

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER: 20-521

PHARMACOLOGY REVIEW(S)

JUL 24 1996

**DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
CORRESPONDENCE DATED 6/20/96**

NDA 20-521

REVIEWER : Young S. Choi, Ph.D.

DATE OF SUBMISSION :

Date Originated : 6/20/96.

Date FDA Received : 6/24/96.

Date Assignment Received : 7/10/96

Date Review Completed : 7/24/96

INFORMATION TO BE CONVEYED TO APPLICANT : Yes (), No (x).

**APPLICANT : ONY, Inc., Amherst, NY and Forest Inc., New York, NY
(Contact person : Michael Resin, Ph.D. (212) 421-7850).**

**NAME OF DRUG : Calf Lung Surfactant Extract Intratracheal Suspension (CLSE).
Trade : Infasurf (INF).**

CATEGORY : Pulmonary surfactant : Calf lung surfactant extract.

**CLINICAL INDICATION : For the prevention and treatment of respiratory distress
syndrome (RDS) in neonates.**

ROUTE OF ADMINISTRATION : Intratracheal instillation.

**DOSE : 3 mL/kg (= 105 mg phospholipid(PL)/kg), may be repeated 12 hours apart, up to 3
doses. Therefore, a total of 315 mg/kg within 24 hours.**

**DOSAGE FORM : 6 mL suspension containing 210 mg PL (= 35 mg/mL) in 0.9% NaCl for
a single use vial.**

PREVIOUS REVIEWS AND DATES :

Date of Submission : Review Date : Reviewer:

7/27/95(RS)

6/18/96

Y. S. Choi

COMMENTS :

This submission contained responses to Dr. Joseph Sun's telephone request to submit a clearance study in preterm lambs that was originally submitted to on January 6, 1992.

SUMMARY AND EVALUATION :

The applicant requested that the study on clearance of INF in 126 day gestation lambs should not be incorporated into the NDA since the information provided on 1/6/92 was on a preliminary pilot study, it was never verified and contained a mistake (in actuality, total alveolar PL was measured chemically, and the study did not use labeled DPPC as the protocol indicated and the sponsor did not know of this change). Also, the results that indicated 30% of the instilled INF remained in the alveolar space 24 hours later has already been incorporated in Section 5.3.1.1. of the NDA.

CONCLUSIONS :

1. Since the subject study has already been mentioned briefly in the original NDA review, and the submission does not change the final results of that study, the applicant's responses do not change the status of the NDA.
2. This NDA is approvable from the standpoint of preclinical data.

LETTER TO THE APPLICANT : None at this time.

/S/

Young S. Choi, Ph.D.
Pharmacologist

/S/

July 24, 1996

cc:
Original (NDA 20-521)
HFD-570/Division file
HFD-570/MO/Pina
HFD-570/Sun
HFD-570/Choi
HFD-570/CSO/Kuzmik
R/D by Y. S. Choi/7/24/96
Revised by Y. S. Choi/ / /96
Init. by J. Sun/ / /96
F/T by Y. S. Choi/ / /96, WP 0827T

**DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
ORIGINAL REVIEW (RS)**

NDA 20-521**REVIEWER : Young S. Choi, Ph.D.****DATE OF SUBMISSION :**

Date Originated : 7/27/95.

Date FDA Received : 7/31/95.

Date Assignment Received : 8/2/95

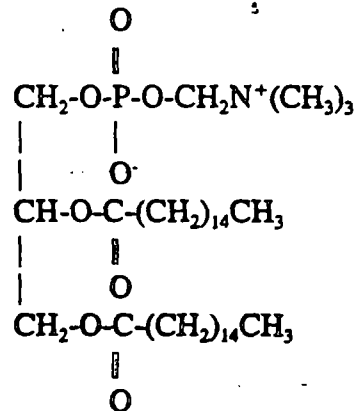
Date Review Completed : 6/17/96

INFORMATION TO BE CONVEYED TO SPONSOR : Yes (x), No ().

SPONSOR : ONY, Inc., Amherst, NY and Forest Inc., New York, NY
(Contact person : Michael Resin, Ph.D. (212) 421-7850).

NAME OF DRUG: Generic : Calf Lung Surfactant Extract Intratracheal Suspension (CLSE).
Trade : Infasurf (INF).
Chemical : Mixture of phospholipids (PL),
surfactant specific apoproteins (SP-B and SP-C).

