10-羟基-2-癸烯酸对腹腔巨噬细胞的吞噬活性及其产生细胞因子的影响

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关键词 10-羟基-2-癸烯酸；腹腔巨噬细胞；肿瘤坏死因子；白细胞介素-1；培养的细胞

目的：研究 10-羟基-2-癸烯酸(HDA)在体外对大鼠腹腔巨噬细胞活性的影响. 方法：测定 HDA 对吞噬活性，抗癌细胞因子 TNF 和 IL-1 产生的影响. 结果：HDA (50, 100 mg·L^{-1})能增强巨噬细胞吞噬活性，HDA 在 50, 100, 200 mg·L^{-1} 时能促进 TNF 和 IL-1 产生. 结论：HDA 上调巨噬细胞的吞噬活性，促进 TNF 和 IL-1 产生，在抗肿瘤和免疫调节中起一定作用.

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Artificial reconstituted pulmonary surfactant in prevention and treatment of respiratory distress syndrome in neonates[1]

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KEY WORDS respiratory distress syndrome; pulmonary surfactants; newborn infant

AIM: To test an artificial reconstituted pulmonary surfactant (APS) for prevention and treatment of respiratory distress syndrome (RDS). **METHODS:** A membrane-formed method combined with supersonic dispersing was used to prepare APS. A pulsating bubble surface tension measurement was established to compare surface properties of APS with natural pulmonary surfactant (NPS). A preliminary clinical trial was made for prevention and treatment of RDS. **RESULTS:** The APS reduced surface tension from 44.0 mN/m to <1.0 mN/m *in vitro*. The changes of APS lipid contents were <5 % of labeled content at 37 ℃. Clinical trial showed that the APS prevented RDS in 20/20 and cured RDS in 2/2 premature neonates. **CONCLUSION:** The APS had good surface properties similar to NPS.

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Respiratory Distress Syndrome (RDS) is often formed in neonates, especially in low-birth-weight premature infants. The pulmonary immaturity is associated with deficiency of the pulmonary surfactant (PS). Replacement therapy used the exogenous PS (natural or artificial reconstituted) is very satisfactory[1-3]. Through analyzing the components of natural PS (NPS), we prepared an artificial reconstituted PS (APS) preparation containing mainly dipalmitolphosphatidylcholine (DPPC) and phosphatidylglycerol (PG). This paper was to compare the surface properties and effects of APS and NPS (calf PS).

MATERIALS AND METHODS

DPPC (TLC pure, 99.0 %, Sigma USA); PG (TLC pure, 99.5 %, made in our Lab); NPS (extracted from neonatal calf lung lavage); other chemicals were of AR; Coulter Counter (England); a pulsating bubble surface tensionor (modified by our Lab from a static air bubble surface tensionor[4]); ZFG81 Rotating Evaporator (Shanghai Medical Appatatus and Instruments Factory, China); CPS-1A Supersonic Micronizer (Shanghai Supersonic Apparatus Factory, China); mice (bred by Shanghai Medical University).

Lipid suspension[5] DPPC and PG were dissolved in a

small volume of chloroform/ethyl ether mixture (3∶2, vol/vol). The flask was put on a rotating evaporator, the organic solvent was removed under vacuum at <45 ℃ to form a lipid membrane on the wall of the flask. A volume of 0.9 % NaCl was added and shaked to detach the lipid membrane from the wall. The liquid phase was sonicated to form a lipid suspension (lipids 100 g·L^{-1}) which was sealed in 2-mL ampules under N_2, sterilized (100 ℃, 30 min), and stored at 4 ℃.

Pulsating bubble surface tensionor for measuring surface tension APS solution (usual 100 μL) was added to the gas-liquid interface of the air bubble. According to the shape and volume of the bubble during pulsating cycles, the minimal surface tension and the balance surface tension were calculated to compare the surface properties between APS and NPS[6].

Stability tests Stability tests of APS were proceeded at 37 ℃ and 4 ℃. Sampling times were 0, 10, 20, and 27 d at 37 ℃, 0, 1, 3, 5, 8, 10, and 12 months at 4 ℃, respectively. Samples were used for analyzing content and surface properties.

Particle measurement A Coulter Counter was used for measuring the lipid particle in a suspension at 25 ℃.

Toxicity test[7] Mice (5 ♂, 5 ♀) were injected ip APS 2.0 mL·kg^{-1} (100-fold of the clinical dose). All of tested mice should not die in 48 h during testing.

Clinical trials The low-birth-weight preterm infants or diabetes mellitus (DM) mothers' infants (n = 20) for prevention of RDS were delivered a dose of APS intratracheally within 2 h of birth. Two infants with RDS were used for treatment trial. During 30 s-instillation, the infant was held in various positions to facilitate APS instilled into each lobar and segmental bronchus. After completion of this treatment each infant was ventilated with a respirator.

