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1 Verantwoording

Aanvraag dierproef DEC-UM

Titel: A novel method for surfactant administration as improvement in lung surfactant replacement therapy

1. Doel van de proef.

Preterm babies may suffer from the immaturity of the lung. This immaturity results into a lifethreatening disease of respiratory distress syndrome (RDS). The lungs are deficient in surfactant (surface active agents), which is a complex mixture of phospholipids and proteins that reduces the surface activity. Exogenous surfactant administration has been developed in preterm sheep and has been successfully implemented in clinical medicine. Current surfactant therapy mandates intubation, a difficult and potentially dangerous procedure.

The aim of the current proposal is to test a new device that will allow surfactant administration without the necessity of intubation.

2. Maatschappelijke relevantie en/of wetenschappelijk belang

The main morbidity of premature birth is related to the immaturity of the lung. The pulmonary surfactant is a mixture of phospholipids and proteins that lowers surface tension and maintains alveolar expansion at end expiration. Clinical trials have shown that the earliest delivery of surfactant provides the best clinical outcomes. Making surfactant delivery earlier and with a less invasive technique available is of relevance for the preterm infants and for national health care.

3. Alternatieven

The proposal will test in the best available animal model the proof of principle for this innovative approach. The components need to be tested in vivo before applying them to ventilated preterm babies. The choice of the animal model is based on body size, costs, use of similar equipment as in the neonatal intensive care units and availability.

It is vital that the results of an animal model can be easily translated to the clinical situation. Therefore we have established an animal model of immature lung-development in preterm lambs. In contrast to that of mice and rats, the lung maturation of sheep is closely similar to that of humans (see figure 1).



Figure 1. Time-points are shown when lung alveolarization, an important hallmark of lung maturation, occurs in different species. Lung maturation in sheep occurs prenatally, as in humans. Lung maturation in

rodents takes place after birth.

4. Ethische afweging

Respiratory Distress Syndrome (RDS) is a frequent and relevant condition in perinatology with high morbidity and mortality rates. Although surfactant treatment already improved neonatal outcome and quality of life for preterm babies suffering from RDS, there is still need for intubation of the baby to administer the surfactant. As intubation is a difficult and dangerous procedure which can only be performed in specialized centers, improving surfactant replacement strategies is therefore of great

Unfortunately, it is not possible to perform the proposed experiments in a rodent model (or any other small animal model) due to species differences in lung development. During the experiments, animals will be regularly assessed for pain and discomfort by skilled, experienced personnel and when necessary, pain relief, anaesthesia or euthanasia will be given. As alternatives administration of surfactant will increase the survival rate and improve the quality of life of preterm infants, the outcomes of this study will outweighs the discomfort of the animals in this experiment.

3 Wetenschap

5. Wetenschappelijke onderbouwing

Surfactant replacement therapy is standard of care. In the past 20 years surfactant replacement therapy has become available worldwide. It is a well studied therapy with a large dose-response and known kinetics. Clinical studies revealed that surfactant replacement therapy must be administered as early as possible. However, current techniques that mandate endotracheal intubation inadvertently delays surfactant therapy. In addition, surfactant and the skilled personal are only available at maximum care centres.

The new surfactant is completely synthetic. No component is of animal origin. The major component is a recombinant surfactant protein B and C with phospholipids. This surfactant was successfully tested after resuspension in water in intubated preterm lambs. A nebulised preparation has recently been developed and successfully tested in vitro for surface tension lowering properties.

The nebulised form of the surfactant allows administration to spontaneously breathing patients. This is in line with recent clinical studies that favour spontaneous breathing with continuous positive airway pressure (CPAP) to keep the airways open and to reduce the work of breathing. The new concept that will be tested in this study makes surfactant replacement therapy available to spontaneously breathing patients.

CHIESI has developed the surfactant and nebulising device with administers the synthetic surfactant. The suggested study is a proof of principle that this new, nebulised surfactant works in an immature lung, with dose finding and long term effect study. The results are used to design a clinical trial.

