^{99m}Tc-ENS: A New Radiopharmaceutical for Aerosol Lung Scintigraphy. Comparison Between Different Freeze-Dried Formulations

Gabriela P. Calmanovici, José R. Boccio, Alexis E. Lysionek, Maria J. Salgueiro, Alfredo Hager, Tomas De Paoli, Maria L. Calcagno, Jorge Nicolini, Mercedes Tarlati, Ricardo A. Caro and Marcela B. Zubillaga

Radioisotope Laboratory and Departments of Physics and Mathematics, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, and Bacon Laboratories SAIC, Buenos Aires, Argentina

Exogenous natural surfactant (ENS) labeled with ^{99m}Tc (^{99m}Tc-ENS) is a new radiopharmaceutical for pulmonary aerosol scintigraphy. In this study, different freeze-dried formulations were evaluated to develop a suitable and long-storage method for the ENS, the nonradioactive precursor of this radiopharmaceutical. **Methods:** Two freeze-dried formulations were evaluated: the sterile ENS suspension-stannous chloride altogether lyophilized (chlorlioENS) and the lyophilized sterile ENS suspension with the addition of stannous chloride as a solid drug (lioENS). These precursors were stored at room temperature for 3 mo and then labeled with ^{99m}Tc. For comparative purposes, the sterile ENS suspension with the addition of stannous chloride labeled with ^{99m}Tc (^{99m}Tc-chlorENS) was also studied. The quality controls for each radiopharmaceutical were performed by an ascending paper chromatography to determine the labeling yield percentages. The study was performed in 30 female Sprague Dawley rats, which inhaled each radiopharmaceutical by nebulization. Twenty-five minutes after the aerosol inhalation, the animals were killed to extract their organs and measure their activity in a gamma spectrometer. The data are given as the percentage of activity concentration (C%) for each organ. **Results:** The physicochemical properties of lioENS were adequate for a freeze-dried product. The labeling yields for ^{99m}Tc-lioENS and for ^{99m}Tc-chlorENS were always greater than 95% even after nebulization. The results of the biologic distribution studies showed that the activity concentration found in lungs for these radiopharmaceuticals were 95.7% ± 2.6% and 96.7% ± 2.6% respectively, results that do not differ statistically. On the other hand, the activity concentration found in lungs for the ^{99m}Tc-chlorlioENS (31.3% ± 11.1%) and its labeling yield percentages (<10%) are statistically different ($P < 0.05$) from the results obtained with the two radiopharmaceuticals mentioned above. **Conclusion:** Taking into account the lioENS physicochemical properties, its long shelf life and that ^{99m}Tc-lioENS shows the same radiochemical and radiopharmacological behavior of the ^{99m}Tc-chlorENS, it can be concluded that the ^{99m}Tc-lioENS can be used for aerosol lung scintigraphy.

Key Words: exogenous natural surfactant; ^{99m}Tc-labeled exogenous natural surfactant; pulmonary aerosol scintigraphy; freeze drying

J Nucl Med 1999; 40:1080–1083

The diagnosis of respiratory diseases is important to study to prevent respiratory disorders. Until recently, the only diagnostic methods available for ventilation lung scintigraphy have been performed with nonspecific radiopharmaceuticals, such as ^{99m}Tc-diethylenetriamine pentaacetic acid (DTPA), ^{133}Xe or ^{81}Kr.

