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TECHNICAL NOTE

^{99m}Tc-ENS, a New Radiopharmaceutical for Aerial Lung Scintigraphy: Comparative Studies in Rats

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ABSTRACT. The biological behavior of ^{99m}Tc-labeled exogenous natural surfactant (^{99m}Tc-ENS) was studied and compared to ^{99m}Tc-diethylenetriaminepentaacetate (^{99m}Tc-DTPA) and $^{99m}TcO_4^-$. The labeling yield percentages for ^{99m}Tc-DTPA and ^{99m}Tc-ENS were higher than 95%. Biodistribution studies performed after aerosolization showed that the percentage of activity concentration in lungs for ^{99m}Tc-ENS was 98.7 ± 1.3%, for ^{99m}Tc-DTPA 77.8 ± 20.6%, and 22.4 ± 7.5% in the case of $^{99m}TcO_4^-$. These results suggest that this new radiopharmaceutical shows an optimal lung concentration, and therefore it can be considered for clinical trials. NUCL MED BIOL 25;5:511–513, 1998. **© 1998 Elsevier Science Inc.**

KEY WORDS: Lung, Surfactant, Rats, ^{99m}Tc, ^{99m}Tc-ENS, Scintigraphy

INTRODUCTION

It is well known that respiratory diseases affect many people all over the world. The most important way of preventing them is an early and correct diagnostic procedure of the respiratory disorder.

For this purpose it should be taken into account that the available non-radioisotopic imaging methods as chest X-ray, CAT, or MRI are nonspecific as they evaluate only macroscopic anatomical disorders. More functional diagnostic procedures are ventilation-perfusion scintigraphy, an important study for the diagnosis of pulmonary embolism, because it gives a good correlation between ventilated and perfused areas (15, 16) and the evaluation of the blood-air barrier permeability, principally for the diagnosis of the adult respiratory distress syndrome (ARDS) (2, 9, 10, 12). This last diagnostic study can be assessed by the pulmonary leak index, for the evaluation of microvascular permeability (10) or by the pulmonary clearance of inhaled ^{99m}Tc-diethylenetriaminepentaacetate (^{99m}Tc-DTPA) for the evaluation of the epithelial permeability (2, 9, 12). It should be noted that previous radiopharmaceuticals for aerial lung scintigraphy, such as ^{133}Xe (3), ^{81m}Kr (15), ^{99m}Tc-DTPA (14), and ^{99m}Tc-technegas (5, 17) are nonspecific for lung scintigraphy.

Exogenous natural surfactants (ENS) are used with success in the treatment of the respiratory distress of newborns (RDS) (11, 13) and seems a promising approach for the treatment of the adult respiratory distress syndrome (ARDS) (6, 7) since pulmonary surfactants are the phospholipid-rich mixture of proteins and lipids that coat the lining of the alveoli. For these reasons we studied at our laboratory a new radiopharmaceutical with the purpose of evaluating the lung ventilation, labeling the exogenous natural surfactant with ^{99m}Tc (^{99m}Tc-ENS) (U.S. Patent Application: 08/742, 977). This radiopharmaceutical is administered by inhalation as a fine aerosol, using a nebulizer. To evaluate the new radiopharmaceutical's specificity for its target organ, the lung, we performed biodistribution studies in rats using ^{99m}Tc-ENS and compared the results with those obtained with ^{99m}Tc-DTPA and $^{99m}TcO_4^-$.

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MATERIALS AND METHODS

Radiopharmaceuticals

$^{99m}TcO_4^-$. $^{99m}TcO_4^-$ was eluted from a molybdenum generator (Bacon Laboratories, Ultra-Technekow® FM, Mallinckrodt®. Activity: 18,500 MBq) as sodium pertechnetate. This isotope was administered in the uncombined form ($^{99m}TcO_4^-$) or was used for the labeling of the DTPA and the ENS.

UNCOMBINED $^{99m}TcO_4^-$. Sodium pertechnetate (296 MBq [8 mCi]) was added to 3 mL of saline solution to obtain an activity concentration of 99 MBq/mL (2.7 mCi/mL).

^{99m}Tc-DTPA. The ^{99m}Tc-diethylenetriaminepentaacetate was prepared by adding 592 MBq (16 mCi) of sodium pertechnetate to a glass flask containing 5–8 mg of DTPA sodium salt and 0.3–0.5 mg of dehydrated stannous chloride (Bacon Laboratories). Then 6 ml of saline solution was added, reaching a final activity concentration of 99 MBq/mL (2.7 mCi/mL).