STRUCTURE : The main component is dipalmitoyl phosphatidylcholine (DPPC), and its structure is shown below :



CATEGORY : Pulmonary surfactant : Calf lung surfactant extract.

CLINICAL INDICATION : For the prevention and treatment of respiratory distress syndrome (RDS) in neonates.

RELATED INDs/NDAs/DMFs/DRUGs :

ROUTE OF ADMINISTRATION : Intratracheal instillation.

DOSE : 3 mL/kg (= 105 mg PL/kg), may be repeated - 12 hours apart, up to 3 doses.
Therefore, the total of 315 mg/kg within 24 hours.

DOSAGE FORM : 6 mL suspension containing 210 mg PL (= 35 mg/mL) in 0.9% NaCl for a single use vial.

COMPOSITION : Main components included phospholipids,
and surfactant specific apoproteins B and C (SP-B and SP-C).

Each mL INF contains 35 mg PL (mg phosphatidylcholine, DPPC)
and mg SP-B and SP-C, and

PRECLINICAL STUDIES SUBMITTED/REVIEWED :

I. PHARMACOLOGY :

1. COMPARATIVE EFFICACY STUDY IN PREMATURE LAMBS :
Page 050010, Vol. 1.18.
2. EFFICACY OF INF IN PREMATURE LAMBS : Page 050036, Vol. 1.18
3. DOSE-RESPONSE STUDY IN PREMATURE LAMBS : Page 050057, Vol. 1.18..

II. PHARMACOKINETICS :

1. CLEARANCE/DISTRIBUTION STUDY OF INF IN ADULT RABBITS :
2. CLEARANCE/DISTRIBUTION STUDY OF INF IN PRETERM LAMBS :
3. HYPEROXIC LUNG INJURY REDUCES EXOGENOUS SURFACTANT
CLEARANCE IN VIVO : Page 050384, Vol. 1.18.

III. TOXICOLOGY :

1. ACUTE INTRATRACHEAL TOXICITY STUDY IN NEWBORN RABBITS :
Page 050168, Vol. 1.18.
2. 7 DAY INTRATRACHEAL TOXICITY STUDY IN NEWBORN PIGS.
Page 050191, Vol. 1.18.
3. SENSITIZATION STUDY IN GUINEA PIGS : Page 050281, Vol. 1.18.
4. ANTIGENICITY STUDY IN RABBITS : Page 050177, Vol. 1.18.
5. MUTAGENICITY STUDY (AMES TESTS) : Page 050321, Vol. 1.18.

SUMMARY AND EVALUATION :

The subject of this NDA is Infasurf (INF), also referred to as calf lung surfactant extract (CLSE), for the treatment and prevention of respiratory distress syndrome (RDS) of premature infants or with low birth weight.

Premature infants with RDS have been shown to be deficient in endogenous surfactant due to prematurity of the Type II cells in the lung; thus, INF will supplement the deficiency in surfactant to help these premature infants to survive.

Mechanism of action for pulmonary surfactant (decreased alveolar surface tension and stabilization of the alveoli during expiration) in premature animals as well as humans has been well established as with 2 previously marketed products.

The following 11 preclinical studies were submitted for this NDA:

- 3 efficacy studies in premature lambs,
- 5 toxicity studies (acute in newborn rabbits, 7 day study in newborn piglets, sensitization studies in guinea pigs, antigenicity study in rabbits, and Ames mutagenicity assays),
- 3 distribution/clearance studies in adult rabbits (2) and preterm lambs.

Most studies were performed by the sponsor's own labs.

In all three pharmacological studies in premature lambs with low PL content and artificial ventilation, INF at proposed single dose of 100 PL mg/kg was shown to produce reasonable efficacy, by showing improved pulmonary gas exchange and oxygenation, and/or lung compliance, and prolonged survival of these premature animals when compared to the untreated controls. Untreated premature lambs died due to respiratory failure within a few hours in spite of mechanical ventilation. Exogenous surfactant administration kept them alive much longer, some up to 24 hours with repeated dosings in some.

