ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Aug; 20 (8): 691 – 695
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Original Research

Comparison of effects of surfactant and inhaled nitric oxide in rabbits with surfactant-depleted respiratory failure[1]

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KEY WORDS adult respiratory distress syndrome; lung compliance; nitric oxide; phosphatidylcholines; pulmonary surfactants; respiratory therapy; bronchoalveolar lavage; anoxia; respiratory insufficiency; phospholipids

ABSTRACT

AIM: To compare effects of pulmonary surfactant and inhaled nitric oxide (iNO) in improvement of survival and blood oxygenation in ventilated rabbits with acute hypoxic respiratory failure induced by repeated bronchoalveolar lavage (BAL). **METHODS**: After BAL all the rabbits had more than 50 % reduction of dynamic lung compliance (C_{dyn}), 50 % increment of resistance of respiratory system (R_{rs}), and an increase of mean oxygenation index (OI) from 1 to 22. The rabbits were then randomly allocated to groups receiving (1) mechanical ventilation only (Control), (2) iNO 0.8 $\mu mol \cdot L^{-1}$ (20 ppm) (NO), (3) intratracheal bolus surfactant phospholipids at 100 $mg \cdot kg^{-1}$ (Surf), and (4) combined surfactant at 100 $mg \cdot kg^{-1}$ with inhaled NO at 0.8 $\mu mol \cdot L^{-1}$ (Surf + NO). All the rabbits were ventilated with standardized tidal volume (8 – 10 $mL \cdot kg^{-1}$) for another 8 h or until early death. **RESULTS**: The rabbits in both control and NO groups had the lowest survival rate, deterioration of lung mechanics and OI, whereas those in the Surf and Surf + NO groups had modestly improved C_{dyn}, R_{rs}, and OI. Only rabbits in the Surf + NO group had significantly improved survival rate and alveolar expansion. **CONCLUSION**: Surfactant with or without iNO is more effective compared to the control and iNO groups in rabbit, suggesting that iNO is not effective unless a method to recruit alveoli is applied.

INTRODUCTION

Inhaled nitric oxide (iNO), a new therapy to selectively dilate pulmonary resistant vessels and improve blood oxygenation, has been introduced into respiratory and critical care medicine since early 90's, mainly for persistent pulmonary hypertension of the newborns (PPHN) and hypoxic respiratory failure in children and adult patients. Its clinical effects are variable[1,2], partly because the underlying diseases in these patients are complex, often accompanied with acute lung injury (ALI) in which the pathogenesis is associated with inflammatory damage, surfactant deficiency and dysfunction, impairment and dysfunction of antioxidant system, and other mechanisms. The selective vasodilatation of iNO depends on efficient intrapulmonary diffusion of NO, and response of GTP-cGMP pathway that results in protein phosphorylation, reversed calcium influx and relaxation of intrapulmonary vascular smooth muscle cells[3]. Pulmonary surfactant is a phospholipid-protein complex produced by the type Ⅱ alveolar epithelial cells. It reduces surface tension of alveolar lining layer, stabilises alveoli during respiration, and facilitates alveolar aeration. Exoge-

[1] Project supported in part by grants from China Medical Board of New York (No 95-620), Shanghai Educational Development Foundation (No 95SG10), Shanghai Bureau of Health Research Funds (No 97BR023), and a travel grant by Shanghai Medical University.
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Received 1998-12-21 Accepted 1999-05-20

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Aug; 20 (8)
E-mail aps@server.shcnc.ac.cn Phn Fax 86-21-6474-2629

nous surfactant is routinely used for neonatal respiratory distress syndrome (RDS), and its application for infant and adult with ALI and hypoxic respiratory failure is under extensive investigation[4,5]. Current clinical studies have shown that, in collapsed lung region, diffusion of NO into intrapulmonary vasculature may be hampered by high alveolar surface tension, edema fluid and hypoxic impairment of the vasculature. In order to enhance the effect of iNO, we supplied surfactant intratracheally to facilitate diffusion of NO in the collapsed rabbit lungs induced by repeated bronchoalveolar lavage (BAL) with saline, and evaluated lung function, blood gas exchange, and survival rate.

