerrane. Baxter, US). A volume control ventilator (AX-500/400, Shenzhen Comen medical, China) I:E ratio 1:3, tidal volume 10mL/kg, was used to support oxygenation with 100% oxygen. Throughout the study, acetate Ringer's solution was infused at 5-10 ml/kg/h via the cephalic vein. Vital parameters were assessed every 15 min including heart rate (HR, beats/min), respiratory rate (RR, breaths/min), oxygen saturation (SpO2, %), mean diastolic blood pressure (MAP, mmHg), end-tidal carbon dioxide (ETCO2, mmHg) and electrocardiography. Immediately the patients were anesthetized, blood samples were collected for biochemical analysis, and this was repeated every 12h.

Histopathological and Histochemical Studies

The target organs, including lungs, liver, heart, and kidneys were harvested from the pig that died during anesthesia. The pathologist was blind from the experimental setup. Tissue samples were resected and fixed for 24h in 10% buffered formalin, then processed following the routine histopathological protocol. Tissue sections were H&E-stained to evaluate degeneration, changes and infiltration patterns of inflammatory cells, i.e., mononuclear cells, and neutrophils. A light microscope was used (Olympus BX60 Olympus Corporation, Essex, UK). Ten high power fields (HPF) in each sample were investigated under the microscope and images were captured using Image-Pro Insight version 9.1 (Media Cybernetics, MA, US). Cellular grading was as follows: grade 0 (-) = 0% of histopathological changes, grade 1 (+) = 1-25% of histopathological changes, grade 2 (++) = 26-50% of histopathological changes, grade 3 (+++) = >50% of histopathological changes (Lvova et al. 2012; Mets et al. 2015; Suyapoh et al. 2021).

Data Analysis

The physiological data and blood chemistry data were presented as mean±SD (Tabe 1) and mean±SE (Fig. 1 and 2). The Prism 8.4.3 Graph pad (GraphPad Software, Inc., California, USA) was used to process the data. Histological images were investigated by a pathologist and compared between pigs that died at 18h (Group I), 24h (Group II), 36h (Group III), and 48h (Group IV) after anesthesia.

RESULTS

The approximate survival times were 18h for one pig (Group I), 24h for two pigs (Group II), 36h for one pig (Group III), and 48h for two pigs (Group IV). The physiology data and blood chemistry data were analyzed from the time of measurement (6h) to the time just before death (Table 1). The physiological data were analyzed with reference to normal ranges throughout the time of anesthesia, including RR, HR, MAP, $ETCO_2$, SpO_2. Blood chemistry analysis took into account blood glucose, serum bicarbonate (HCO_3^-), serum lactate, and pH.

Cardiorespiratory Effects

These parameters fluctuated in all groups between 6 and 48h after the induction of anesthesia. In Group I, Group II, and Group IV, HR increased to 118.6±11.56 beats/min before cardiac arrest. These results contrasted the HR of Group III which was 67.50±2.12 before cardiac arrest. MAP data were in line with HR data. The MAP of all pigs was between 41.50±13.44 and 60.23±9.33mmHg,

Table 1: Physiological and chemical profiles (mean±SD) show final data before death

Profile	Time of death (Final data)				Reference Values*
	Group I	Group II	Group III	Group IV	
Respiratory rate	12.56±2.25	22.47±3.26b	14.00	21.15±5.75b	10-15breaths/min
Heart rate	112.2±12.87b	116.2±23.93b	67.50±2.12a	118.6±11.56b	90-107beats/min
Mean arterial blood	42.81±2.90a	55.41±12.72a	41.50±13.44a	60.23±9.33a	86-123mmHg
End-tidal carbon dioxide ($ETCO_2$)	34.33±1.18a	36.28±4.14	42.50±3.54	64.35±5.79b	35-45mmHg
Oxygen saturation (SpO_2)	99.56±0.51	97.62±4.77	100.0	99.75±0.54	95-100%
Blood glucose	45a	105	17a	117.3±4.60	75-136mg/dL
Serum bicarbonate (HCO_3^-)	29.8	32.7±2.12	25.7a	37.1±3.25b	28-35mmol/L
Serum lactate	2.07b	1.07±0.43	9.76b	1.25±0.21	0.5-1.5mmol/L
pH	7.47	7.59±0.05b	7.13a	7.39±0.02	7.38-7.48

The values bearing "a" were lower while values with "b" were higher than the maximum reference values. *(Hannon et al. 1990; Cooper et al. 2014; Solevåg et al. 2014; Morgaz et al. 2015; Malavasi 2015).

which indicated hypotension, and MAP remained in the critical range to the end apart from in Group III, where MAP increased from 47.75±3.19mmHg to 98.69±49.52 mmHg after 24h of anesthesia and then dropped to 41.5±13.44mmHg before cardiac arrest (Fig. 1).

