CLINICAL EVALUATION OF GLY-GLY-ALANINE AND SAME GROUP PEPTIDES LABELED WITH $^{99m}$Tc

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ABSTRACT

In this study, seven different (six of them with –NH and one of them with –NHSH side chain) peptides (Gly-l-Hystidine, Gly-l-Methionine, Gly-l-Tyrosine, Gly-Gly-l-Alanine, Gly-l-leucine amide, Gly-l-Glutamic acid, Gly-l-Gly-amide) were selected. For the positive control, tetruglycin (Gly-Gly-Gly-Gly) was chosen and for each of them, $^{99m}$Tc labeling studies have been performed using both direct and ligand exchange methods. Among these peptides, two of them (Gly-Gly-l-Alanine (GGA) and Gly-l-leucyl amide(GLA)) were labeled with the yields that were close to the USP acceptance criterions. Quality control has been performed by reverse phase HPLC analysis method with the use of a Beckman-Gold Radio-HPLC system. Latter on, radiopharmaceutical distribution and organ uptake characteristics of these peptides ($^{99m}$Tc-GGA and $^{99m}$Tc-GLA) on rabbits were studied by using gamma camera imaging technique. Also, animal bio-distribution with GGA and inflammation imaging studies with GLA on rat models with sterile inflammation have been performed. Results indicated that in direct radio-labeling method developed for the small molecule peptides, labeling yields and retention times for $^{99m}$Tc-GGA and $^{99m}$Tc-GLA radio-complexes were % 75.09 at 12 minute and % 74.20 at 17.5 minutes, respectively in reverse phase Radio-HPLC analysis. For the rest of the five peptides, direct and ligand exchange labeling methods were unsuccessful to produce a radio-labeled peptide that has an acceptable yield in terms of the radiopharmaceutical quality.

Gamma camera imaging studies have indicated that both of the peptides ($^{99m}$Tc-GGA and $^{99m}$Tc-GLA) have demonstrated remarkable rapid blood clearance and dynamic discharge through kidneys. It is possible to say that based on the initial images and renogram data, dynamic kidney imaging can be obtained right after 30 minutes of the application and static kidney scintigrams can be taken by performing late imaging with $^{99m}$Tc-GGA radio-peptide complex. On the other hand, $^{99m}$Tc-GLA complex may have a potential use especially for very clear dynamic kidney and infection imaging studies. In bio-distribution study on rabbits, in terms of radio-peptide retention, the most important organ and body fluid samples were taken and then counted (first with collimated and then with non-collimated gamma camera counting system) with TENELLEC well type detector system. When compared to the other body organs, it was observed that kidneys showed the most active retention and the most of the applied radio-peptide were thrown out through urine. Also, In the infection imaging studies on mouse models
with colitis using the same radio-labeled peptides, \(^{99m}\text{Tc-GLA}\) proved to be more appropriate as indicated by the scintigrams.

**INTRODUCTION**

Recently, labeling of small molecule peptides with radionuclids, which can be used in tumor and infection imaging, has gained enormous importance and become a very intensive research area (1). Although \(^{99m}\text{Tc labeled Monoclonal Antibody (Mab) and their fragments (Fab, Fab2)}\) have been known and used for tumor and infection imaging for the last 10 years, their blood clearance and target organ accumulation rates are limited. In the diagnosis phase, the need for the agents with better blood clearance and target organ accumulation rates has been indicated by the clinicians. With radiolabeled small peptides it is possible to obtain better target specific accumulation behavior, rapid clearance, quality imaging with high accuracy compared to all other known agents (2).