MATERIALS AND METHODS

Surfactant Preparation of porcine lung surfactant phospholipid extract was performed by lung lavage, centrifugation, chemical extraction, filtration and saline suspension as reported elsewhere[6]. Stock surfactant preparation was 100 mg phospholipids in 2.5 mL of steriled normal saline.

Nitric oxide Stock gas (Shanghai BOC, Shanghai, China) was obtained as NO at 40 $\mu mol \cdot L^{-1}$ (1000 ppm), balanced in nitrogen (purity >99.999 %), containing less than 0.5 % nitrogen dioxide (NO_2) relative to NO. It was supplied to the inspiratory line of the ventilator circuit about 20 cm proximal to the endotracheal tubing connector. Details of the NO gas flow control and monitoring was reported elsewhere[6].

Animal management Thirty healthy adult New Zealand white rabbits (2.6 ± 0.2 kg) were sedated with im diazepam (2 $mg \cdot kg^{-1}$), and anaesthetised with iv 1 % pentobarbital sodium (2 $mL \cdot kg^{-1}$). Rabbits were tracheotomized, intubated, and ventilated mechanically with a ventilator (Newport Wave E-200) set at pressure control mode to provide a tidal volume (V_T) of 8 – 10 $mL \cdot kg^{-1}$, a frequency of 25/min, inspiration-to-expiration time ratio of 1:2, and inspired fraction of oxygen (FiO_2) of 0.21. Baseline blood pH, PaO_2, and $PaCO_2$ were measured using an automated blood gas analyser. Baseline dynamic compliance (C_{dyn}) and resistance of the respiratory system (R_{rs}) were measured with a pneumotachograph GM 250 Navigator (Newport), and were expressed as $mL \cdot cmH_2O^{-1} \cdot kg^{-1}$ and $cmH_2O \cdot L^{-1} \cdot s^{-1}$, respectively (1 cm H_2O = 98.0665 Pa).

Bronchoalveolar lavage (BAL) The rabbits were disconnected from the ventilator, and a volume of 40 $mL \cdot kg^{-1}$ of body weight of 0.9 % NaCl at 37 ℃ was infused into and withdrawn from the lungs 3 times, then collected, and the animal was reconnected to the ventilator. This procedure was repeated twice at 10 min interval, and a total of 9 washes was applied. More than 85 % of the instilled BAL fluid was collected from each animal. The BAL fluid was immediately centrifuged at 200 × g and 4 ℃ for 10 min to remove cell debris, and the supernatant was stored at −20 ℃ for biochemical analysis.

From the first lavage, ventilator frequency was increased to 40/min, peak inspiratory pressure (PIP) was raised to 25 cmH_2O or higher, and positive end-expiratory pressure (PEEP) at 4 cmH_2O was applied to keep V_T at 8 – 10 $mL \cdot kg^{-1}$ and $PaCO_2$ at 4.7 – 6.0 kPa. After last BAL, the rabbits were ventilated for 30 – 60 min until PaO_2 decreased to less than 16 kPa and a reduction of C_{dyn} by more than 30 % from the corresponding baseline level. This moment was regarded as treatment time 0 h and the rabbits were then subjected to the experimental protocols. Lactated Ringer's solution and bicarbonate sodium were provided intravenously when necessary.

Experiment protocols The rabbits were randomly allocated to four treatment groups receiving (1) mechanical ventilation only (Control); (2) inhalation of 0.8 $\mu mol \cdot L^{-1}$ (20 ppm) NO (NO); (3) intratracheal instillation of a bolus surfactant at 100 mg phospholipids $\cdot kg^{-1}$ body weight (Surf); and (4) combined treatment as for the groups 2 and 3 (Surf + NO). All the rabbits were subsequently ventilated for another 8 h, or until early death, and values for C_{dyn}, R_{rs}, and arterial pH, PaO_2, $PaCO_2$ were measured each hour. Oxygenation index (OI, $FiO_2 \times MAP \times 100 / PaO_2$) was used for evaluation of blood oxygenation, where MAP is mean airway pressure (in cmH_2O) recorded from the ventilator. At the end of the period of ventilation, rabbits were sacrificed by an overdose of 5 % pentobarbital sodium and the animal lungs were processed.