The pigs were ventilated using a ventilator set at 10 breaths/min, but they were able to breathe spontaneously at more than that rate. The RR of all the pigs was between 12.00±0.43 and 22.47±3.26 breaths/min from 6h to the time before respiratory arrest (Fig. 1). $ETCO_2$ is an indicator of carbon dioxide production and elimination out of the body. It is therefore influenced by ventilation. $ETCO_2$ increased to 64.35±5.79 mmHg in Group IV before respiratory arrest, whereas other groups were within the reference range from the beginning to the end. In addition, peripheral SpO_2 showed within the reference range (Fig. 1).

Blood Glucose and Blood Chemistry

Before death, blood glucose and blood chemistry changed suddenly in the first 12-36h of anesthesia (Fig. 2). In Group I and Group III, blood glucose levels, an indicator of body metabolism, decreased from the normal values of 121 and 70mg/dL, respectively at 6h before arrest to 45 and 17mg/dL. Serum lactate, an indicator of anaerobic respiration, was within the reference range from 6h to the time just before death in all pigs apart from Group I and III, in which serum lactate increased to 2.07 and 9.76 mmol/l, respectively. The pH of Group III decreased to 7.13, which suggests the onset of metabolic acidosis (Fig. 2).

Signs of Respiratory Pathologies

This study comprised a histopathological examination of tissue samples of lung, liver, heart, and kidneys. Lesions were semi-quantitatively evaluated to provide profiles of cellular degeneration and inflammatory cell infiltration. The major pathological changes presented in lung and liver tissues while changes in heart and kidney tissues remained within normal limits.

Generally, cellular degeneration was evaluated from observation of the peri-nucleolar space and cytoplasmic blebs (Fig. 3A). Peri-nucleolar space was observed mainly in the pneumocyte of the alveolar duct. It was identified by a clear or empty area within the pneumocyte cytoplasm (Fig. 3A, pink spots in color-inverted images). The grading level for pigs in Groups I, II and III was low (+), moderate (++), and moderate (++), respectively. Alveolar wall cytoplasmic blebs were characterized by outward bulging of the cell membrane of pneumocytes. In Groups II and III cytoplasmic bleb was graded moderate (++) and in Group I there was no lesion development (Fig. 3B). Pulmonary emphysema and enlargement of the airspaces were observed mainly in the peripheral area of the lung and occasionally around the bronchus. Atelectasis of the lung, partial collapse of the air sac, was noticed within the pulmonary parenchyma and air spaces. The grading level of these changes in Groups II, III and IV was moderate (++), but was low (+) in Group I (Fig. 3B). Congestion was identified by the engorgement of red blood cells in vascular areas. It was presented only in Group I, at the low grade (+) at short-term anesthesia (Fig. 3B). Inflammatory cell infiltration into the lung parenchyma was observed in all groups at the low grade (+) over all the period of anesthesia. Changes in bronchiolar tissue were assessed along with the treatment. There were no significant changes in the airways of pigs in Groups I, II and III (Fig. 3B).

