

## Basic Sciences: Radiopharmacy, Radiopharmacology

### Solid phase labelling of technetium-99m glutamic acid: Radiopharmacological studies

*E. Campos, C. Kremer, A. León, G. Martinez, J. Gaudiano*

Department of Radiochemistry, Faculty of Chemistry and Nuclear Medicine Centre, Faculty of Medicine, University of Uruguay, Montevideo, Uruguay

#### Abstract

Amino acids labelled with Carbon-11 and Nitrogen-13 have uses in the localization of tumours, and in the scintigraphy of the pancreas and myocardium. The present study describes the behaviour of glutamic acid labelled with Technetium-99m in healthy and tumour bearing animals.

Under optimal conditions, a labelling efficiency of 26 % is obtained, with a radiochemical purity of 96 % and no detectable colloids. Zinc concentrations in the final preparation are well below those at which chemical toxicity becomes apparent. Previous studies suggest the structure as that of an oxotechnetium complex  $(\text{TcO}_2(\text{glu}_2))^{4-}$  ( $\text{pH} = 6$ ).

Biodistribution studies have been performed in normal mice. Paper electrophoresis shows that the complex is excreted unchanged via the kidneys. In animals with adenocarcinoma at various stages of differentiation, tumour to blood ratios of between 0.4 and 2.0 are observed. Blood clearance and renal excretion in human subjects are reported and uptake in human breast carcinoma is demonstrated.

**Key words:** Tumor imaging, Tc-99m-amino acids, solid phase labelling, Tc-99m-glutamic acid.

#### Introduction

The potential of amino acids as tumour seeking agents is described in several publications [11], [9], [6], [13]. Of the amino acids, glutamic acid can be identified as showing preferential localization in different types of tumour cells [11], [9], [3]. Amino acids also have chemical structures which allow them to act as ligands in the formation of coordination complexes.

In a previous study, a Technetium-99m glutamate complex using stannous chloride as reducing agent was synthesized. Uptake of this agent has been observed in tumours induced in Wistar rats (adenocarcinoma and malignant fibrous histiocytoma [1]. Studies in patients with metastatic bone diseases, previously demonstrated with Technetium-99m pyrophosphate, showed uptake at the sites of the lesions [3]. Physico-chemical checks and biodistribution studies, however, revealed the presence of varying amounts of colloidal material in the final formulation, later confirmed by double labelling methods [2]. Thus, solid phase reduction techniques [4] were adopted to produce Technetium-99m glutamate free from colloids. The use of zinc as reducing agent produces no detectable colloid and very low levels of zinc are present in the final formulation. Structural studies have been undertaken on the rhenium analogue of the labelled complex. Understanding of the synthetic process and knowledge of the chemical structure of the radiopharmaceutical are considered valuable in rationalising the development of this class of agents, and in the elucidation of structure-activity relationships.

All reagents used were of analytical reagent grade.

### 1. Technetium-99m labelling of glutamic acid using zinc as reducing agent

To 6 ml aqueous glutamic acid solution ( $11 \text{ mg ml}^{-1}$ ) were added 1.5 ml Technetium-99m generator eluate (100 mCi; 3.7 GBq) and 200 mg powdered zinc. After stirring for 20 min on a Vortex mixer, the suspension was allowed to stand for a further 25 min, and filtered through a membrane filter, 0.22  $\mu\text{m}$  pore size.

### 2. Testing of labelled material

2.1. Chromatography. Ascending paper chromatography was performed on Whatman no. 2 paper using acetone and 0.9% sodium chloride solution as developing solvents.

2.2. Biodistribution and renal clearance studies in healthy animals. Biodistribution studies were performed in 30 normal mice. Technetium-99 glutamate was administered by intravenous injection, and the animals killed by neck dislocation between 5 and 75 min post injection. Technetium-99m activity in the selected tissues was assayed in a well counter with a Thallium-activated sodium iodide crystal detector. Data obtained were processed using a Fortran program in a digital PDP 11/23 computer system. Renal clearance curves in Wistar rats anaesthetised with thiopental were obtained using a TMC 404C analyser and gamma spectrometer allowing storage of kinetic data over a 2 hour period. Results were confirmed using a gamma camera with on-line computer, performing a standard renogram study over a 30 min period acquiring frames every 10 sec.