For pharmacokinetics, distribution and clearance of INF was studied in normal lungs of adult rabbits (2 studies) and premature lambs (126th day of gestation) after 125 mg/kg INF administered intratracheally (IT). At 6 hours postdosing, 30% of the administered dose was present in the lungs of adult rabbits; and at 24 hours post dosing, 20% of the administered dose was present. Less than 1% of the administered dose in adult rabbit was found in the liver, with negligible radioactivity in other tissues. In premature lambs, at 24 hours post dosing, 30% of the administered dose was in alveolar space, with the remaining material "becoming associated" with the lung tissue. Only a trace of radioactivity was reported in the blood or liver of premature lambs at 24 hours post dosing. Half time clearance of labeled INF

from normal rabbits was reported as approximately 12 hours for the lung lumen. In another study of hyperoxic lung injury model, groups of New Zealand adult rabbits were treated with a bolus of 125 mg INF after exposed to room air (controls) or 100% oxygen for 48 hours or 64 hours. More than 75% of administered surfactant remained in the lungs up to 24 hours in both groups. Forty eight hour oxygen exposure did not produce "visible signs" of lung injury, but 64 hour exposure did. Up to 48 hours, there were no significant differences between 2 groups (air and oxygen) for clearance of exogenous surfactant or Type II cell function (synthesis rate of phosphatidylcholine, PC). Oxygen treated group had significantly more labeled surfactant in the lungs. Thus significantly greater levels of labeled PC was found in the alveolar wash, but much lower synthesis rate of PC in Type II cells than controls. Rate of clearance in the control animals was 2.4% of INF/hour, but it was reduced to 1.3% per hour after exposure to 100% oxygen. Therefore, prolonged oxygen exposure caused lung injury and decreased clearance of exogenous INF from the lungs (half time clearance would be much longer), probably due to Type II cell dysfunction.

In an acute toxicity study in new born rabbits, a group of 39 one day old pups from 13 litters received a single dose of 100 mg/kg INF in 0.15 mL of 0.9% saline via percutaneous IT injection, 40 pups received 0.15 mL 0.9% saline, and 17 pups had no injection. The pups were kept for 2 weeks (11 from untreated, 27 from saline, 28 from INF) or 4 weeks (2 from untreated, 6 from saline and 4 from INF) before sacrifice. Clinical signs, survival and body weight changes were monitored. Mortality in the first 3 days after injection was 4 in the control group, 7 each in saline control and INF group, showing no significant differences between groups for mortality or survival rate (80% for untreated controls, 77% for saline controls and 70% for INF group). Pups from all groups grew normally for 4 weeks. Therefore, mortality rate within the first 3 days, body weight gain, lung weight, and lung histopathology showed no statistically significant differences between groups including the control group. Therefore, INF was not superior over untreated or saline control group in this study, although INF group showed slightly larger (3% over untreated) average weight at week 4. In two subsequent litters, 10 pups each were injected daily for 3 days with similar dose of INF and saline. None of the pups survived the 3 daily injections, showing intolerance to percutaneous intratracheal injections for 1-3 day old pups, but the deaths were not drug-related.

In a 7 day intratracheal toxicity study, groups of newborn (less than 48 hours old) piglets were treated by injection of 100 mg/kg/day (2.9 mL/kg) INF into the trachea, once/day, for 7 consecutive days, along with a group of untreated controls and vehicle (saline) control group. Total of 4 of 16 INF treated piglets died whereas none died in the untreated group : 2 died of suffocation (too rapid injection on day 1 and replaced), another one died of tissue change at the injection site on day 7, and one died on day 2 with liver hemorrhage due to trauma". One from vehicle control group, was killed moribund on day 3. Some piglets in 2 treated groups became apneic and required assistance with breathing, probably due to large volume and too rapid administration of INF or saline, and labored breathing was also noted sporadically from

day 3 through 22 in all 2 treated groups. Total of 6 (4/4 M + 1/1 F on day 8 plus 1/1 F on day 22) of 16 INF treated pigs showed slight to marked "irritation" (hemorrhage/congestion/edema with inflammatory infiltrates) at the injection sites, with swelling from day 2 to day 7 (they also received antibiotics), while none was reported in saline control group. All 2 treated groups (INF or Saline) had depressed body weight gain by days 8 and 22, especially M. No significant drug related changes were reported for INF treated groups for hematology or clinical chemistry values for blood samples collected at necropsy, nor any significant gross or histopathological findings reported except for the injection sites of INF.

Although INF did not produce any significant systemic toxicity in acute and 7 days treatment, overall outcome of INF treatment was not significantly superior to untreated piglets based on mortality rate (some deaths which may not be drug-related vs none in untreated controls) and irritation reaction at the injection sites of INF (vs none in the controls) in a 7 day toxicity study. Based on our experience with surfactant treated newborns, these findings are not considered unusual.