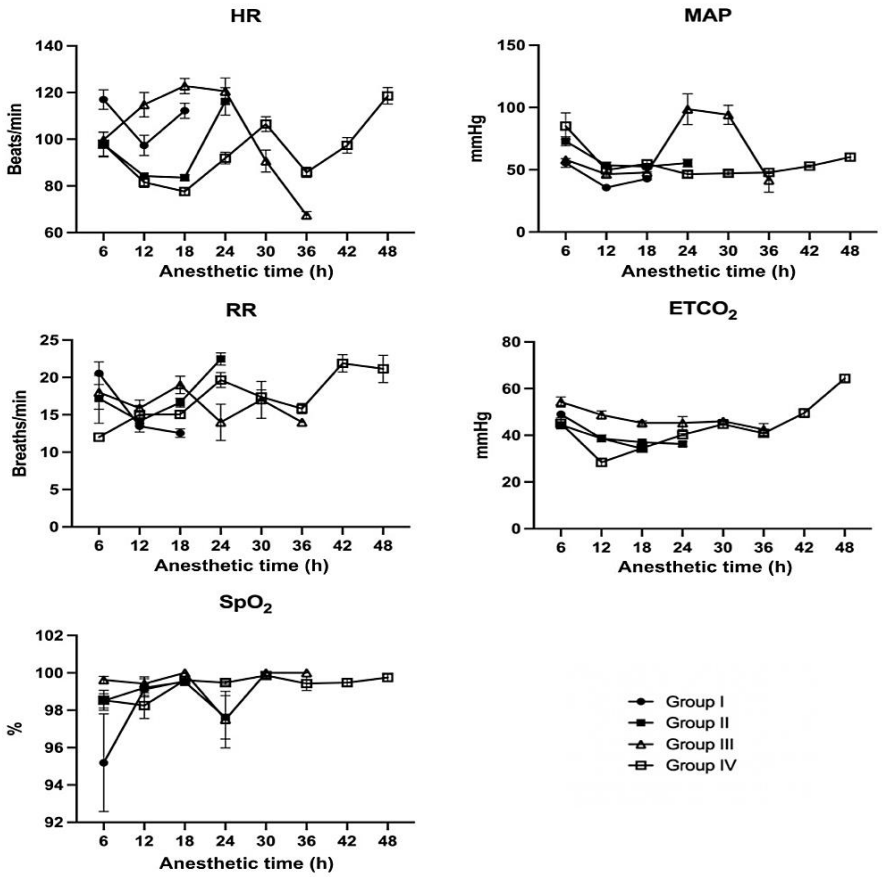


Fig. 1: Dynamic changes in cardiac and respiratory parameters (mean±SE) include heart rate (HR), mean arterial blood pressure (MAP), respiratory rate (RR), End-tidal carbon dioxide ($ETCO_2$), and oxygen saturation (SpO_2). MAP was within the critical reference throughout anesthesia, whereas $ETCO_2$ gradually increased to above the reference limit, especially in group IV.

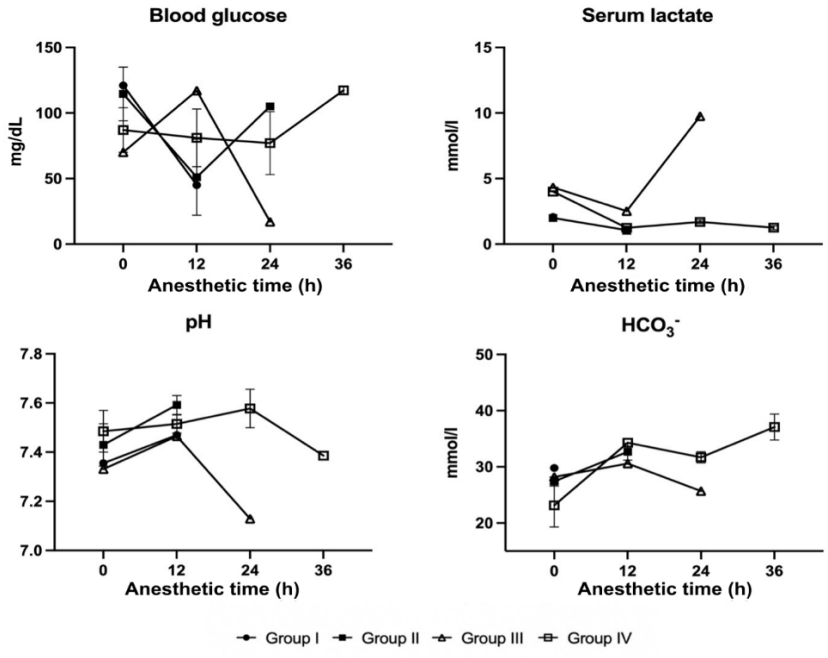
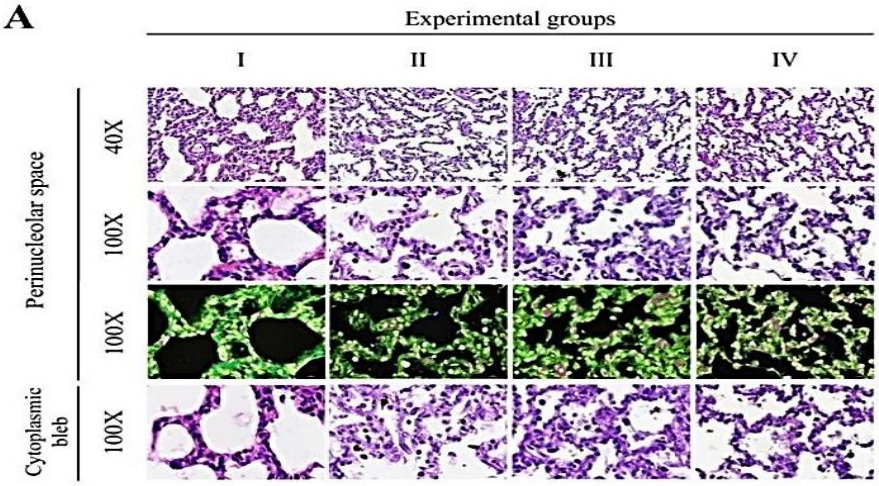