^{99m}Tc-ENS. The ^{99m}Tc-labeled exogenous natural surfactant was obtained by the following procedure: 2.5 mg of surfactant (Baby Fact P/ GEMEPE SA) containing 0.5 mg of stannous fluoride (FW 156.7, Sigma Chemical) was labeled with 296 MBq (8 mCi) of sodium pertechnetate, with a final activity concentration of 99 MBq/mL (2.7 mCi/mL). The activity of the radiopharmaceuticals was measured in an ionization chamber (RADX model 255 Remote).

Quality Controls

To test the radiochemical purity of the radiopharmaceuticals, an ascending paper chromatography on Whatman paper was performed, using acetone (Merck) as solvent, according to Castiglia *et al.* (4) and Waldman *et al.* (18).

Animals

Thirty female Sprague-Dawley rats, weighing between 240 and 290 g, were randomized in three groups of 10 animals each, placed in stainless steel cages (315 mm × 445 mm × 240 mm high) and maintained with standard food and water *ad libitum* with cycles of 12 h of light and darkness.

Administration of Radiopharmaceuticals

The rats were anesthetized with 300 mg/kg of chloral hydrate AR (Mallinckrodt®). Each radiopharmaceutical was placed in the chamber of a comp-air nebulizer (Omron NE-C08 Nebulizer Comp-air™) to obtain a fine aerosol with particle sizes ranging between 0.5 and 5 μm. A special mask adapted for the shape of each rat nose was used to administer this radioaerosol to the rats for 5 min. After each nebulization, the mask, the chamber, and every nebulizer accesory were decontaminated, washed, and controlled in order to prevent later contamination.

Biodistribution Studies

Twenty-five minutes after the aerosol inhalation, the animals were sacrified to extract their organs, which were 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 cm × 5 cm NaI(Tl) standard well crystal, which was previously set to optimal electronic conditions. All measurements were carried out with constant geometry with an efficiency equal to 5%.

To obtain results independent on the inhaled radioactivity and the organ mass, the data were given as the percentage of activity concentration (C%) of each organ, using the following expression:

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

where: A (cpm) is the measured activity in the organ; m (g) is the mass of the organ; Σ[A (cpm)/m (g)] is the sum of the activity concentrations of all the organs.

Statistical Studies

Results are given as mean ± SD. For comparative studies we evaluated the results by the Kruskal-Wallis test, followed by the Dunns' test, fixing a $p < 0.05$ as the limit for the significance (19).

RESULTS AND DISCUSSION

As it has been pointed out, our aim in the present work was to compare the biodistribution of ^{99m}Tc-ENS to that of ^{99m}Tc-DTPA and $^{99m}TcO_4^-$.

The labeling yield percentage was always higher than 95% for the ^{99m}Tc-ENS and the ^{99m}Tc-DTPA, even after the aerosolization procedure (Table 1), indicating that the labeling procedure was adequate. The radioaerosol obtained in the aerosolization procedure

TABLE 1. Quality Control of the Radiopharmaceuticals. Labeling Yield Percentage for ^{99m}Tc-ENS and ^{99m}Tc-DTPA before and after Aerosolization

Product	Before aerosolization	After aerosolization
^{99m}Tc-ENS	98.2 ± 2.6%	97.9 ± 2.2%
^{99m}Tc-DTPA	99.2 ± 0.4%	99.2 ± 0.5%

The difference between the labeling yield percentage of the radiopharmaceuticals, before and after the aerosolization, was not statistically significant.