6. Wetenschappelijke beoordeling

This DEC request was reviewed and approved by J

Proefdier

7. Proefdier keuze

7a. Soort, stam / herkomst / eindbestemming

The experiments will be performed with pregnant sheep, retrieved from a farm contracted by the CPV. Twin pregnancies will be preferred to reduce the number of ewes. The experiment will be done with preterm lambs at 131-138d gestational age (term is 150 d). For the lambs, it concerns terminal experiments.

Lambs are the most appropriate animals with which to address our hypothesis, for a number of reasons:

- Development of the lungs in sheep has been sufficiently studied to allow informed experimental design and integration of findings with those from previous studies. The current surfactant replacement therapy was developed in preterm lambs.
- 2. Preterm lambs are large enough to use similar equipment for ventilation as in the neonatal intensive care unit, provide sufficient blood and tissue to allow enough biomolecular analyses, for thorough assessment of the biological systems being investigated, in individuals.

7b. Sexe

Both sexes of lambs will be used.

7c. Aantallen:

We have successfully used group numbers of 8-12 animals for evaluations of postnatal lung maturation and surfactant metabolism

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5 Dierproef

8. Experiment

- Preterm lambs will be born via c-section at 131-138 d gestational age. The ewe will receive pain medication before the operation
- 1. Comparison to gold standard: This study will be performed as a positive control to demonstrate the quality and efficacy of the new surfactant both in resuspended and nebulised form. In turn, the new surfactant in resuspended and nebulised form will also be compared to the golden standard surfactant.

Preterm lambs will be intubated, sedated, and a central line will be inserted. Animals will be randomized to the following three experimental groups: Curasurf® (Golden standard surfactant) resuspended, 200mg/kg intratracheally: n=15Synthetic surfactant resuspended, 200 mg/kg intratracheally; n=15Synthetic surfactant nebulised, 200 mg/kg intratracheally; n=15

The animals will be ventilated for 48 h. The major outcome is oxygenation which is tested every 30 min by arterial blood gas analysis.

• 2. Feasibility of delivery to spontaneously breathing animals: This experiment will test the hypothesis that nebulised surfactant in spontaneously breathing animals will be effective. Three different doses will be tested. If a successful surfactant therapy is possible with the two lower doses we will not test the higher dose and save the animals

Preterm lambs will be dried, put on CPAP administered by a ventilator and a central line will be inserted. Animals will be randomized to the following three experimental groups: Synthetic surfactant nebulised, 50 mg/kg; n=15Synthetic surfactant nebulised, 200 mg/kg; n=15

The animals will be maintained for 3 h. The major outcome is oxygenation which is tested every 30 min by arterial blood gas analysis. During these experiments we can determine the optimal dosage and ventilation strategies for the animals which we will then test in a long term study (see aim 3).

• 3. Long term effects: This experiment will test the hypothesis that nebulised surfactant in spontaneously breathing animals will be effective for a long period of time. The appropriate dose is determined in the previous set of experiments.

Preterm lambs will be dried, put on CPAP administered by a ventilator and a central line will be inserted. Animals will be given synthetic surfactan nebulised, at the lowest dose that was determined to effective and with optimal ventilation settings which were determined in the 3h study previously described. As all parameters will be optimized in aim 1 and 2, we can ensure an optimal treatment protocol which will lead to less variation between animals in the long term study. The animals will be maintained for 48 h. The animals will receive a continuous intravenous sedation during this period and total parenteral feeding. The major outcome is oxygenation which is tested every 60 min by arterial blood gas analysis.

This experiment will be done with n=15 animals.

At the end of the experiment the animals will euthanized for tissue collection. The lung will be lavaged with saline (bronchoalveolar lavage fluid, BALF) to recover the surfactant in the alveoli. The cells in the BALF will be analysed for cell type, number and content of surfactant components. The remaining lung tissue will be homogenized and analysed for surfactant pool size and amounts of the instilled synthetic surfactant protein.

9. Experimentele condities 9a. Anesthesia

Ewe: The c-section is done according to veterinary practice under aseptic conditions. The ewe will receive a spinal analgesia with lidocaine. In addition, a local anesthesia of the skin will be done with lidocaine.

Epidural anesthesia (C-section)

- 1. 5 mL epidural lidocaine 2% injection
- 2. 20 mL local lidocaine 2% skin injection
- 3. midazolam (Dormicum: 5 mg/mL) 5 mg i.v.