2.3. Toxicity testing. Levels of zinc in the final formulation were estimated using atomic absorbance on a Perkin-Elmer model 380 spectrophotometer. Analysis was performed using an air/acetylene flame, wavelength 214 nm and slit 0.7 nm. Exact dilutions of the formulation were prepared to allow detection of the zinc under optimal conditions of linearity and sensitivity. Standard solutions of zinc in the concentration range 0.5 to 1.0 ppm contained identical concentration of glutamic acid and sodium chloride.

### 3. Structural studies on the labelled material

3.1. Spectrophotometric study of the reduction of perrhenate. It was decided to study the effects of initial pH, and time of reaction on the zinc reduction of perrhenate. To 10 ml of ammonium perrhenate,  $2.5 \times 10^{-4} \text{ M}$ , previously adjusted to the chosen pH, was added 1 gram zinc powder. Reaction proceeded at 60°C with uninterrupted stirring for the chosen time. After filtration, the UV spectrum of the filtrate was recorded on a Shimadzu UV-210 A spectrophotometer. The solid residue dissolved in hot, concentrated hydrochloric acid and treated to boiling for 5 min. The UV spectrum of an appropriate dilution of the resulting solution was recorded.

3.2. Synthesis of the rhenium analogue of Technetium-99m glutamic acid. 250 mg glutamic acid was dissolved in 10 ml  $2.5 \times 10^{-4} \text{ M}$  ammonium perrhenate solution, and the pH adjusted to 10.0 1 g zinc powder was added, and the reaction allowed to proceed at 60°C for 6 hours with continuous stirring. The suspension was allowed to stand for a further 24 hours at room temperature, and filtered. The UV spectrum of the filtrate was recorded against reference solutions of water and saturated glutamic acid solution. The filtrate was processed as described above. A control solution was prepared using water in place of ammonium perrhenate solution.

3.3. Electrophoretic study of the structure of the labelled compound. Paper electrophoresis of the labelled compound was performed at selected pH values. Whatman no. 1 was used as support medium, and separation performed at a potential of 300 V for 90 min. Stability of the labelled complex at the pH values used had been previously verified using paper chromatography. For comparison of electrophoretic behaviour, other metal complexes ( $\text{Ni}(\text{glu})_2$ ,  $\text{Co}(\text{glu})_2$  and  $\text{Cu}(\text{glu})_2$ ) were synthesized and analysed. The

stoichiometric quantity of glutamic acid was added to a solution of the corresponding salt ( $\text{NiSO}_4$ ,  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  or  $\text{CoCl}_2 \cdot 2\text{H}_2\text{O}$ ) [10], [8], [7]. The effect of the buffer on electrophoretic migration was evaluated using a ligand producing a complex whose charge is independent of pH variation.  $\text{K}(\text{Co}(\text{EDTA}))$  was chosen because of its deep colour and ease of localization.

Nickel, cobalt and copper complexes and glutamic acid itself were visualized using a 0.2% ethenolic solution of ninhydrin.

#### 4. Preliminary tests on stability

In vitro stability of the final preparation was determined using paper chromatography. Prior to storage, the preparation had been purged with nitrogen and was stored in an inert atmosphere.

#### 5. Biodistribution studies in mice bearing spontaneous adenocarcinoma

The technique used was the same as that already described for normal mice, using time intervals between 45 and 60 min. Anatom-pathological studies were performed by the pathologic anatomy department, College of Medicine. A slightly modified version of the Ulber technique was adopted in the preparation of whole-body autoradiographs.

#### 6. Clinical studies in humans

Two series were studied totalling 20 patients. The first series comprised 6 male patients, 4 with lung adenocarcinoma, one with tuberculosis of the lung, and one with acute pneumonia. Of the 14 females making up the second group, all had breast tumours, 4 benign and 10 adenocarcinoma. Each patient received between 3 and 10 mCi (111 to 370 MBq)  $\text{Tc}^{99\text{m}}$ -glutamic acid. The first group of patients were imaged continuously for 120 min and then at 3, 5, 7 and 24 hours post injection. An optimum time for imaging of 40 min post injection was established, and subsequent studies involved the acquisition of static images at this time.

