Y-90 Labeled RGD peptide for Tumor Targeting

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1. Introduction

Targeting of tumor angiogenesis-related receptors is a promising approach to advancing cancer diagnosis and treatment. $\alpha_v\beta_3$ integrin is a receptor that mediated cellular interaction with extracellular matrix and plays a crucial role in tumor growth, metastasis and angiogenesis. Radiolabeled cyclic-RGD peptides can be applicable for noninvasive tumor imaging of αvβ3 integrin expression and targeted therapy. In this study, we prepared a new DOTA conjugated cyclic-RGD peptide, DOTA-Maleimido-cyclic(RGDfC), and radiolabeled with Y-90 for therapy through the targeting of tumor $\alpha_v\beta_3$ integrin expression.

2. Methods and Results

2.1 Synthesis of DOTA-Maleimido-cyclic(RGDfC)

For the synthesis of DOTA-Maleimido-cyclic(RGDfC), cyclic(RGDfC) was synthesized by applying standard Fmoc strategy. Briefly, Trityl resin conjugated Fmoc-Aspartic acid allylester was used as a solid supporter of the reaction. After deprotection of Fmoc group using 20% piperidine in DMF, it was coupled with Fmoc protected amino acid under the reaction condition of HBTU, HOBT, DIPEA, DMF. After deprotection of allyl group of aspartic acid using Pd(0) catalyst. Cyclisation was accomplished by the coupling between amine of phenylalanine and carboxylic acid of aspartic acid by applying a conventional amide coupling method.
After cleavage of cyclic((RGDfC) from the resin, DOTA-maleimide was conjugated and purified using RP-HPLC to form a stable thioether compound, DOTA-Maleimido-cyclic(RGDfC).

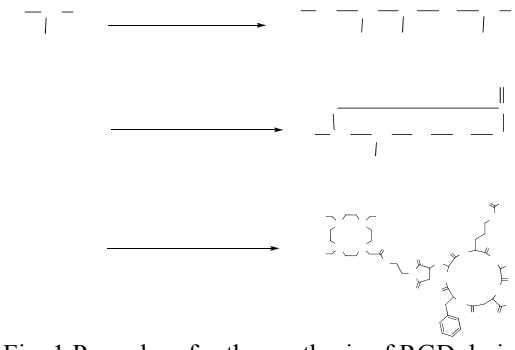

Fig. 1 Procedure for the synthesis of RGD derivatives

2.2 Radiolabeling of Y-90

The filmless autoradiographies of silica gel or polyacrylamide gel were carried out using Cyclone Storage Phosphor System (PerkinElmer, Wellesley). The radioactivity was measured using an ionizing chamber (Capintec 115R, BIODEX Atomlab 200) by setting the calibration value for ^{90}Y that was corrected and calibrated by the manufacturer.
For radiolabeling with [90Y] YCl_3, a reaction vial of DOTA Maleimido-cyclic(RGDfC), 10-6M was prepared in 1 M acetate buffer(0.4ml) containing stabilizer (5mg of ascorbic acid and 6mg of dihydroxybenzoic acid). [^{90}Y] YCl_3 (0.5mCi/ 0.1ml) was added to the reaction vial and incubated at 90 ℃ for 30min. The radio chemical purity was identified by using Cyclone Storage Phosphor System.
Y-90 was labeled with DOTA Maleimido-cyclic(RGDfC) with high labeling yield(>98%) by the reaction at 90 C for 30 min.

2.3 Biodistribution of Y-90 labeled DOTA Maleimido-cyclic(RGDfC)

Biodistribution studies were performed using female balb/C mice and nude mice implanted with Calu6 human lung cancer cells, subcutaneously. For this study, female BALB/c nude mice (Orient.co), aged 6 to 8 weeks, were injected with 5 x 10^6 Calu6 (human non- small cell lung carcinoma) cells subcutaneously. The mice were used for in vivo biodistribution studies 2 weeks post inoculation of tumor cells, when tumors reached a weight of approximately 0.2g. The 0.111MBq (2uCi) of 90Y-DOTA Maleimido-cyclic(RGDfC) was injected intravenously into the tumor bearing mice. For biodistribution studies, the mice were sacrificed 2, 24h (n=5) after injection and the radioactivities in the tumor, kidney, liver, spleen, heart, small and large intestine, lung, stomach and blood were determined using Liquid scintillation counter (Perkin Elmer) and expressed as percentage of the injected dose per gram tissue (% ID/g).
Y-90 -DOTA Maleimido-cyclic(RGDfC) showed fast renal clearance in normal mice and specific uptake in athymic mice. Tumor uptake was 1.48±0.24 %ID/g(2 h post injection) and 0.80±0.08 % ID/g(24 h postinjection) and the blood-to-tumor ratio was 14.47 and 13.56, respectively.

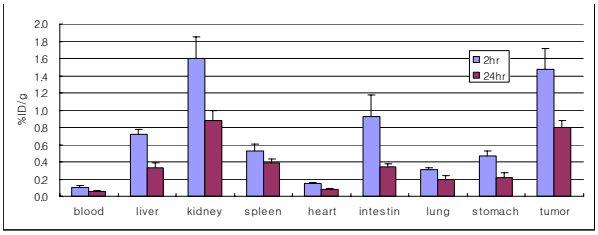

Fig. 2 Biodistribution of 90Y-DOTA-RGD in Calu6 bearing mice at 2 and 24hr p.i

3. Conclusion

We prepared a new Y-90 labeled cyclic-RGD peptide which shown specific uptake in Calu6 human lung cancer cells xenografted in athymic mice. The prepared RGD peptide may be useful candidate for noninvasive evaluation of tumor $\alpha_v\beta_3$ integrin expression and for the targeted therapy of cancer. In future, we will perform well-designed research for the evaluation of its potent as a tumor-targeting therapeutics.

4. Referance

[1] Wangler, B. et al., Application of tris-allyl-DOTA in the preparation of DOTA-peptide conjugates, *Tetrahedron Letters*, 47 (33), p.5985-5988, Aug 2006

[2] Dijkgraaf, Ingrid et al., Effects of linker variation on the in vitro and in vivo characteristics of an 111In-labeled RGD peptide., *Nuclear medicine and biology*, 34 (1), p.29-35, Jan 2007

[3] Indrevoll, B. NC-100717: A versatile RGD peptide scaffold for angiogenesis imaging, *Bioorganic & Medicinal Chemistry Letters*, 16 (24), p.6190-6193, Dec 2006