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Biodistribution of a new isotope tracer in an experimental tumor model

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SUMMARY

The biodistribution and kinetics of N-acetylcysteine labelled with the radioisotope Technetium-99m was assessed in healthy controls and in Walker 256 carcinosarcoma bearing rats. The biodistribution of ^{99m}Tc-NAC was characterized by rapid blood clearance and high kidney uptake.Initial tumor concentrations were low but reached reasonable tumor/blood and high tumor/muscle ratios by 4 and 24 hours after injection. The results indicate that ^{99m}Tc-NAC is a promising tracer for tumor imaging in this experimental model, and deserves further investigation.

INTRODUCTION

The Walker 256 carcinosarcoma is a well-known laboratory mammary strain that is easily implanted in the rat[1] , where its growth can be documented and biodistribution studies performed.

There is great interest in the development of efficient tumor-imaging radiopharmaceuticals. Amino acids as a class attract considerable interest as potential carriers of radionuclides, both due to their wide distribution across tissue barriers and fluid compartments, and owing to their physiologic role in protein synthesis and other metabolic processes.

N-Acetylcysteine (NAC) is an antioxidant amino acid and cysteine precursor, currently used as a mucolytic agent and as antidote during paracetamol poisoning [2].

NAC was labelled with technetium-99m because of favorable physical and radiation characteristics (photon energy - 140 keV, half-life - 6.02 h)[3] which could provide adequate visualization of the malignancy.

In previous studies ^{99m}Tc-N-Acetylcysteine has been suggested as a renal imaging agent[4]. In the present paper the possible interest of the drug for diagnostic imaging in cancer is analysed, along with the uptake and kinetics of the amino acid in the described model.

MATERIALS AND METHODS

N-Acetylcysteine (NAC) (USPXXII) was provided by Oxford Nutrition (U.K.) and $NA^{99m}TcO_4$ was eluted from the Mo-99 generator of the Institute of Energetic and Nuclear Research/National Committee of Nuclear Energy, (IPEN/CNEN) São Paulo.

Preparation and radiochemical analysis of ^{99m}Tc-NAC

The ligand NAC (10 mg) was dissolved in 1 mL of distilled water. A phosphate buffer solution pH 12 and a solution containing 26 µg of Sn (II) prepared in HCl 0,1 N, both previously nitrogenated, were 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.

Radiochemical purity of the final solution was determined by silica-gel thin layer chromatography (ITLC-SG) using acetone and saline as solvents.

Animal experiments

Biodistribution studies were perfomed in male *Wistar* rats (250-300 g). These animals were stratified in two groups, namely healthy controls and tumor-bearing subjects. Sixty animals were investigated in each group, divided in lots of six rats according to time of sacrifice after tracer injection.

Each animal, was weighed and anesthetised (urethane), receiving an intravenous injection of the radiotracer (100 µCi/3.7MBq). At 1,5,10,15,30,60,90,120,960 and 1440 min. after injection, the animals were sacrificed by decapitation. Blood samples were collected in heparinized tubes and all principal organs and tissues excised and weighed. The specific radioactivity was measured in a NaI(Tl activated) gamma well counter. Tissue activity was expressed as percentage of injected dose per gram wet weight (mean±SD). The percent of dose/g of organ was determined by comparing tissue radioactivity with suitably

diluted aliquots of the injected dose. Samples of blood were centrifuged and plasma activity was also determinated. Additional determinations were performed including urinary and fecal activity, binding to plasma proteins[5] and binding to erythrocytes.

RESULTS AND CONCLUSIONS

^{99m}Tc-NAC was produced with excellent radiochemical purity using the stannous procedure (greater than 98%). The preparation was simple and convenient,. with good reproducibility of the labelled compound.

