NEW BIOMEDICAL TECHNOLOGIES

Pharmacological Properties and Therapeutic Activity of Russian-Manufactured Pulmonary Surfactants

O. A. Rozenberg, L. N. Danilov*, V. A. Volchkov, E. S. Lebedeva*, V. F. Dubrovskaya, A. A. Val'kovich, O. V. Klestova, Yu. A. Kirillov, A. A. Seiliev, A. A. Shaldzhyan, L. V. Loshakova, A. E. Shul'ga, and A. G. Zhuikov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 126, No. 10, pp. 455-458, October, 1998
Original article submitted November 10, 1997

Three pulmonary surfactant preparations: from human amniotic fluid, from bronchoalveolar lavage fluid, and from cattle lung tissue homogenate were tested in preclinical studies. The preparations are nontoxic, possess no mutagenic, teratogenic, and allergic activities and do not modify visceral morphology after repeated injections. After a single intratracheal administration the drugs normalize arterial blood oxygenation in 15-30 min and arrest the respiratory distress syndrome in dogs, which is confirmed roentgenologically and clinically.

Key Words: *respiratory distress syndrome; surfactant; pharmacological properties; therapeutic activity*

The respiratory distress syndrome (RDS) is one of the major causes of neonatal and adult mortality [3,5]. About 30,000 babies with RDS are annually born in Russia; in the USA 150,000 RDS cases are recorded annually in adults. In RDS 15-30% newborns and 50-70% adults die [3,5,7]. In preterm newborns RDS is caused by immaturity of type 2 alveolocytes and the resultant primary deficiency of pulmonary surfactant (PS) [2]. In RDS of adults, PS deficiency is secondary, developing as a result of structural and functional disorders in the air-blood barrier. It often develops after multiple injury, sepsis, shock lung, radiation injury, etc. [4,7]. Application of natural and synthetic PS in recent decade together with progressive resuscitation technologies markedly decreased neonatal mortality from RDS [4]; effective use of PS in adults with RDS has been reported [9,11].

Natural and synthetic PS preparations have been widely used all over the world: survana (USA), surfactant-TA (Japan), curosurf (Italy), alveofact (Germany), exosurf (UK) [8].

We developed a technologically inexpensive method for preparing natural PS and characterized their physicochemical properties. Three preparations were studied: human PS isolated from parturients' amniotic fluid, PS from bronchoalveolar lavage fluid (PS-BLF), and PS prepared by water-salt extraction of finely dispersed cattle lung (PS-WSE).

The pharmacological and therapeutic properties of these PS preparations are studied.

Department of Medical Biotechnology, Central Roentgenoradiological Institute, Ministry of Health; Department of Experimental Pathology, State Research Center of Pulmonology, Ministry of Health, St. Petersburg

0007-4888/98/0010-01048$20.00

MATERIALS AND METHODS

Acute toxicity was assessed after a single intravenous or intraperitoneal injection to mice and intratracheal administration to rats. In rats, chronic toxicity was assessed after 10 daily inhalations of the agents in total doses 10-50 times higher than the therapeutic doses. Pulmonary and visceral morphology, allergogenicity, effects on immune response, mutagenicity and teratogenicity, and blood cell count were studied. Blood levels of cholesterol, glucose, urea, bilirubin, protein levels, alkaline phosphatase, asparagine and alanine transaminases activities were measured before and during experiment. Urinary sugar, proteins, urobilinoids, specific weight, and pH were determined.

Respiratory distress syndrome in dogs was induced by effective bronchoalveolar lavage [6]. Mongrel dogs weighing 12-15 kg aged 4-5 years were used. Experiments were carried out under romitar and thiopental narcosis. After injection of myorelaxants (tubarin and ditilin) the animals were transferred to forced ventilation of the lungs. X-ray examination of the lungs was carried out during the inspiration phase. Forty min after drug administration the dogs were transferred to spontaneous respiration.

For forced ventilation, adequate respiration volume and rate were chosen, and the mean inspiration pressure was 20 cm H_2O. The lungs were ventilated with air. Arterial blood gases were examined using an AVL-995 gas analyzer (Biomedical Instruments).

