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A stable model of respiratory distress by small injections of oleic acid in pigs

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Abstract *Objective:* Development of a stable model of respiratory distress in pigs with oleic acid, fulfilling clinical criteria of the adult respiratory distress syndrome (ARDS).
Design: Eight pigs (9.1 ± 0.7 kg) were anesthetized with pentobarbital, paralyzed with tubocurarine and mechanically ventilated with an F_{IO_2} of 0.6, an I:E ratio of 2:3 and a PEEP of 0.2 kPa. Oleic acid (dissolved 1:1 in 96% alcohol) was administered in a series of multiple injections of 0.1 ml until P_{aO_2} was lower than 8 kPa.
Measurements and results: Careful titration of the oleic acid injections on guidance of the P_{aO_2} established a reproducible respiratory distress (P_{aO_2} = 7.3 ± 0.8 kPa), in which gas exchange and hemodynamic variables were stable for at least 4 h. The number of oleic acid injections (22 ± 11, mean and SD) varied between the animals.
Conclusions: With the use of multiple injections of oleic acid, a stable model of early respiratory distress in pigs can be achieved, in spite of individual differences in sensitivity. Such a stable model allows for a diversity of studies on early respiratory distress.

Key words Oleic acid · Lung injury · Respiratory distress · Albumine · Bolus injections

Introduction

Oleic acid has often been used to induce an experimental model of respiratory distress in animals [1–8]. In these studies oleic acid was given either as a single bolus or as a continuous infusion, and the amount administered differed between studies. Mortality was up to 30% [3, 7]. In most studies, respiratory distress criteria according to Petty [9] and Murray [10] were not fulfilled and interventions were studied without a proper description of the model.

We aimed at a regimen of multiple injections of oleic acid to induce an early stage of respiratory distress in pigs that fulfilled the clinical criteria of the adult respiratory distress syndrome (ARDS) [9, 10]. Furthermore, gas exchange and hemodynamic variables had to be stable for several hours. Such a model of experimental respiratory distress can be used for studies on basic mechanisms and therapeutic interventions.

Methods

Surgical procedures and ventilatory conditions

Eight Yorkshire pigs (9.1 ± 0.7 kg) were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg·kg^{-1}) and

placed in a supine position on a thermo-controlled operation table to maintain body temperature. Anesthesia was maintained by a continuous infusion of pentobarbital sodium ($8.5\ mg \cdot kg^{-1} \cdot h^{-1}$) via an ear vein. After tracheostomy the pigs were connected to a volume controlled ventilator.

A single-lumen polythene catheter was inserted through the right common carotid artery into the aortic arch for measuring arterial blood pressure (P_{ao}) and sampling of blood. Three catheters were inserted via the right external jugular vein: (1) a 5F Swan-Ganz catheter was inserted into the left pulmonary artery to monitor pulmonary arterial pressure (P_{pa}) and pulmonary blood temperature and to sample mixed venous blood; (2) a double-walled catheter was inserted into the right atrium for injection of room temperature saline during the thermodilution procedures; (3) a four-lumen catheter was inserted into the superior vena cava to measure central venous pressure (CVP) and to infuse fluids and anesthetics. All catheters for measuring blood pressure were continuously flushed at a flow rate of $3\ ml \cdot h^{-1}$ with normal saline containing a low dose of heparine ($10\ IU \cdot ml^{-1}$ infusion fluid) to avoid clotting in the catheters. A catheter was also placed in the urinary bladder to avoid urine retention.

Tubocurarine was given at a rate of $0.2\ mg^{-1} \cdot kg^{-1} \cdot h^{-1}$ after the surgical procedure to suppress spontaneous breathing. Mechanical ventilation and wash-in and wash-out procedures to determine lung volume were performed with a computer-controlled two-bellow ventilator [11]. Ventilation was set at 10 breaths per min and tidal volume (V_T) adjusted to a P_{aCO_2} of 5.0–5.6 kPa. The inspiratory to expiratory ratio was 2:3, the inspiratory fraction of oxygen (F_{IO_2}) was 0.6 and the positive end-expiratory pressure (PEEP) was 0.2 kPa. These settings were kept constant throughout the experiments.

Measured and estimated data

Gas exchange, acid-base indices and hemoglobin

Oxygen and carbon dioxide tensions and acid-base indexes of blood were determined with an automatic blood gas analyzer (Radiometer ABL3). Hemoglobin (Hb) concentration, arterial (S_{aO_2}) and mixed venous O_2 saturation were determined with an oxymeter (Radiometer OSM2). S_{aO_2} in blood after dextran infusion was corrected according to the manual of the OSM2. Respiratory gases, including helium (He), were analyzed by a mass spectrometer (MGA 1100, Perkin-Elmer).

Arterial oxygen content (C_{aO_2}), venous admixture ($\dot{Q}_s$) in percentage of total pulmonary blood flow ($\dot{Q}_t$) and physiological dead space (V_D) in percentage of V_T were calculated according to standard equations [12–14]. The parameters of the oxygen saturation curve were adapted to pig blood [15, 16]; we also used pig blood data from our laboratory.