Biochemical assays In order to quantitate amount of surfactant phospholipids in alveolar space, BAL fluid samples were extracted with chloroform/methanol (2:1, vol/vol) to isolate the phospholipids in

the chloroform phase. Disaturated phosphatidylcholine (DSPC) was separated from other phospholipids[7]. DSPC and total phospholipids (TPL) were determined according to the method described by Bartlett[8]. Values for TPL are presented as $mg \cdot kg^{-1}$, and those for DSPC as percentage of TPL (DSPC/TPL). Total proteins (TP) in BAL fluid were measured[9], and are presented as $mg \cdot kg^{-1}$. The DSPC and TP ratio was expressed as mg/g.

Measurements of nitrite/nitrate and methemoglobin (MetHb) Blood and urine nitrite/nitrate at baseline, treatment time 0 and 4 h were measured using a modified method using Griess reagent[10], and values are expressed as $\mu mol \cdot L^{-1}$ of serum or urine. MetHb was determined[11], and expressed as percentage of total hemoglobin (Hb).

Histologic and morphometric examination of lungs Lungs from 4 – 7 rabbits of each group were fixed by pulmonary artery perfusion with 4 % formaldehyde[6]. Sections stained with hematoxylin and eosin were examined by light microscopy for evidence of lung injury, as described elsewhere[1,12]. Lung expansion was quantified by the point-counting method, and expressed as volume density (V_V) of aerated alveolar spaces[1,13]. Fifty fields of each lung section were examined from each animal (magnification: × 300), and field-to-field variability was determined by calculating the coefficient of variation of V_V (CV[V_V]). A low value of CV[V_V] indicates homogeneity of alveolar aeration.

Statistics Data are presented as $\bar{x} \pm s$. Survival rate was examined with chi-square and Fisher's exact test. Analysis of variance (ANOVA) and Student-Newman-Keuls post hoc test were applied to parametric data. Within-group differences were determined with the Wilcoxon signed-rank test.

RESULTS

General conditions of the rabbits There was no significant difference of baseline values of physiological parameters across the groups. The survival rate in the Surf + NO group was higher than that in the control group ($P < 0.05$) (Tab 1).

Lung function measurements Values for OI, C_{dyn}, and R_{rs} at baseline level and during the treatment period are shown in Tab 2. BAL resulted in all the rabbits a significant reduction of C_{dyn} and an increase of R_{rs} by approximately 50 %, respectively, from corresponding baseline levels, and increase of mean values of OI from 1 to 22 in 60 min ($P < 0.05$). During the treatment period, there was no improvement of OI in the NO group. In contrast, OI in both Surf and Surf + NO groups were improved moderately, with

Tab 1. General conditions of the rabbits, and ratio of disaturated phosphatidylcholine (DSPC) and total proteins (TP) in bronchoalveolar lavage fluid. $\bar{x} \pm s$. $^bP < 0.05$ *vs* **NO group.**

Group		Control	NO	Surf	Surf + NO
n		8	6	8	8
Body weight/kg		2.5 ± 0.2	2.7 ± 0.2	2.6 ± 0.1	2.6 ± 0.2
Survival	3 h	5	6	8	8
	6 h	0	1	7^b	7^b
	8 h	0	1	5	6^b
DSPC/TP /$mg \cdot g^{-1}$		159 ± 106	104 ± 46	105 ± 48	139 ± 102

Tab 2. Oxygenation index, dynamic compliance and resistance of respiratory system at baseline and different treatment time in experimental rabbits. $n = 6 - 8$ **at baseline and 0 – 1 h;** $n = 5 - 8$ **at 3 h;** $n = 1 - 6$ **at 8 h.** $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **control.** $^eP < 0.05$, $^fP < 0.01$ *vs* **NO group (ANOVA).** $^hP < 0.05$ *vs* **corresponding values at baseline (within-group test).**

