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Research Article

Quality control methods for ^{99m}Tc-labeled exogenous natural surfactant (^{99m}Tc-ENS)

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Summary

To standardize the quality control for ^{99m}Tc-ENS, the following methods were studied: (1) physical properties and pH, (2) radiochemical purity (chromatographic studies on Whatman-1 paper, or instant thin-layer chromatography and solvent extraction using different solvents and (3) rat biodistribution studies by intratracheal injection. The tolerance limits were fixed for each method. The radiopharmaceutical stability was also evaluated. The results showed that ^{99m}Tc-ENS was a white suspension with a pH between 4.0 and 6.0. The limit for radiochemical impurities in Whatman-1 paper/acetone was fixed at lower than 2% and the established limit for the organic aliquot in cyclohexane extraction was greater than 2%. In the biodistribution studies, the limits for activity concentration were fixed at greater than 90% for lungs, less than 9% for the gastrointestinal system and less than 1% for the sum of the other organs studied. After a storage time of 6 h at room temperature or in a refrigerator, ^{99m}Tc-ENS physical properties and pH, radiochemical and biodistribution results were within the established values. In conclusion, the quality control methods for ^{99m}Tc-ENS are tests on physical properties and pH, radiochemical purity by Whatman-1 paper/acetone chromatography and cyclohexane extraction and biodistribution studies in rats. The stability of this radiopharmaceutical is at least 6 h at room temperature.

Key Words: surfactant; ^{99m}Tc-ENS; quality control; lung ventilation scintigraphy; radiopharmaceutical

Introduction

Exogenous natural surfactant (ENS) labeled with ^{99m}Tc (^{99m}Tc-ENS) has been studied for aerial lung scintigraphy. The quality of the images obtained with

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this radiopharmaceutical are comparable to those of ^{99m}Tc-DTPA,[1] the radiopharmaceutical most commonly used for this study in our country. However, ^{99m}Tc-ENS has demonstrated more specificity in animal lungs than ^{99m}Tc-DTPA.[2]

As it is well known, the safety and efficacy of drugs are important to be asserted. This requires a well-established quality assurance protocol. These concepts also hold true for radiopharmaceuticals, most of them being prepared from kits and labeled with ^{99m}Tc from a ^{99}Mo-^{99m}Tc generator. All the products involved in the preparations are licensed with detailed quality control procedures. It should be borne in mind that the chemical reactions behind simple kit procedures are complex, involving stoichiometry, side reactions and possible impurities.[3] In the particular case of ^{99m}Tc-ENS, the freeze-dried ENS + gentisic acid + stannous chloride is the non-radiactive precursor to be labeled with ^{99m}Tc in Nuclear Medicine Centers.[4] To preclude any undesirable effect of the radiopharmaceutical to the patient, the preparation of radiopharmaceuticals should include quality assurance parameters such as physical properties and pH, radiochemical, radionuclidic and chemical purity, biodistribution, sterility and apyrogenicity.[3] Biodistribution tests are performed to make sure that the radiopharmaceutical is directed to the organ studied. It is generally carried out by intravenous injection since this is the route of administration to the patient; however, for ^{99m}Tc-ENS each rat received an intratracheal injection of this radiopharmaceutical since lung aerial scintigraphy is performed by nebulization.

Another important point to take into account is the stability of the prepared radiopharmaceutical, which in some cases determines that the radiopharmaceutical can be only used in a very short period post-preparation.[5]

The aim of this paper is to standardize the quality control methods of ^{99m}Tc-ENS and to study the stability of this radiopharmaceutical.

Experimental

Radiolabeling procedure

^{99m}Tc-ENS: $^{99m}TcO_4^-$ solution (eluted from a ^{99}Mo-^{99m}Tc generator, Radiofarm®. *Activity*: 18500 MBq) was added to vials containing a freeze-dried powder with the following composition: 2.5 mg of ENS, 1 mg of gentisic acid, 0.5 mg of stannous chloride.

$^{99m}TcO_4^-$ (used as reference) was used as eluted from the generator.