Lamb:

Preterm lambs which are included for mechanical ventilation will be sedated under aseptic conditions. The anterior neck is locally anesthetized (2% lidocaine, 0.5 mL subcutaneously) and, after tracheotomy, an endotracheal tube is securely tied into place. A 4.5 mm (ID) tube is used for preterm lambs. Each lamb is put on a radiator bed, similar to those used in the neonatal intensive care unit and ventilated for 3. A continuous sedation is given with midazolam (0,3 mg/kg/h) and ketamine (4 (-6) mg/kgBW/h.). Rectal temperature is regularly checked. A neonatologist will be available for the whole period of ventilation. Arterial and venous umbilical catheters will be inserted.

Sedation of ventilated lamb

- 1. ketamine (10mg/kg body weight)
- 2. midazolam (0,3 mg/kg/h)
- Spontaneously breathing animals: Lambs will be born as previously described, dried, fluid will be suctioned out of the pharynx. Arterial and venous umbilical catheters will be inserted. Preterm lambs have at this age a sufficient respiratory drive on continuous positive airway pressure (CPAP) of 5 cm H₂O which will be given. In clinical medicine no sedation or anesthesia is given to preterm babies on CPAP. Spontaneously breathing preterm lambs will be assessed for pain and distress which is usually not encountered.

9b. Pijnbestrijding

Ewe: After C-section, the ewe will receive antibiotics (Augmentin). During recovery, the ewe will be regularly assessed for pain or discomfort and when necessary Fynadyne will be administered as pain relief.

Intubated lambs: The intubated lambs will be kept under sedation throughout the experiment by means of fentanyl $(5\mu g/kg/h)$ and midazolam (0,3mg/kg/h). The depth of the anesthesia/analgesia will be regularly assessed and adjusted by experienced staff.

Spontaneously breathing lambs: Our experience show that spontaneously breathing lambs of this gestational age under CPAP do not need analgesia or anesthesia which is in accordance with the clinical situation in the neonatal intensive care unit where preterm infants on CPAP also do not require anesthesia/analgesia. However, lambs will be regularly assessed for discomfort and pain by experienced staff en when necessary, fentanyl $(5\mu g/kg/h)$ will be administered.

9c. Euthanasie en Humane eindpunten

• Lambs are euthanized at the end of the experiment or if a human end point has been reached by a lethal overdose of pentobarbital (150-200 mg/kg).

• Ewe: Human endpoints:

- Infection/sepsis
- Untreatable pain or agitation
- Lambs: Human endpoints:
 - o untreatable pneumothorax
 - o uncorrectable severe respiratory acidosis
 - untreatable pain or agitation
 - uncorrectable hypovolemia
 - multi-organ failure
 - infection/ sepsis

Zorg

10a. Ongerief

Ewe

- 1. Transport: gering (01), duration 10 min
- 2. C-section under epidural anesthesia: matig (03), duration 20 min
- 3. Recovery of caesarean section: gering/matig (02), duration max. 2 days

Preterm lamb (intubated)

- 1. Intubation: matig (03), duration 10 min
- 2. ventilation under sedation: gering/matig (02), duration max. 2 days
- 3. euthanasia: gering/matig (02), duration 2 min

Preterm lambs (spontaneously breathing)

- 1. Ventilation under analgesia: matig (03), duration max. 2 days
- 2. Euthanasia: gering/matig (02), duration 2 min

Total discomfort

- matig (03) for ewes
- matig (03) for preterm lambs

10b. Welzijnsevaluatie

The condition of the lambs will be continuously monitored for any form of discomfort and pain. As our group is highly skilled in these experiments, adjustments in anesthesia/analgesia will be made correctly and when necessary. Experiences from previous experiments have shown that lambs respond will towards our procedures of C-section, catherization, sedation and maintenance of these animals during long term studies. During the experiments, lambs will be assessed on the following criteria every 15 minutes by skilled personnel:

- clinical impression
- temperature
- blood pressure
- heart rate
- blood gas
- glucose
- respiratory settings
- cardiac function (echography)
- brain function (EEG)

11. Verzorging en huisvesting

We will minimise the impact of experimentation on the animals to be used by employing the following strategies:

- The ewe will be kept as long as possible in the natural environment.
- The ewe will be given prophylactically antibiotics and will recover over a week and will be re-used for a different experimental protocol.