Group	Control	NO	Surf	Surf + NO
	Oxygenation index			
Baseline	1.5 ± 0.3	1.2 ± 0.5	1.2 ± 0.2	1.3 ± 0.4
0 h	22.5 ± 9.8^h	19.9 ± 7.4^h	22.1 ± 6.7^h	21.0 ± 8.8^h
1 h	24.2 ± 5.6	31.3 ± 9.0	14.2 ± 5.4^{bf}	12.0 ± 7.3^{ct}
3 h	24.9 ± 8.1	29.4 ± 8.4	18.3 ± 9.5	15.9 ± 7.4^e
8 h	–	23.9 ± 0	16.5 ± 5.3	10.4 ± 6.2
	Dynamic compliance/$mL \cdot cmH_2O^{-1} \cdot kg^{-1}$			
Baseline	1.01 ± 0.15	1.05 ± 0.25	1.11 ± 0.19	1.09 ± 0.21
0 h	0.52 ± 0.17^h	0.51 ± 0.15^h	0.60 ± 0.11^h	0.57 ± 0.09^h
1 h	0.30 ± 0.18	0.40 ± 0.10	0.68 ± 0.20^e	0.48 ± 0.22
3 h	0.40 ± 0.09	0.32 ± 0.10	0.56 ± 0.15^f	0.55 ± 0.12^f
8 h	–	0.26 ± 0	0.51 ± 0.05	0.54 ± 0.05
	Respiratory resistance/$cmH_2O \cdot L^{-1} \cdot s^{-1}$			
Baseline	34.6 ± 12.6	32.9 ± 6.3	38.4 ± 6.6	38.3 ± 10.0
0 h	66.9 ± 20.8	49.2 ± 12.7^h	54.3 ± 10.8^h	49.0 ± 15.3^h
1 h	109.6 ± 51.0	64.3 ± 19.8^b	57.6 ± 18.0^b	50.8 ± 23.1^c
3 h	78.8 ± 21.7	69.0 ± 24.7	55.1 ± 10.5	54.5 ± 8.4
8 h	–	29.0 ± 0	62.2 ± 7.9	57.7 ± 12.5

Baseline was the moment when induction of lung injury by bronchoalveolar lavage was not initiated.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Aug; 20 (8)
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a tendency in favor of Surf + NO group at 6 – 8 h of treatment. However, average levels of C_{dyn} and R_{rs} for Surf and Surf + NO groups during treatment remained unchanged compared with the corresponding values at time 0 h.

Chemical analysis of BAL fluid In all the groups, mean values of TPL were about 9 – 12 mg·kg^{-1} whereof approximately 50 % was DSPC. TP in BAL fluid were about 40 – 55 mg·kg^{-1}, and values for DSPC/TP in BAL fluid were presented in Tab 1. No marked difference was found between the groups for these parameters.

Measurements of nitrite/nitrate and methemoglobin Values of MetHb in each group were all less than 1 % of the total hemoglobin. There were no substantial changes for both serum and urinary nitrite/nitrate levels during iNO in both NO and Surf + NO groups (Tab 3).

Tab 3. Nitrite/nitrate (the sum of nitrite and nitrate in μmol·L^{-1}) in serum and urine. Numbers in parenthesis. $\bar{x} \pm s$.

Group	Control	NO	Surf	Surf + NO
Serum nitrite/nitrate				
Baseline	155 ± 48 (7)	104 ± 58 (6)	74 ± 54 (8)	83 ± 45 (8)
0 h	122 ± 32 (6)	103 ± 29 (6)	105 ± 80 (8)	113 ± 43 (8)
4 h	137 (1)	129 ± 31 (5)	102 ± 70 (7)	122 ± 28 (7)
Urinary nitrite/nitrate				
Baseline	613 ± 278 (7)	686 ± 386 (6)	519 ± 360 (8)	766 ± 568 (8)
0 h	330 ± 297 (4)	642 ± 226 (6)	648 ± 448 (7)	504 ± 228 (8)
4 h	714 (1)	596 ± 271 (5)	583 ± 417 (7)	417 ± 103 (7)

Histological and morphometric findings In all the four groups, atelectasis, edema, hyaline membranes, intraalveolar patchy hemorrhage and infiltration of neutrophils in the lungs were found. In the Surf and Surf + NO groups, there were modestly improved aeration of alveoli and less severe edema, hyaline membranes, hemorrhage and infiltration of neutrophils compared to those in the Control and NO groups. Aeration of alveoli was improved in the Surf + NO group as reflected by increased V_V and low values for CV(V_V), but in the NO and Surf groups there was only modest improvement of CV (V_V) (Tab 4).