Lung volume and compliance

End-expiratory lung volume (V_{EE}) was estimated with use of an open He (4–5%) wash-in and wash-out technique [17]. The compliance of lungs and thorax (C_{rs}) was estimated with use of an inspiratory pause method [18]. Airway pressure (P_T) was measured in the tracheal cannula. Inspiratory volumes of 6, 12 and $18\ ml \cdot kg^{-1}$ were inflated at intervals of 2 min during normal mechanical ventilation. Each of these three inflations was followed by an inspiratory pause of 3 s. The gradual volume decrease during the inspiratory pause (ΔV_{Hg}) was recorded with the use of a mercury cord fixed around the thorax at 5 cm cranial from the sternal xyphoid. The mercury cord was calibrated with the three inflation volumes superimposed on the V_{EE}. Lung volume ($V_{EE} + V_T - \Delta V_{Hg}$), and airway pressure at the end of the inspiratory pause served as the data for the compliance estimation. A third-degree polynomial pressure-volume curve was fitted through these data and through end-expiratory volume and pressure. This P–V curve revealed an approximately linear part between the volumes 4 and $8\ ml \cdot kg^{-1}$ above V_{EE}. The slope of this part ($\Delta V/\Delta P$) was used as the estimate of C_{rs}. Throughout the experiments changes in thoracic volume were monitored with the mercury cord. Estimates of C_{rs} before and after the oleic acid injections were compared at the same thoracic volume level. Assuming a constant chest wall compliance [6], the changes in C_{rs} indicated changes in lung compliance.

Hemodynamic data and oxygen delivery

P_{ao}, P_{pa} and CVP were measured continuously with use of Baxter disposable fluid-filled pressure transducers, type Uniflow. Pressure values were referred to ambient air pressure and to a zero-level at the height of the manubrium and averaged over a ventilatory cycle. Transducers were calibrated by application of pressure to this reference zero-level under guidance of a mercury manometer.

Cardiac output ($\dot{Q}_t$) was determined by the thermodilution technique. The average of four determinations equally spread over the ventilatory cycle was used to estimate mean cardiac output [19].

Oxygen delivery (D_{O_2}) was calculated according to $D_{O_2} = C_{aO_2} \times \dot{Q}_t$.

Data acquisition

Throughout the experiments all blood pressures, ECG, P_T, air flow ($\dot{V}$), and the resistance of the mercury cord were continuously recorded on a Gould recorder type RS 3800. During the estimations of $\dot{Q}_t$ all other hemodynamic signals were sampled at a frequency of 250 Hz. The sampling period was 18 s, i.e., 3 ventilatory cycles. P_T, $\dot{V}$ and the mercury cord signal were sampled for 9 s at 100 Hz for the estimations of C_{rs}. During the open wash-in and wash-out procedures the in- and expiratory gases were sampled at a frequency of 50 Hz. All sampled signals were stored on computer discs for off-line analyses.

Experimental procedures and observations

Protocol of the experiment

After the surgical procedures, a stabilization period of at least 30 min followed. Baseline observations were then taken over a period of 1 h. $\dot{Q}_t$ and gas exchange variables were determined at the beginning and at the end of this hour. V_{EE}, C_{rs} and chest X-rays were obtained once.

After the baseline measurements, $10\ ml \cdot kg^{-1}$ isotonic dextran-40 solution was given over a period of 30 min via the Swan-Ganz or the double-walled injection catheter. Immediately after the dextran infusion, the circulatory and gas exchange variables were measured again. To control the solvent of the oleic acid, 16 injections of 0.1 ml of 96% alcohol-saline solution (1:1) were given at 90-s intervals via the Swan-Ganz catheter. Immediately after these injections, the measurements were repeated.

After the control observations with alcohol, commercial oleic acid (Unichema International, specific gravity 0.89), dissolved 1:1 in 96% alcohol, was administered through the Swan-Ganz catheter into the right atrium. Injections of 0.1 ml oleic acid took 1 s and were given at intervals of 90 s until a P_{aO_2} below 8 kPa was achieved.

Occasionally, we lengthened this interval and decreased the amount of oleic acid to 0.05 ml when a preceding injection caused a significant effect on the circulation. After each injection the catheter was flushed with 1 ml saline (40 °C). In pilot experiments we also applied larger amounts of oleic acid (0.2–1 ml), which immediately caused cardiovascular shock and death. Furthermore, P_{aO_2} changed only slightly until the 10th injection. Therefore, in this study P_{aO_2} was measured after the first 10 injections, every two or four injections and thereafter at the end of each single injection.