Lyophilized PS preparations were emulsified in normal saline (0.9% NaCl) before use, and 10-15 ml emulsion was administered into the trachea through an intubation tube under x-ray control into the left and right lungs.

To elucidate the fate of exogenously administered PS, phospholipid content and composition [10] in lavage fluid and electron-microscopic picture [1] of rat lungs were studied after intratracheal administration of the preparations in a dose of 40 mg/kg. The studies were carried out over time from 15 min to 7 days after administration (phospholipids) and from 15 min to 6 h (electron microscopy).

RESULTS

The acute, chronic, and specific toxicity tests confirmed the safety of the agents. We failed to determine the LD_{50}, because intravenous and intraperitoneal injections of doses 10-50 times higher than the therapeutic dose to mice did not cause their death or changed their behavior. Only 0.2-0.4 ml of the preparation emulsion can be administered to rats intratracheally, which is no more than 50-100 mg/kg. Such doses were tolerated without disorders in respiratory function or behavioral changes. No mutagenic, teratogenic, or allergic effects of the studied agents were observed, nor local irritations or disorders in blood clotting.

In preliminary experiments with estimation of the amount of PS washed out in individual portions of BLF, a protocol of total BLF was developed, ensuring 90% removal of PS from the lungs and leading to RDS which was diagnosed clinically and by x-ray examination. For inducing RDS by such a method, dogs should be lavaged with 8-10 portions of normal saline in succession, 400 ml/kg each (about 80% lung volume per portion).

After lavage, the dogs developed dyspnea and tachicardia at rest and in exercise; the animals were dull. X-ray examination of the chest showed lavage atelectasis. Immediately after lavage both partial oxygen pressure (Po_2) and saturation with oxygen (HbO_2) in arterial blood hemoglobin decreased,

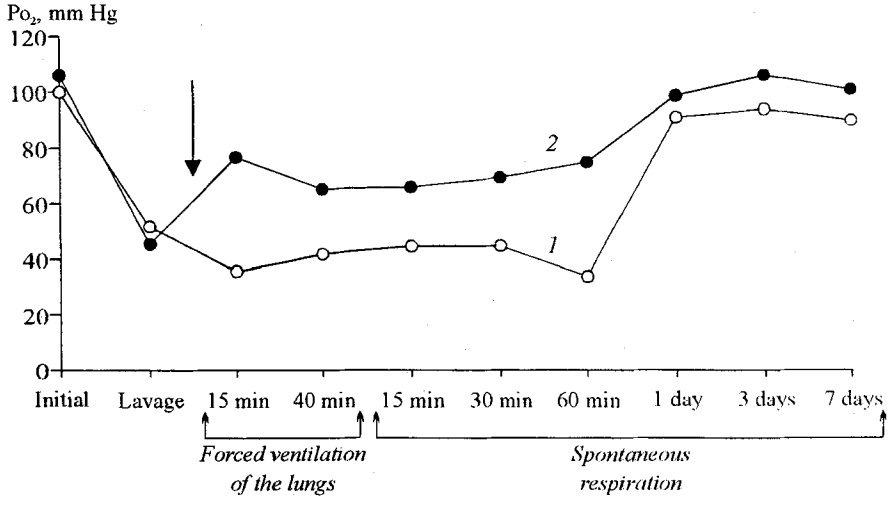

Fig. 1. Changes in oxygen pressure in arterial blood of a dog in control (*1*) and after a single administration of human surfactant preparation in a dose of 15 mg/kg (2). Arrow shows the moment of drug administration.

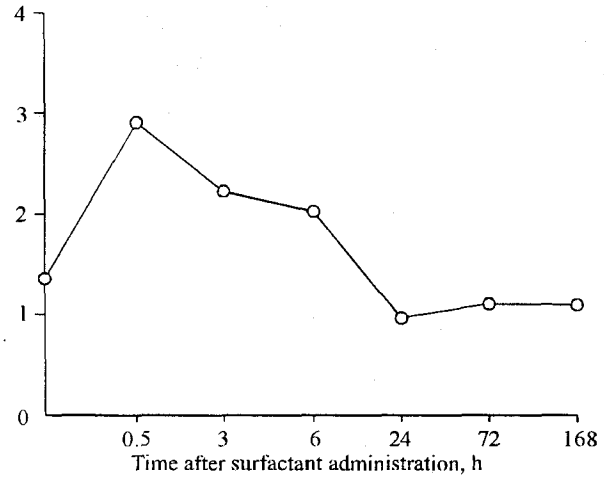

Fig. 2. Phospholipid content in bronchoalveolar lavage fluid of rats after a single intratracheal administration of human surfactant. Ordinate: phospholipid content, mg.

