Radiolabeling efficiency and stability study on Lutetium-177 labeled bombesin peptide

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Abstract. Bombesin is a 14-amino-acid peptide having the ability to specifically bind gastrin releasing peptide receptors (GRPR) which show over-expression in many types of cancer cells. Therefore, bombesin analogs have been complexed with radionuclides and reported as radiopharmaceuticals for cancer diagnosis and therapy. Lutetium-177 (Lu-177) is a beta emitting radionuclide that decays with a half-life of 6.65 days. The medium beta energy and the relatively long half-life of Lu-177 make it one of the ideal radionuclides used in targeted radionuclide therapy. As the oxidation state of this radioisotope is 3+, it requires multidentate chelators such as DOTA to form stable complex. In this work, the commercially available conjugated peptide, DOTA-[Pro\textsuperscript{1},Tyr\textsuperscript{4}]-bombesin, was labeled with Lu-177 for preliminary formulation as a therapeutic radiopharmaceutical. The aim was to evaluate the radiolabeling efficiency using various amounts of the peptide and the stability in human serum for 7 days. The radiolabeling was performed in sterile water for injection with 5 mCi of Lu-177, adjusted to pH 5.5 to 6.0 by 0.5 M sodium acetate, and incubated at 100\textdegree C for 30 min. It was found that the radiochemical yield was more than 99% when using 20 µg of the peptide, and the complex was stable for a week. Moreover, human serum was used to simulate in vivo condition. The results showed high complex stability with more than 98% remaining intact after 7 days.

1. Introduction
Peptide-based radiopharmaceuticals have been successfully used for the localization and the staging of diseases in molecular imaging technique for over two decades [1]. A higher density of peptide receptors found on tumor cells than in normal tissues benefits peptide-based radionuclide targeting. The radiolabeled peptides will specifically bind the receptors, accumulate in the tumors and reveal themselves as hot spots on images [2]. Moreover, the unbound radiolabeled peptide will be rapidly cleared from the blood pool and non-target tissues resulting in the high target-to-background ratio. Therefore, superior-quality images will be constructed, and tumors can be identified.

Bombesin is a tetradecapeptide initially isolated from frog skin by Erspamer and coworkers [3]. It binds bombesin receptors which are often expressed in several tumors such as breast, ovarian, prostate, lung, colon and skin tumors [4]. It was reported that bombesin analogs could be radiolabeled with both beta and gamma emitting radionuclides [5].

For radionuclide therapy, \textsuperscript{177}Lu has been of interest due to its suitable energy (beta energy 498 keV and gamma energy 208 keV) and half-life (6.65 days), as well as ease of production in high yield via
a cyclotron by $^{176}\text{Yb}(d,p)^{177}\text{Yb} \rightarrow ^{177}\text{Lu}$ [6]. $^{177}\text{Lu}$ can also be produced in nuclear reactor by direct irradiation of enriched $^{176}\text{Lu}$ or indirect irradiation of $^{176}\text{Yb}$. It has been developed as radiopharmaceuticals by complexing with peptide-conjugated-multidentate chelators such as DTPA and DOTA.

In this study, the labeling condition of DOTA-[Pro$^1$,Tyr$^4$]-bombesin with $^{177}\text{Lu}$ has been reported. The stability of the labeled peptide has been investigated and the stability in human serum has been evaluated so as to predict its degradation behavior in biological condition.

2. Materials and methods

2.1. Chemicals and quality control technique

DOTA-[Pro$^1$,Tyr$^4$]-bombesin in TFA salt was purchased from ABX. Lu-177 as $^{177}\text{Lu}$-lutetiumchloride solution in 0.05 M HCl was purchased from IDB Holland BV. All other chemicals and materials were of analytical grade. Thin layer chromatography was used for the chemical quality control of the labeled compound. Radiochemical purity was performed by Instant Thin Layer Chromatography Silica Gel impregnated glass fiber strips (ITLC-SG) using a mixture of NH$_2$OH/EtOH/H$_2$O (2:10:20) as mobile phase. In this system, the labeled compound moved with the mobile phase to solvent front, while free $^{177}\text{Lu}$ and impurities remained at the origin [5]. The chromatograms were analyzed by radio-ITLC equipped with NaI detector. All experiments were performed in triplicate.