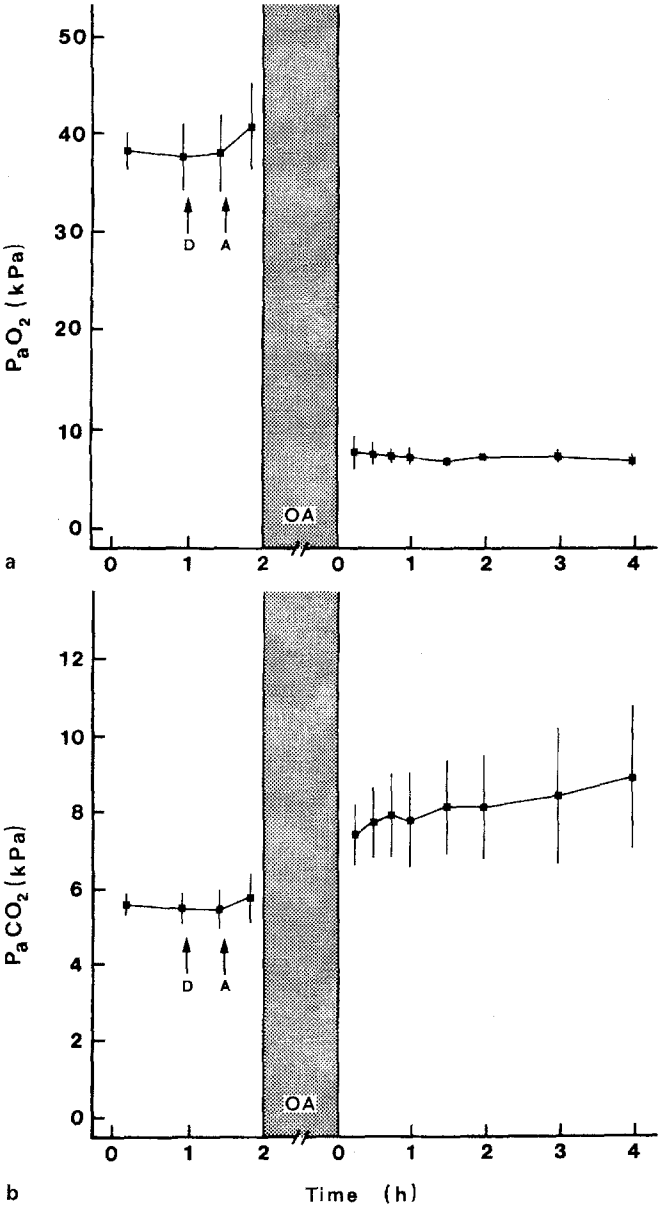


Fig. 1a, b Effects of oleic acid on gas tensions. Baseline observations were made in the first hour (*D* start of dextran infusion (30 min), *A* start of alcohol-saline injections (25 min), *OA* oleic acid injection). The interrupted X-axis indicates the time difference in oleic acid administration for each animal. *Zero time* corresponds to the time of the last oleic acid injection. Mean values of six animals; at $t = 4$ h, $n = 5$. *Vertical bars* SD. **a** Arterial oxygen tension; **b** arterial carbon dioxide tension

Zero-time of the respiratory-distress observation period was set at the moment of the last oleic acid injection. All measurements of gas exchange and cardiac output were performed at 15, 30, 45, 60, 90, and 120 min and every hour thereafter. All continuously monitored signals were also sampled at these time intervals. Estimations of V_{EE} and C_{rs} were calculated 90, 120 and 180 min after the last injection of oleic acid. In some experiments estimations of V_{EE}, $\dot{Q}_s/\dot{Q}_t$, V_D/V_T and C_{rs} failed because of the formation of bloody froth in the tracheal cannula or because of technical problems with the mass spectrometer. At the end of the experiments chest X-rays were obtained again.

Lung weight and morphology

At the end of the experiments, the animals were killed with pentobarbital sodium (0.07 $g \cdot kg^{-1}$), and the lungs were immediately fixed. The results of these morphological studies have been published elsewhere [20].

Criteria of respiratory distress

A criterion of respiratory distress, mentioned by Petty [9], is a P_{aO_2} of 6.7 kPa with an F_{IO_2} of 0.6 at zero PEEP. This P_{aO_2} value was used as a target during the oleic acid injections and during the period after these injections. Murray [10] suggested a scoring system to characterize the severity of the disease based on a chest roentgenogram, hypoxemia (P_{aO_2}/F_{IO_2}), positive end-expiratory pressure and C_{rs}. To adapt the Murray C_{rs} scoring system to our pigs, we recalculated the values per Kilogram of body weight, assuming an average weight of 70 kg in Murray's patients. This scoring system was used to evaluate our model.

Statistical analysis

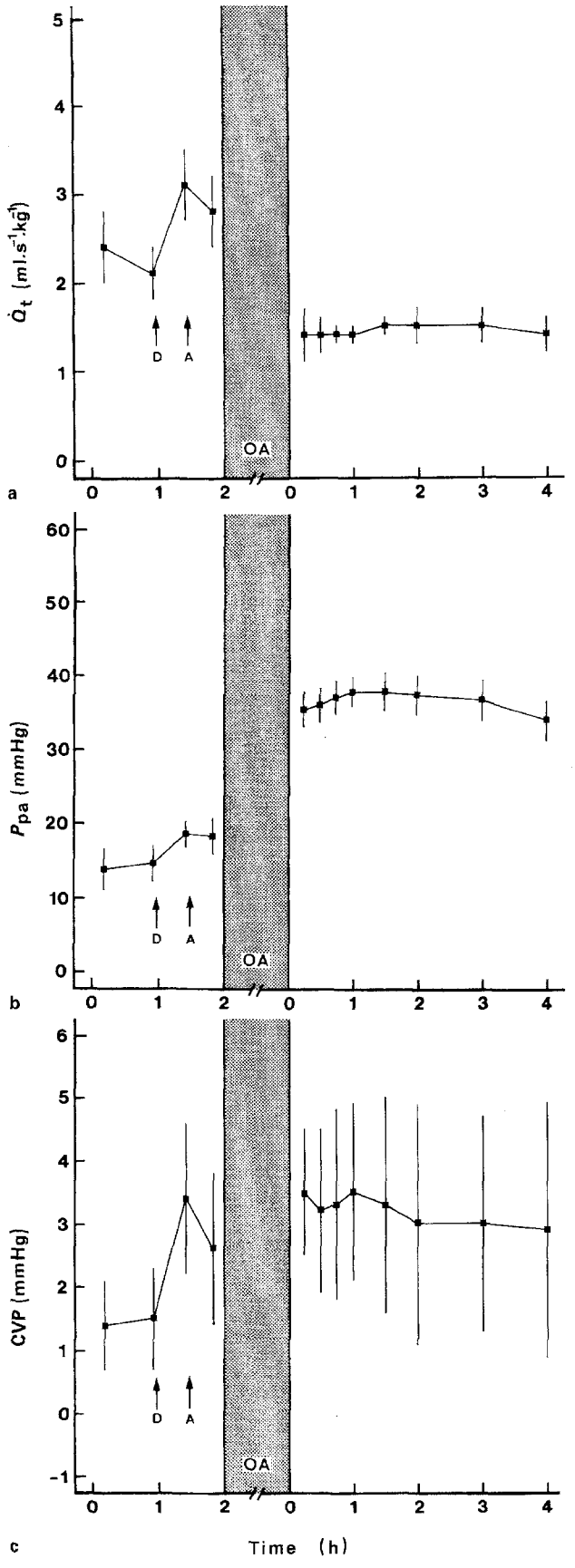
The results of the first 4 h after oleic acid administration (Figs. 1, 2) were analyzed using standard repeated measures analysis of variance (SPSS-Manova). Student *t*-tests (statgraphics) for paired samples were used in Table 1 and to compare C_{rs} and V_{EE} before and after oleic acid application. We regarded differences with a *P*-value ≤ 0.05 as statistically significant. Data are presented as mean values $\pm$ 1 SD.