Fig. 2: Dynamic changes in metabolism and blood chemistry (mean±SE) include blood glucose, serum lactate, pH and serum bicarbonate. Pigs were given a general anesthetic until death at various hours. Low blood glucose was found in groups I and III, whereas increases in serum lactate were shown in group III.

A



B

Organ samples	Experimental groups			
	I	II	III	IV
Pulmonary parenchyma				
Degeneration				
• Peri-nucleolar space	+	++	++	+++
• Cytoplasmic bleb	-	++	++	+++
Emphysema	+	++	++	+++
Atelectasis	+	++	++	++
Congestion	+	-	-	-
Inflammatory cell infiltration	+	+	+	+
Airway				
Degeneration				
• Clara cell loss of bleb	-	-	-	++
• Epithelial bleb	-	-	-	++
• Sloughing of epithelium	-	-	-	++
Inflammatory cell infiltration	-	-	-	-

Fig. 3: Pigs were anesthetized and supported with mechanical ventilation. Observed pathological changes of the lung and airway included cellular degeneration, emphysema, atelectasis, congestion, and inflammatory cell infiltration. Images (A) show alveolar cell degeneration, including peri-nucleolar space and cytoplasmic bleb using cytochemistry (H&E) and inverted pictures of four groups: original magnifications = ×40 and ×100. Group I was a pig that died on 18h, Group II included two pigs that died on 24h, Group III was a pig that died on 36h, and Group IV included two pigs that died on 48h. In Group I, mild peri-nucleolar spaces without cytoplasmic bleb were observed. The increasing number of peri-nucleolar spaces (pink) and moderate number of cytoplasmic blebs were seen in Group II, III and IV. The highest number of peri-nucleolar spaces with cytoplasmic bleb was seen in the last section. The table (B) lists dynamic changes of each pathological lesion and group.

Overall, histopathological examination of Group IV showed a high grade (+++) of cytological and tissue changes. The degeneration of the lung was primarily detected in the airway at grade 2 (++) and extended to pulmonary parenchyma at grade 3 (+++). Bronchiolar cell degeneration, including Clara cell loss of bleb, epithelial bleb, and sloughing of the epithelium was observed (Fig. 3B). Semi-quantitatively, peri-nucleolar space and cytoplasmic bleb of the pneumocyte were significantly changed (Fig. 3A). These changes were seen with a low level of inflammatory cell infiltration, e.g., neutrophils and mononuclear cells, including macrophages, and