Imaging was performed using a large field of view gamma camera, and results stored on computer, to allow subsequent processing or quantification.

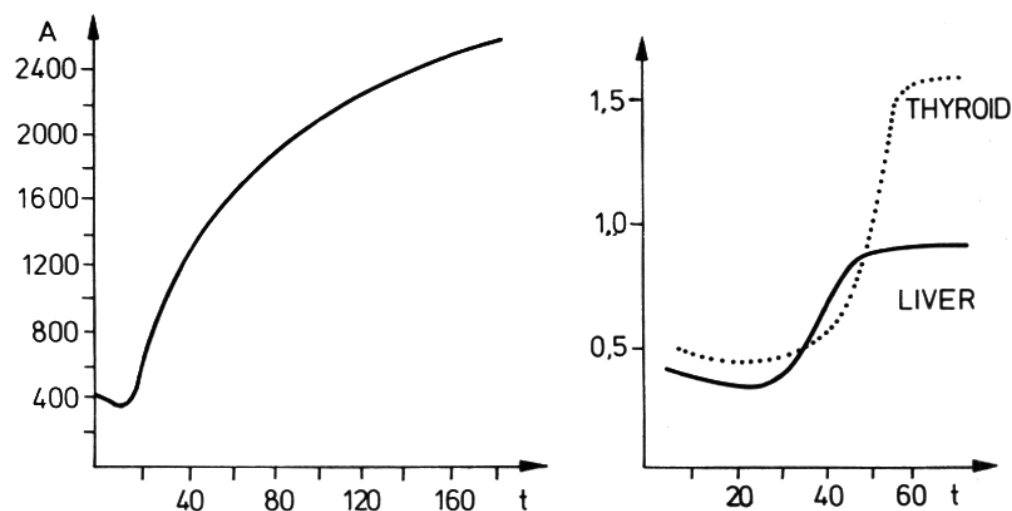


Fig. 1. Time activity curve from region of interest in bladder. Ordinate: Counts/gram of wet tissue in region of interest. Abscissa: time in frames of 10 sec.

Fig. 2. Ordinate: Ratio of activity (gram of wet tissue to activity) gram of blood. Abscissa: time in min.

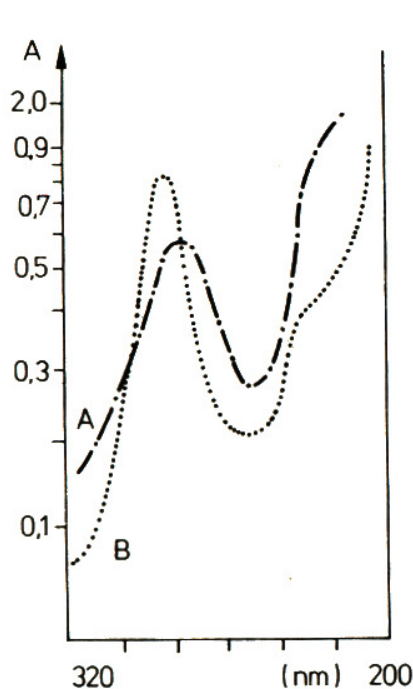


Fig. 3. Spectra obtained in the synthesis of the Re analogue A-filtrate. B-diluted solution of the solid residue treated with concentrated HCl.