Results

Control observations

Dextran infusion and alcohol-saline injections

The effects of dextran infusion are presented in Table 1. The main effects are a decrease in Hb concentration and an increase in $\dot{Q}_t$, P_{ao}, P_{pa}, CVP and oxygen delivery. No changes occurred after the single injections of the alcohol-saline solution. After the whole series of injections Hb concentration was increased slightly and $\dot{Q}_t$ and CVP were decreased (Table 1). All other variables remained constant.



Oleic acid injections

The administration of 0.1 ml of oleic acid injections had extensive hemodynamic effects, as demonstrated in an individual example (Fig. 3). These effects varied in severity between injections within a single animal as well as between animals. The injections caused an immediate rise of P_{pa} and CVP, and a decrease in P_{ao} and arterial pulse pressure. When severe circulatory reactions were observed, resulting in a high CVP and a low P_{ao}, the interval between the injections was lengthened until these variables were partly recovered and stable again. When an injection caused a mean $P_{ao} < 40$ mmHg, a critical level below which the coronary flow is dependent on pressure [21], we diminished the next injection by 50% (0.05 ml). One of the eight animals died after the 12th injection. The oleic acid injections established a stable hypoxemia in six of the seven remaining animals. The P_{aO_2} of the seventh animal recovered partly within 30 min after the last injection. This animal was eliminated from the study. The number (22 ± 11, mean $\pm$ SD), the time (75 ± 38 min) and the total dose (0.12 ± 0.07 ml·kg^{-1}) of oleic acid injections indicate a large spread in individual sensitivity with regard to causing a respiratory distress of similar severity.

Characteristics of the oleic acid model

The effects of oleic acid on gas exchange and hemodynamic variables 1 h after the last injection are presented in Table 1. During the distress period two animals died due to circulatory shock, one after 3.5 h and the other after 4 h. The standard bicarbonate (HCO_3^-) concentrations were 22 and 18 mmol·l^{-1}, respectively in those animals. One experiment ended after 5 h because of technical reasons. The remaining three animals were studied for 6 h.

We restricted the presentation of averaged data and the statistical testing ($n = 5$, SPSS Manova) to a period of 4 h after the last oleic acid injection. For $\dot{Q}_s/\dot{Q}_t$ and V_D/V_T statistical testing was limiting to the first 2 h because of missing values after this period.

Gas exchange, acid-base indices and hemoglobin

P_{aO_2} decreased profoundly during the series of oleic acid injections (Table 1) and remained stable during the

Fig. 2a–c Hemodynamic variables. Abbreviations, time scale, vertical bars and observation numbers as in Fig 1. **a** Cardiac output; **b** pulmonary arterial pressure; **c** central venous pressure

Table 1 Control data and data after oleic acid administration. Values are mean ± SD; (N) at 45 min after oleic acid and $n = 5$ due to technical reasons (P_{aO_2} arterial P_{O_2}, P_{aCO_2} arterial P_{CO_2}, S_{aO_2}, arterial oxygen saturation, $\dot{Q}_s/\dot{Q}_t$ venous admixture, V_D/V_T physiological dead space, *Hb* hemoglobic concentration, $\dot{Q}_t$ cardiac output, P_{ao} arterial pressure, P_{pa} pulmonary arterial pressure, CVP central venous pressure, D_{O_2} oxygen delivery, $P_{T,p}$ peak tracheal pressure, *pH* arterial pH, HCO_3^- standard, bicarbonate concentration)

	Baseline $n = 6$	Dextran $n = 6$	Alcohol/NaCl $n = 6$	Oleic acid 60 min $n = 6$
P_{aO_2} (kPa)	38.8 ± 3.6	39.1 ± 4.1	42.0 ± 4.5	7.3 ± 0.8*[5]
P_{aCO_2} (kPa)	5.5 ± 0.4	5.4 ± 0.5	5.7 ± 0.6	7.8 ± 1.2*[5]
S_{aO_2} (%)	99.2 ± 1.1	99.2 ± 1.6	98.9 ± 1.6	72.0 ± 7.5*[5]
$\dot{Q}_s/\dot{Q}_t$ (%)	7.2 ± 2.0	11.2 ± 2.*[2]	–	42.8 ± 6.0 (N)*[4]
V_D/V_T (%)	33.8 ± 10.8	28.0 ± 8.2	–	58.5 ± 3.1 (N)*[4]
Hb (mmol · l^{-1})	6.5 ± 0.5	5.0 ± 0.4*[2]	5.3 ± 0.5*[2,4]	7.6 ± 1.2*[5]
$\dot{Q}_t$ (ml · s^{-1} · kg^{-1})	2.1 ± 0.3	3.1 ± 0.4*[2]	2.8 ± 0.4*[2,3]	1.4 ± 0.3*[5]
P_{ao} (mmHg)	99.7 ± 10.9	112 ± 14.7*[1]	112 ± 13.1*[1]	97.8 ± 13.9
P_{pa} (mmHg)	14.7 ± 2.4	18.7 ± 1.7*[2]	18.2 ± 2.4*[2]	37.6 ± 2.0*[5]
CVP (mmHg)	1.5 ± 0.8	3.4 ± 1.2*[2]	2.6 ± 1.2*[1,4]	3.5 ± 1.4*[5]
D_{O_2} ml · s^{-1} · kg^{-1})	32.7 ± 4.4	37.5 ± 6.7*[1]	35.3 ± 5.8	17.9 ± 1.2*[5]
$P_{T,p}$ (kPa)	2.1 ± 0.5	2.1 ± 0.5	2.2 ± 0.6	3.6 ± 0.3*[5]
pH	7.46 ± 0.04	7.47 ± 0.04	7.45 ± 0.04	7.32 ± 0.06*[5]
HCO_3^- (mmol · l^{-1})	28.8 ± 1.7	29.1 ± 1.8	28.7 ± 1.5	25.6 ± 2.1*[5]