The lambs will be maintained by experienced animal technicians who perform physical exams and electrolyte controls.

12. Deskundigheid

has 9 years of experience in large and small animal models. He has developed the sheep model of CPAP in his post-doc in the USA.

Our research team has broad experience in sheep experiments. C-section delivery will be performed by skilled certified researchers. The intubation and introduction of catheters will be done by neonatologists and/ or skilled researchers.

13. Standard Operation Procedures (SOP) SOP is enclosed.

Relevante literatuur

Kramer, B.W. (2007) The respiratory distress syndrome (RDS) in preterm infants. Intensivmed. 44: 403-408

Jobe, A.H., Kramer, B.W., Moss, T.J., Newnham, J.P., Ikegami, M. (2002) Decreased indicators of lung injury with continuous positive expiratory pressure in preterm lambs. Pediatr. Res. 52: 387-392 Kramer, B.W., Jobo, A.H., Dada, M. G. S.

Kramer, B.W., Jobe, A.H., Bachurski, C.J., Ikegami, M. (2001). Surfactant protein A recruits neutrophil granulocytes into the lungs of ventilated preterm sheep. Am. J. Respir. Crit. Care Zimmermonn J. J. Jones D. J. Two.

Zimmermann LJ, Janssen DJ, Tibboel D, Hamvas A, Carnielli VP. Surfactant metabolism in the neonate. Biol Neonate 2005; 87:296-307.

Kramer B.W. Antenatal inflammation and lung injury: prenatal origin of neonatal disease. Journal of Perinatology 28: S21-27.

SOP 1: Protocol for delivery of preterm lambs

1. The ewe is kept in a group pen, with free access to water, to reduce the rumen volume. This period also provides opportunity for closer pre-anaesthesia examination of animals in order to assess whether they are healthy and fit for inclusion in our studies.

2. The ewe is held immediately prior to delivery in a group holding pen. This is identical to the movement and holding used prior to shearing.

3. The ewe is then placed on a laparotomy trolley and moved to operation room.

4. An intravenous line is placed and the ewe will be given midazolam (5mg i.v.) for sedation.

5. The ewe is held by a person with experience handling sheep. Spinal anaesthesia with lidocaine will be performed: An 18 G spinal needle is inserted through the intravertebral space at the level of the iliac crests, and is introduced into the subdural space. The stylet is withdrawn from the needle and the needle opening is observed for leakage of cerebrospinal fluid. If fluid leakage is not observed the needle is withdrawn and reintroduced. Lidocaine (60mg, 3ml) is slowly injected into the subdural space. The absence of muscle tone in the ewe's hind limbs is used to confirm effective spinal anaesthesia. Spinal anaesthetic is given by experienced personnel or by novices under the direct instruction and supervision of an experienced person. Amoxicilline 500 mg is given intravenously. If the ewe is agitated midazolam 5 mg is given intravenously.

6. The absence of muscle tone in the ewe's hind limbs is used to confirm effective spinal anaesthesia.

7. The ewe is then placed on a laparotomy trolley and is taken to an adjacent room for Caesarean delivery of the fetus. The Caesarean delivery is done under aseptic conditions.

8. The ewe's abdomen is anesthetized with subcutaneous injections of 2% lidocaine in the left abdomen, avoiding the superficial epigastric and mammary veins, to gain access to the abdominal cavity. A hand is introduced into the abdomen to palpate the uterus and identify the location of the fetal head. The uterus is gently manipulated and the fetal head and overlying uterus are exteriorized through the maternal abdominal incision. The uterus and fetal membranes are then incised, with care taken to avoid placentomes and all but the smallest blood vessels. The fetus is then delivered headfirst, through the maternal abdominal incision. Before closure of the uterus, oxytocine 0,1 mL/kg is given in utero.

9. The uterus, the abdominal muscles and wall, the abdominal skin are closed by individual, running sutures under aseptic conditions. The ewe receives a bolus of 500 mg amoxicilline intravenously and oxytetracycline. The ewe returns to pen with free access to food and water.