Table 1 - Biodistribution of ^{99m}Tc-NAC (% Injected Dose/g)

Time	1 min.		5 min.		10 min.	
Organs	Normal	Tumor	Normal	Tumor	Normal	Tumor
Kidneys	4.46±1.61	3.02±0.77	7.62±0.93	5.12±2.17	10.55±4.54	6.28±1.68
Liver	0.67±0.16	0.52±0.12	0.42±0.08	0.28±0.07	0.38±0.10	0.31±0.06
Heart	1.14±0.32	0.86±0.03	0.56±0.12	0.33±0.09	0.30±0.04	0.34±0.10
Lungs	1.24±0.39	1.03±0.24	0.77±0.26	0.42±0.17	0.50±0.13	0.41±0.10
Spleen	0.51±0.06	0.42±0.11	0.34±0.14	0.21±0.07	0.21±0.07	0.21±0.06
Stomach	0.64±0.18	0.32±0.07	0.42±0.15	0.25±0.07	0.33±0.12	0.28±0.05
Large int.	0.62±0.08	0.36±0.09	0.45±0.12	0.26±0.13	0.30±0.08	0.27±0.08
Small int.	0.47±0.09	0.31±0.08	0.31±0.07	0.20±0.06	0.30±0.08	0.19±0.03
Muscle	0.32±0.03	0.25±0.07	0.27±0.06	0.15±0.07	0.15±0.04	0.14±0.03
Tumor		0.20±0.05		0.18±0.07		0.25±0.07
Blood/ml	2.47±0.59	1.89±0.10	1.56±0.34	0.83±0.15	0.92±0.23	0.82±0.25
Plasma/ml	6.10±1.50	4.22±0.68	3.14±1.12	1.75±0.67	1.98±0.60	1.70±0.47

Time	15 min.		30 min.		60 min	
Organs	Normal	Tumor	Normal	Tumor	Normal	Tumor
Kidneys	14.34±7.23	9.94±2.69	17.66±6.17	10.26±3.36	20.21±3.27	10.83±2.98
Liver	0.33±0.13	0.26±0.03	0.29±0.04	0.26±0.06	0.29±0.06	0.25±0.11
Heart	0.28±0.17	0.21±0.06	0.19±0.03	0.16±0.03	0.15±0.02	0.12±0.07
Lung	0.46±0.18	0.32±0.06	0.29±0.09	0.25±0.02	0.23±0.04	0.16±0.09
Spleen	0.18±0.06	0.15±0.02	0.14±0.05	0.12±0.02	0.13±0.03	0.11±0.08
Stomach	0.29±0.10	0.27±0.03	0.24±0.06	0.19±0.05	0.25±0.11	0.19±0.11
Large int.	0.29±0.10	0.20±0.06	0.22±0.11	0.17±0.06	0.23±0.07	0.16±0.08
Small int.	0.28±0.17	0.19±0.04	0.290.08	0.19±0.09	0.31±0.10	0.20±0.08
Muscle	0.13±0.06	0.08±0.03	0.09±0.03	0.06±0.01	0.08±0.02	0.05±0.03
Tumor		0.19±0.07		0.19±0.10		0.19±0.04
Blood/ml	0.75±0.20	0.54±0.08	0.50±0.07	0.40±0.07	0.42±0.07	0.27±0.12
Plasma/ml	1.63±0.72	1.04±0.28	1.13±0.36	0.85±0.16	0.86±0.11	0.66±0.16

