Iodixanol inhibits exogenous surfactant therapy in rats with acute respiratory distress syndrome

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Iodixanol inhibits exogenous surfactant therapy in rats with acute respiratory distress syndrome. J. Kesecioglu, M.J. Schultz, J.J. Haitsma, G.J. den Heeten, B. Lachmann. ©ERS Journals Ltd 2002.
ABSTRACT: Optimal alveolar distribution of exogenous surfactant is an important determinant of its beneficial effect. This distribution can be determined by suspending surfactant in a radiological contrast medium before intratracheal instillation, followed by radiological imaging. Iodixanol is reported to be a safe contrast medium that causes no lung injury when instilled intratracheally.

In this study, the effects of surfactant suspended in saline were compared with surfactant suspended either in 4:1 saline-iodixanol (64 mg iodine·mL^{-1}) or in 1:1 saline-iodixanol (160 mg iodine·mL^{-1}), on oxygenation and lung mechanics in a rat model of adult respiratory distress syndrome (ARDS) induced by lung lavage.

After the induction of ARDS, surfactant instillation improved oxygenation, total lung volume at inflation with a distending pressure of 35 cmH$_2$O, lung volume at transpulmonary pressure of 5 cmH$_2$O and Gruenwald index. The effects of surfactant suspended in 4:1 saline-iodixanol were similar to those of surfactant alone. However, instillation of surfactant suspended in 1:1 saline-iodixanol resulted in significantly lower values in all measured parameters. Surface tension was the lowest in surfactant suspended in saline alone and addition of iodixanol led to an increase in surface tension in a dose-dependent manner.

In conclusion, iodixanol at the higher dose caused an inhibition of the exogenous surfactant effect, characterized as a lack of improvement in oxygen tension in arterial blood, low total lung compliance, volume at 5 cmH$_2$O end-expiration and Gruenwald index. This effect of iodixanol was probably due to its high surface tension, especially if a high concentration was used. Surfactant suspended in a lower concentration of iodixanol seems a better alternative, allowing for radiological imaging of the distribution of surfactant when intratracheally instilled.
Eur Respir J 2002; 19: 820–826.

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Neonatal respiratory distress syndrome is characterized by immaturity of the lungs, resulting in relative absence of pulmonary surfactant [1]. In neonates with this syndrome, administration of exogenous surfactant intratracheally is the primary life-saving treatment [2]. In acute respiratory distress syndrome (ARDS), acute respiratory failure from altered ventilation/perfusion ratios, leading to hypoxaemia, hypo- or hypercapnia and decreased lung compliance [3], is directly correlated with the disturbance of the pulmonary surfactant system [4–8]. Recent studies on intratracheal administration of exogenous surfactant in patients with ARDS have yielded controversial results. Although a multicentre study showed that there were no benefits to surfactant administration [9], others have demonstrated an improvement in oxygenation [10–14] and some randomized studies with small numbers of patients showed a trend towards improved mortality [15].

The beneficial effects of surfactant therapy are dependent on factors such as the dose and method of intratracheal administration. These factors determine the extent of distribution of surfactant in the alveoli. While the dose of surfactant to be given is usually known [15], there is no data currently available on the distribution of surfactant in the bronchoalveolar system, except as *post mortem* analysis in lung tissue [16]. A possible method to determine this distribution *in vivo* is by combining the surfactant with a contrast medium before intratracheal administration, followed by radiological imaging. It is important that the contrast medium used does not interfere with surfactant activity by increasing the surface tension at the alveolar level, as this may further impair optimal distribution of exogenous surfactant.

There are contradictory reports on the seriousness of adverse effects when commonly used contrast media are aspirated in the lungs [17–22]. However, it has been shown that nonionic and isotonic agents are well tolerated and cause fewer pulmonary complications than ionic and hyperosmolar agents [17–19, 23, 24]. LARSEN *et al.* [25] reported that single intratracheal instillation of the nonionic, iso-osmolar, dimeric contrast medium iodixanol was unlikely to cause any

significant tissue damage or lead to respiratory complications in healthy rat lungs. However, there is no data on the effect of contrast media when instilled or accidentally ingested into the ARDS lung.