Peptides are small molecules that is easy to synthesize, less susceptible to immune system rejection and have fast blood clearance which results in high T/B (target/background) ratio in a short time period. In addition to this, bonding affinity of the peptides to receptors are much higher than Mab fragments. These characteristics of the peptides make them attractive for diagnostic and treatment applications (3). \(^{99m}\text{Tc labeled chemotactic peptides, Platelet factor-4 based small molecule peptides, Tuftsin receptor antagonists used for imaging of infection foci, Somatostatine analogues used for imaging of tumors, and Platelete glucoproteine receptor (GP IIb/IIIa) used for thrombous imaging are the ones that have gained most attraction (2).**

**MATERIALS AND METHODS**

1.) Application Conditions for RP-HPLC

Analysis of the selected peptides (Gly-1-Hystidine, Gly-1-Methionine, Gly-1-Tyrosine, Gly-Gly-1-Alanine, Gly-1-Leucine amide, Gly-1-Glutamic acid, Gly-1-Gly-amide purchased from SIGMA) which were planned to be radiolabeled with \(^{99m}\text{Tc}\) were performed with a reversed phase HPLC (Beckman System Gold equipped with Nouveau Gold software, Beckman-166 UV detector and 50 µL injection lup) system at Imperial Cancer Research Fund Nuclear Medicine Research Laboratory, London, UK. A Beckman Ultrasphere ODS 5 µm (4.6 x 250 mm) column and acetonitrile (ACN) in 0.01 N phosphate buffer at pH 6 were used. Figure.1 illustrates the gradient mobile phase flow. As shown, the composition of the mobile phase was 5% ACN and 95% phosphate buffer during the first 5 minutes. Between 5th and 25th minutes, the composition of the mobile phase were gradually changed in a way where mobile phase became essentially 95% ACN and 5% phosphate buffer. From 25th to 30th minutes, the composition of the mobile phase was reversed back to initial status (5% ACN and 95% phosphate buffer) and kept the same from this point until the end of the analysis. Flow rate of the mobile phase was set to 1.0 mL/minute. Absorbance measurements were made at 220 nm with UV detector and radioactivity detections were carried out with a multi-channel analyzer system.
2) Direct Labeling Method and Radio-HPLC Application

Direct labeling of the peptides were performed in small sterile glass vials of 2 mL volume. 200 µL portions of the peptide samples were taken from commercial peptide samples prepared in 0.5 N phosphate buffer to give an overall concentration of 1.0 mg/mL and dissolved in 1.0 M HCl. Then 4 µL (113 mg/mL) of freshly prepared SnCl₂ solution were added. When peptide sample and SnCl₂ solutions were thoroughly mixed, 200.0 µL pertecnetate (⁹⁹ᵐTcO₄⁻) (4-8 mCi) sample were added. Finally, 100.0 µL 0.5 M of Na₃PO₄ solution was added and glass vial was closed. Now, labeling of the peptide can be accomplished by either waiting about an hour at room temperature or in about 10 minutes in a water bath at 90°C (4). This radiolabeled peptide solution was cooled to room temperature and 5 µL portion of this injected to the HPLC system for analysis.

3) Biodistribution Studies on Animals

In order to investigate the biological behaviours of the successfully labeled peptides ⁹⁹ᵐTc-GGA and ⁹⁹ᵐTc-GLA biodistribution and infection imaging studies were performed on rabbits and rats. Biodistribution studies were performed on rabbits with both labeled peptides whereas infection imaging studies were done on rats with only ⁹⁹ᵐTc-GLA. For this, four Albino rabbits (two male and two female) and three Winster male rats were chosen. Organ imaging and radio-clearance potential of these peptides were studied by a gamma camera (TOSHIBA GCA 602A). For organ imaging, first peptides were prepared and applied to ear vein of the anesthetized animals. Subsequently animals were put under gamma camera and dynamic images were collected. After two hours, static images were collected for about 10 minutes on the same animal. Once the imaging processes were completed, animals were sacrificed by an overdose of calcium in order to obtain individual body fluids and organ uptake. Each organ (kidneys, column, skin-muscle, spleen, heart, bladder, liver, lungs, and bowel) and body fluids (blood and urine) were counted by a well type (TENNELEC) counter equipped with a NaI(Tl) crystal for biodistribution. To
determine the percent Injected Dose (I.D.%) per gram of the organs, each organ was carefully isolated and weighted. Radio-clearance data were obtained simultaneously from dynamic images with the help of the software of the gamma camera.