Exogenous natural surfactants (ENSs) contain phospholipids, neutral lipids and proteins. They are prepared from mammalian lungs. Since 1989, ENSs as well as synthetic surfactants have been available for treatment of respiratory distress syndrome in the neonate (*1*), and they are still being studied (*2–4*). They are also being studied for treatment of respiratory distress syndrome in the adult (*1,5,6*) as well as other pathologies (*7,8*). The principal property is to spread spontaneously on the air-alveoli interface, reducing the tendency of the alveoli to collapse (*9*). Taking these characteristics into account, we used sterile ENS suspension and stannous fluoride as a reducing agent, which has shown an optimal percentage of activity concentration in lungs, to develop a new radiopharmaceutical, ENS labeled with ^{99m}Tc (^{99m}Tc-ENS) (*10*). This agent would allow a lower nebulization time as well as higher activity concentration in lungs. This characteristic is important in physiopathologic situations such as the intensive care unit, where patients are often on respirators (*11*). Similar studies performed with inhaled surfactants such as Exosurf demonstrated that a mixture of ^{99m}Tc-DTPA with synthetic surfactant appears to be a reasonable method for evaluating surfactant deposition (*12*). Other researchers have demonstrated that Exosurf initially retards the ^{99m}Tc-DTPA aerosol clearance, but ^{99m}Tc-DTPA transalveolar clearance returns to baseline rates within 1–2 h (*3*). Coleman et al. (*12*) evaluated the feasibility of using ^{99m}Tc-DTPA as a radioactive tracer for aerosolized synthetic surfactant (dipalmitoilphosphatidylcholine, cetyl alcohol and tyloxapol), and Suga et al. (*3*) evaluated the effect of aerosolized synthetic surfactant on pulmonary ^{99m}Tc-DTPA clearance. In a previous study (*10*), we demonstrated that ENS can be labeled with ^{99m}Tc with a high yield, allowing the use of ^{99m}Tc-ENS as a radiopharmaceutical for aerosol lung scintigraphy.

Received Jun. 12, 1998; revision accepted Jan. 4, 1999.

For correspondence or reprints contact: Bioch. Gabriela P. Calmanovici, Radioisotope Laboratory, School of Pharmacy and Biochemistry, University of Buenos Aires, Junin 956, Buenos Aires, 1113 Argentina.

An important consideration when providing labile products such as ENS to nuclear medicine centers is ensuring the preservation of their physicochemical properties for several months before labeling. This was performed by stabilizing the stannous ligand complex by freeze-drying against air oxidation and hydrolysis. Even though some kits such as liquid or frozen solutions under inert atmosphere have been used, the freeze-dried kits have advantages in their long shelf life, the procedural reliability and the ease of reconstitution into a clear solution or suspension in case of labeled colloids or particles, suitable for parenteral administration. The shelf life of some kits is approximately 6 mo (*13*), assuming that their behavior does not change for a long period of time.

The purpose of this study was to develop a kit of homogeneous, pure and stable ENS. The final form and quality of a freeze-dried product depend on the way the freeze-drying is conducted (*14*), and lyophilization is a complex multistage process that must be carefully adjusted to each case (*15*). Therefore, in each case, different freeze-dried formulations were evaluated and the physicochemical controls of the products were performed. The radiopharmacological and radiochemical behavior of the ^{99m}Tc-ENS obtained using the ENS-freeze-dried formulations as nonradioactive precursors were compared with that of the ^{99m}Tc-ENS obtained with a sterile ENS suspension. In all the cases, stannous chloride was used as the reducing agent.

MATERIALS AND METHODS

Freeze-Dried Formulations and Freeze-Drying Processes

Two freeze-dried formulations were evaluated. In both of them, the freeze-drying cycle was performed in the same way. The different formulations are described below.

ENS-Stannous Chloride Altogether Lyophilized (chlorlioENS). A 2.5-mg sterile ENS suspension stored at 4°C (Baby Fact P; GEMEPE SA, Buenos Aires, Argentina) and 0.5 mg stannous chloride (mol wt 189.6; Sigma Chemical Co., St. Louis, MO) were placed in a flask to be freeze-dried.

ENS Lyophilized with Stannous Chloride as Solid Drug (lioENS). A 2.5-mg sterile ENS suspension stored at 4°C (Baby Fact P; GEMEPE SA) was placed in a flask to be freeze-dried. After the freeze-drying process, 1 mg stannous chloride (mol wt 189.6; Sigma Chemical Co.) was added to this flask.