The authors hypothesized that iodixanol could be a suitable contrast medium to combine with surfactant to allow radiological imaging of the distribution of surfactant. It is known that different agents can alter the *in vivo* and *in vitro* properties of exogenous surfactant [26]. Therefore, the aim of the study was to find an iodixanol concentration to combine with surfactant, which would not alter the exogenous surfactant properties, as measured by gas exchange and lung mechanics *in vivo* and by surface tension *in vitro*, in a surfactant-deficient animal model.

Materials and methods

Exogenous surfactant

The surfactant (HL 10; Leo Pharmaceutical Products, Copenhagen, Denmark) used in these experiments was a freeze-dried natural surfactant isolated from minced pig lungs. It consisted of ~90–95% phospholipids and 1% hydrophobic proteins (surfactant protein (SP)-B and SP-C), the remainder being other lipids such as cholesterol, glyceride and free fatty acids. SP-A (the largest surfactant-associated protein, with a molecular weight of 26–38 kDa) was absent in this surfactant preparation. On the day of the experiments, fresh surfactant suspensions were prepared by diluting the freeze-dried surfactant in sterile saline or iodixanol mixtures.

Iodixanol mixtures

Iodixanol (Nycomed, Oslo, Norway) is a nonionic, iso-osmolar, dimeric, water-soluble contrast medium. All concentrations of iodixanol are isotonic with blood and have the same osmolarity as blood. On the day of the experiments, sterile saline was mixed 1:1 and 4:1 with iodixanol. The stock solution of iodixanol contained 320 mg iodine$\cdot$mL^{-1}.

Animal study

This study was approved by the Animal Care and Use Committee of the Erasmus University Rotterdam.

The study was performed in 30 male adult Sprague-Dawley rats (body weight 240–350 g). After induction of anaesthesia with nitrous oxide, oxygen and isoflurane (65, 33, 2% respectively) a tracheotomy was performed and a catheter was inserted into the carotid artery. Anaesthesia was maintained with sodium pentobarbital (60 mg$\cdot$Kg$^{-1}\cdot$h^{-1} *i.p.*) and muscle relaxation was attained with pancuronium bromide (0.5 mg$\cdot$Kg$^{-1}\cdot$h^{-1} *i.m.*). The lungs were ventilated with a Servo Ventilator 300 (Siemens-Elema, Solna, Sweden) at the following ventilator settings: pressure-controlled ventilation, inspiratory oxygen fraction (F_I,O$_2$) 1.0, ventilation frequency

30$\cdot$min^{-1}, peak airway pressure 14 cmH$_2$O, positive end-expiratory pressure (PEEP) 2 cmH$_2$O and inspiratory:expiratory ratio 1:2.

After reaching steady state (oxygen tension in arterial blood (P_a,O$_2$) >500 mmHg), the rats were randomly divided into five groups, each consisting of six rats. Sham rats (Group I) were ventilated for 120 min with the ventilatory settings mentioned earlier. In Groups II–V, ARDS was induced by lung lavage, as described by LACHMANN *et al.* [4]. In brief, lungs were lavaged six to seven times with warm saline (37°C, 30 mL$\cdot$kg^{-1}) to produce a P_a,O$_2$ <80 mmHg at a peak inspiration pressure of 26 cmH$_2$O and PEEP of 6 cmH$_2$O. The ventilator settings were unchanged throughout the entire observation period. Approximately 5 min after P_a,O$_2$ <80 mmHg, Group II (control) received no treatment, Group III received surfactant suspended in sterile saline, Group IV received surfactant suspended in a 4:1 saline-iodixanol mixture (iodixanol concentration 64 mg iodine$\cdot$mL^{-1}) and Group V received surfactant suspended in a 1:1 saline-iodixanol mixture (iodixanol concentration 160 mg iodine$\cdot$mL^{-1}). Rats in Groups III–V were treated with a surfactant dose of 150 mg$\cdot$Kg^{-1}. The surfactant preparation used in all groups was suspended in saline or saline-iodixanol mixture at a concentration of 40 mg$\cdot$mL^{-1}. This resulted in a total volume of ~3.7–4 mL$\cdot$kg bodyweight^{-1}. Mixtures used in all rats were incubated together for 30 min at 37°C.