RESULTS AND DISCUSSION

It was observed that radiolabeling of small molecule peptides could not be possible unless their metal complexes have a stable complex geometry even if the peptides contains appropriate bonding sites. Therefore, it was concluded that labeling of five of the seven peptides investigated in this study was unsuccessful for this reason. The remaining two peptides were labeled successfully with a yield around 75% as shown on Figure 2 and Figure 3 which illustrate HPLC chromatograms of $^{99m}$Tc-GGA and $^{99m}$Tc-GLA, respectively. In both figures, the first peak on the left side of the chromatogram belongs to the ($^{99m}$TcO$_4$)$^-$ the second one (black area) corresponds to negative control ($^{99m}$Tc-Phosphate complex) and the third peak is for the labelled peptide. It is also interesting to note that there is another small peak on the right side of the peptide peak in Figure 2 and one on the left side of labeled peptide peak in Figure 3. These peaks indicate the presence of the radio-isomer of these labelled peptides. Figure 4 shows the ITLC chromatogram of the $^{99m}$Tc-GGA. It was found out that the labeling yield of the peptide was around 99%. However, as indicated in Figures 2 and 3, the actual labeling yields were found to be around 75 % for bot of the peptides. This indicates that HPLC is more sensitive and can detect the presence of possible radio-isomers otherwise could not be observed with ITLC. This can be an important issue when the radio-isomers show different biochemical behaviour that the main labeled peptide. However, results from animal imaging studies demonstrated that these radio-isomers were showed the same biological behaviour with main labeled peptide as shown in Figure 5a and 5b. Extensive kidney uptake of these peptides were very clear as can be seen on the figure. Kidney uptake Renograms of these peptides were showed the same results as illustrated in Figure 6a and 6b. Figure 6a is the renogram of $^{99m}$Tc-GGA and Figure 6b is for the $^{99m}$Tc-GLA. Because $^{99m}$Tc-GLA is a relatively smaller peptide, infection imaging potential of this peptide was also studied on rats. Figure 7 shows the image of a rat model that sterile infected by 4% acetic acid administrated by rectal way. Organ uptake of $^{99m}$Tc-GLA complex in rabbit organs and body fluids are shown in Figure 8. As shown in this figure, uptake of the kidneys is remarkably higher when compared to the other organs. This clearly indicates that this labeled peptide may have a potential as a kidney imaging agent.

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**Figure 2.** HPLC chromatogram of $^{99m}$Tc-GGA complex in the presence of negative control and pertechnetate.

**Figure 3.** HPLC chromatogram of $^{99m}$Tc-GLA complex in the presence of negative control and pertechnetate.
Figure 4. ITLC chromatogram of $^{99m}$Tc-GGA

![ITLC chromatogram of $^{99m}$Tc-GGA](image)

**Figure 5.**

- **a.** Static gamma camera image of $^{99m}$Tc-GLA injected to Albino rabbit and
- **b.** ROI processed dynamic image of $^{99m}$Tc-GLA injected to Albino rabbit to obtain renogram.

![Static gamma camera image of $^{99m}$Tc-GLA](image)

Late image of Tc-GGA 2 hours

Lymph nodes

kidneys

bladder

Dynamic image of $^{99m}$Tc-GGA

1-Right Kidney
2-Left Kidney
3-Bladder
4-Liver
5-Background
Figure 6. a) Renogram of $^{99m}$Tc-GGA injected to Albino rabbit and b.) Renogram of $^{99m}$Tc-GLA injected to Albino rabbit.

Figure 7. Static gamma camera image of $^{99m}$Tc-GLA injected to Winster Rat with infection.
Figure. 8. Plot of percent injected dose (I.D.%) versus organs and body fluids of an Albino rabbit.

REFERENCES