while partial carbon dioxide pressure increased (Fig. 1). Dullness and dyspnea persisted for 3 days, Po_2, HbO_2, and Pco_2 normalized by the end of the first day, and lung tissue saturation with air normalized only on the 7th-8th days after lavage.

The respiratory distress syndrome was arrested by a single intratracheal administration of PS two weeks after the control experiment. Bronchoalveolar lavage was carried out according to the same protocol and the drugs were administered after 30 min. Fifteen-thirty minutes after PS administration, Po_2 (Fig. 1) and HbO_2 markedly increased, while arterial blood Pco_2 decreased (data not shown). Po_2 and HbO_2 normalized 30-60 min after drug administration, while in the control, blood gases normalized only 24 h after bronchoalveolar lavage. Roentgenograms of the chest 1 h after drug administration show recovery of saturation of the lungs with air, and by the end of 24 h after PS administration there was no difference between intact and treated animals. PS preparations differed by effective therapeutic doses. For human PS and PS-BLF this dose was 15 mg/kg and for PS-WEL 50 mg/kg. Specific activity of PS-WEL was studied in a wide dose

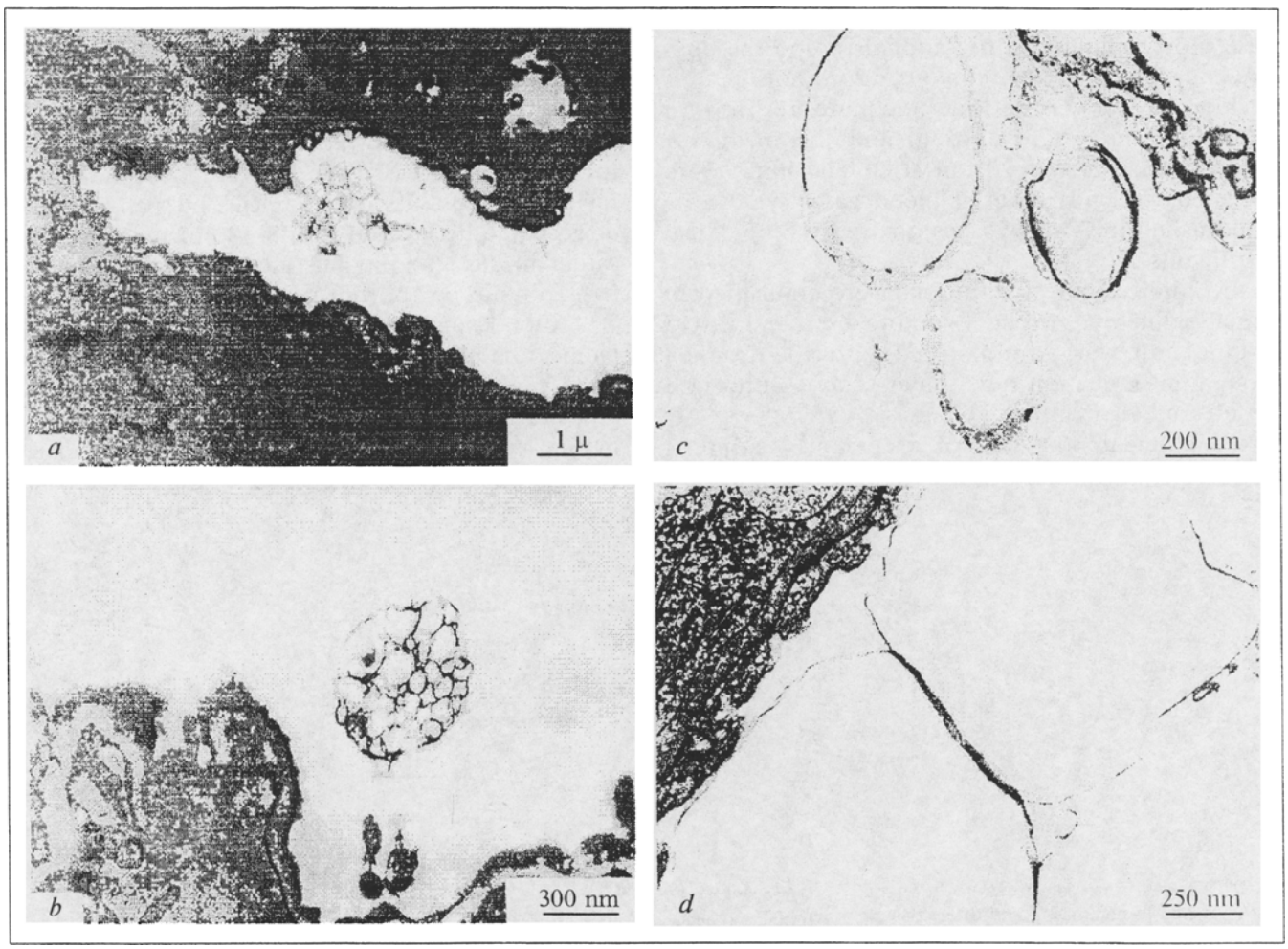

Fig. 3. Structural changes in exogenous surfactant in alveolar cavities 15 min (*a*, *b*), 60 min (*c*), and 360 min (*d*) after administration. ×12,000 (*a*), 37,000 (*b*), 48,000 (*c*), and 42,000 (*d*).

range: from 15 to 100 mg/kg; the doses 50-100 mg/kg were effective. In some experiments the therapeutic effect of PS-WEL was observed 3-4 h after administration, while the effects of PS from the amniotic fluid and BLF manifested itself immediately after administration.

A detailed study of chronic toxicity after 10-day daily inhalations in doses of 100-400 mg/kg showed no pathological changes in blood cell count, biochemistry of the blood and urine, structural disorders in the lungs and other viscera.