Blood samples for measurement of P_a,O$_2$ and carbon dioxide tension in arterial blood (P_a,CO$_2$) were taken from the carotid artery at steady state and at 15, 30, 60, 90 and 120 min in Group I rats. In Groups II–V, these measurements were performed before lung lavage and 5 min after the last lavage (directly followed by treatment) and at 15, 30, 60, 90 and 120 min post-treatment. At the end of the experiment, animals were killed with an overdose of intra-arterial administered sodium pentobarbital. Subsequently, pressure/volume curves were recorded in all groups using the syringe technique (see later), followed by bronchoalveolar lavage (BAL) for determination of total-protein concentration.

Pressure/volume curves

The static pressure/volume curves were recorded with the syringe technique. After the thorax and diaphragm were opened, the tracheostomy catheter was connected to a pressure transducer with a syringe attached to it (Validyne model DP 45–32; Validyne Engineering, Northridge, CA, USA) and pressures were recorded on a polygraph (Grass model 7B; Grass Instrument, Quincy, MA, USA). The lungs were first ventilated with nitrogen for 3–5 min. Subsequently, using a syringe filled with nitrogen, the lungs were first inflated (within 10 s) to an airway pressure of 35 cmH$_2$O which was maintained for 5 s, and then deflated to an airway pressure of 0 cmH$_2$O. The lungs were then reinflated in steps of 0.5 mL until an airway pressure of 35 cmH$_2$O was reached. Each inflation step took 1–2 s followed by a 5-s pause to allow
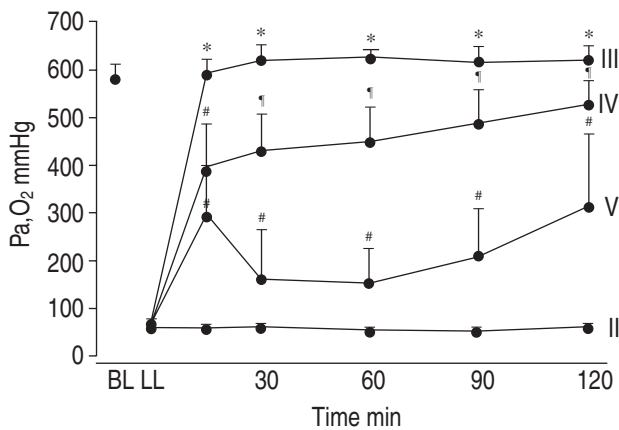
Fig. 1.–Changes in mean arterial oxygen tensions (P_{a,O_2}) of the study groups before lung lavage (BL), after lung lavage (LL) and 15, 30, 60, 90 and 120 min thereafter. Data are presented as mean±SD. Group II: control; III: treated with surfactant; IV: treated with surfactant suspended in 4:1 saline-iodixanol; V: treated with surfactant suspended in 1:1 saline-iodixanol. *: p<0.05 versus Groups II, IV and V; #: p<0.05 versus Group; ¶: p<0.05 versus Groups II and V.

pressure equilibration. The lungs were then deflated in the same way until an airway pressure of 0 cmH$_2$O was reached. The volume left in the syringe was recorded. Total lung capacity was defined as lung volume at inflation with a distending pressure of 35 cmH$_2$O (TLC35).

The Gruenwald index, which characterizes the surfactant system *in situ*, was calculated from the pressure/volume curve, defined as:

$$((2 \times V5) + V10)/(2 \times V_{max}) \qquad (1)$$

where $V5$, $V10$ and V_{max} are the lung volumes at transpulmonary pressures of 5, 10 and 35 cmH$_2$O from the deflation limb, respectively [27].

