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Improved Labeling Technique and Radiochemical Purity Determination of ^{99m}Tc-*N*-acetylcysteine

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A simple procedure for the labeling of *N*-acetylcysteine with ^{99m}Tc and the results of optimization of the labeling procedure are described. The best results are obtained using 10 mg of the ligand and 26 μg of Sn(II), at an alkaline pH. In these conditions, the radiochemical yield is found to be greater than 98%, with good reproducibility. The complex obtained is recommended for further studies in biological systems.

Introduction

Amino acids, as a class, attract considerable interest as potential carriers of radionuclides because of their participation in many vital processes associated with the living system. Studies of amino acids, labeled with positron emitters, demonstrated that the uptake of radioactivity in tumors provided good visualization, which correlated with the malignancy of the lesions (Bading *et al.*, 1986; Schober *et al.*, 1988)

There are many reports about the labeling of cysteine and its derivatives like homocysteine with ^{99m}Tc. They are considered renal scintigraphic agents and show a high uptake in tumors (Takeda *et al.*, 1990; Wang and Liu, 1995). However, cysteine is known to be toxic even when administered in moderate amounts (Greenstein and Winitz, 1961). *N*-acetylcysteine (NAC) was never studied as a tumor imaging agent and even in other settings available literature is scarce and sometimes confusing.

Subramanian *et al.* (1976) first labeled acetylcysteine with ^{99m}Tc for renal imaging. They studied the effect of pH and stability after preparation of the complex, as well as the biological distribution in a few organs of mice. No attempt was made to determine the radiochemical yield of the complex, or to separate labeled technetium (^{99m}Tc – NAC) from non-reacting colloid ($^{99m}TcO_2$).

Johannsen *et al.* (1978) labeled cysteine and several of its derivatives, including *N*-acetylcysteine, using different labeling agents, and characterized the complexes. Details of the procedure and optimization for *N*-acetylcysteine were not explained and it was mentioned that sometimes the formation of the desired complex failed and a polymer was obtained instead. A similarly brief report of the labeling of homocysteine and related molecules, including NAC, is available from Takeda and Okada (1989), again with only passing interest in *N*-acetylcysteine. The aim of the present study was to improve the labeling routine of NAC with ^{99m}Tc under various experimental conditions, in order to obtain a stable and reproducible complex appropriate for further studies.

Materials and Methods

N-acetylcysteine (USP XXII) was provided by Oxford Nutrition (U.K.) and $Na^{99m}TcO_4$ was eluted from a ^{99}Mo generator (Institute of Energetic and Nuclear Research/National Committee of Nuclear Energy, São Paulo, Brazil). Three types of buffers were used to reach the required pH in the mixture of the labeling procedure, namely 0.2 M Na_2HPO_4/0.1 M citric acid for a final pH 4, 0.1 M H_2KPO_4/0.1 M NaOH for a final pH 6 and 7, and 0.1 M Na_2HPO_4/0.1 M NaOH for a final pH 8 and 9.

Labeling of NAC with ^{99m}Tc-basic procedure

The ligand (10 mg) was dissolved in 1 mL of distilled water. The buffer solution, previously nitrogenated, was added to obtain the selected pH. A solution containing 500 μg of Sn(II)/mL was

prepared in 0.1 M HCl, also previously nitrogenated, and 100 μL was added to the solution above, followed by 37 MBq/mL of $Na^{99m}TcO_4$. The mixture was stirred and allowed to stand for 30 min at room temperature. Subsequently, it was filtered through a 0.22 μm millipore membrane.

Optimization of reaction parameters

The parameters of the ligand concentration (3, 5, 10, 15 mg/mL of NAC), mass of the reductor (26, 50, 100, 260 μg of Sn(II)), pH of the reaction mixture (4, 6, 7, 8, 9) and the incubation period (30, 60, 90, 120 min) were optimized by varying them one at a time, so as to obtain a maximum yield and radiochemical purity of the final product ^{99m}Tc – NAC.

