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 (chlorioENS) and the lyophilized sterile ENS suspension with the addition of stannous chloride as a solid drug (ioiENS). 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-chlorioENS 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 ioiENS were adequate for a freeze-dried product. The labeling yields for $^{99m}$Tc-ioiENS and for $^{99m}$Tc-chlorioENS 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 was 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 the $^{99m}$Tc-chlorioENS (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 ioiENS physicochemical properties, its long shelf life and that $^{99m}$Tc-ioiENS shows the same radiopharmaceutical and physicochemical behavior of the $^{99m}$Tc-chlorioENS, it can be concluded that the $^{99m}$Tc-ioiENS can be used for aerosol deposition. **Key Words:** exogenous natural surfactant; $^{99m}$Tc-labeled exogenous natural surfactant; pulmonary aerosol scintigraphy; freeze drying.


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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-diethylentriamine pentaacetic acid (DTPA), $^{133}$Xe or $^{11}$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-alkveoli 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 (dipalmitylophosphatidylcholine, cetyl alcohol and tylaxol), 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.
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 radiopharmaceutical and radiochemical behavior of the 99mTc-ENS obtained using the ENS-freeze-dried formulations as nonradioactive precursors were compared with that of the 99mTc-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 Allogether Lyophilized (chlorioENS). 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 (iio-ENS). 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.

Radiochemicals

The radiochemicals were obtained using 99mTcO4− 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). 99mTc-HEDP and 99mTc-HEDP (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 99mTc (99mTc-chloroENS). 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/m², thickness 0.33 mm, medium flow rate), using acetic (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 (Nutrimento® Rodents Diet No. 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 (Omnir NE-C08 nebulizer comp-air®; Omnim 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 aerosol 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 Na(TI) 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 radiopharmaceutical purity was given as the labeling yield percentage:

\[ \text{% labeling yield} = \frac{A (cpm)}{A (cpm) \text{ total}} \times 100 \]

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

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

\[ C\% = \left( \frac{A(\text{cpm}) \times 100}{\text{m(g)} \times \sum[A(\text{cpm})/m(\text{g})]} \right) \]

where \( A(\text{cpm}) = \) the measured activity in the organ, \( m(\text{g}) = \) the mass of the organ and \( \sum[A(\text{cpm})/m(\text{g})] = \) the sum of the activity concentrations of all the organs.

**Statistical Studies**

Results are given as mean ± SD. For comparative biologic distribution studies, we evaluated the results by the Kruskal-Wallis one way analysis of variance by ranks (18), followed by the Dunns test. Chromatographic studies were evaluated by two-factor experiment with repeated measures on one factor (19) and by Wilcoxon matched-pairs signed-rank test (20). In all the tests, significance was set at 0.05 level.

**RESULTS**

The physicochemical properties of the freeze-dried precursors of the radiopharmaceuticals are described as follows: the IioENS was a fine white powder, and its pH after reconstitution was 5.5. The chlorioENS was a pale brown fine powder, and its pH after reconstitution was also 5.5.

The labeling yield percentages are shown in Table 1. It can be observed that the labeling yields for \(^{99m}\text{Tc}\)-chlorioENS and \(^{99m}\text{Tc}\)-IioENS were significantly different (\( P < 0.05 \)), but they were always higher than 95% before the aerosolization (99.8% ± 0.1% and 99.0% ± 0.9%, respectively) as well as after the aerosolization (99.6% ± 0.3% and 98.3% ± 1.7%, respectively). The labeling yield percentages of each radiopharmaceutical before and after the aerosolization did not differ significantly. The \(^{99m}\text{Tc}\)-chlorioENS labeling yield percentage differed significantly from the previously mentioned radiopharmaceuticals (\( P < 0.05 \)). The difference between its labeling yield percentages before the aerosolization procedure (1.5% ± 0.6%) and after this procedure (4.4% ± 2.6%) was significantly different (\( P < 0.05 \)).

The results of the biologic distribution studies are shown in Figure 1. It can be observed that in lungs the activity concentrations do not differ significantly for the \(^{99m}\text{Tc}\)-chlorioENS (96.7% ± 2.6%) and the \(^{99m}\text{Tc}\)-IioENS (95.7% ± 2.6%). On the other hand, the activity concentration found in lungs when \(^{99m}\text{Tc}\)-chlorioENS was used (31.3% ± 11.1%) differs significantly (\( P < 0.05 \)) from the activity concentrations of the other radiopharmaceuticals. The activity concentrations found in all organs for \(^{99m}\text{Tc}\)-chlorioENS differ significantly from activity concentrations found in all organs for other radiopharmaceuticals.