Bronchoalveolar lavage fluid

The BAL samples of all rats were prepared as follows. All BAL samples were centrifuged for 5 min at 2,000×*g* to remove cell material. Protein concentration was determined with a photospectometer (Beckman DU 7400; Fullerton, CA, USA) at 595 nm using the method of BRADFORD [28] (Bio-Rad, Munich, Germany) with bovine serum albumin protein assay (Sigma, St Louis, MO, USA) as standard.

Surface tension

In Groups III–V the surface activity of surfactant alone and the two surfactant-iodixanol mixtures with different concentrations was measured with the Wilhelmy balance (E. Biegler GmbH, Mauerbach, Austria) by applying incremental doses of 10, 20 and 50 µL of the mixtures to the surface of a saline-filled trough [29]. Surface area was compressed and expanded with a cycling time of 3 min·cycle^{-1} maximum and minimum surface area of 64 and 12.8 cm^2, respectively (100% and 20%). Maximal and minimal surface tensions were measured after three cycles at 100% and 20% surface area, respectively, and are expressed as milli-Newton (N)·m^{-1}.

Statistical analysis

Comparisons were analysed with repeated measures analysis of variance (ANOVA). If a difference was found, a *post hoc* test was performed (Tukey-Kramer). Statistical significance was set at p<0.05. All data are expressed as mean±SD.

Results

Arterial blood gas values in Groups I–V

In sham rats (Group I), P_{a,O_2} values obtained at steady state and 15, 30, 60, 90 and 120 min thereafter were 657±19, 603±2, 580±15, 616±9, 605±16 and 609±8 mmHg, respectively. After induction of ARDS, P_{a,O_2} values in control rats (Group II) remained low during the entire study, whereas administration of surfactant in Group III caused a rapid and complete improvement in P_{a,O_2} values. A slower improvement in P_{a,O_2} values in rats treated with the surfactant-iodixanol mixtures was seen in Groups IV and V (fig. 1). From 15 min on, P_{a,O_2} values obtained in Group III were significantly higher than those in the control rats (Group II) and rats that received surfactant-iodixanol mixtures (Groups IV and V) (p<0.05). From 30 min on, P_{a,O_2} values in rats in Group IV were significantly higher than those in Group V (p<0.05).

P_{a,CO_2} values were significantly higher in Group II from the time of lung lavage onwards compared to all other groups (p<0.05) (table 1). P_{a,CO_2} values were

Table 1. – Data on oxygen tension in arterial blood of all study groups

Group	BL	LL	15 min	30 min	60 min	90 min	120 min
I	23±3		24±3*	28±1*	30±2*	28±3*	29±3*
II	31±6	70±24#	77±27#	76±31#	83±36#	84±36#	70±20#
III	38±15	47±7¶	36±10¶	31±5¶	33±6¶	29±5¶	30±6¶
IV	47±9	63±8	51±5	49±6	50±4	44±4	44±7
V	37±9	53±13	41±10	46±13	50±12	49±12	48±13

Data is presented as mean±SD. BL: before lavage; LL: repeated lavage with saline to induce lung injury; Group I: sham; Group II: control; Group III: treated with surfactant; Group IV: treated with surfactant suspended in 4:1 saline-iodixanol; Group V: treated with surfactant suspended in 1:1 saline-iodixanol. *: p<0.05 versus Group II, IV and V; #: p<0.05 versus Group I, III, IV and IV; ¶: p<0.05 versus Group IV and V.