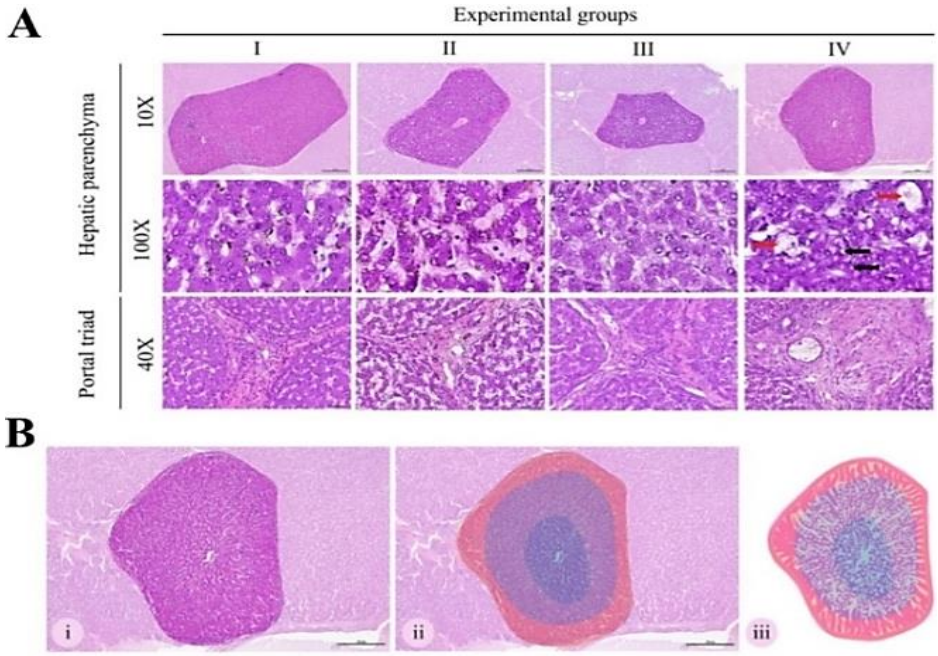
hemosiderinophages (hemosiderin-laden macrophages). Ten per cent of neutrophils accumulated along the peribronchiolar area and inside the bronchiolar lumen.

Signs of Liver Pathologies

Hepatobiliary injuries in response to short to moderate-term anesthesia, such as centrilobular-midzonal degeneration, cholestasis, congestion, sinusoidal dilatation, portal degeneration, and inflammation were evaluated (Fig. 4A-C). Centrilobular-midzonal degeneration was indicated by vacuolar ballooning areas within hepatocytes established in the zone of the hepatic lobule, including the intermediary zone (purple) and the perivenous zone (blue) (Fig. 4B). The change was first observed only in Group III at the moderate grade (++) (Fig. 4A). Semi-quantitatively, congestion and cholestasis displayed a similar trend, showing a low grade (+) in all terms of anesthesia (Fig. 4C). Sinusoidal dilatation from anesthesia in Groups I, II and III was low (+), low (+), and moderate (++), respectively. Inflammatory cell infiltration and portal system degeneration was not detected in these groups (Fig. 4C).

Zonal degeneration was significant in Group IV. Ballooning and vacuolar degeneration presented at moderate grade (++) and high grade (+++), respectively. The pattern and detail of centrilobular-midzonal degeneration are shown in Fig. 4Bi-iii. Dilatation of the hepatic sinusoid was observed at the high grade (+++), whereas congestion, cholestasis, inflammation, and portal injuries presented at the same grades as Group I, II and III (Fig. 4A, C).

These results suggest that prolonged assisted ventilation during anesthesia induced cellular injuries in the lungs and airway, causing hypoxia, resulting in severe centrilobular-midzonal degeneration of the hepatic lobule. shown that isoflurane directly inhibited insulin secretion



C

Organ samples	Experimental groups			
	I	II	III	IV
Hepatic parenchyma and vasculature				
Centrilobular-midzonal degeneration				
• Vacuolar	-	-	++	+++
• Ballooning	-	-	+	++
Cholestasis	+	+	+	+
Inflammatory cell infiltration	-	-	-	-
Congestion	+	+	+	+
Sinusoidal dilatation	+	+	++	+++
Portal system				
• Inflammatory cell infiltration	-	-	-	-
• Cholangiocyte degeneration	-	-	-	-