**DISCUSSION**

We have previously demonstrated that it is possible to label a sterile suspension of ENS with \(^{99m}\text{Tc}\), using stannous fluoride as a reducing agent, to perform aerosol ventilation scintigraphy (10). In this study (10), the sterile ENS suspension was labeled with \(^{99m}\text{Tc}\), using stannous chloride as a reducing agent (\(^{99m}\text{Tc}\)-chlorioENS). The biodistribution results of \(^{99m}\text{Tc}\)-chlorioENS were similar to those found previously. As has been pointed out, our purpose in this study was to determine the radiopharmaceutical and radioclinical behavior of \(^{99m}\text{Tc}\)-ENS prepared with lyophilized and nonlyophilized ENS, using stannous chloride as a reducing agent in both cases. Analyzing the studied freeze-dried formulations, it can be observed that ENS physicochemical properties were adequate, the labeling yields obtained when this radiopharmaceutical precursor was labeled with \(^{99m}\text{Tc}\) (\(^{99m}\text{Tc}\)-IioENS) were higher than 95% even after the nebulization procedure (Table 1) and its percentage of activity concentration in lungs was 95.7% ± 2.6% (Fig. 1). These results do not differ significantly from those obtained with \(^{99m}\text{Tc}\)-chlorioENS (Table 1, Fig. 1), demonstrating that this freeze-dried formulation is adequate for clinical use of this radiopharmaceutical. This high activity concentra-
tion in lungs can be explained by the characteristic biophysical properties of the surfactant, which determines its lining on the alveolar surface (5). It is important to point out that both aerosolized radiopharmaceuticals are adequate for radioaerosol diagnosis, because their particle sizes (between 0.5 and 5 μm) and tracer-ligand bindings (labeling yields higher than 95% even after nebulization) are adequate (10,17). Moreover, according to Dijk et al. (2), neither the surfactant composition nor its biophysical properties are altered by nebulization with a jet nebulizer, results that are in accordance with our results.

On the other hand, the labeling yield for the 99mTc-chloroioENS was lower than 5% (Table 1), and the percentage of activity concentration in lungs was 31.3% ± 11.1% (Fig. 1), showing a comparable value to that of the lung activity concentration obtained with 99mTcO4− (22.4% ± 7.5%) (10), which is nonspecific for the organ under study (10). Its biodistribution is nonspecific throughout all the other studied organs. Moreover, this radiopharmaceutical behavior is similar to that of the 99mTcO4− (10). These results may be due to the oxidation of the stannous ion (Sn2+) in an aqueous solution in the flask before the lyophilization process (21,22), and they are in agreement to the low labeling yield of the product. Therefore, the so-called 99mTc-chloroioENS may be supposed to be mainly 99mTcO4−. These results indicate that this formulation is not recommended to be freeze-dried.

It is important to point out that the radiochemical and radiopharmaceutical behavior depends on the freeze-dried formulation as well as the freeze-drying procedure. On the other hand, it must be noted that when the sterile ENS suspension is freeze-dried alone (ioENS), these properties are not affected, because the radiopharmaceutical and biochemical behavior of 99mTc-ioENS does not differ significantly from the one obtained with 99mTc-chloroENS, showing that the freeze-drying procedure does not affect the 99mTc-ENS radiopharmaceutical and biochemical characteristics. This last observation is very important with regard to the clinical use of this radiopharmaceutical, because the freeze-dried products are more stable than the products in solution (27).

CONCLUSION

Lio-ENS shows suitable physicochemical properties to be used as precursor of 99mTc-ENS. The radiopharmaceutical obtained with this precursor (99mTc-lioENS) shows a radiopharmaceutical and radiobiological behavior that is suitable for pulmonary aerosol scintigraphy. It should be noted also that the shelf life of the nonradioactive precursor of 99mTc-ENS (lipo-ENS) is at least 3 mo, which is an adequate period for its management at nuclear medicine centers.

REFERENCES


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