Tab 4. Morphometric analysis of alveolar expansion (V_V) and coefficiency of variation (field-to-field variability) of V_V[CV(V_V)]. $\bar{x} \pm s$.
$^bP < 0.05$, $^cP < 0.01$ *vs* control.

Group	Control	NO	Surf	Surf + NO
n	6	5	4	7
V_V	0.53 ± 0.05	0.58 ± 0.07	0.61 ± 0.03	0.63 ± 0.05^b
CV(V_V)	0.39 ± 0.08	0.30 ± 0.11^b	0.22 ± 0.03^c	0.22 ± 0.07^c

DISCUSSION

In the present study, repeated BAL in adult rabbits consistently induced respiratory failure within 60 min of mechanical ventilation, with impairment of lung mechanics and gas exchange, and the characteristic changes of ALI and ARDS. Subsequent use of surfactant or a combined surfactant and iNO treatment showed effects in increased survival rate and improved blood oxygenation. However, this combined treatment showed no striking improvement compared with the effects of surfactant treatment alone. Much less effects were found in the rabbits treated by iNO, which is quite different from that seen in our previous study[6] in which iNO improves blood oxygenation in oleic acid-induced rabbit ARDS. In the present study, endogenous surfactant was removed by repeated BAL, and lung damage and atelectasis were present as verified by morphological examination, which should account for the ineffectiveness of iNO. In contrast, both surfactant and combined surfactant and iNO treatment tended to be more effective. It should be possible that instilled surfactant opens collapsed lungs, facilitating diffusion of NO gas to resistant vasculature in the lung interstitial compartment, as evidenced by increased alveolar V_V and low CV (V_V), significantly improved blood oxygenation, and prolonged survival time in both groups compared with the rabbits in the control and NO groups. Thus surfactant played a major role, improved diffusion of NO to expanded alveolar spaces, enhanced intrapulmonary blood flow, and reversed intrapulmonary ventilation-perfusion mismatching in this animal model, but overall effect was modest when comparing each other between both surfactant treated groups. Use of iNO alone for ARDS remains controversy, and recent multicenter trials have failed to reveal its long term efficacy for survival[14,15]

Nevertheless, evidence for improvement of blood oxygenation using iNO is definitive from these studies. Mechanism of ALI is in part associated with volotrauma and hyperoxic damage of the lungs. Using iNO alone for ALI and ARDS may not be sufficient when dealing with diffusive lung damages. We therefore anticipate that a combined use of iNO and intratracheal administration of surfactant to enable NO distribution has no substantial side effect, and there is variable response to combined surfactant and iNO treatment in terms of types of animal model.

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气道滴入肺表面活性物质和吸入一氧化氮治疗经气道肺泡灌洗诱发兔急性呼吸衰竭的效果比较[1]

R563.805

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关键词 成人型呼吸窘迫综合征；肺顺应性；一氧化氮；磷脂酰胆碱类；肺表面活性剂；呼吸疗法；支气管肺泡灌洗；低氧；呼吸功能不全；磷脂类

目的：比较单独或联合应用气道滴入肺表面活性物质(Surf)和吸入一氧化氮(iNO)对急性呼吸窘迫综合征的疗效. **方法**：成年兔反复气道生理盐水灌洗，去除内源 Surf，经机械通气诱发急性肺损伤和呼吸衰竭，随机分组给予：iNO 0.8 $\mu mol \cdot L^{-1}$；Surf 磷脂 100 $mg \cdot kg^{-1}$；联合应用 iNO 和 Surf；或不给药对照. **结果**：机械通气 8 小时生存率以联合治疗组为最高，Surf 组及联合治疗组的氧合指数(OI)、肺顺应性、气道阻力均有所改善. 联合治疗组的肺膨胀有显著改善. **结论**：对因 Surf 缺乏而萎陷和损伤的肺，单纯 iNO 不显治疗效果，但应用 Surf 或与 iNO 联合应用，具有提高生存率和改善血氧及肺膨胀的效果.

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