Hydrolyzed-reduced ^{99m}Tc compound ($^{99m}TcO_2$) (used as reference): $^{99m}TcO_4^-$ solution was injected into a vial containing 0.5 mg of stannous chloride (Sigma Chemical Co.).

The final activity concentration was 0.5 MBq/ml for radiochemical studies and 555 MBq/ml for biodistribution studies. The content of each vial was

shaken vigorously for 60 s and the sealed vial was allowed to stand at room temperature for at least 10 min prior to the analysis.

Quality control

Physical properties and pH. The examination of the physical characteristics before and after reconstitution of each formulation sample were examined and the sample pH was measured using pH test papers (Merck, pH: 1–10 and pH: 0.5–5.0).

Radiochemical purity

Chromatographic studies: An aliquot (10–15 µl) of ^{99m}Tc-ENS was spotted on Whatman-1 paper (RJM Sales, Inc), or silica gel-impregnated glass fiber strips (instant thin layer chromatography, ITLC™ SG, Gelman Sciences). The Whatman-1 paper and ITLC strips were developed with saline solution, acetone, butanol, chloroform or cyclohexane (Sigma Chemical Co., GR/AR grade). The distance of solvent migration was 10 cm in all the cases. To determine the front relation (Rf) strips were dried and cut into 1 cm sections and counted. This procedure was performed in 10 different samples of each labeled compound. After the analysis and comparison of the Rf values in each chromatographic system and choosing the optimal one, 10 samples of ^{99m}Tc-ENS preparation were analyzed to determine the tolerance limits for $^{99m}TcO_4^-$ impurities.

Solvent Extraction studies. An aliquot (500 µl) of ^{99m}Tc-ENS was mixed with 800 µl of chloroform, chloroform: methanol (2:1), phenol (saturated in Tris HCl and stabilized with α-hydroxyquinaline), cyclohexane or butanol and then centrifuged at 14 000 rpm for 10 min. After extraction, an aliquot (500 µl) of each organic fraction was measured. This procedure was performed in 10 samples of each labeled compound. The percentage of activity in each organic aliquot was calculated as $\%A_{org} = [(A_{org}/A] \times 100$, where A_{org} is the activity in the organic phase aliquot, A_0 the activity in the radiopharmaceutical preparation aliquot, $\%A_{org}$ the percentage of activity in the organic phase aliquot. The adequate extraction solvent was chosen according to the results obtained and the limit tolerance percentage for ^{99m}Tc-ENS extraction in that solvent aliquot was established according to statistical procedures.

Biodistribution studies. The experiments performed with animals adhered to ethical standards and were conducted according to local animal care regulations. Ten Sprague Dawley rats (250–300 g) were each anesthetized with 300 mg/kg of chloral hydrate AR (Mallinckrodt®) and received an intratracheal injection of ^{99m}Tc-ENS (0.3–0.5 ml). This procedure was designed taking into account the techniques used by other authors for the

administration of pulmonary surfactant.[6,7] After 30 min post-injection the rats were killed using a lethal dose (800 mg/kg) of chloral hydrate and organs of interest (lungs, gastrointestinal system, heart, blood, liver, spleen and kidneys) were excised, blotted dried and counted. The results were given as percentage of activity concentration.[2] The limit percentage for activity concentration was established for at least two of the three rats which were intratracheally administered with the radiopharmaceutical.[8]

Radiopharmaceutical stability. To study the radiopharmaceutical stability, five samples of ^{99m}Tc-ENS, stored at room temperature (20–25°C) or in a refrigerator (4–8°C) were analyzed by the chosen methods for quality control at preselected times (0.5, 3 and 6 h). The values obtained were compared to the established limits for each methodology.

Measurements

The activity was measured in an ionization chamber (RADX model 255 Remote). The samples of the radiochemical purity and biodistribution studies were measured in a monochannel gamma spectrometer, with a relative error of less than 1%.