Table 2. – Data from all study groups, showing lung volume at inflation with a distending pressure of 35 cm H_2O (TLC35), Gruenwald index and total protein concentration (TPC)

Study Group	I	II	III	IV	V
TLC35 mL·kg^{-1}	49.9±1.8	35.3±0.8*	52.0±4.6[#]	51.2±4.6[#]	42.4±4.0*,[#],[¶]
Gruenwald index	1.04±0.08	0.41±0.01*	0.83±0.08*,[#]	0.82±0.07*,[#]	0.61±0.09*,[#],[¶]
TPC mg·mL^{-1}	0.25±0.02	1.27±0.37*	0.82±0.03*,[#]	0.88±0.15*,[#]	0.91±0.10*,[#],[¶]

Data are presented as mean±SD. Group I: sham; Group II: control; Group III: treated with surfactant; Group IV: treated with surfactant suspended in 4:1 saline-iodixanol; Group V: treated with surfactant suspended in 1:1 saline-Iodixanol. *: $p < 0.05$ *versus* group I; [#]: $p < 0.05$ *versus* Group II; [¶]: $p < 0.05$ *versus* Group III and IV.

significantly lower in Group III from 30 min onwards compared to Groups IV and V ($p < 0.05$) (table 1). There was no significant difference in $P\mathrm{a},\mathrm{CO}_2$ values between Groups IV and V.

Pressure/volume curves in Groups I–V

Data derived from the pressure/volume curves of all groups are given in table 2 and figure 2. TLC35 was significantly higher in Groups I, III, IV and V than in the control group ($p < 0.05$) (table 2). Rats that received surfactant suspended in 1:1 saline-iodixanol had significantly lower TLC35 than rats in Groups I, III and IV. Significantly higher $V5$ were measured in Groups III and IV compared to Groups II and V ($p < 0.05$) (fig. 2). The Gruenwald Index was significantly lower in Groups II and V than in the other groups (table 2).

Surface tension

Surface tension values of surfactant and surfactant-iodixanol mixtures are shown in figure 3. Surfactant dissolved in saline had the lowest minimum surface tension compared to the other mixtures. Surfactant suspended with 1:1 saline-iodixanol had a significantly higher minimum surface tension than surfactant alone or surfactant suspended with 4:1 saline-iodixanol. Increasing the concentration of surfactant resulted in a further decrease in minimum surface tension of surfactant alone and surfactant mixed with 4:1 saline-iodixanol. Surfactant mixed with 1:1 saline iodixanol was not affected by an increase of surfactant concentration.

Protein content of bronchoalveolar fluid

The protein concentrations measured in BAL fluid of sham rats and Groups II–V are given in table 2. All treated groups had significantly higher protein concentrations than sham rats. Group II had the highest concentration of proteins and this value was significantly higher than that in all other groups.

Discussion

In this study, iodixanol was used as a contrast agent because it has been reported to be a safe agent when aspirated or directly instilled in the lungs [25]. Aspiration of a contrast media used for the roentgen
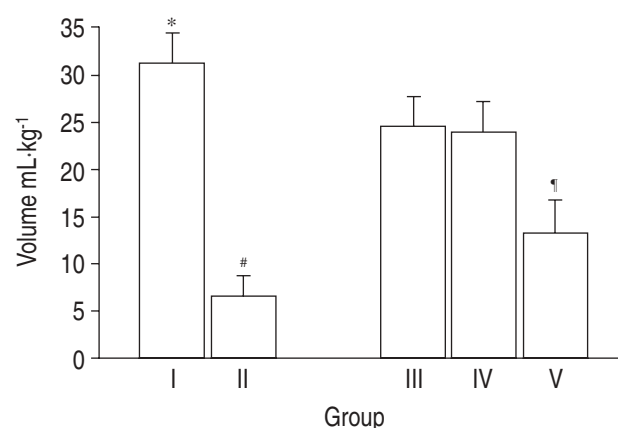