*[1] $P \leqslant 0.05$, *[2] $P < 0.01$ compared to baseline, *[3] $P \leqslant 0.05$, *[4] $P < 0.01$ compared to dextran, *[5] $P < 0.01$ compared to alcohol-NaCl

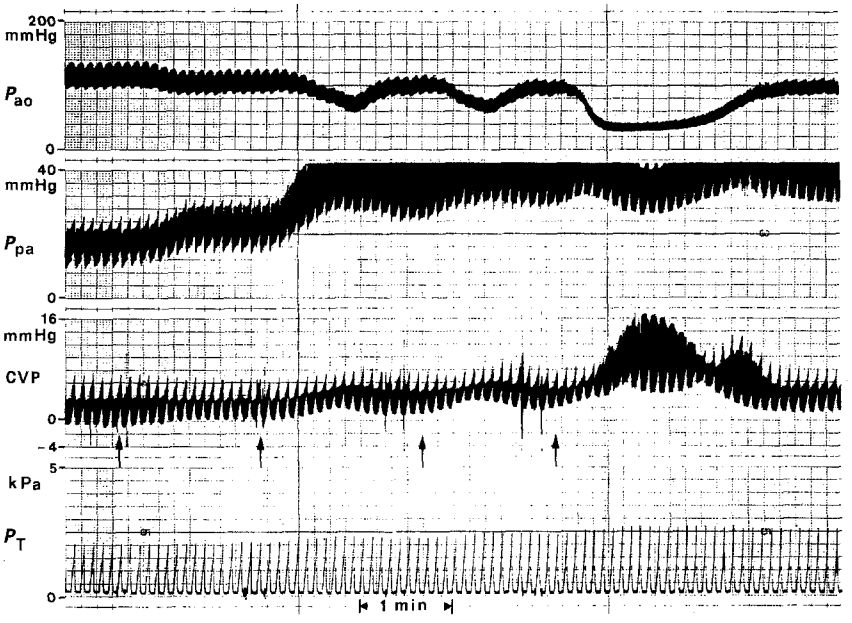


Fig. 3 Hemodynamic effects of oleic acid injection. Four out of eight signals are shown (P_{ao} arterial pressure, P_{pa} pulmonary arterial pressure, CVP central venous pressure, P_T tracheal pressure). *Each arrow* indicates an injection of 0.1 ml oleic acid. The time interval after the second injection was lengthened because of severe hemodynamic reactions

distress period (Fig. 1a). After the 4-h period, the individual P_{aO_2} values were similar. The decreased S_{aO_2}, the increased $\dot{Q}_s/\dot{Q}_t$, the increased P_{aCO_2} (Fig. 1b), the decreased pH and HCO_3^- concentration, and the doubled value of V_D/V_T after the oleic acid administration did not change significantly during the distress period.

Hb concentration, which was reduced after volume expansion with dextran, increased during the period of oleic acid injections to a value above its baseline level (Table 1). In the distress period Hb concentration remained the same.

Lung volume and compliance

V_{EE} was decreased from 21.0 ± 2.6 ml · kg^{-1} ($n = 6$) in baseline to 10.9 ± 2.9 ml · kg^{-1} ($P < 0.05$, $n = 4$) at 90 min after the last oleic acid injection. Estimations at 120 min ($n = 3$) and 180 min ($n = 3$) were similar to the values at 90 min after oleic acid administration.

C_{rs} was decreased from 18.3 ± 3.2 ml · kPa^{-1} · kg^{-1} in baseline to 8.7 ± 2.8 ml · kPa^{-1} · kg^{-1} ($P < 0.05$, $n = 5$) at 90 min after the last oleic acid injection. Individual values of C_{rs} in three animals at 120 min and 180 min

were similar to their values at 90 min. Tracheal pressure at peak inflation ($P_{T,p}$) was increased after oleic acid administration and rose gradually during the 4-h distress period to 4.0 ± 0.7 kPa ($P = 0.02$).

Hemodynamic data and oxygen delivery

Oleic acid administration decreased cardiac output ($\dot{Q}_t$) significantly to a value below baseline in spite of the dextran administration (Table 1, Fig. 2a). The oleic acid injections did not change P_{ao}, but increased P_{pa} and CVP (Table 1, Fig. 2b, c) and reduced D_{O_2} to about 50%. All of these variables remained stable throughout the distress period.