The maximal clinical dose is 105 mg/kg, up to 3 doses, a total of 315 mg/kg, over a 24 hour period, but 2 toxicity studies utilized only a single dose of 100 mg/kg/day. The dose of INF used in both acute and 7 day toxicity studies, 100 mg/kg, is only 1/3 of the clinically recommended maximal daily dose in premature infants.

Since sufficient clinical data would support safety of this product, only one subchronic toxicity is considered sufficient for this NDA, although toxicity studies in two species are usually required for NDA approval.

In a sensitization study, groups of Hartley albino guinea pigs were injected SC with 0.2 mL INF, 2% milk protein or 2% egg albumin solutions as positive controls for 7 consecutive days, along with an untreated control group. After 10 days, they were challenged with IP injection (0.2 mL) of either INF, milk protein or egg albumin. Guinea-pigs were observed immediately following challenge for signs of Type I systemic anaphylaxis. After the challenge, none from the untreated control group or INF group showed Type I systemic anaphylactic reactions except one female out of 10 in INF group. This is not surprising considering INF is of bovine origin, containing a small amount of surfactant specific apoproteins, SP-B and SP-C. All guinea pigs in 2 positive control groups showed signs of Type I systemic anaphylaxis (ear/nose twitch, pedaling, cyanosis and/or respiratory distress), including one fatal anaphylactic reaction in egg albumin group.

In an another antigenicity study of INF in rabbits, rabbit serum immunized intradermally (ID) with INF and adjuvant for 4 weeks clearly demonstrated significant antibody production, although a single IT dose of INF to newborn and adolescent rabbits produced only very low levels of antibodies in serum in some animals. For 4 months, their lungs looked normal and they showed normal behavior.

Antibody production have been demonstrated in animals for other surfactants of animal origin and also in human infants after another surfactant or milk, but pathological significance of this finding is not apparent.

A mutagenicity study was conducted

along with appropriate negative and positive control groups. The results of INF in Ames assays were negative.

CONCLUSIONS :

1. 100 mg/kg INF produced good efficacy in premature animals by improving oxygenation and lung compliance.
2. Half time clearance of labeled INF in adult rabbits was approximately 12 hours, and slightly longer in premature lambs. Prolonged 100% oxygen exposure injured lungs, and clearance of INF was reduced in injured lungs.
3. The results of acute IT toxicity studies in newborn rabbits and 7 day IT toxicity study in newborn piglets did not show INF being significantly superior to untreated controls or vehicle control group, based on mortality rate, survival rate, and incidence of pneumothorax. INF did not produce any significant unexpected toxicity nor systemic toxicity except for the irritation reaction (6/16) and swelling (days 2 to 7) at injection sites in the 7 day IT toxicity study in piglets.
4. Effects of INF in premature animals and newborn animals are different due to differences in amount of baseline surfactant levels in the lungs between prematured and matured animals.
5. INF showed a very slight potential in Type I hypersensitivity testing in guinea pigs. INF also produced measurable antibody formation in serum of rabbits when administered with adjuvant.
6. Since sufficient clinical data support safety of this product, this NDA is considered approvable.

RECOMMENDATIONS :

1. This NDA is approvable based on preclinical data.
2. Some modification of the labeling is needed. See the letter to the applicant.

[Redacted] /S/

Young S. Choi, Ph.D.

Pharmacologist

[Redacted] /S/

June 17, 1996

cc:

Original (NDA 20-521)

HFD-570/Division file

HFD-570/MO/Pina

HFD-570/Sun

HFD-570/Choi

HFD-570/CSO/Kuzmik

R/D by Y. S. Choi/6/11/96

Revised by Y. S. Choi/6/17/96

Init. by J. Sun/6/17/96

F/T by Y. S. Choi/ 6/17/96, WP 0809T-3

APPEARS THIS WAY
ON ORIGINAL

NDA 20-521

Page 8

LETTER TO THE APPLICANT :

We have reviewed your NDA resubmission dated 7/27/95 and have the following comments on the preclinical section :

The section of Carcinogenesis, Mutagenicity and Impairment Fertility in the labeling needs the following modification : Carcinogenicity or reproductive studies were not performed with Infasurf. Results of a mutagenicity study (Ames assay) of Infasurf were negative.