Fig. 2. – Lung volumes at transpulmonary pressures of 5 cmH$_2$O from the deflation limb of the pressure/volume curve of the study groups. Data are presented as mean±SD. Group I: sham; II: control; III: treated with surfactant; IV: treated with surfactant suspended in 4:1 saline-iodixanol; V: treated with surfactant suspended in 1:1 saline-iodixanol. *: $p < 0.05$ *versus* Groups II, III, IV and V; [#]: $p < 0.05$ *versus* Group III, IV and V; [¶]: $p < 0.05$ *versus* Group III and IV.
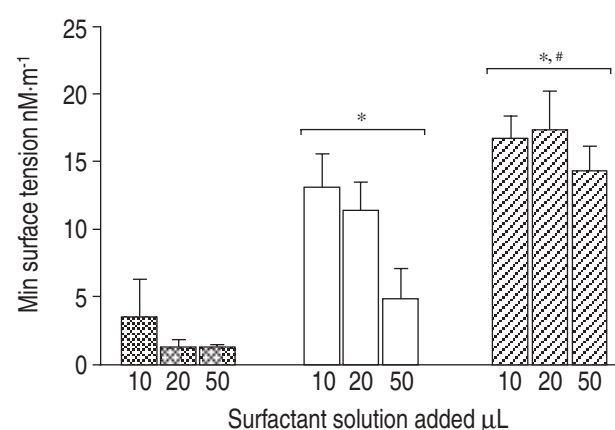

Fig. 3. – Mean surface tension values of surfactant suspended in saline (■), 4:1 saline-iodixanol (□) and 1:1 saline-ioxidinal (▨), made with three different surfactant doses of 10, 20 and 50 μL. Data are presented as mean±SD. Values with three different surfactant doses were compared between groups. *: $p < 0.05$ *versus* surfactant suspended in saline; [#]: $p < 0.05$ *versus* surfactant suspended in 4:1 saline-iodixanol.

examination of the upper gastrointestinal tract may cause severe respiratory insuffficiency. Studies have therefore been performed in rats [17–19, 22, 30, 31], guinea pigs [23], rabbits [20, 21] and dogs [18] to assess the damage of aspiration of commonly used water-soluble contrast media. As a result, it has been established that although some of these contrast media are not suitable to use for intrabronchial diagnostic procedures, other nonionic media did not cause harm to the respiratory system [17–24, 31]. Similarly, LARSEN et al. [25] reported that nonionic iso-osmolar contrast media, including iodixanol, when instilled intratracheally did not induce lung tissue damage or lead to respiratory complications.

The results of this study show that in rats with induced ARDS, iodixanol administered intratracheally, inhibits the effect of exogenously administered surfactant in a dose-dependent manner. This effect was indicated by a lack of improvement of P_{a,O_2} and low TLC35, V_5 and Gruenwald index values and was more prominent when surfactant was dissolved in higher concentrations of saline-iodixanol. This data contrasts with the findings of LARSEN et al. [25] concerning the effects of iodixanol. An important factor contributing to this contrast is the difference in methodology used. All the previous studies used animals with healthy lungs [17–24, 30, 31]. The same was true for LARSEN et al. [25] who used rats with healthy lungs and showed that iodixanol did not cause tissue damage or lead to respiratory complications. This study was performed on rats with injured lungs who needed mechanical ventilation with high pressures and an F_{i,O_2} of 1.0 to survive. The respiratory effects of iodixanol would probably not cause significant symptoms and changes in the histopathology of a healthy lung, especially if this effect was transient. Conversely, even minor damage can have deleterious effects on lung function and the oxygenation of ARDS lungs, as seen in the ARDS model in this study.

Another difference was that in the previous studies, animals were either intubated or a temporary tracheostomy was performed for the instillation of the contrast media, and breathing was spontaneous thereafter [17–25, 30, 31]. Instillation of saline was commonly used for control animals. Although the amount of saline used was significantly lower than in animal ARDS models, it is not the ideal placebo to use for intratracheal instillations [17, 21, 30], as saline, due to its high surface tension, impairs lung function. LARSEN et al. [25] based their judgement of contrast media on the histopathological examination, which was performed, at the earliest, at 6 h and, at the latest, on day 7. They showed that the majority of animals treated with iodixanol were unaffected compared to the control group, which received saline. In this study, all animals were anaesthetized, had an arterial cannula, received muscle relaxants and were mechanically ventilated. This allowed for ventilation of the lungs in steady state and for frequent blood gas measurements to be performed to determine the immediate changes of lung function during the trial. It was noted that surfactant improved the oxygenation. Adding iodixanol to surfactant (especially at a higher concentration) decreased the improvement of P_{a,O_2} in injured lungs. More importantly, P_{a,O_2} values were constantly high in both the sham and surfactant groups, but constantly low in the untreated group. This showed that the lower P_{a,O_2} values in Groups IV and V were completely dependent on the iodixanol concentration used. In both iodixanol groups, a time-dependent gradual increase of P_{a,O_2} was observed. This improvement did not reach the level of the sham or surfactant group within the study period of 120 min, especially in Group V. However, it was suggested that the iodixanol effect was temporary, probably depending on its rate of metabolism. If no permanent damage occurred in the lungs, histopathological examinations performed at a later stage would certainly be normal.