Time	90 min.		120 min.		1440 min.	
Organs	Normal	Tumor	Normal	Tumor	Normal	Tumor
Kidneys	21.87±4.46	14.27±3.46	15.90±1.52	13.77±1.84	3.86±0.79	4.97±0.97
Liver	0.26±0.08	0.25±0.11	0.22±0.06	0.25±0.14	0.04±0.01	0.04±0.01
Heart	0.10±0.04	0.12±0.06	0.05±0.01	0.10±0.05	0.09±0.00	0.01±0.00
Lung	0.20±0.03	0.16±0.06	0.11±0.02	0.14±0.08	0.02±0.01	0.02±0.00
Spleen	0.10±0.05	0.12±0.08	0.05±0.00	0.09±0.04	0.02±0.00	0.03±0.01
Stomach	0.17±0.10	0.15±0.07	0.08±0.03	0.09±0.04	0.01±0.00	0.02±0.00
Large int.	0.16±0.13	0.12±0.04	0.11±0.03	0.12±0.06	0.02±0.01	0.02±0.00
Small int.	0.28±0.06	0.21±0.10	0.21±0.02	0.20±0.07	0.01±0.00	0.02±0.01
Muscle	0.05±0.02	0.05±0.02	0.04±0.01	0.05±0.02	0.005±0.00	0.006±0.00
Tumor		0.26±0.02		0.20±0.02		0.04±0.00
Blood/ml	0.30±0.07	0.29±0.08	0.17±0.03	0.22±0.10	0.025±0.00	0.025±0.01
Plasma/ml	0.66±0.21	0.60±0.25	0.29±0.03	0.54±0.21	0.03±0.006	0.02±0.00

The highest concentration of the injected dose was found in the kidneys (one minute uptake of 4.46 ± 1.61 % in normal and 3.02 ± 0.77 % in tumor-bearing animals). Peak levels were reached after 90 minutes for both controls (21.87 ± 4.46%) and cancer organisms (14.27 ± 3.46%). All other organs had much lower results, starting with the lungs (respectively 1.24 ± 0.39 %ID/g and 1.03 ± 0.24 %ID/g after the first minute). The activity in all organs except for the kidneys decreased with time (Table I).

Early tumor concentrations were relatively low but reached an acceptable level along the studied period. As other values rapidly diminished with time, the tumor/blood and tumor/muscle ratios were estimated as 5.2 and 0.89 at 90 minutes and 6.67 and 1.60 at 24 hours respectively. Plasma protein binding was 54.93 ± 6.22% and binding to erythrocytes was 4.58 ± 0.57%. Urine was the main excretory medium with a loss of 56.74 ± 3.92% (controls) and 50.73 ± 3.41 % (tumor-bearing) six hours after injection. Preliminary imaging studies done in gamma camera after four hours permitted clear identification of the tumor mass.

Cysteine and other sulfur-amino acids have been screened as radiotracers in various settings including tumor models, but with few definitive conclusions. Specifically we have not found in the literature more than occasional references to N-acetylcysteine. The successful standardization of ^{99m}Tc-NAC in the conditions of this study, with over 98 % radiochemical purity, enabled the performance of complete biodistribution studies, expanding the knowledge regarding this amino acid.

The prevailing impression regarding N-acetylcysteine, is that it is a natural renal agent, along with other members of this family of molecules, due to the high renal uptake, that dwarfs biodistribution to all other viscera. Yet, this is a long-lived substance, which maintained high concentrations in the kidneys after 24 h (4.97 ± 0.97 % dose/g of organ). Moreover, it had affinity for cancer tissue, with increased uptake between one and 90 minutes, and only slow loss of radioactivity within 24 h. As tracer concentration in blood and all other tissues (except kidneys) steeply diminished after four hours, the tumor/blood and tumor/muscle ratio became increasingly more favorable for imaging procedures. This was preliminarily confirmed during scintigraphic studies done by four hours. Indeed, between 4-24 hours, tumor activity (per gram of tissue) was only exceeded by that of the kidneys, and stayed at far higher levels than in most organs, thus assuring easy differentiation in diagnostic studies.

In the condition of this study, it is concluded that:

1) The tracer ^{99m}Tc-NAC was produced by a simple and convenient technique, with a high degree of radiochemical purity.
2) The biodistribution profile of the drug was ascertained between 1 and 1440 minutes, in both normal controls controls and tumor-bearing animals.
3) Traditional high uptake by the kidneys was confirmed along with a heretofore unreported tropism for experimental mammary cancer tissue (Walker carcinosarcoma).
4) Late measurements (4-24 h) indicated favorable tumor/blood and tumor/organ ratios, permitting the successful application of diagnostic imaging procedures.

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