Criteria of respiratory distress

The stable P_{aO_2} below 8 kPa in our model approximated the physiological criterion of P_{aO_2} for ARDS ($P_{aO_2} < 6.7$ kPa, $F_{IO_2} \geq 0.6$, PEEP = 0 kPa), as defined by Petty [9]. Evaluation of our oleic acid model with the scoring system described by Murray [10], revealed a moderate-to-severe respiratory distress (averaged score of all animals 2.4 ± 0.1). The chest X-rays showed alveolar edema in all lung lobes of the six animals (value = 4), the hypoxemia score (P_{aO_2}/F_{IO_2}) was below 100 in all animals (value = 4), and PEEP was below 0.5 kPa (value = 0). C_{rs} was between 5.8 and 8.6 $ml \cdot kPa^{-1} \cdot kg^{-1}$ in three animals (value = 2) and between 8.6 and 11.5 $ml \cdot kPa^{-1} \cdot kg^{-1}$ (value = 1) in the other three animals.

Discussion

Dextran infusion and alcohol-saline injections

Volume expansion has been applied by several authors, but we could not find a standardized regime. The volume expansion was performed either before [22], or during and after [23], or only after the administration of oleic acid [24]. To evaluate the effects of oleic acid, we avoided interventions in parallel with oleic acid administration and therefore infused dextran prior to the injections of oleic acid. The volume expansion increased cardiac output to 150% of its baseline value.

The series of alcohol-saline injections did not affect gas exchange or other pulmonary functions. We regard the slight increase in Hb and the decrease in $\dot{Q}_t$ and CVP to be an effect of counteracting neuro-humoral control mechanisms on the preceeding volume expansion [25]. The total amount of injected alcohol was on average 0.2 $ml \cdot kg^{-1}$, with a maximal amount in one experiment of 0.3 $ml \cdot kg^{-1}$. For a human adult this would be about 21 ml alcohol, corresponding to the consumption of one-and-a-half glasses of wine. Undoubtedly, alcohol was metabolized during application of the series of injections, leading to a lower dose than mentioned. In mechanically ventilated dogs 48 ml alcohol applied in 1 h did not affect gas exchange or hemodynamic variables at an F_{IO_2} of 0.3 [26]. If we assume a body weight of 24 kg per dog, this amount is 2 ml/kg, which is a much larger dose than our maximal dose of 0.3 ml/kg. Alcohol has to be given in a dose five times larger than the maximal dose in this study to potentiate the anesthetic action of pentobarbital [27]. We consider the amount of alcohol applied to our animals to be too small for such an effect. Based on the previously mentioned literature and the fact that the application of dextran and alcohol-saline did not affect gas exchange, we assume the oleic acid is the sole causative agent for the respiratory distress in our pigs.

The regime of oleic acid administration

The toxic effects of oleic acid can be neutralized by albumine [28]. Presumably, fatty acids have only a toxic effect on the alveolar-capillary membrane, leading to disturbances in gas exchange if the concentration of the free fatty acids exceeds the binding capacity of albumine in the blood. Individual variations in this binding capacity might be an explanation for the wide range of injections (12–42), necessary to obtain a similar respiratory distress. Another reason for this range could be individual variations in the distribution of oleic acid to the lungs. However, in a former pathological study we found a homogeneous distribution of the lesions in all lung lobes [20], suggesting a homogeneous distribution of oleic acid. Individual differences in the formation of oleic acid micelles in the blood or in the degree of protein binding were not investigated.

The large difference in individual sensitivity to oleic acid makes a single bolus injection or a continuous infusion less suitable to establish a reproducible model in different animals. An excessive amount of oleic acid could lead to severe pulmonary edema, circulatory shock and death of the animal. Some authors reported a mortality of 25–30% during application of oleic acid [3, 7]. In another study in pigs in which oleic acid was given as a continuous infusion in 30 min [8], a dose similar to the one we applied caused an unacceptably high mortality. Kruse-Elliot and Olson [4] used a continuous infusion of oleic acid (20 $ml \cdot kg^{-1} \cdot h^{-1}$) in pigs throughout the entire experiment. They reported a hypoxemia after 1 h, i.e., P_{aO_2} was 6.7 kPa with

ventilation at room air, which remained constant for a period of 4 h. However, in another group of pigs they found a mortality of 100% within 2.5–3 h during such an infusion. According to our data, it is not necessary to continue an infusion of oleic acid once respiratory distress is established. The advantage of our regimen of multiple small injections is that a relatively high dose ($0.12\,ml \cdot kg^{-1}$) can be applied to induce respiratory distress, while maintaining a low mortality (one out of eight in our experiments).

We recommend observing stability for at least 30 min, because in one animal P_{aO_2} started to recover in less than 30 min after the last oleic acid injection. When recovery of P_{aO_2} occurs, oleic acid administration can be continued before studying some intervention.

The distress model

Mechanisms of injury

The oleic acid induced lung injury is probably primarily caused by a direct toxic effect on the endothelial wall [29, 30]. A mechanism might be the potency of oleic acid to inhibit the activity of the Ca-pump and the $(Na^+ + K^+)$-ATPase [31, 32], leading to dysfunction of the cell membrane. Electronic microscopical investigation in a previous study [20] revealed signs of degeneration of the endothelium, like swelling and vacuolization. Mediators like leukotrienes, phospholipase A or cyclooxygenase metabolites and the increased number of leukocytes [20, 33] and aggregated thrombocytes [34] seen in the lungs after oleic acid may contribute to the pathogenesis of this type of lung injury [3, 4, 29].