RESULTS

Most of lipid particle diameters in the APS preparation were <10 μm (>99.0 %, measured by a Coulter Counter). Sterilization had no effect on the content and surface properties of the APS preparation (content of lipids: from 103.5 % to 104.5 %, minimal surface tension: from 0.70 to 0.69 milli Newton per meter (mN/m), and balance surface tension: from 42.12 to 42.33 mN/m).

The surface properties of APS were similar to those of NPS. APS reduced surface tension from 44.0 mN/m to <1.0 mN/m during continuous cycling. Surface tension-reduced abilities between APS and NPS showed no marked difference (P > 0.05) (Tab 1).

Tab 1. Surface properties of APS and NPS. n = 6 measuring times, $\bar{x} \pm s$, $^aP > 0.05$ *vs* NPS.

	Surface tension (mN/m) APS	NPS
	1st Cycle	
$\sigma_{balance}$	44.32±0.13[a]	44.26±0.18
$\sigma_{minimum}$	1.00±0.04[a]	1.17±0.10
	10th Cycle	
$\sigma_{balance}$	44.09±0.14[a]	44.46±0.20
$\sigma_{minimum}$	0.98±0.05	1.20±0.11
	After cycled for 8 h (30 cycles per min)	
$\sigma_{balance}$	44.99±0.15[a]	46.59±0.19
$\sigma_{minimum}$	0.96±0.04[a]	1.08±0.14

The surface properties of APS did not change during 27-d accelerating tests at 37 ℃, the minimal surface tension was reduced to <1.0 mN/m, and the content change of major components (lipids) was <5 % of labeling content (Tab 2). These suggested that the APS could be kept at 4 ℃ for about 12 months (recommended storage condition is 4 ℃).

Tab 2. Stability of APS preparation. n = 3 measuring times. $\bar{x} \pm s$.

Time	$\sigma_{balance}$ (mN/m)	$\sigma_{minimum}$ (mN/m)	Lipids Content (%)
37 ℃			
0 day	42.33±0.14	0.70±0.04	104.6±1.0
10-day	40.16±0.13	0.70±0.03	104.1±1.0
20-day	43.18±0.11	0.96±0.02	103.5±1.0
27-day	44.20±0.10	0.96±0.03	101.2±1.1
4 ℃			
0 month	43.19±0.10	0.71±0.03	104.6±1.0
1-month	42.24±0.13	0.69±0.04	104.3±1.2
3-month	43.94±0.14	0.72±0.05	104.1±0.9
5-month	44.20±0.09	0.99±0.04	104.0±1.0
8-month	43.09±0.10	0.97±0.03	102.6±1.1
10-month	43.41±0.08	0.98±0.04	101.3±1.0
12-month	43.21±0.06	0.96±0.03	101.2±1.0

In the normal storage test at 4 ℃, APS remained its original surface properties and content within 12 months (Tab 2).

In the acute toxicity test, all of the 10 mice were survived in 48 h.

RDS did not form in all of the 20 preterm neonates. Two neonates with RDS were intratracheally treated with APS. The respiratory

distresses were improved evidently within 20 h, and cured in 48 h.

DISCUSSION

The membrane-formed method combined with supersonic dispersing can disperse phospholipids (DPPC and PG) to form a uniform lipid suspension (APS preparation). The method of preparing APS is easible, effective, and suitable to resconstitute PS.

PS major components are phospholipids, apoproteins, and some hydrocarbons. When alveolar breathes, PS can reduce alveolar surface tension to avoid alveolar from collapsing, reduce pulmonary injury, and increase lung compliance[8,9].

Many APS or NPS containing major components of PL had been explored for clinical prevention study of RDS abroad. After the safety of acute toxicity of the APS (made in our Lab) was passed, the APS was used for clinical prevention and treatment of RDS. Clinical trial results showed that APS could provent preterm infants from formation of RDS (the morbidity in the same cases is about 20 %[10]), and treat RDS infants, effectively.

Results of the clinical trial support that RDS mechanism is due to lung premature of neonatals, especially in low-birth-weight preterm babies. This study made it possible to prevent and treat RDS clinically in China, and provided reliable basis for prevention and treatment of adult respiratory distress syndrome.

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187-184

人工重组肺泡表面活性剂对新生儿呼吸窘迫综合征的防治[1]

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关键词 呼吸窘迫综合征；肺泡表面活性剂；新生婴儿

目的：研制用于预治新生儿呼吸窘迫综合征(RDS)的人工重组肺泡表面活性剂(APS)。**方法**：采用成膜法结合超声分散制备 APS。脉冲气泡表面张力测定法用于评价 APS 与天然 PS 的表面性能。用气管滴注给药临床防治新产儿的 RDS。**结果**：制得 APS 粒径 10 μm 以下的在 99 %以上。制剂体外可有效降低表面张力(从 44.0 mN/m 至 1.0 mN/m 以下)。初步临床试验显示 APS 能有效预防 RDS 发生(20/20)和治疗 RDS(2/2)。**结论**：APS 制剂体外具有与天然 PS 相似的良好表面性能。