Fig. 4: Pigs were anesthetized and supported with mechanical ventilation. Observed pathological changes of the hepatic parenchyma and portal system included hepatocyte degeneration (vacuolation, and ballooning), congestion, cholestasis, sinusoidal dilatation, and inflammatory cell infiltration. Group I was a pig that died on 18h, Group II comprised two pigs that died on 24h, Group III was a pig that died on 36h, and Group IV comprised two pigs that died on 48h. Images (A) show hepatocyte degeneration using cytochemistry (H&E), moderate and high grades of vacuolation were observed in group III and IV, respectively. Unremarkable changes in the portal area were observed in all groups. Original magnifications = ×10, ×40, ×100. Zonal hepatocellular degeneration (B). Photomicrograph of affects hepatocytes within defined areas (centrilobular-midzonal zone) of the hepatic lobule. The degeneration pattern is characterized by the presence of vacuolar-ballooning degeneration of the hepatic parenchyma (Bi), Zonation of the liver, including periportal area (orange), midzonal area (purple), and centrilobular area (blue) (Bii), the illustration of centrilobular-midzonal degeneration show vacuolization of blue and purple area of liver (Biii). Original magnifications = ×10. The table (C) lists the dynamic changes of each pathological lesion and group.

DISCUSSION

Our study showed that a decrease in glucose occurred during the first 36h of anesthesia. Previous studies have from pancreatic beta cells (Desborough et al. 1993) and impaired glucose tolerance during anesthesia (Tanaka et al. 2011; Tanaka et al. 2005). Moreover, prolonged inhalation-maintained anesthesia may induce changes in splenic hemodynamics which affect hepatic blood flow (Bernard et al. 1992) and pancreatic perfusion, resulting in both impaired hepatic glucose uptake and insulin output (Kim et al. 2016).

Isoflurane inhalation induced hepatic injury (Gunza and Pashayan 1992) and increased liver apoptosis, in part by regulating the expression of IGF-1 (Zhu et al. 2017). Liver dysfunction leads to hypoproteinemia and hypoglycemia that can impair gluconeogenesis and eventually glycogen storage (Bednarski et al. 2011). Hepatobiliary pathologies such as hepatocyte degeneration, cholestasis and oedema were found in all pigs, but far more in the pig that died 48h after anesthesia. Prolonged mechanically assisted ventilation during anesthesia coincided with hepatocellular damage that was associated with local ischemia caused by hypoxia from lung damage or reduced hepatic blood flow due to hypotension, which presented in all the pigs in our study.

Glucose is the main metabolic fuel for the brain under normal physiological conditions (Mathew and Thoppil 2021). Reduced blood glucose is a significant indicator of metabolic failure before death. Hypoglycemia-induced bradycardia and sudden death (Nordin 2014; Reno et al. 2018) has been associated with ST wave change, lengthening of the QT interval (Robinson et al. 2003; D'Imperio et al. 2021), and cardiac repolarization (Koivikko et al. 2008; Andersen et al. 2021). Thus, we suggest that blood glucose must be monitored for all patients who undergo assisted ventilation, and hypoglycemia should be treated immediately with intravenous glucose.

The highest serum lactate level we recorded was 9.76mmol/l and the lowest pH was 7.13 at 12h before death. Mortality is strongly correlated with blood glucose and serum lactate levels. A study of critically ill patients (Chen et al. 2019) found that the mortality rate was approximately 23% when serum lactate was higher than 2.3mmol/l and approximately 10% when blood glucose was lower than 70mg/dL (Freire Jorge et al. 2017). During the first 24h after admission, the coincidence of low glucose and high serum lactate was associated with a high risk of acute kidney injury, liver dysfunction, and hospital mortality (Freire Jorge et al. 2017). The results of our study support these findings. The decrease in blood glucose level as a result of ischemia (Desouza et al. 2003; Paelestik et al. 2017) led to lactate production and accumulation, which induced tissue acidosis and cellular damage (Bakker et al. 1996; Rehni et al. 2018). Hyperlactatemia is a marker for tissue hypoperfusion (Régnier et al. 2012; Alegría et al. 2017) or hypoxia, which indicate the onset of the anaerobic glycolysis process (Garcia-Alvarez et al. 2014; Semler and Singer 2019). Venous blood gas analysis of 302 dogs (Kohen et al. 2018) showed that plasma lactate concentration levels between 3.3 and 7.7mmol/l coincided with a mortality rate of 45.4%. In a study of 185 cats (Kohen et al. 2018), serum lactate concentration between 3.5 and 8.7mmol/l was correlated with a mortality rate of 44.6%. Patients with blood lactate concentrations of more than 2.5mmol/l should be closely evaluated for signs of deterioration (Kohen et al. 2018).