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After 3 days of recovery the ewe is allowed in paddock, stitches won't be removed. Sutures will be removed by skilled personnel when still present 10 days after the C-section.

SOP 2: Protocol for mechanical ventilation in sheep

1. The cord is clamped, blood is taken from maternal side of the cord in 50 ml syringe with heparine. This blood sample will be used for autologous blood transfusion if the lamb is anemic. The lamb is sedated (ketamine, 10 mg/kg and xylazine, 0.1 mg/kg IM) by intramuscular injection under aseptic conditions before separation from the mother.

2. The anterior neck of the lamb is locally anesthetized (2% lidocaine, 0.5 mL subcutaneously). A 4.5 (ID) cuffed endotracheal tube (of appropriate size for the trachea) is then introduced 5-6 cm into the trachea, towards the lungs, and is tied using the second cotton tie.

3. After the lamb has been weighed it is placed on a bed under a radiant warmer. The lamb's rectal temperature is constantly monitored, by use of a small thermostat probe, and the radiant warmer adjusted to maintain rectal temperature between 36 and 38 °C. A continuous sedation is given with midazolam (0,3 mg/kg/h) and ketamin (4-6 mg/kgBW/h).

4. The lamb's endotracheal tube is immediately connected to a mechanical ventilator set to deliver 40 breaths/minute with an inspiratory time of 0.7s, with a positive end-expiratory pressure of 5 cmH2O. The peak inspiratory pressure is set initially at 25 or 35cm H2O (depending on gestational age) and is adjusted after connection to achieve a tidal volume of 5-10ml/kg. Inspired gas will have an O2 content adjusted to obtain a normal oxygenation.

5. Surfactant is applied according to dosing protocol via the nebulising device connected in ventilation setting.

6. An umbilical arterial catheter is inserted, via the cut end of the umbilical cord, and is inserted so that its tip lies in the descending aorta. This catheter is used for sampling fetal blood at regular intervals for blood gas analysis and may be used for continuous administration of ketamine and midazolam to prevent spontaneous ventilation or body movement. An umbilical line is placed to provide venous access for the sedation medication and for parenteral feeding (8 mg/kg/min glucose).

7. The lamb will be ventilated for a period of 3 hours. During this time period the researchers (pediatricians, specialised in neonatology) will attend the lambs. During this period arterial blood gas measurements are made at regular intervals to adjust respiratory settings. Peak inspiratory pressure of the ventilator is adjusted (up or down, as necessary) to target a PCO2 of 50 mmHg. A peak inspiratory pressure of 40 cm H2O is not exceeded. Supplemental

boluses of midazolam will be given if there is any sign of spontaneous breathing or body movement.

8. At the end of the period of ventilation, the inspired O2 concentration is increased (if necessary) to 100%, the lamb is deeply anaesthetised with intravascular pentobarbitone (30 mg/kg), the chest is gently compressed by hand to expel excess gas, and the endotracheal tube is clamped. A period of 4-5 minutes is allowed for residual O2 in the lungs to be absorbed, in order for the lungs to collapse.

9. While deeply anaesthetised, the lamb is exsanguinated prior to the performance of postmortem measurements and tissue collection.

SOP 3: Protocol for spontaneously breathing sheep

- 1. The ewes will receive 24 h before delivery an injection of epostane, an inhibitor of prostaglandine production.
- 2. Lambs will be born as previously described, dried, fluid will be suctioned out of the pharynx. Preterm lambs have at this age a sufficient respiratory drive on continuous positive airway pressure (CPAP) of 5 cm H₂O which will be given. The lamb is put on the back and a 2.0-3.0 uncuffed endotracheal tube (of appropriate size for the lamb) is then introduced 5-6 cm into the nose, towards the lungs, and is tied using the second cotton tie.
- 3. Spontaneously breathing preterm lambs will be assessed for pain and distress which is usually not encountered.