**APPEARS THIS WAY
ON ORIGINAL**

NOTE : Portions of this review were excerpted from the sponsor's submission.

REVIEW OF PRECLINICAL DATA :

COMMENTS : Most studies were submitted and previously reviewed and they will be summarized below. Two studies (mutagenicity and hyperoxic lung injury in adult rabbits) are new studies and they will be reviewed in this NDA.

I. PHARMACOLOGICAL STUDIES :

A. A CONTROLLED CLINICAL COMPARISON OF FOUR DIFFERENT SURFACTANT PREPARATIONS IN SURFACTANT-DEFICIENT PRETERM LAMBS :

COMMENT : This study was submitted originally on 4/4/91

Methods : Premature lambs from a twin pregnancy were surgically delivered prematurely on day 126 ± 1 gestational age (full term = 137-145 days). Five groups of 5-6/group of lambs were treated as follows with bolus via endotracheal tube, just before the onset of mechanical ventilation after delivery, except Exosurf that was administered 1-3 minutes after initiation of ventilation, in 2 aliquotes.

Group: Treatment :

- 1: Untreated control : ventilated without any surfactant.
- 2: "Natural" Sheep Surfactant (NSS, 100 mg phospholipids/kg, 5 mL/kg).
- 3: Survanta (SUV, 100 mg phospholipids/kg, 4 mL/kg)
- 4: Infasurf (INF, 105 mg phospholipids/kg, 3 mL/kg)
- 5: Exosurf (EXO, 67.5 mg phospholipids/kg, 5 mL/kg).

Ten mL of fetal lung fluid were collected before and after treatment from each lamb for defemination of PL and protein content. Each lamb was reported as receiving a single dose. Some lambs received repeated doses, if the lamb survived 12 hours. Each lamb was monitored, and pulmonary function and body weight were determined; and after death, pressure-volume curves of lungs were also determined.

RESULTS :

- a). All lambs surgically delivered on day 126 ± 1 gestation had premature lungs as

shown by low PL content.

- b). Untreated premature lambs died due to respiratory failure within a few hours in spite of mechanical ventilation, but exogenous surfactant administration kept them alive much longer, some up to 24 hours with repeated dosings.
- c). Survival, oxygenation, and lung compliance for INF were better than or equal to NSS, followed by SUR, then EXO, the poorest. Pressure-volume curves of lungs after death showed similar results for each of these surfactants.
- d). After surfactant treatments, PL concentration in the lung lavage fluid of all 4 surfactant treated groups was over 8 times greater than that of untreated control group, although the values were not significantly different between surfactant treated groups.
- e). Protein concentration of lung lavage fluids also was not significantly different between groups, including the control group. This is not surprising, since premature lungs have air leaks as well as edema.
- f). All surfactants produced pneumothorax. This (pneumothorax) is also a well established adverse finding in premature laboratory animals as well as premature human infants, along with intracranial hemorrhage.
- g). Average body weight of premature lambs in EXO group was slightly smaller than that of SUR group or NSS.

The quantitative comparison of all these effects between different surfactants is difficult in this study, primarily due to use of different doses of surfactants and different time of surfactant administration in relation to the initiation of ventilation in these premature lambs. Thus, the design and procedure used in this study are not adequate to make a valid conclusion that any one of these surfactants has greater efficacy, and hence safety. However, INF produced good signs of efficacy in this premature lamb model.

2. EFFICACY OF INF IN PREMATURE LAMB :

COMMENTS : This study was submitted in the amendment of 7/20/87

Methods : A total of 36 premature lambs had instillation of INF into the lungs either 15 or 100 mg/kg INF (not 30 mg/kg as reported in summary table) before breathing, and they were kept with ventilation 6 to 48 hours with blood pressure, heart rate and temperature monitoring.

RESULTS : No hyperthermia, no hypotension or no systemic toxicity, with an improvement of lung compliance and blood gas exchange were reported.

3. DOSE-RESPONSE STUDY IN PREMATURE LAMB :

COMMENT : This study was submitted in the amendment dated 1/6/92.

Methods: Lambs delivered surgically either on day 127 gestation (n=18) or day 133 gestation (n=14) were intubated while they were still connected to the umbilical cord, and 4-8 lambs each were treated with either 15 mg/kg or 100 mg/kg INF via endotracheal tube, along with 5 controls each. Subsequently, the umbilical circulation was cut, and mechanical ventilation was started with 100% O₂, and various parameters were monitored up to 12 hours.