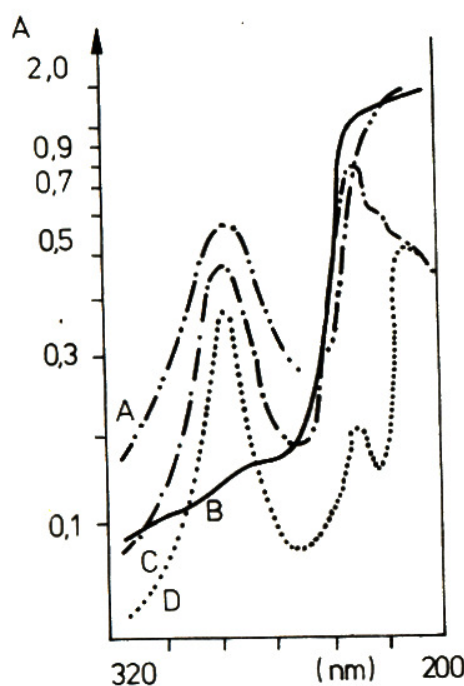


Fig. 4. UV spectra of: A-filtrate of the solution containing Re, reference water; B-filtrate of the control solution, reference  $\text{ReO}_4^-$ ; C-filtrate of the solution containing Re, reference: aqueous solution of glutamic acid, same concentration; D-filtrate of the solution containing Re, reference: filtrate of the control solution.

## Results

Labelling yields of 26 % are obtainable with radiochemical purity in excess of 96 % and no evidence of colloid formation.

Biodistribution studies in normal mice show a high renal clearance (Fig. 1). Liver uptake increases with time, and there is a subsequent increase in uptake in thyroid and stomach (Fig. 2). The compound is not selectively concentrated in brain or pancreas. Renal clearance in Wistar rats is almost the same as that of DTPA, and similar performance observed in human subjects. Zinc concentrations were found to lie between 100 and 160 ppm, regardless of the initial activity of pertechnetate used in the labelling. Study of the UV spectra of perrhenate solution shows rapid disappearance of the characteristic band at 227 nm [12] with no appearance of new ones. This phenomenon is unrelated to initial pH. UV spectra of the solutions of filtrate dissolved in hydrochloric acid all show peaks at 281 nm (Fig. 3a). Under optimal conditions for reaction of perrhenate with glutamic acid, a band of 276 nm (Figs. 3b and 4a) is observed, which is not present when water is used instead of perrhenate solution (Fig. 4b). The band is more noticeable if the spectrum is plotted using glutamic acid solution (Fig. 4c) or the corresponding control solution (Fig. 4d) as reference. Solutions of the filtrate produced from the reaction between perrhenate and glutamic acid produce spectra similar to those shown in Fig. 3a.

Results of electrophoretic studies on the molecular structure of the labelled molecule are shown in table.

Under the conditions studied, the molecular complex appears stable up to 48 hours post labelling. Over this period the free pertechnetate level rises from approximately 1 % to 4–5 %.

Activity cleared in the urine of Wistar rats behaves electrophoretically in the same way as the original injected material.

Turnover to blood ratios expressed as activity per gram of tissue in mice with adenocarcinomas of widely-differing degrees of differentiation vary between 0.4 and 2.0.

The 6 patients with lung pathology all showed high concentration of the radiopharmaceutical in lesions demonstrated by chest X-ray. Uptake is lower in the two cases of infective disease. It seems clear that the highest concentration of radiopharmaceutical seen in the 4 patients with lung adenocarcinoma are external to the lesions. Patients with mammary pathology showed variable degrees of tumour uptake [5].



Table  
Electrophoretical Determinations

Run	pH	Compound	Net charge	Migration (cm)
1	4.4	Ni(glu) <sub>2</sub>	2-	2.7-3.5
2	6.0	Ni(glu) <sub>2</sub>	2-	2.5-3.3
3	3.0	Ni(glu) <sub>2</sub>	0	0
4	4.4	Cu(glu) <sub>2</sub>	2	2.6-3.7
5	6.0	Cu(glu) <sub>2</sub>	2-	3.1-3.6
6	3.0	Cu(glu) <sub>2</sub>	0	0
7	4.4	Co(glu) <sub>2</sub>	2-	2.8-3.3
8	6.0	Co(glu) <sub>2</sub>	2-	2.9-3.5
9	3.0	Co(glu) <sub>2</sub>	0	0
10	4.4	Co(EDTA)	1-	6.7-7.0
11	6.0	Co(EDTA)	1-	6.5-6.8
12	3.0	Co(EDTA)	1-	6.7-7.1
13	4.4	glu	1-	4.3-5.0
14	6.0	glu	1-	5.7-6.4
15	3.0	glu	0	0
16	4.4	<sup>99m</sup> Tc-glu	-	7.2-7.6
17	6.0	<sup>99m</sup> Tc-glu	-	7.2-7.4
18	3.0	<sup>99m</sup> Tc-glu	-	3.5-4.0
19	2.0	<sup>99m</sup> Tc-glu	-	3.6-4.0
20	10.0	<sup>99m</sup> Tc-glu	-	7.4-7.7

