# $^{99m}$Tc-N-ACETYLCYSTEINE: BIODISTRIBUTION IN RATS WITH TUMOR

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## ABSTRACT

The amino acid N-Acetylcysteine was labelled with Tc-99m by a simple and convenient technique with more than 98 % of radiochemical purity. The biodistribution and the uptake of the radiopharmaceutical was ascertained in healthy controls and in Walker 256 carcinosarcoma-bearing rats between 1 and 1440 minutes. Biodistribution results indicated rapid blood clearance and significant radioactivity in all principal organs and tissues, without marked differences between controls and tumor-bearing organisms. The highest uptake corresponded to the kidneys, but sufficient tropism for experimental mammary cancer tissue (Walker carcinosarcoma) was confirmed.

## I. INTRODUCTION

On account of elevated growth rate, most tumor cells have an increased demand for amino acids.

Relatively high accumulation of amino acids in tumors is believed to be related to the enhanced protein synthesis and/or transport of amino acids [1].

The amino acid N-acetylcysteine (NAC), which contains three functional groups, carboxyl, sulfhydryl and amine is an efficient chelating moiety for $^{99m}$Tc.

N-acetylcysteine is an antioxidant and nucleophile amino acid, as well as a cysteine precursor. It is currently used as a mucolytic agent and in the treatment of paracetamol intoxication. It's also suggested as chemoprotective adjunt in cancer therapy [2].

Nearly 80% of all radiopharmaceuticals used in nuclear medicine are $^{99m}$Tc-labelled compounds. The reasons for such a preeminent position of $^{99m}$Tc in clinical use are its extremely favorable physical and radiation characteristics. The 6 hours physical half-life and the monochromatic 140 keV photons give images of superior spatial resolution [3].

NAC was labelled with Tc-99m by Subramanian et al. (1976) as a renal scanning agent [4].

Walker 256 carcinosarcoma is a well-known laboratory mammary strain that is easily implanted in rats [5], where its growth can be documented and biodistribution studies performed.

The principal goal of this investigation was to assess the uptake of $^{99m}$Tc-NAC in organs and tissues of healthy control animals and tumor-bearing rats. The possible application of the agent in diagnostic imaging for cancer based on these results was also examined.

## II. 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 (100OCi/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.

## III. 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.

The biodistribution of $^{99m}$Tc-NAC is characterized by rapid blood clearance. Ten minutes after injection only 17.22±3.36 and 15.88±4.25 %Dose remain in the blood for control and tumor rats respectively.

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) (Figure 1a). 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 (Figure 1a,b,c,d ).

The tumor uptake was 0.20±0.05 %Dose/g initialy and remain stable until 240 minutes after administration of drug.. 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.

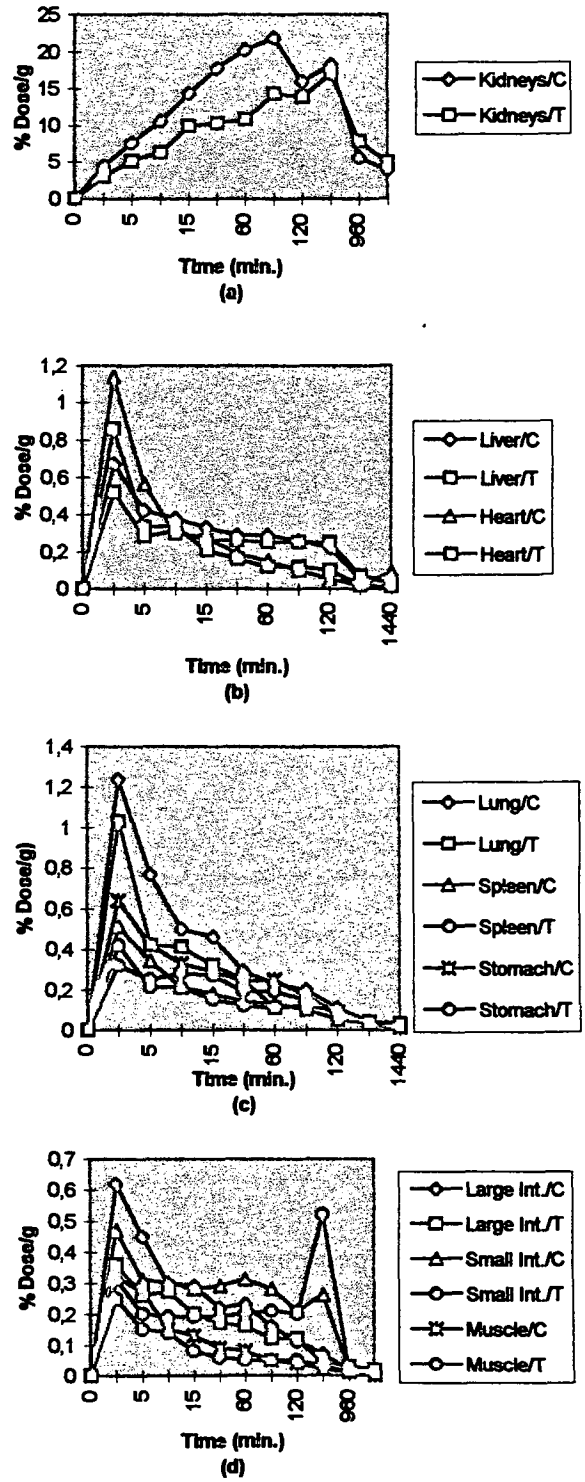


Figure 1 - Biodistribution of $^{99m}$Tc-NAC in Control Rats and Rats with Tumor.
C = Controls T = Tumor-bearing

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 conditions 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) Traditional high uptake by the kidneys was confirmed along with a heretofore unreported tropism for experimental mammary cancer tissue (Walker carcinosarcoma).
3) Late measurements (4-24 h) indicated favorable tumor/blood and tumor/organ ratios.

## ACKNOWLEDGMENTS

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