RESULTS :

- a). Both 15 and 100 mg/kg INF were efficacious for both 127 and 133 day gestation lambs up to 12 hours, and resulted in significant improvement of oxygenation and lung compliance (\uparrow PaO₂ and \downarrow PaCO₂), \downarrow wet lung weight ratios (\downarrow water content), and \downarrow pneumothorax when compared to the respective controls.
- b). The \uparrow PaO₂ was reported as almost immediate in both groups, but the increase was approximately 50% greater after 100 mg/kg than 15 mg/kg.
- c). Pressure-volume (P-V) curves of the lungs showed a dose-related increase in lung volume over the respective controls in both 127 and 133 day gestation lambs. Therefore, 15 and 100 mg/kg showed a dose-related efficacy response.

II. PHARMACOKINETICS :

1. CLEARANCE/DISTRIBUTION OF INFASURF IN ADULT RABBITS AND PREMATURE LAMBS :

COMMENTS : Two clearance/distribution studies in normal adult rabbits and premature lambs were originally submitted in amendment of 1/6/92 and they were reviewed together.

Methods : 125 mg DPPC-INF in 4 mL saline, containing 35 mg PL/mL with radioactivity of 0.8 μ Ci/mL was administered intratracheally to 24 normal adult rabbits or an unknown number of premature lambs (which

were surgically delivered on day 126 of gestation). It was not clear how many or if any control group was included in any of the studies. They were killed at 6 and 24 hours post dosing, and samples were taken of bronchoalveolar lavage material, lung tissue, blood, liver, kidney, heart, and adipose tissue. Radioactivity of phospholipids (DPPC) was determined from tissue homogenates.

RESULTS :

- a). Tritium labeled INF cleared rapidly from the lungs of adult rabbits and "slightly" more rapidly from the lungs from preterm lambs. This pattern has been established with other surfactants.
- b). At 6 hours postdosing, 30% of the administered dose was reported as present in the lungs of adult rabbits; and at 24 hours post dosing, 20% of the administered dose was reported as present in alveolar space of adult rabbits. Less than 1% of the administered dose in adult rabbits was found in the liver, with negligible radioactivity in other tissues.
- c). In premature lambs, at 24 hours post dosing, 30% of the administered dose was in alveolar space, with the remaining material "becoming associated" with the lung tissue. Only a trace of radioactivity was reported in the blood or liver of premature lambs at 24 hours post dosing.
- d). "Half time clearance" of labeled INF from normal rabbits was reported as approximately 12 hours for the lung lumen.

2. HYPEROXIC LUNG INJURY REDUCES EXOGENOUS SURFACTANT CLEARANCE IN VIVO :

COMMENT : This study is a new study submitted to this NDA.

Methods : Groups of 72 New Zealand White adult rabbits were treated IT with a bolus dose of 125 mg labeled INF in 4 mL saline after they were exposed to room air (controls) or 100% oxygen for 48 hours or 64 hours. Subgroups of rabbits were sacrificed at 10 minutes, 6 hour and 24 hours post dosing for determination of radiolabeled phospholipid in alveolar wash, lung tissue, alveolar Type II cells and liver, heart and kidneys.

RESULTS :

- a). Forty-eight hour oxygen exposure did not produce "visible signs" of lung injury, but 64 hour exposure did (increasing protein content in alveolar wash).

- b). More than 75% of administered surfactant remained in the lungs up to 24 hours in both groups.
- c). Oxygen up to 48 hours had no significant effect on clearance of exogenous surfactant or Type II cell function as measured by synthesis of phosphatidylcholine (PC).
- d). Oxygen treated group had significantly more labeled surfactant in the lungs, and its alveolar wash contained significantly greater levels of labeled PC than the controls ($44 \pm 9\%$ vs $27 \pm 5\%$ in controls at 6 hours and $27 \pm 2\%$ at 24 hours, vs $6 \pm 1\%$ in controls).
- e). Type II cells in oxygen treated group showed much lower rate of synthesis for PC than that of controls : at 6 hours, 24 ± 2 cpm/ 10^6 cells vs 38 ± 7 cpm/ 10^6 cells in controls and at 24 hours, 42 ± 5 cpm/ 10^6 cells vs 70 ± 12 cpm/ 10^6 cells in controls.
- f). Control animals cleared approximately 2.4% of INF/hour from the alveolar space. Exposure to 100% oxygen reduced this clearance rate to 1.3%/hour, thus resulted in increased half time clearance to "approximately 20 hours", probably due to Type II cell dysfunction.
- g). Other tissues except the lungs had radioactivity below the detectable limits and/or background.