2.2. Investigation of labeling conditions

Labeling conditions were investigated with various amounts of DOTA-[Pro$^1$,Tyr$^4$]-bombesin peptide (1 to 30 µg). To the solution of 1 µg/µL peptide in water, 10 µL of 10% (w/v) ascorbic acid as stabilizer and 5 mCi of $^{177}\text{Lu}$ were added; then the pH was adjusted to 5.5 to 6.0 by 0.5 M sodium acetate in a total volume of 300 µL. The reaction mixture was incubated at 100°C for 30 min then cooled to room temperature. Radiolabeling efficiency was evaluated by radio-ITLC-SG. The optimal condition was found and used for further study.

2.3. Stability study

2.3.1. Shelf-life. To an aliquot of 20 µg of DOTA-[Pro$^1$,Tyr$^4$]-bombesin dissolved in water, 10 µL 10% (w/v) ascorbic acid was added; the mixture was labeled with 5 mCi $^{177}\text{Lu}$ at pH 5.5 to 6.0 in 300 µL total volume. After incubation at 100°C for 30 min and being cooled to room temperature, the radiochemical purity was monitored by radio-ITLC-SG at 1, 3, 6, 24, 48, 72, 96, 120, 144 and 168 h.

2.3.2. Serum stability. Serum stability test was performed in human serum. To a solution of 50 µL labeled compound from the same labeling condition as 2.3.1, 450 µL human serum was added, and the mixture was incubated at 37°C. Radio-ITLC-SG was used to analyze for the % remain intact at 1, 3, 6, 24, 48, 72, 96, 120, 144 and 168 h.

3. Results and discussion

3.1. Radio-ITLC-SG chromatogram

The analysis of labeled compound was conducted on the basis that the labeled compound moves with the mobile phase to the solvent front whilst the unreacted $^{177}\text{Lu}$ and other impurities remain at the baseline as shown in figure 1. Other types of mobile phase were reported such as 0.1 M citrate/citric acid buffer pH 5.0 to determine free $^{177}\text{Lu}$ [7] and 10% (w/v) ammonium hydroxide solution : methanol (1:1) to determine colloidal impurities [8]. However, the method used in this experiment is recommended as it could separate all impurities from the labeled compound in one system.
Figure 1. Radio-ITLC-SG chromatogram of (a) free $^{177}$Lu and other impurities and (b) the labeled compound.

3.2. DOTA-[Pro$^{1}$,Tyr$^{4}$]-bombesin labeled Lu-177

The labeling of DOTA-[Pro$^{1}$,Tyr$^{4}$]-bombesin with $^{177}$Lu was performed without purification. Labeling yield was greater than 99% when using no less than 20 µg of the peptide as shown in figure 2. Therefore, this amount of the peptide was used to label with $^{177}$Lu for stability study.

Figure 2. Radiolabeling yields versus peptide amounts of $^{177}$Lu-DOTA-[Pro$^{1}$,Tyr$^{4}$]-bombesin.
3.3. Stability study

3.3.1. Shelf-life. As $^{177}$Lu has a half-life of 6.65 days, the shelf-life of $^{177}$Lu-DOTA-[Pro$^1$,Tyr$^4$]-bombesin was investigated for a period of 7 days. The labeled compound was kept at room temperature and analyzed by radio-ITLC-SG as described above. The results revealed high stability of $^{177}$Lu-labeled peptide as % remain intact was more than 95% after a week, see figure 3. It was also reported that the stability of the labeled compound was checked while it was stored at 2-8°C [9]. However, the results showed no significant differences.

![Figure 3. Stability test of $^{177}$Lu-DOTA-[Pro$^1$,Tyr$^4$]-bombesin for 7 days.](image)

3.3.2. Serum stability. Serum stability experiment was performed in order to forecast in vivo stability of the labeled compound. After incubation in human serum, $^{177}$Lu-labeled compound was measured at several time intervals as shown in figure 4. Although the metabolic degradation was found as % intact decreased, the remaining labeled compound was higher than 95% over 7 days which demonstrated a high stability.

![Figure 4. Serum stability test of $^{177}$Lu-DOTA-[Pro$^1$,Tyr$^4$]-bombesin for 7 days.](image)

4. Conclusion
This study presented the optimal radiolabeling condition of bombesin peptide with the therapeutic radionuclide. The labeling of DOTA-[Pro$^1$,Tyr$^4$]-bombesin with $^{177}$Lu was successful and stable for 7 days. The in vitro stability study showed very promising data. For future work, other in vitro studies such as cell binding and cytotoxicity would be considered before moving onto biodistribution study in animals.
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