Gas exchange and acid base data

In our animals a moderate-to-severe respiratory distress, according to the Murray criteria [10], was induced. P_{aO_2} decreased to a level close to the physiological criteria of ARDS described by Petty [9] and remained stable until the end of the experiments. $\dot{Q}_s$ was increased after administration of oleic acid, indicating the presence of low ventilation-perfusion ratios [23]. The increase P_{aCO_2} indicates that the effective alveolar ventilation was decreased after oleic acid induced lung injury; we found a doubling of V_D in our animals. The decrease in pH immediately after introduction of oleic acid was due to the increase in P_{aCO_2} and the decrease in HCO_3^- concentration. In the two animals that died after 3.5 and 4 h, the sudden decrease in P_{ao} coincided with low values of HCO_3^- concentration (22 and 18 $mmol \cdot l^{-1}$, respectively). We assume that oxygen delivery would have been insufficient at that time, leading to metabolic acidosis.

Pulmonary data

Edema was undoubtedly the main reason for the decrease in V_{EE}. Edema was evidenced by the increased weight of the lungs and heart; by the infiltrates seen on the chest X-rays and by the morphologic examination [20]. Impaired surfactant function, leading to an increase of surface tension in the alveoli causing atelectasis [35], contributed to the decrease in V_{EE}.

We compared C_{rs} before and after oleic acid administration at the same thoracic volume, implying a similar effect of thoracic recoil forces. Therefore, the decreased C_{rs} after oleic acid administration indicated an increased stiffness of the lungs, which we attribute to pulmonary edema and impaired surfactant function. Hall et al. [35] also found an altered static P–V curve after administration of oleic acid in rabbits.

Hemodynamic variables and heart function

The successive single injections of 0.1 ml oleic acid caused acute rises in P_{pa} (Fig. 3), which in turn probably caused the rise in CVP. The pulmonary hypertension was mainly caused by vasoconstriction of the pulmonary muscular arteries [20]. We attribute the fall in P_{ao}, immediately after an oleic acid injection, to a decrease in $\dot{Q}_t$ caused by the increased CVP [36]. P_{ao} always recovered within a few minutes, which is in the time domain of the neuro-humoral control mechanisms [37].

The decrease in $\dot{Q}_t$ after oleic acid administration was also observed by other authors [4, 22]. It has been suggested [22] that a decrease in venous return as a result of a decrease in plasma volume is the main mechanism for the reduction of $\dot{Q}_t$ during oleic acid pulmonary edema. We consider the increase in P_{pa} to be an additionally important mechanism in the increase in CVP and in the resulting reduction of $\dot{Q}_t$. A moderate rise in P_{pa} does not necessarily lead to a decrease in $\dot{Q}_t$ [38]. However, in our experiments the rise in P_{pa} was extensive. An additional mechanism that might have contributed to the rise in CVP after oleic acid administration in our pigs could be a decreased myocardial contractility [39].

Oxygen delivery

DO_2 was reduced after oleic acid administration as a result of the decrease in $\dot{Q}_t$ and CaO_2. In two animals

severe metabolic acidosis developed after 3.5–4 h, indicating an insufficient supply of oxygen to the tissues. The decrease in D_{O_2} after oleic acid is probably close to a critical value below which maintenance of life is impossible. If this is true, the development of models with a more severe distress will be nearly impossible.

Conclusions

A stable model of respiratory distress can be induced in pigs by injecting a series of multiple small injections of oleic acid. Careful titration of the number of injections to decrease P_{aO_2} in each animal leads to a reproducible model of early respiratory distress. Such titration adapts the amount of oleic acid to the individual differences in sensitivity. Our experimental model fulfilled the criteria of respiratory distress and was characterized by (1) a stable P_{aO_2} of about 7 kPa, (2) severe pulmonary hypertension, (3) decreased $\dot{Q}_t$ resulting in a decreased DO_2, (4) decreased V_{EE} and C_{rs}, (5) radiographic features of the respiratory distress syndrome, and (6) a stable period of at least 4 h, allowing studies on basic mechanisms and therapeutic interventions.