III. TOXICITY STUDIES :

1. ACUTE TOXICITY STUDY IN NEWBORN RABBITS :

COMMENTS : This study was submitted in the amendment of 7/20/87

Methods : From 13 litters, a group of 39 one day old pups received a single dose of 100 mg/kg INF in 0.15 mL of 0.9% saline via percutaneous intratracheal injection, 40 pups received 0.15 mL 0.9% saline, and 17 pups had no injection (not 20-46/group as reported in a summary table). The pups were kept for 2 weeks (11 from untreated, 27 from saline, 28 from INF) or 4 weeks (2 from untreated, 6 from saline and 4 from INF) before sacrifice. Clinical signs, survival and body weight changes were monitored. In the subsequent litters, 10 pups each were injected daily for 3 days with 100 mg/kg INF and saline.

RESULTS :

Pups that died in the first 3 days after injection : 4 in the control group, 7 each in saline control and INF group, showing no significant differences between groups for mortality or survival rate (80% for untreated controls, 77% for vehicle controls and 70% for INF). Pups from all groups grew normally for 4 weeks : Average body weight of the untreated controls at week 2 was approximately 10% larger than other 2 groups (the values from 27 saline controls were slightly larger than those from 28 INF group), but at the week 4, the average body weight was INF(3%) > untreated > saline (8% ↓), showing no significant differences between groups in body weight gain. Lung weights at weeks 2 and 4 showed no significant differences between 3 groups, and histopathology of all lungs from INF or controls were interpreted as "normal".

In two subsequent litters, 10 pups each were injected daily for 3 days with similar dose of INF and saline. None of the pups survived the 3 daily injections, showing intolerance of percutaneous intratracheal injections for 1-3 day old pups, which was not drug-related.

2. SEVEN DAY INTRATRACHEAL TOXICITY STUDY IN NEWBORN PIGS :

Study No. : 403A-601-930-90 : Study Duration : 2/19/90-3/23/90).

COMMENT : This study was originally submitted in the amendment dated 6/5/90

Lab Performing the Study :

GLP Statements : Yes, submitted.

Methods : 2 groups (4 M + 4 F/group) of newborn (less than 48 hours old) piglets were treated with 100 mg/kg/day (2.9 mL/kg) INF by injection into the trachea, once/day, for 7 consecutive days. One group was killed on day 8 and the other group was kept an additional 14 days without any treatment, then killed on day 22. A group of 4 M + 4 F piglets served as untreated controls, and they were killed on day 22. Another group of 8 M + 8 F piglets was treated with 2.9 mL/kg 0.9% sodium chloride solution (vehicle control). One half of vehicle control group was killed on day 8; and the remaining half, on day 22. All were killed by cardiac puncture under pentobarbital anesthesia after overnight fasting and blood collection. All piglets were examined grossly, and selective tissues were examined for histopathology.

RESULTS :

Total of 4 of 16 INF treated piglets died whereas none died in the untreated group : 2 died of suffocation (too rapid injection on day 1 and replaced), another one died of "tissue change at the injection site" on day 7, and one died on day 2 with liver hemorrhage "due to trauma". One from vehicle control group, was killed moribund on day 3. "Several" piglets in 2 treated groups became apneic and required assistance with breathing, probably due to large volume and too rapid administration of INF or saline, and labored breathing was also noted "sporadically" from day 3 through 22 in all 3 treated groups. Total of 6 (4/4 M + 1/1 F on day 8 plus 1/1 F on day 22) of 16 INF treated pigs showed slight to marked "irritation" (hemorrhage/congestion/edema with inflammatory infiltrates) at the injection sites, with swelling from day 2 to day 7 (they also received antibiotics), compared to none in saline control group. All treated groups (INF or Saline) had depressed body weight gain by days 8 and 22, especially M. No significant drug related changes were reported for INF treated groups for hematology or clinical chemistry values for blood samples collected at necropsy, nor any significant gross or histopathological findings except for the injection sites of INF.