Faculty of Health, Medicine

and Life Sciences

Dierexperimenten Commissie



voorzitter p/a Secretariaat DEC-UM Postbus 616 NL-6200 MD Maastricht Telefoon: /

Uw referentie:

Aan:

Onze referentie :

Maastricht, 19-07-2011

Geachte Onderzoeker,

Uw projectaanvraag: "The efficacy of a novel lung surfactant replacement therapie", is op de DEC vergadering van 15 juli 2011 besproken.

De DEC heeft een aantal vragen en opmerkingen:

- De titel op het voorblad stemt niet overeen met de titel van de aanvraag. De DEC verzoekt dit aan te passen.
- De DEC merkt op dat het totale aantallen dieren bij punt 7c niet overeen stemt met het voorblad. De DEC verzoekt dit in overeenstemming te brengen.
- De DEC verzoekt bij punt 7c de uitval als percentage aan te geven.
- De DEC verzoekt de variatie en het verwachte verschil tussen het gemiddelde, in percentages aan te geven. Nu heeft de DEC de indruk dat het verwachte verschil 60% is, en daarmee zou het aantal dieren per groep 4 zijn.

Gelieve eventuele vragen te beantwoorden in een brief en indien noodzakelijk Uw project aan te passen en duidelijk de aanpassingen grijs te markeren.

Uw project staat bij de DEC geregistreerd onder nummer 2011-097, gelieve dit nummer in verdere correspondentie te vermelden.

Hoogachtend,

Voorzitter DEC-UM

Aan : 1, voorzitter p/a Secretariaat DEC-UM Postbus 616 NL-6200 MD Maastricht Telefoon:

Gaande wijzigingen DEC aanvraag 2011-097

Geachte

Naar aanleiding van uw brief op 19 juli 2011, hebben we de opmerkingen die werden aangehaald aangaande DEC aanvraag 2011-097 aangepast.

- De titel op het voorblad en de DEC aanvraag luidt nu : A novel method for surfactant administration as improvement in lung surfactant replacement therapy
- Het aantal dieren op het voorblad is nu gewijzigd naar 45 preterme lammeren die verdoofd worden voor intra-tracheale instillatie van surfactant en 60 preterme lammeren die lichte verdoving krijgen voor de toediening van surfactant in genebuliseerde vorm. Dit maakt in totaal 105 preterme lammeren waarvoor er 105 zwangere schapen nodig zijn.
- De uitval in sectie 7c is nu vermeld als volgt: Loss of animals for inclusion in the experiments is considered to be low [less than 5% for loss or less than one animal per 14; less than 10% for experimentation or less than 2 animals per 14]
- De variatie en het verwachte verschil zijn nu duidelijk vermeld in sectie 7c als volgt: To measure a significant difference in the new administration strategy of surfactant, we used a power calculation (α: 0.05, Π: 0.8) to estimate the number of animals per group. We expect a relevant difference of 35% and a variance of 30% in groups.

De doorgevoerde wijzigingen in het protocol zijn in het grijs gemarkeerd. Hoogachtend,



Faculty of Health, Medicine and Life Sciences

Aan:

Ons kenmerk

Doorkiesnummer

Maastricht 05-09-2011

DEC-UM Voorzitter DEC-UM

p/a secretariaat DEC-UM

Secretariaat DEC-UM

Bezoekadres

Postadres Postbus 616 6200 MD Maastricht

| Verantwoordelijk | onderzoeker | (VO) |): |
|------------------|-------------|------|----|
|------------------|-------------|------|----|

Namens de Vergunninghouder van de DEC-UM, delen wij u mede dat voornoemd project aan de ethische toetsingscriteria voor proefdiergebruik voldoet.

Project: The efficacy of a novel lung surfactant replacement therapie.

De DEC maakt geen bezwaar tegen uitvoering van dit project zoals aangevraagd en geeft een **positief advies**.

| Projectnummer: | 2011-097 | | | | |
|----------------|--------------------------|--|--|--|--|
| Diersoort: | schaap | | | | |
| Aantal dieren: | 105 ooien en 105 lammere | | | | |
| Einddatum: | 01-09-2015 | | | | |

Uw project staat bij de DEC en CPV geregistreerd onder bovenstaand nummer. Gelieve dieren, die voor dit project bestemd zijn, ook onder dit nummer aan te vragen.

Voorzitter DEC-UM

Vicevoorzitter DEC-UM