Online data supplement

Patients with ARDS show incomplete restoration of alveolar surface activity upon recombinant SP-C-based surfactant treatment: putative role of neutral lipids

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METHODS

Study synopsis

All ARDS patients participated in a randomized, multicenter, controlled, phase I/II pilot study, investigating the safety and efficacy of an intrabronchial administration of a recombinant SP-C-based surfactant (Venticute). Table 1 shows the study synopsis of the Venticute trial.

Table 1

Investigational drug:	recombinant surfactant protein C (rSP-C) based surfactant (Venticute;in the pharmaceutical formulation 1 mg rSP-C is applied with 50 mg phospholipids)
Phase:	I/II
Objectives:	- to assess the safety and efficacy of Venticute (maximum total dose 4 mg rSP-C plus 200 mg phospholipids per kilogram lean body weight) in the treatment of adults with the Acute Respiratory Distress Syndrome in comparison to standard therapy - to assess the composition and function of surfactant recovered from bronchoalveolar lavage (BAL) fluid
Design:	randomized, multicenter, parallel group, controlled pilot study
Study population:	Subjects diagnosed with ARDS with one or more identifiable ARDS risk factors (e.g. sepsis, aspiration, trauma or surgery, multiple blood transfusions, pancreatitis, pneumonia) Patients must have been diagnosed for ARDS within 120 hours prior to study entry
Study groups:	Group 1 (ARDS-standard care): receives standard ARDS treatment but no Venticute Group 2 (ARDS-surfactant treatment): receives standard ARDS treatment plus Venticute (1 mg rSP-C + 50 mg phospholipids/kg lean body weight up to four times)
Dosing scheme:	Patients receive Venticute by intratracheal instillation. Patients are alternately positioned in the left or right lateral decubitus position, and the calculated drug-volume is given in aliquots of up to 25 ml via intratracheal catheter during a brief pause of the mechanical ventilation.

Treatment overview:	Patients receive an initial dose of Venticute. Up to 3 additional doses may be given. Allowed time points of administration are 4, 8, 12, 16, and 20 hours after the initial dose. Subsequent doses will be given every four hours up to a maximum of 4 doses in total. If a patient is not retreated, he/she should be retreated at the next possible time point provided the retreatment criteria are still met.
Treatment period:	24 hours
Observation period;	up to 28 days
Primary variables:	- Primary efficacy variable: excess-area under the Pa_{O2}/FI_{O2} curve during 24 hours after study time t = 0 (start of first administration) - Number of days with unassisted breathing up to day 28

Table 1

Study synopsis of the Venticute trial

Study design

The study commenced in April 1998 at the following European/South African Clinical Centers: University Hospital of Geneva, Switzerland; University Hospital Zurich, Switzerland; Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; University Hospital, Tubingen, Germany; University Hospital of Regensburg, Regensburg, Germany; Medical Clinic II, Justus-Liebig-University, Giessen, Germany; Klinikum Grosshadern, Ludwig-Maximilians-University, Munich, Germany; University Hospital RWTH Aachen, Germany; Charite, Campus Virchow-Klinikum, Humboldt-University, Berlin, Germany; Hannover Medical School, Hannover, Germany; University Hospital of Mannheim, Germany; University Hospital of Bonn, Germany; University Hospital Carl Gustav Carus, Technical University, Dresden, Germany; Henri Mondor Hospital, Creteil, France; Hopital Sainte Marguerite, Marseille, France; Guy's and St. Thomas' Hospital, London, United Kingdom**;** Milano-Bicocca University, San Gerardo Hospital, Monza, Milano, Italy; Erasmus MC, University Medical Center Rotterdam, The Netherlands; St. Clara Hospital, Rotterdam, The

Netherlands; University of the Orange Free State, Bloemfontein, South Africa; Unitas Hospital, Lyttleton, South Africa; Wilgers Hospital, Pretoria, South Africa; University Hospital of Wales, Cardiff, Wales. The study was completed in May 1999. The study was approved by local institutional review boards at each participating institution, and informed consent was obtained from all patients or their legal representatives. General inclusion criteria were ARDS as defined by the American-European Consensus Criteria for not longer than 120 hours since diagnosis (early ARDS), age > 18 years, completion of a physical examination including vital signs, and a positive end-expiratory pressure (PEEP) ≥ 5 cm H_20. In addition, the patients had to have at least one of the following predispositions for ARDS: burn injury, trauma or surgery, polytransfusion, witnessed aspiration of gastric contents, sepsis syndrome, pancreatitis, direct toxic injury of the lung (e.g. inhalation injury), or pneumonia. Exclusion criteria included the following: previous episode of ARDS or bacterial pneumonia demanding artificial ventilation that resolved and then reoccured during the current hospitalization; any preexisting lung disease with a FEV_1 or FVC ≤ 65% predicted; primary cancer of the lung or cancer metastatic to the lung; AIDS or known HIV infection; ARDS due to predisposition other than listed in the inclusion criteria; women of child-bearing age unless pregnancy has been excluded; prediction, based on clinical judgement, that the patient would not survive at least two days for non-respiratory reasons; inability to tolerate bronchoscopy and bronchoalveolar lavage (e.g. endotracheal tube too small); neutrophil count ≤ 1,000 per µl; platelet count < 10,000/mm^3; INR > 2,5 or PTT > 60 seconds in the absence of anticoagulation; total bilirubin > 5mg/dl; mean blood pressure < 60 mm Hg; cardiac index < 1.5 l/min/m^2; elevated intracranial pressure (≥ 20cm H_20) or depression of Glasgow coma score to ≤ 5 in the absence of sedatives; Pa_{O2}/FI_{O2} < 60mmHg at the time of enrollment; treatment with NO inhalation; diffuse bilateral infiltrates present for longer than 8 consecutive days. Two primary efficacy variables were defined prospectively. The first was the excess area under the Pa_{O2}/FI_{O2}-versus-time curve for the 24-hour period beginning 1 hour after