COMMENTS : Although INF did not produce any significant systemic toxicity after 7 days treatment; overall outcome of INF treatment was not significantly superior to untreated piglets in this study based on mortality rate, survival rate and irritation reactions at the injection sites of INF. However, based on our experience with surfactant treated newborns, the finding on mortality rates in toxicity studies are not considered unusual.

No coagulation time, differential for WBC, urinalysis, or organ weights were done/reported in this study.

3. SENSITIZATION STUDY IN GUINEA PIGS :

COMMENT : This study was originally submitted in amendment dated 6/5/90

Methods : 2-5 M + 2-5 F/group of Hartley albino guinea pigs were injected SC with 0.2 mL INF, 2% milk protein or 2% egg albumin solutions for 7 consecutive days, along with an untreated control group. After 10 days, they were challenged with IP injection (0.2 mL) of either INF (INF and untreated groups) or milk protein or egg albumin. Guinea-pigs were observed immediately following challenge for signs of Type I systemic anaphylaxis.

RESULTS :

After the challenge, none from the untreated control group and INF group showed Type I systemic anaphylactic reactions except one F out of 10 in INF group. This F from INF group showed a noticeable head shake and hyperactivity, indicating some reactivity of INF. All guinea pigs in 2 positive control groups showed signs of Type I systemic anaphylaxis (ear/nose twitch, pedaling, cyanosis and/or respiratory distress), including one fatal anaphylactic reaction in egg albumin group. Necropsy of the dead guinea pig showed edema around larynx, suggesting asphyxiation as the cause of death. No significant differences in weight gain were shown between groups although egg albumin treated M gained somewhat less weight when compared to other groups.

COMMENTS:

- a). The positive result is not surprising considering INF is of bovine origin, containing a small amount of surfactant specific apoproteins, SP-B and SP-C.
- b). The challenge by IP injection in this test is generally much less effective, when compared to IV injection, that usually produces 100% anaphylaxis and/or 100% mortality in the egg albumin group.

4. ANTIGENICITY STUDY IN RABBITS :

COMMENT: The original report of this study was submitted in amendment dated 7/20/87 as a part of a summary report.

Methods: Three rabbits (adolescent) were treated with intradermal (ID) injection of 100 mg/kg INF (?dose was not clear in the original submission) with complete adjuvant for 4 weeks, and 4 rabbits received a single IT dose of INF. Groups of newborn rabbits (1 or 2 day old) were treated with a single dose of INF 100 mg/kg injection into the trachea (IT). Serum was obtained at week 4, and anti-CLSE antibodies were determined by :
until reading of ≥ 0.1 at 405 nm was obtained.

RESULTS :

- a). Serum from all 3 adolescent rabbits treated ID with INF plus adjuvant showed significant antibodies over the preimmune shelf control levels. One of 4 adolescent rabbits after a single IT dose of INF had 4 times the antibodies by week 6 over the preimmune self control level, and the antibody level was still 2 times the preimmune level at week 19 showing persistence.

- b). Serum from newborn rabbits treated IT-with INF showed very low levels of antibodies in "some" animals. The serum from newborns after a single IT dose of INF at 1:80 dilution showed significantly more antibodies (24%) than vehicle (4%) whereas untreated showed none (0%).
- c). There were no significant differences between groups in body weight at weeks 2 or 4, lung histology was normal, and all animals showed normal behavior during 19 week observation period.

5. MUTAGENICITY STUDIES :

MUTAGENICITY ASSAY : Sponsor Project Number : T/3700/0009 :

COMMENT : This study is a new study submitted for this NDA.

GLP Statements : Yes, submitted.

Criteria for Valid Test : All cultures must demonstrate the characteristic mean number for spontaneous revertants in the vehicle control as shown below :

RESULTS :

- a). Results of dose-ranging study showed no cytotoxicity with 10 concentrations of INF from [redacted] but slight precipitate was noted with INF [redacted]

- b). Results of mutagenicity assays were negative except slight precipitate formed at all concentrations of INF, with/without metabolic activation. Positive control groups showed at least 4 to 147 fold increases of mean revertants/plate.

IV. LABELING :

The submitted labeling for preclinical part needs following modification under the section on Carcinogenesis, Mutagenicity and Fertility : Carcinogenesis and reproductive studies have not been performed. Results of a mutagenicity study (Ames assay) were negative.

**APPEARS THIS WAY
ON ORIGINAL**