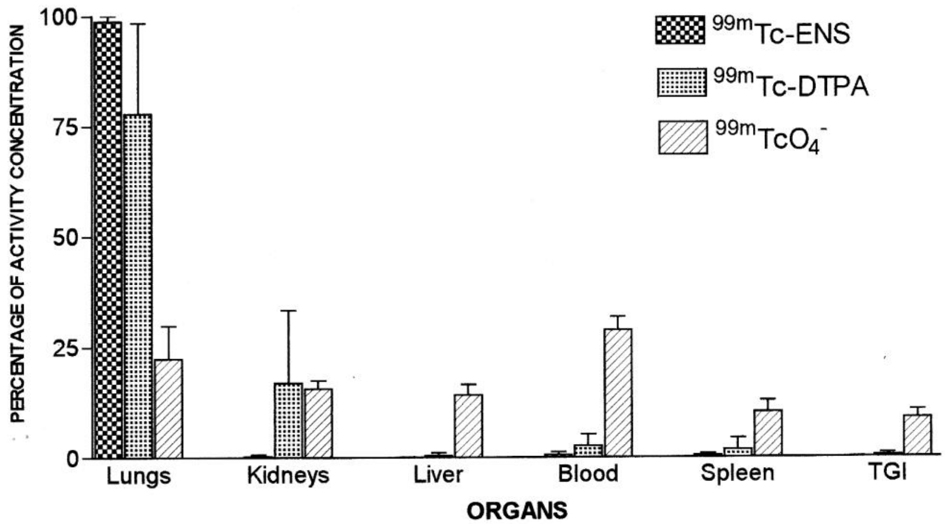
was suitable for radioaerosol diagnosis, as its required conditions, particle size, and tracer-ligand binding were optimal (18). A particle size between 0.5 and 5 μm was obtained, which is associated to a good deposition pattern. Particles smaller than 0.5 μm are generally exhaled and particles larger than 5 μm are deposited in the higher air tract (1, 8). As Waldman *et al.* (18) demonstrated that, with an ultrasonic nebulizer, chemical breakdown takes place for ^{99m}Tc-DTPA, which is not the case with a jet nebulizer, we used a jet nebulizer to prevent this problem.

Biodistribution studies for all the products are shown in Figure 1. It can be observed that in lungs the ^{99m}Tc-ENS has an activity concentration of 98.7 ± 1.3%, whereas ^{99m}Tc-DTPA and $^{99m}TcO_4^-$ show activity concentrations of 77.8 ± 20.6% and 22.4 ± 7.5%, respectively. The difference between the results obtained with all the products is statistically significant ($p < 0.05$). It should be noted that the biodistribution result of ^{99m}Tc-DTPA show a high standard deviation in lungs, which indicates the uncertainty of its accumulation in these organs. These results can be explained by taking into account the different physicochemical properties of the products under study.

Huchon *et al.* (12) concluded that low molecular weight solutes cross respiratory membranes faster than do high molecular weight ones. However, the physicochemical properties of a particular radiolabeled solute affects its clearance (12). This agrees with our study: $^{99m}TcO_4^-$, the smallest molecule, has the lowest activity concentration in lungs. Moreover, it has been demonstrated that this molecule has a higher diffusion rate of the air-blood barrier than does ^{99m}Tc-DTPA (14). The same behavior has been observed in our study.

In the case of ^{99m}Tc-ENS we observed that almost all the radiopharmaceutical remains in lungs, with a small standard deviation indicating a reproducible biodistribution pattern. This result may be attributed to the fact that ^{99m}Tc-ENS is fixed onto the alveolar surfactant layer because it is homologous with its components. It is interesting to analyze the percentage of activity concentration found in the kidneys, taking into account that they are responsible for the elimination of the products. For ^{99m}Tc-ENS this value is very low (0.3 ± 0.4%), indicating that up to 25 min after the nebulization the radiopharmaceutical is practically not eliminated. However, the values obtained for $^{99m}TcO_4^-$ (15.6 ± 1.9%) and ^{99m}Tc-DTPA (16.9 ± 16.5%) are statistically different from that of ^{99m}Tc-ENS, indicating that they cross the blood-air membrane and are consequently eliminated through urine. On the other hand, the biological behavior of inhaled $^{99m}TcO_4^-$ shows a nonspecific distribution in all the organs.

The biological behavior of ^{99m}Tc-ENS demonstrates that almost all the radiopharmaceutical concentrates in lungs, whereas its activity concentration is very low in all other organs. This last observation is due to the high specificity of the ^{99m}Tc-ENS. Our



Product	Lungs (%)	Kidneys (%)	Liver (%)	Blood (%)	Spleen (%)	TGI* (%)
^{99m}Tc-ENS	98.7±1.3	0.3±0.4	0.1±0.1	0.6±0.6	0.4±0.4	0.1±0.1
^{99m}Tc-DTPA	77.8±20.6	16.9±16.5	0.5±0.6	2.6±2.6	1.7±2.6	0.5±0.4
$^{99m}TcO_4^-$	22.4±7.5	15.6±1.9	14.1±2.4	28.8±3.0	10.2±2.6	9.0±1.7

* TGI is gastointestinal system (gut + stomach).

FIG. 1. Biodistribution studies expressed as percentage of activity concentration of the different products: ^{99m}T(^{99m}Tc-DTPA, and ^{99m}Tc-ENS. The table shows the mean values ± SD.

results suggest that this new radiopharmaceutical may be effective for the diagnosis of ventilatory related pulmonary disorders.

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