

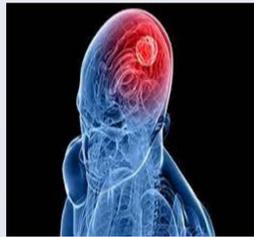
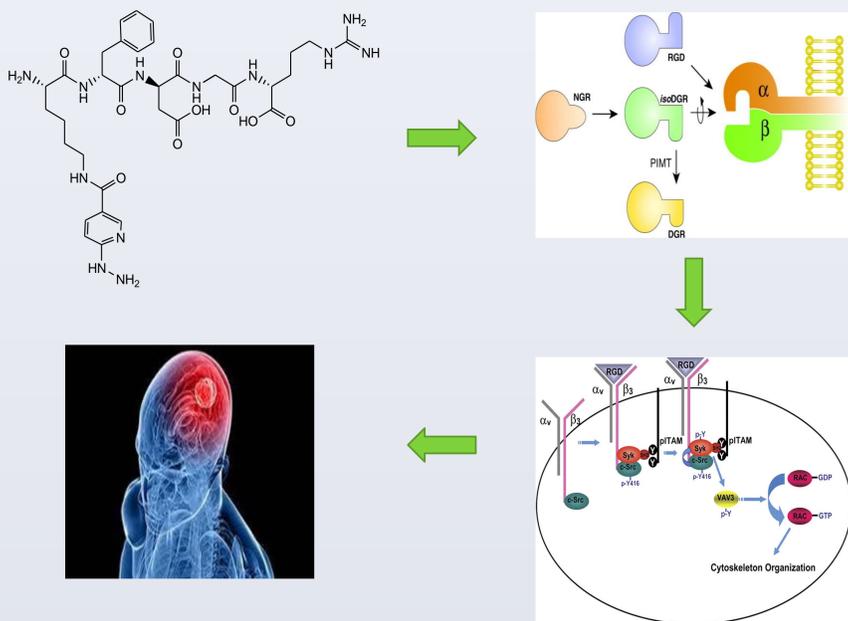
Radiochemical Evaluation and *In Vitro* Assessment of the Targeting Ability of a Novel ^{99m}Tc-HYNIC-RGD for U87MG Human Brain Cancer Cells

Hanieh Karimi , Nourollah Sadeghzadeh , Saeid Abediankenari , Farzaneh Rezazadeh , Fereshteh Hallajian

Department of Radiopharmacy, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Iran

Abstract

Labeled RGD peptide that specifically targets $\alpha\beta_3$ integrin has great potential for the early diagnosis of malignant tumors. $\alpha\beta_3$ integrin receptors appear specifically more on the surface of glioblastoma (malignant glioma) cells rather than normal cells. One of the peptides that has been used in recent years is RGD, which is formed of a sequence of amino acids, such as arginine, glycine and asparagine. Asparagine, but because of the instability of the RGD linear form in an *in vivo* environment, the RGD cyclic form is studied [1,2]. One of the well-known techniques is the amino acid retro-inversion method, which causes greater stability and higher affinity. This method involves the replacement of D- amino acids rather than L- amino acids, so that the peptide structure is completely in reversal. In this topology, the side-chain of new peptides and the mother peptide is quite similar, except that the replacement of D- amino acid rather than L- amino acid gives it a very special stability, because mammals' protease enzymes are not able to identify peptide type D [3].

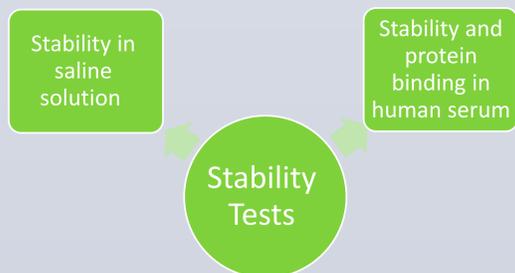


objectives

The aim of this study was to identify a novel RGD that can be radiolabeled with ^{99m}Tc with *in vitro* assessment of its targeting ability for U87MG human brain cancer cells. According to the properties of the RGD peptide that are desired, in this study the form of D (DGR) peptide with HYNIC as bifunctional chelator agent and lysine as the spacer is labeled with ^{99m}Tc radioisotope. After the assessment of radiochemical purity and stability, the absorption rate in U87MG human brain cancer cells as high-receptor expressing $\alpha\beta_3$ is considered.

Materials and Methods

Radiolabeling of peptide HYNIC conjugate was performed by adding 25 μ g (25 μ l) of the stock solution new HYNIC-BB derivative (1 mg/ml in water), 10 mg of tricine, and 2.5 mg of EDDA in 0.5 mL of water at PH=7. 40 μ g SnCl₂ (20 μ l of 2 mg/ml SnCl₂, 2H₂O in nitrogen-purged 0.1 M HCl) was added to this solution. Finally, 370-740 MBq of ^{99m}TcO₄ in 0.5 mL saline was added to the solution and incubated for 10 min. at 95 °C [3]. The reaction mixture was analyzed after cooling it down to room temperature.