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References

1. Ashbaugh DG, Uzawa T (1968) Respiratory and hemodynamic changes after injection of free fatty acids. J Surg Res 8: 417–423
2. Blomqvist H, Berg B, Frostell C, Wickerts CJ, Hedenstierna G (1990) Net fluid leakage (L_N) in experimental pulmonary oedema in the dog. Acta Anaesthesiol Scand 34: 377–383
3. Julien M, Hoeffel JM, Flick MR (1986) Oleic acid lung injury in sheep. J Appl Physiol 60: 433–440
4. Kruse-Elliott K, Olson NC (1990) Role of leukotrienes during oleic acid-induced lung injury in pigs. J Appl Physiol 68: 1360–1367
5. Leeman M, Lejeune P, Closset J, Vachiéry J-L, Mélot C, Naeije R (1990) Nature of pulmonary hypertension in canine oleic acid pulmonary edema. J Appl Physiol 69: 293–298
6. Slutsky AS, Scharf SM, Brown R, Ingram RH (1980) The effect of oleic acid-induced pulmonary edema on pulmonary and chest wall mechanics in dogs. Am Rev Respir Dis 121: 91–96
7. Zetterström H, Jakobson S, Janeras L (1981) Influence of oncotic pressure on lung water accumulation and gas exchange after experimental lung injury in the pig. Acta Anaesthesiol Scand 25: 117–124
8. Sum-ping ST, Symreng T, Jebson P, Kamal GD (1991) Stable and reproducible porcine model of acute lung injury induced by oleic acid. Crit Care Med 19: 405–408
9. Petty TL (1982) Adult respiratory distress syndrome: definition and historical perspective. Clin Chest Med 3: 3–7
10. Murray JF, Matthay MA, Luce JM, Flick MR (1988) An expanded definition of the adult respiratory distress syndrome. Am Rev Respir Dis 138: 720–723
11. Jansen JRC, Hoorn E, Goudoever J van, Versprille A (1989) A computerized respiratory system including test functions of lung and circulation. J Appl Physiol 67: 1687–1691
12. Berggren SM (1942) The oxygen deficit of arterial blood by non-ventilating parts of the lung. Acta Physiol Scand. [Suppl] 11: 7–89
13. Anthonisen NR, Fleetham JA (1987) Ventilation: total, alveolar, and dead space. In: Fishman AP, Farhi LE, Tenny SM, Geiger SR, (eds) Handbook of Physiology, Sect 3. The respiratory system, vol IV. Gas exchange. American Physiological Society, Bathesde, pp 113–129
14. Enghoff H (1938) Volumen Inefficax. Bemerkungen zur Frage des schaedlichen Raumes. Uppsala Lakaref Foerh 44: 191–218
15. Bartels H, Harms H (1959) Sauerstoff-dissoziationskurven des Blutes von Säugetieren. Pflügers Arch 268: 334–365
16. Kelman GR (1966) Digital computer subroutine for the conversion of oxygen tension into saturation. J Appl Physiol. 21: 1375–1376
17. Huygen PEM, Gültuna I, Ince C, Zwart A, Bogaard JM, Feenstra BWA, Bruining HA (1993) A sensitive ventilation inhomogeneity index from multiple breath indicator gas wash-out tests, applied in mechanically ventilated patients. Crit Care Med 21: 1149–1158
18. Bone RC (1976) Diagnosis of causes for acute respiratory distress by pressure-volume curves. Chest 70: 740–746
19. Jansen JRC, Versprille A (1986) Improvement of cardiac output estimation by the thermodilution method during mechanical ventilation. Intensive Care Med 12: 71–79
20. Grotjohan HP, Van der Heijde RMJL, Wagenvoort CA, Wagenvoort N, Versprille A (1993) Pulmonary vasoconstriction in oleic acid induced lung injury. A morphometric study. Int J Exp Pathol 74: 347–355
21. Berne RM, Rubio R (1979) Coronary circulation. In: Berne RM, Sperelakis N, Geiger SR (eds) Handbook of physiology, Sect 2, The cardiovascular system, vol I. The heart. American Physiological Society, Bethesda, pp 875–951
22. Henning RJ, Heyman V, Alcover I, Romeo S (1986) Cardio-pulmonary effects of oleic acid-induced pulmonary edema and mechanical ventilation. Anesth Analg 65: 925–932
23. Halden E, Hedstrand U, Torsner K (1982) Oleic acid lung damage in pigs. Acta Anaesthesiol Scand 26: 121–125
24. Gemer M, Dunegan LJ, Lehr JL, Bruner JD, Stetz CM, Don HF, Hayes JA, Drinker PA (1975) Pulmonary insufficiency induced by oleic acid in the sheep. A model for investigation of extracorporeal oxygenation. J Thorac Cardiovasc Surg 69: 793–799
25. Guyton AC, Taylor AE, Granger HJ (1975) Circulatorory physiology. II. Dynamics and control of body fluids. Saunders, Philadelphia, pp 316–329

26. Doekel RC, Weir EK, Looga R, Grover RF, Reeves JT (1978) Potentiation of hypoxic pulmonary vasoconstriction by ethyl alcohol in dogs. J Appl Physiol 76–80
27. Ramsey H, Haag HB (1946) The synergism between barbiturates and ethyl alcohol. J Pharmacol Exp Ther 88: 313–322
28. Davis BD, Dubos RJ (1915) The binding of fatty acids by serum albumin, a protective growth factor in bacteriological media. Proc Soc Exp Biol Med 13: 215–228
29. Hofman WF, Ehrhart IC (1984) Permeability edema in dog lung depleted of blood components. J Appl Physiol 57: 147–153
30. Jefferson NC, Necheles H (1948) Oleic acid toxicity and fat embolism. Proc Soc Exp Biol Med 68: 248–250
31. Lamers JMJ, Stinis HT, Montfoort A, Hülsmann WC (1984) The effect of lipid intermediates on Ca^{2+} and Na^{+} permeability and $(Na^{+} + K^{+})$-ATPase of cardiac sarcolemma, a possible role in myocardial ischemia. Biochim Biophys Acta 774: 127–137
32. Pine RW, Vincenzi FF, Carrico CJ (1983) Apparent inhibition of the plasma membrane Ca^{2+} pump by oleic acid. J Trauma 23: 366–371
33. Derks CM, Jacobovitz-Derks D (1977) Embolic pneumopathy induced by oleic acid. A systematic morphologic study. Am J Pathol 87: 143–158
34. Spragg RG, Abraham JL, Loomis WH (1982) Pulmonary platelet deposition accompanying acute oleic acid induced pulmonary injury. Am Rev Respir Dis 126: 553–557
35. Hall SB, Notter RH, Smith RJ, Hyde RW (1990) Altered function of pulmonary surfactant in fatty acid lung injury. J Appl Physiol 69: 1143–1149
36. Guyton AC, Lindsey AW, Abernathy B, Richardson T (1957) Venous return at various right atrial pressure and the normal venous return curve. Am J Physiol 189: 609–615
37. Kirchheim HR (1976) Systemic arterial baroreceptor reflexes. Physiol Rev 56: 100–176
38. Liebmann PR, Patten MT, Manny J, Shepro D, Hechtmann HB (1978) The mechanism of depressed cardiac output on positive end-expiratory pressure (PEEP). Surgery 83: 594–598
39. Schuster DP, Perez JE, Trulock EP, Williamson JR, Biello DR, Kenzora JL, Amundsen T, Lange LG (1986) Cardiac dysfunction during acute lung injury induced by oleic acid in dogs. Am Rev Respir Dis 133: 519–525