The mechanism of the iodixanol effect on the lungs can be explained by the results obtained from the measurements of surface tension of the mixtures instilled. As expected, surfactant had a very low surface tension. When the amount measured was increased from 10 to 50 μL, surface tension decreased even further. Surfactant combined with 4:1 saline-iodixanol at an amount of 10 μL, had much higher surface tension, although this improved significantly when the amount of surfactant was increased to 50 μL. In contrast, surfactant mixed with 1:1 saline-iodixanol not only had the highest surface tension but was also unaffected by the increase in the amount of surfactant. This model of ARDS caused by lung lavage is characterized by surfactant deficiency. In the absence of surfactant, the stability of the fluid balance in the lungs is disturbed, leading to lung oedema. This is evidenced by the increase of total protein concentration measured in BAL fluid in all rats who had lung lavage compared to sham animals. Furthermore, after lung lavage, the surface tension at the air/liquid interface is much higher than normal and hence higher pressures are required to maintain lung aeration. Administration of surfactant improved lung function by decreasing the surface tension of the interface between alveoli and air. There was also an improvement of lung function with the mixture of surfactant with 4:1 saline-iodixanol. However, when surfactant was mixed with 1:1 saline-iodixanol, its beneficial effect was mostly inhibited, resulting in high surface tension at air/liquid interface and, therefore, only minor improvement of oxygenation.

This situation was also confirmed by the *post mortem* lung-mechanic data. TLC35 was significantly lower in the high-dose iodixanol group (Group V) than in the lower-dose iodixanol (Group IV) and surfactant group Groups (III). This showed that at the same pressure level, the lungs of the rats in Groups III and IV could receive much higher volumes due to lower surface tension at the air-liquid interface and higher lung compliance compared to lungs of rats in Group V. Similarly, V_5 values were higher in groups receiving surfactant and surfactant dissolved in 4:1 saline-iodixanol mixture compared to rats receiving surfactant dissolved in 1:1 saline-iodixanol mixture. This meant that at an expiratory pressure value of 5 cmH₂O, more volume of air was available in the rat lungs of Groups III and IV, showing that the lungs

were more open and compliant at expiration than in Group V and thus allowing prolonged gas exchange at end expiration. Finally, the Gruenwald index results characterizing the surfactant system *in situ*, confirmed that the surfactant was less effective when dissolved in a high concentration of iodixanol.

In conclusion, iodixanol causes a temporary inhibition of exogenous surfactant, administered to the lungs of rats with adult respiratory distress syndrome. This effect is more prominent when surfacant is dissolved in a higher concentration of saline-iodixanol. The inhibitive effect of iodixanol is probably due to its high surface tension, which also affects the distribution of the surfactant. These results suggest that although no damage is shown to occur with intratracheal instillation of iodixanol in healthy lungs, it may have a deleterious effect on injured lungs if instilled or accidentally ingested. Use of a lower concentration has less effect on respiratory functions and has a lower surface tension. Therefore, it is unlikely that it will significantly affect the distribution of the instilled surfactant. For this reason, this suspension appears to be a safer alternative to be used for intratracheal administration, radiodiagnostic procedures and experimental studies on the distribution of surfactant in the lungs.

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