In this study, MAP showed a decline from the beginning of anesthesia until death, perhaps due to hypoglycemia or prolonged isoflurane inhalation. Isoflurane can induce changes in heart rate and MAP that reduce both cardiac output (the product of heart rate and stroke volume) and total peripheral resistance (TPR) through a reduction of the sympathetic nervous system (Constantinides et al. 2011). The sympathetic nervous system initiates the mechanism of cardiovascular stabilization, which stimulates catecholamine release leading to positive inotropic cardiac function, increased heart rate (Schwertz et al. 2004; De Backer and Foulon 2019), increased oxygen demand, and increased blood glucose level (Barth et al. 2007; López Garcia de Lomana et al. 2022). The hemodynamic balance of physiological activity depends on tissue oxygen metabolism (Shen et al. 2021). If certain conditions that cause tissue and organ damage cannot be resolved, organ failure and death occur (McKinley et al. 2016).

The primary adverse effect of isoflurane is respiratory depression (Gargiulo et al. 2012; Cavalcante et al. 2018), followed by lung injury caused by systemic vasodilation and reversed pulmonary constriction (Putensen et al. 2002). Rats exposed to 1.5% isoflurane for 4h showed highly increased incidence of neurogenic pulmonary edema to 100%, due to the release of vascular endothelial growth factor in bronchial epithelium (Kandatsu et al. 2005). Strosing et al. (2016) reported that ventilated mice that inhaled isoflurane for 6h presented histologic features of lung injury. Dogs with injured lungs that received low concentrations of isoflurane (0.25, or 0.5 vol%) during mechanical ventilation showed decreased systemic blood flow and oxygen delivery (Putensen et al. 2002).

In the present study, longer durations of anesthesia resulted in an increase in lung injury. Emphysema, atelectasis, and inflammatory cell infiltration showed in all the pigs, but the pig that died at 48h showed a higher level of $ETCO_2$, which has been associated with inadequate ventilation or decreased CO_2 elimination during anesthesia (Peltekova et al. 2010; Solhpour et al. 2022), which led to respiratory failure in our study. A study of patients who showed postoperative pulmonary complications (PPCs) acquired during prolonged (≥24h) anesthesia with mechanical ventilation (Pedersen et al. 1992) found that the most frequent lesions on the PPCs were pathologies of atelectasis and pneumonia, which presented on 2.6% and 50% of the subjects, respectively. Correct mechanical ventilation relies on pressure and volume control. Continuous ventilation with a high tidal volume (>700 ml) and high positive end-expiratory pressure (PEEP) (>30 cmH_2O) was associated with acute respiratory distress syndrome (ARD), which developed at 48h after exposure (Gajic et al. 2005). Moreover, longer isoflurane-maintained anesthesia induced more alveolar macrophage aggregation, but less phagocytic activity (Kotani et al. 1998). Long term exposure to isoflurane is also related to airway inflammation (Oshima et al; 2021

Odeh et al. 2022). IL-8 is one of the key chemokines involved in the migration of epithelial granulocytes, which promote pulmonary inflammation and asthma (Cromwell et al. 1992). We found more epithelial pathology lesions of the airway in the pig that died 48h after anesthesia was induced. Longer exposure of epithelial cilia to isoflurane resulted in less frequent ciliary beating, as shown by a rat tracheal epithelial model (Matsuura et al. 2006).

Conclusion

MAP was below the reference range in all pigs before death. Severe hypoglycemia was found in the pigs that died between 18 and 36h after anesthesia was induced. Therefore, blood glucose might be a good predictor of early endpoint death. Only the pig that died at 48h showed a high $ETCO_2$. All the pigs showed pulmonary degeneration and emphysema lesions, but a longer duration of anesthesia resulted in more pathological changes in lungs, airway, and signs of life. Therefore, blood glucose, MAP, and $ETCO_2$ were the essential parameters that we conclude should be closely monitored during anesthesia. The first system to fail during assisted ventilation under anesthesia might be the glucose metabolism and the second, the respiratory system.

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Author Contributions

SL, SPV, KD, and JS contributed to the concept planning. JS, SL, SD, CW, NA, and VL collected data. JS, KD, and WS performed the analysis of data and interpretation. JS, KD, and WS prepared the initial draft of the manuscript. All authors approved the final submission.

Competing Interests

The authors declare that they have no competing interest.

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