Figure 2 shows the content of phospholipids in lavage (mean values of three independent experiments), and Figure 3 is a photograph of the rat lungs after a single intratracheal administration of human PS. The phospholipid content in lavage drops to the initial level 6-12 h after administration, and electron microscopy shows successive changes in the structure of administered PS from aggregations of vesicles to bilayer vesicles adhering to alveolar surface. Similar results were observed with surfactants from cattle lungs.

From these results it can be concluded that human and cattle PS are safe and highly effective preparations that can be used for arresting surfactant-deficient DRS in experimental animals.

Clinical trials of human PS, which are in progress now, confirm the experimental findings.

REFERENCES

1. V. F. Dubrovskaya, R. P. Stepanov, and T. T. Shishko, *Arkh. Patol.*, **54**, No. 11, 16-20 (1992).
2. M. E. Avery and J. Mead, *Am. J. Dis. Child.*, **97**, 51-56 (1959).
3. M. E. Avery and T. A. Merritt, *N. Engl. J. Med.*, **234**, No. 13, 910-912 (1991).
4. H. L. Halliday, *Drugs*, **51**, No. 2, 226-237 (1996).
5. B. A. Holm and S. Matalon, *Anesth. Analg.*, **69**, No. 25, 805-818 (1989).
6. B. Lachmann, B. Robertson, and J. Vogel, *Acta Anaesthesiol. Scand.*, **24**, 231-236 (1980).
7. J. F. Lewis and A. H. Jobe, *Am. Rev. Respir. Dis.*, **147**, 218-223 (1993).
8. H. Segerer and M. Obladen, *Pediatr. Rev.*, **5**, 67-82 (1990).
9. R. G. Spragg, N. Gillard, P. Richman, *et al.*, *Chest*, **105**, No. 1, 195-202 (1994).
10. V. E. Vaskovsky, E. Y. Kostetsky, and I. M. Vasendin, *J. Chromatogr.*, **114**, No. 1, 129-141 (1985).
11. D. Walmarth, A. Gunther, A. Ghofrani, *et al.*, *Am. J. Respir. Crit. Care Med.*, **154**, 57-62 (1996).