Radiochemical analysis

Radiochemical purity of the final solution was examined using chromatographic methods, with Whatman 3MM and Whatman no. 1 papers, as well as with thin layer chromatography silica gel (TLC-SG) in 1.5 × 13 cm strips using acetone and saline as solvents. The chromatographic system provides consistent results. Pertechnetate ($^{99m}TcO_4^-$) moves with the solvent front in acetone ($R_f = 1.0$) while hydrolyzed reduced technetium ($^{99m}TcO_2$) and the labeled compound (^{99m}Tc – NAC) remain at the starting point of the strip. With saline solvent, $^{99m}TcO_2$ remains at the origin ($R_f = 0$) and the complex moves with $^{99m}TcO_4^-$ to the front of the strip. The labeling yield and the quantities of ($^{99m}TcO_4^-$) and $^{99m}TcO_2$ are derived from the two sets of chromatograms developed in acetone and saline.

Results and Discussion

Typical labeling yields of ^{99m}Tc – NAC prepared under various conditions are shown in Table 1. With the increase of final pH, higher yields were achieved with a maximum value of 98.76% at pH 9. As NAC is strongly acidic, a pH 12 buffer was necessary to reach the reported pH 9 in the solution.

Table 1. Representative effects of ligand mass, reductor mass and pH on radiochemical purity of ^{99m}Tc-NAC

Parameters		^{99m}Tc-NAC %	$^{99m}TcO_4^-$ %	$^{99m}TcO_2$ %
NAC† (mg)	3	95.56	0.77	3.67
	5	96.50	0.33	3.17
	10	98.43	0.22	1.35
	15	88.69	2.65	8.66
Sn (II)‡ (μg)	26	98.73	0.69	0.58
	50	98.53	0.58	0.89
	100	98.69	0.46	0.85
	260	93.57	0.75	5.68
pH§	4.0	69.18	0.13	30.69
	6.0	78.29	0.12	21.59
	7.0	86.38	0.22	13.4
	8.0	96.10	0.34	3.56
	9.0	98.76	0.42	0.82

† With 200 μg of $SnCl_2.2H_2O$, at pH 9, 30 min incubation period.
‡ With 10 mg of NAC, at pH 9, 30 min incubation period.
§ With 50 μg of Sn (II) and 10 mg of NAC, 30 min incubation period.

Studies of the effect of pH on the labeling of NAC with ^{99m}Tc made by Subramanian *et al.* (1976), in the range 3–8.4, indicated results opposite to our results. They obtained the best binding at a lower pH and as the pH increased a pink color appeared. In our study, we did not observe any change in the color of the solution, which remained clear throughout the experiment. A comparison with the results of Johannsen *et al.* (1978) could not be done because, as indicated above, these authors furnished only basic information about their technique.

It is known that when the pH is more alkaline proton-dissociated forms tend to appear at the extremities of the molecule involving sulfhydryl (-SH), carboxyl (-COOH) and other radicals, thus creating optimal conditions for the labeling of technetium to the functional groups (Takeda and Okada, 1989). Variation of the mass of Sn(II), from 26 to 260 μg, revealed a progressive increase in $^{99m}TcO_2$ formation. Consequently, the smallest amount was selected (26 μg of Sn(II), equivalent to 50 μg of $SnCl_2.2H_2O$)

Subramanian *et al.* (1976) used a proportion of Sn(II), at least 10 times larger, and only MEK as solvent, so they could not separate ^{99m}Tc – NAC from the colloid $^{99m}TcO_2$. Indeed, in their biodistribution study the uptake by the liver was high, about twice the percentage noticed in our preliminary studies (still unpublished). The pattern of uptake of unbound colloid of technetium, which is strongly hepatotropic, is consistent with the numbers of those authors.

Takeda and Okada (1989) made a study of ^{99m}Tc-homocysteine and some related compounds, including NAC. The yield obtained for *N*-acetylcysteine was only 61.8%, which was considerably less than in the present investigation. Again, the difference could be attributed to lack of optimization of the technical procedure.

In Table 1 the influence of ligand mass on the yield can be assessed. Between 3 and 10 mg, the results continuously improved, but larger masses reduced again the benefit, possibly due to an imbalance between the ligand and Sn(II). Thus, 10 mg was adopted as the ideal quantity. With regard to the incubation period, increases above the standard period of 30 min were not associated with yields higher than 98%, therefore, this value was settled as the appropriate incubation period.

After the analysis of all parameters, the final labeling protocol was defined as follows: 10 mg of NAC, 26 μg of Sn(II), pH 9.0 for the reaction mixture and incubation time of 30 min. This methodology was repeated numerous times, without failures and with yields similar to those shown in Table 1. The complex obtained can be recommended for further studies in biological systems.

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