The freeze-drying cycle was designed taking into account the mode of operation of the freeze-drying equipment (Virtis GPC-3T; Virtis, New York, NY) and the intrinsic properties of the material to be freeze-dried (*14*). The cycle consisted of three stages: freezing, primary drying and secondary drying. When the first stage (freezing) was finished, the product was at −33°C. In the second stage (primary drying), the temperature reached 2°C. Finally, in the third and last stage (secondary drying), it reached 7°C.

The freeze-dried pharmaceuticals were stored at room temperature (21–27°C) for 3 mo. Every week their physical characteristics were examined, and after the reconstitution their pH was measured.

Radiopharmaceuticals

The radiopharmaceuticals were obtained using $^{99m}TcO_4^-$ eluted from a molybdenum generator (Radiofarm®; Bacon Laboratories, Buenos Aires, Argentina; activity 18,500 MBq) as sodium pertechnetate. Their absolute activity was measured in an ionization chamber (RADX model 255 Remote; RADX Corp., Houston, TX).

^{99m}Tc-lioENS and ^{99m}Tc-chlorlioENS (Reconstitution of Freeze-Dried Precursors of Radiopharmaceuticals). Each radiopharmaceutical precursor was reconstituted in a flask by adding 296 MBq sodium pertechnetate and saline solution. The final activity concentration for each radiopharmaceutical was 99.9 MBq/mL.

Sterile ENS Suspension with Addition of Stannous Chloride Labeled with ^{99m}Tc (^{99m}Tc-chlorENS). A 2.5-mg sterile ENS suspension (Baby Fact P; GEMEPE SA) containing 0.5 mg stannous chloride (mol wt 189.6; Sigma Chemical Co.) was labeled with 296 MBq sodium pertechnetate. The final activity concentration was 99.9 MBq/mL.

The quality controls of the radiopharmaceuticals were performed by an ascending paper chromatography on Whatman chromatography paper (RJM Sales, Inc., New York, NY; basis weight 185 g/m^2, thickness 0.33 mm, medium flow rate), using acetone (Merck, Buenos Aires, Argentina) as solvent, according to Castiglia et al. (*16*), Waldman et al. (*17*) and Calmanovici et al. (*10*).

Animal Models

Thirty female Sprague Dawley rats weighing between 220 and 260 g were randomized in three groups of 10 animals each, placed in stainless steel cages (315 × 445 × 240 mm high) and maintained with standard food (Nutrimentos® Rodents Diet N° 3; Nutrimentos SA, Buenos Aires, Argentina) and water ad libitum with cycles of 12 h of light and darkness.

The rats were anesthetized with 300 mg/kg chloral hydrate Analytical Reagent (Mallinckrodt, New York, NY). Each radiopharmaceutical was placed in the chamber of nebulizer-compressed air (Omron NE-C08 nebulizer comp-air®; Omron Healthcare, Inc., Vernon Hills, IL), to obtain a fine aerosol with particle sizes ranging between 0.5 and 5 μm. A special mask adapted to the shape of each rat nose was used to administer this radioaerosol for 5 min. After each nebulization, the mask, the chamber and every nebulizer accessory were decontaminated, washed and checked out to prevent later contamination (*10*).

Twenty-five minutes after the aerosol inhalation, the animals were killed and the lungs, kidneys, liver, blood, spleen and TGI (gastrointestinal system with its content) were extracted, washed and weighed. The activity of each organ was measured in a gamma counter with the same geometry for all the organs, using a monochannel gamma spectrometer with a 5 × 5 cm NaI(Tl) standard well crystal, which was previously set to optimal electronic conditions. All measurements were performed with constant geometry with an efficiency equal to 5% and a relative error less than 1%.

Data Analysis

The radiochemical purity was given as the labeling yield percentage:

$$\% \text{ labeling yield} = \text{A(cpm) at the origin/A(cpm) total,}$$

where A (cpm) total = A (cpm) at the origin plus A (cpm) at the front of solvent.

The activity found in each organ was expressed as the percentage of activity concentration (C%) to obtain results independent of the inhaled radioactivity and the organ mass, using the following