### Discussion

Aspects of the study, for example the labelling yields and labelling efficiencies, are considered self-evident from the results expressed and will not be further discussed. Rather, the discussions will concentrate on those parts of the study related to toxicity, structure/activity relationships in human subjects, and clinical results.

The only element in the final preparation considered likely to present a hazard to human subjects in zinc. In relation to normal or therapeutic levels of zinc in blood (63 and 136 µg%) atomic absorption spectrophotometry shows levels 17 times below the normal value (100 µg%).

It is not possible to draw many valid conclusions from the clinical studies because of limited patient numbers, and the quality of the images observed. The best use of the material seems to be in the diagnosis of neoplastic inflammatory lesions of the breast [5].

From the analysis of UV spectra (Figs. 3 and 4) it can be deduced the perrhenate is effectively reduced by zinc, as demonstrated by the disappearance of the absorbance band at 227 nm. The peak at 281 nm in hydrochloric acid is characteristic of the presence of hexachlororhenate with rhenium in oxidation state +4. The absorbance band seen at 276 nm is that of the rhenium-glutamic acid complex.

The table shows electrophoretic migration determined at different pH values, of the Tc<sup>99m</sup>, Co, Cu and Ni

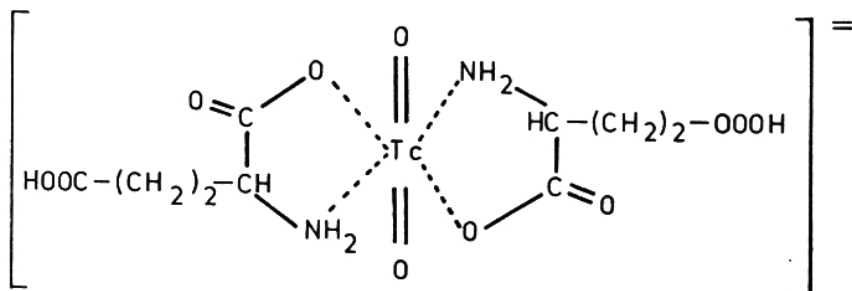
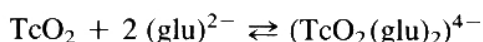


Fig. 5. Structure formula of the Tc-99m-glutamic acid complex.

complexes, of glutamic acid, together with those of the Co (EDTA) ligand. This evidence, together with consideration of the behaviour of glutamic acid as a bidentate ligand [10] and of similarities and differences in aspects of technetium and rhenium chemistry, leads to the proposal of the following structure for the  $\text{Tc}^{99\text{m}}$ -glutamic acid complex (Fig. 5).

Despite the fact that the equilibrium shows in the equation



does not favour the formation of the Tc-glutamic acid complex, once formed it is kinetically stable.

In terms of in vivo behaviour of Tc-glutamic acid, the proposed structure would allow the labelled molecule to attach to the surface of the tumour, with a high affinity for the amino acid, in a manner analogous to that producing a decomposition of the rhenium complex on cationic resins. This process would result in the liberation of  $\text{TcO}_2$  which is subsequently removed from the tumour by the blood. In fact, images obtained in clinical studies, where activity is seen predominantly in the tumour peripheral zones, are seen optimally at 45–60 min post injection, and are not improved at longer imaging times. The same behaviour is observed in mice with spontaneous adenocarcinoma.

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Authors' address:

Prof. Dr. P. Cox, Dr. Daniel den Hoed Kliniek, Postbus 5201, NL - 3008 AE Rotterdam