Statistical studies

The results of the radiochemical purity and biodistribution studies were expressed as mean $\pm$ SD. For comparative radiochemical studies and to test the differences among the different organs activity concentration percentage we evaluated the results by a one-way analysis of variance (ANOVA) with a previous data transformation when necessary.[9] A value of $p<0.05$ was considered to be statistically significant.

The tolerance limits for the radiochemical purity studies for ^{99m}Tc-ENS was established by the Goldstein's one-side tolerance limits for individual observations test ($p = 0.01$, tolerance = 99%). The limit for the organ percentage of activity concentration was performed by the Goldstein's one side tolerance limits for individual observations test ($p = 0.05$, tolerance = 95%).[10]

Results and discussion

It is well known that quality assurance parameters for radiopharmaceuticals are of great importance[3,11] to preclude any undesirable effect to the patient and to obtain reliable results. For this reason in this paper we standardize the methodologies for the quality control of ^{99m}Tc-ENS.

^{99m}Tc-ENS was a white suspension with a pH between 4.0 and 6.0. These results agree with the previous ones.[4]

Most of the radiochemical purity methods are radiocromatographies in specific reagents to separate the studied radiopharmaceutical from the radiochemical impurities.[3,12] However, for some radiopharmaceuticals the analysis of the radiochemical purity is performed by other methods.[13] In our radiochemical study two different techniques were analyzed in order to separate ^{99m}Tc-ENS from $^{99m}TcO_4^-$ and $^{99m}TcO_2$. The chromatographic studies results are shown in Table 1. It can be observed that only $^{99m}TcO_4^-$ migrated with the solvent front (Rf = 0.7–1.0) in Whatman-1 paper/saline solution, Whatman-1 paper/acetone, ITLC/saline solution, ITLC/acetone and ITLC/butanol while ^{99m}Tc-ENS and $^{99m}TcO_2$ remained at the origin (Rf = 0.0–0.1). These chromatographic systems allow us to clearly separate ^{99m}Tc-ENS from $^{99m}TcO_4^-$. Whatman-1 paper/acetone was chosen as the system to determine the $^{99m}TcO_4^-$ impurities. Developing times were fast and comparable to ITLC/ saline or ITLC/acetone but Whatman-1 paper is more available and less expensive than ITLC. The percentage of $^{99m}TcO_4^-$ impurities in ^{99m}Tc-ENS preparation was $0.39 \pm 0.49\%$ and the limit value for $^{99m}TcO_4^-$ impurities is fixed as less than 2%.

Since ^{99m}Tc-ENS cannot be differentiated from $^{99m}TcO_2$ with none of the studied chromatographic systems, a solvent extraction system was studied. Table 2 shows the percentage of activity in each organic-phase aliquot ($\%A_{org}$) for each labeled compound in different organic solvents. It can be observed that in chloroform/methanol, chloroform or phenol, ^{99m}Tc-ENS and $^{99m}TcO_2$ can be partially extracted. In butanol, ^{99m}Tc-ENS and $^{99m}TcO_4^-$ can be partially extracted. However, in cyclohexane the only compound that is partially extracted is ^{99m}Tc-ENS ($\%A_{org} = 4.92 \pm 0.82$), differentiating it from $^{99m}TcO_4^-$ ($\%A_{org} = 0.20 \pm 0.10$) and $^{99m}TcO_2$ ($\%A_{org} = 0.24 \pm 0.16$), which remain in the aqueous phase. For these reasons, cyclohexane extraction was