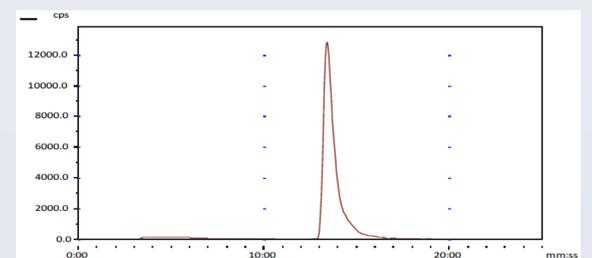


The tendency of radioligand to tumor cells

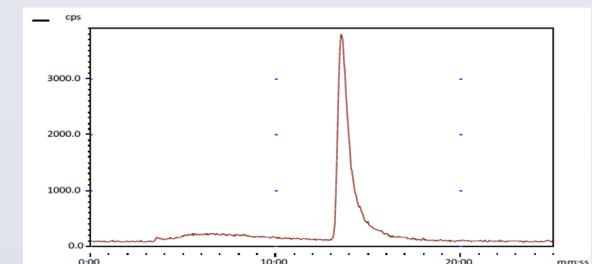
- 1 The U87MG cells in DMEM +10% (v/v) fetal bovine serum (FBS)+1% L-glutamine (2mM) +1% penicillin(100IU/ml)/streptomycin(100 μ g/ml) +1% amphotericin B(0.25 μ g/ml).
- 2 The cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂ and were subcultured weekly detaching with trypsin/EDTA solution (0.25%).
- 3 Medium was removed from the 6-well plates (three unblocked and three blocked groups) contain U87MG cells with density of 1 million cells per well and cells were washed once with 2 ml of medium (DMEM with 1% FBS).
- 4 Furthermore, 1.5 ml medium was added to each well, and the plates were incubated at 37°C for 1 h.
- 5 seven different concentrations (1-40 nmol/L) of radioligand was added to the medium, and the cells were incubated for 2 h at 37°C
- 6 To determine nonspecific binding, we incubated cells in three blocked group with the radioligand in the presence of 150 μ l, 1 μ mol/L cold peptide..
- 7 The cellular uptake was stopped at 1 h by removing medium from the cells and washing twice with 1 ml of ice-cold phosphate-buffered saline (PBS)
- 8 Finally, the cells were solubilized with 1 N NaOH. The culture medium and the receptor-bound were measured in a gamma counter

RESULTS

• Radiochemical Purity: 98%

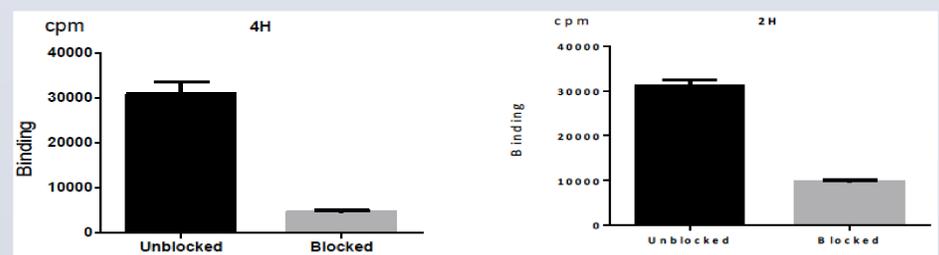


• Stability in human serum : 98%



• Stability in Saline Solution: 94%

• Protein binding of ^{99m}Tc-HYNIC-DGR after 4 hours was less than 20% and 80% free of radio peptide in human serum



• Radioligand specific binding rate in glioblastoma cells was 1.55% after 1 h and it increased 13.96% after 4 h

• rapid internalization (2.28% at 1 h and 14.82% at 4 h)

CONCLUSION

The ^{99m}Tc- (HYNIC)-DGR was prepared with high radiochemical purity and there is a low level of protein-binding using tricine/EDDA. Novel radiopeptide showed a high stability in human serum. In cell studies, specific binding showed the high uptake that increased over time. A good affinity of radioligand to glioblastoma cells has been seen. With these promising characteristics, this radiopeptide demonstrated some potential for targeting $\alpha\beta_3$ integrin receptor-expressing tumors. Therefore, more *in vivo* studies are required.

Some FERENCES

- [1] Decristoforo, C; Faintuch-Linkowski, B. [^{99m}Tc]HYNIC-RGD for imaging integrin alphavbeta3 expression. Nucl Med Biol., 2006, 33(8), 945-52.
- [2] Fani, M.; Psimadas, D.; Zikos, C. Comparative Evaluation of Linear and Cyclic ^{99m}Tc-RGD Peptides for Targeting of Integrins in Tumor Angiogenesis. Anticancer Res., 2006, 26(1A), 431-434
- [3] Giordano, R.J.; Cardó-Vila, M.. From combinatorial peptide selection to drug prototype (I): targeting the vascular endothelial growth factor receptor pathway. Proc. Natl. Acad. Sci. 5117-5112, (11)107, 2010

karimihanieh1@gmail.com