randomization (ARDS-standard care group) or beginning with the first surfactant administration (ARDS-surfactant group). The excess area under the curve was calculated as the area between the horizontal line corresponding to the average of three baseline Pa_{O2}/FI_{O2} values and the linearly connected Pa_{O2}/FI_{O2} measurements during the 24-hour period after t = 0. The second primary efficacy variable was the number of days with unassisted breathing within the 28-day observation period. Secondary variables included the percentage of patients alive at day 28, the percentage of patients alive at day 28 with unassisted breathing, the excess area under the Pa_{O2}/FI_{O2}-versus-time curve during the 120-hour period after study time t = 0, and differences of Pa_{O2}/FI_{O2}-values at different time points after randomization and study time t = 0.

Conduct of the study

During a 6-hour baseline period, clinical and respiratory parameters were recorded. At three hour intervals, three sets of physiological parameters (ventilator settings, arterial blood gases, vital signs) were obtained. Volume-controlled ventilation was adjusted to tidal volumes of 6-10 ml/kg body weight and employed throughout the study. Permissive hypercapnia was allowed. A modified APACHE II score was calculated. Due to the use of sedatives, neurologic evaluation could not be performed consistently and was therefore omitted from this modified score. The first BAL (- 3 hours) for surfactant analyses was obtained. Immediately after the conclusion of the baseline period (within 30 minutes), patients were prospectively randomized (by means of a computer generated randomization list) to receive either standard care (ARDS-standard care; n = 17) or standard care plus surfactant treatment (ARDS-surfactant; n=14). Treatment with the first dose of surfactant (for treated patients) had to start within 2 hours after randomization. Up to three additional doses were administered during the 24 hours after initial treatment (treatment period; see below). During the treatment period, physiological parameters (ventilator settings, arterial blood gases, vital signs) were

recorded at 0, 1, 2, 4, 8, 12, 16, 20, and 24 hours. Patients were subsequently observed for up to 28 days or until hospital discharge (observation period). During this observation period, patients were screened daily for possibility to wean from the mechanical ventilator. Physiological parameters (ventilator settings, arterial blood gases, vital signs) were recorded at 36, 48, 72, 96, and 120 hours after first treatment plus daily (first daily measurement) as long as the patient was intubated, or at least at days 7, 14, and 28. Second and third BAL were obtained at 48 and 120 hours after the first treatment.

Surfactant dosage and administration

The recombinant surfactant protein C (SP-C) based surfactant preparation (Venticute) was placed at our disposal by the sponsor (ALTANA Pharma AG, Konstanz, Germany) as a dry powder for resuspension in 0.9 % NaCl to achieve a final concentration of 1 mg recombinant SP-C and 50 mg phospholipids per ml. In the surfactant treatment group, patients received 1 ml of recombinant SP-C surfactant (containing 1 mg of recombinant SP-C + 50 mg of phospholipid) per kilogram lean body weight. Surfactant was administered through an inner catheter that was inserted through the endotracheal tube and placed 1–2 cm above the carina. Patients were placed in the right lateral decubitus position with the head elevated 30°, and half of the dose was delivered over 20–30 seconds with the ventilator paused at end exhalation. Subsequently, the patient was placed on the left side, and the second half dose was administered. Up to three additional doses were administered at predefined time points (4, 8, 12, 16, or 20 hours after initial treatment). Patients were retreated if the Pa_{O2}/FI_{O2} ratio was between 60 and 240 mm Hg and the patient remained intubated and on mechanical ventilation with a PEEP greater than or equal to 5 cm H_2O. Concomitant medication was allowed according to patient's need. However, not allowed during the whole study was intratracheal or intrabronchial treatment with other experimental drugs, inhalation of NO, or other treatment that was known to directly influence the primary variable Pa_{O2}/FI_{O2}

BAL

BAL was obtained by flexible fiberoptic bronchoscopy from healthy volunteers, patients with cardiogenic pulmonary edema (once) and from ARDS study subjects before (-3 hours = baseline), and at 48 and 120 hours after the first treatment. One segment of the lingula or the right middle lobe was lavaged with a total volume of 200 ml of sterile 0.9 % NaCl in 10 aliquots with a fluid recovery ranging between 50 and 70 %. The fractions of the BAL fluid were pooled, filtered through sterile gauze and centrifuged at 200 x g (10 minutes, 4 °C) to remove cells and membraneous debris. When performing the second and the third lavage, different segments were chosen each time.