Table 1. Standarization of a chromatographic system for ^{99m}Tc-ENS

Chromatographic system					
Stationary phase	Mobile phase	Rf ^{99m}Tc-ENS	Rf $^{99m}TcO_4^-$	Rf $^{99m}TcO_2$	Developing time (min)
Whatman-1 paper	Saline solution	0.07 ± 0.05	$0.78 \pm 0.06_*$	0.06 ± 0.05	25
Whatman-1 paper	Acetone	0.07 ± 0.05	$0.86 \pm 0.07_*$	0.08 ± 0.04	10
Whatman-1 paper	Butanol	0.08 ± 0.04	0.06 ± 0.05	0.07 ± 0.05	100
Whatman-1 paper	Chloroform	0.08 ± 0.04	0.07 ± 0.05	0.07 ± 0.05	30
Whatman-1 paper	Cyclohexane	0.08 ± 0.04	0.06 ± 0.05	0.07 ± 0.05	60
ITLC	Saline solution	0.07 ± 0.05	$0.80 \pm 0.08_*$	0.07 ± 0.05	10
ITLC	Acetone	0.07 ± 0.05	$0.88 \pm 0.08_*$	0.06 ± 0.05	10
ITLC	Butanol	0.07 ± 0.05	$0.76 \pm 0.08_*$	0.06 ± 0.05	41
ITLC	Chloroform	0.07 ± 0.05	0.07 ± 0.05	0.07 ± 0.05	16
ITLC	Cyclohexane	0.07 ± 0.05	0.06 ± 0.05	0.06 ± 0.05	14

Results are expressed as mean ± SD. Rf is the front relation, $^{99m}TcO_2$ is hydrolyzed and reduced ^{99m}Tc techentium, min is minutes, ITLC is instant thin-layer chromatography.
*$p < 0.05$ from Rf $^{99m}TcO_2$ and Rf ^{99m}Tc-ENS.

Table 2. Extraction of ^{99m}Tc-ENS in different solvents

	^{99m}Tc-ENS (%)	$^{99m}TcO_4^-$ (%)	$^{99m}TcO_2$ (%)
Chloroform: methanol 2:1	38.62 ± 12.00	0.20 ± 0.08	18.94 ± 9.15
Chloroform	24.41 ± 5.00	0.51 ± 0.28	14.26 ± 5.78
Phenol	61.74 ± 4.55	11.61 ± 0.87	35.89 ± 4.29
Cyclohexane	4.92 ± 0.82	0.20 ± 0.10	0.24 ± 0.16
Butanol	7.07 ± 0.84	27.82 ± 1.91	0.23 ± 0.05

Results are expressed as mean ± SD of the aliquot percentage extraction. $^{99m}TcO_2$ is hydrolyzed and reduced ^{99m}Tc techentium.

Table 3. Biodistribution standarization for ^{99m}Tc-ENS

Organ	% AC
Lung	97.46 ± 2.10
Gastrointestinal system	2.35 ± 2.10
Heart	0.07 ± 0.05
Blood	0.04 ± 0.02
Liver	0.05 ± 0.03
Spleen	0.08 ± 0.06
Kidneys	0.05 ± 0.05

%AC is percentage of activity concentration. Results are expressed as mean ± SD of percentage of the organ activity concentration.

chosen as the system to discriminate ^{99m}Tc-ENS from $^{99m}TcO_2$ and $^{99m}TcO_4^-$ and the cut-off point was set at 2% in the organic aliquot.

In Table 3 the biodistribution results are shown. The percentage of lung activity concentration (97.4 ± 2.1%) differs significantly from that of the other organs. The percentage of gastrointestinal system activity concentration (2.35 ± 2.1%) differs significantly from that of the other studied organs. The tolerance limit established for at least two of the three studied animals, at 30 min for this administration methodology was greater than 90% for lungs, less than 9% for gastrointestinal system and less than 1% for the sum of the other studied organs (heart, blood, liver, spleen and kidneys). This last limit was established taking into account the fact that the activity in these organs is negligible.

The results of the radiopharmaceutical stability studies, obtained at each time for ^{99m}Tc-ENS stored at room temperature or in a refrigerator, are within the established tolerance limits. Therefore the stability of ^{99m}Tc-ENS is at least 6 h. This agrees with a convenient time for performing the studies which is recommended, an expiration time between 3 and 6 h for prepared radiopharmaceuticals.

Conclusion

The quality control methods for ^{99m}Tc-ENS are physical properties and pH determinations, radiochemical purity analysis with Whatman-1 paper/acetone

chromatography, cyclohexane extraction and biodistribution studies in rats by tracheal instillation. The stability of this radiopharmaceutical is at least 6 h at room temperature.

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