Neutral lipid preparation

A neutral lipid mixture consisting of 5 % monoglycerides (1-Oleoyl-rac-glycerol, Sigma M 7765 or rac-1-Palmitoylglycerol, Sigma M 1640; purity 99 %), 7.5 % diglycerides (1,2-Dioleoyl-rac-glycerol, Sigma D 8394, purity 97 % or 1,2-Dipalmitoyl-sn-glycerol, Fluka 42553, purity > 99 %), 16 % triglycerides (1,2,3-Trioleoyl-glycerol, Sigma T 7140 or Glycerol tripalmitate, Sigma T 5888; purity 99%), 56 % cholesterol (Sigma C 8667, purity > 99 %), 10 % free fatty acids (oleic acid, Sigma O 1008 or palmitic acid sodium salt, Sigma P 9767; purity 99 %) and 5.5 % cholesterolester (cholesteryl oleate, Sigma C 9253 or cholesteryl palmitate, Sigma C 6072; purity > 98 %) (wt/wt/wt/wt/wt/wt) dissolved in chloroform was prepared at a total neutral lipid concentration of 1 mg/ml. Different amounts of this stock solution were dried under nitrogen and resuspended with the rabbit LA pool or Venticute to obtain surfactant preparations with increasing amounts of neutral lipids (0-25 % wt/wt of phospholipids).

RESULTS

Clinical results of the Venticute trial

Table 2

Variable	ARDS standard care	ARDS surfactant treatment
Patients alive on day 28, n (%)	13 (76.5)	10 (71.4)
Patients weaned and alive on day 28, n (%)	5 (29.4)	8 (57.1)
Ventilator-free days to day 28	0.0 (0.0-1.0)	15.5 (0.0-21.5)*
$AUC_{0\text{-}24hours}$, mm Hg · hr	220 (60-702)	402 (178-1390)
$AUC_{0\text{-}120hours}$, mm Hg · hr	4970 (48-7910)	5450 (4500-8060)

Table 2

Clinical Results

Data are presented as median (25-75 percentile)

$AUC_{0\text{-}24\ hours}$ = area under the Pa_{O2}/FI_{O2}-versus-time curve from 0 to 24 hours

$AUC_{0\text{-}120hours}$ = area under the Pa_{O2}/FI_{O2}-versus-time curve from 0 to 120 hours

Significance level is indicated by * p = 0.016 (ARDS surfactant treatment versus standard care)

Impact of neutral lipids on the surface activity of different surfactant preparations in vitro

We investigated the potential impact of neutral lipid mixtures, mimicking the neutral lipid profile in ARDS patients, on the surface acitivity of different surfactant preparations. γads values of the recombinant SP-C surfactant or a natural rabbit LA preparation were only slightly increased in presence of increasing amounts of neutral lipids (Figure 1). Next, we investigated the influence of increasing amounts of single neutral lipids on the surface activity of the recombinant SP-C surfactant. Monoglycerides and cholesterol, were the most effective in inhibiting the γads values (Figure 2).

FIGURE LEGENDS

Figure 1

Influence of neutral lipids on the surface activity of a recombinant SP-C-based surfactant preparation and of the large surfactant aggregate (LA) fraction of natural rabbit lung surfactant

A neutral lipid mixture containing 100 % unsaturated neutral lipids and mimicking the neutral lipid profile in ARDS patients was added in increasing amounts (0-25 % of phospholipids, wt/wt) to 2 mg/ml of a recombinant SP-C surfactant (squares) and to 2 mg/ml of the LA fraction of natural rabbit lung surfactant (circles). Furthermore, a neutral lipid mixture containing 50 % unsaturated neutral lipids was added in increasing amounts to recombinant SP-C surfactant (triangles). The surface tension values after 12 seconds film adsorption (γads) are given. Data are given as median. n = 8 for each concentration. NL = neutral lipids; PL = phospholipids.

Figure 2

Influence of single neutral lipids on the surface activity of a recombinant SP-C-based surfactant preparation

Single neutral lipids were added in increasing amounts (0-15 % of phospholipids, wt/wt) to 2 mg/ml of the recombinant SP-C surfactant. The surface tension values after 12 seconds film adsorption (γads) are given. Data are given as median. n = 8 for each concentration

Monoglycerides (diamonds); diglycerides (up triangles); cholesterol (circles); free fatty acids (down triangles); triglycerides (stars); cholesterolester (squares); NL = neutral lipids; PL = phospholipids.