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Tumor angiogenesis imaging agent: biodistribution of ^{131}I-YG5 and ^{131}I-Boc-YG5

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Abstract The cyclic peptide YG5 and the t-butyloxycarbonyl (Boc)-modified analog (Boc-YG5) were labeled with radioiodine. The radiochemical purity of ^{131}I-YG5 or ^{131}I-Boc-YG5 was almost 100% after purification by RP-HPLC. Biodistribution in BALB/C nude mice bearing MCF-7 tumor was measured. After t-butyloxycarbonyl (Boc)-modification, the ^{131}I-Boc-YG5 was quite resistant to deiodination *in vivo*, resulting in negligible radioactivity accumulation in thyroid. The radiotracer clearance in tumor became faster, the absolute tumor uptake decreased for ^{131}I-Boc-YG5, but the tumor-to-tissue uptake ratios increased. The uptake ratios of tumor to muscle, blood, heart, and lung at 1 h post injection reached 4.73, 1.70, 4.09 and 1.70, respectively. It is demonstrated that Boc-group is an effective prosthetic one to prevent deiodination *in vivo* and improve tumor imaging for radioiodinated NGR.

Key words Radioiodination, Angiogenesis, Biodistribution, NGR, Deiodination, t-butyloxycarbonyl.

1 Introduction

Identified as a cell adhesion motif[1−3], NGR is an effective tumor-homing agent, binding specifically to CD13/APN that is an angiogenic regulator expressed in tumor vasculature undergoing angiogenesis, but not in blood vessels of other normal tissues[4,5]. Compared to RGD, which has been a hot focus in tumor imaging and chemotherapy, NGR has similar affinity on tumor vascular[5,6]. Dox-NGR, NGR-TNF and other anticancer drug conjugated to NGR have revealed the potency of this tumor-homing peptide in chemo-therapy[5–9]. And it is worthwhile to explore the potential of NGR in tumor imaging *in vivo*.

Internalization of small peptides, such as anti-EGFRvIII antibody[10], anti-μ mAb DA4-4 [11] and cyclic RGD containing peptide[12], after their binding to the receptor expressed on the cell membrane, is an important phenomenon. Internalization brings on deiodination of directly radioiodinated small peptides. Many alternative approaches were evaluated for radioiodination of small peptide to prevent deiodination, involving labeled prosthetic group that is coupled to peptide [10−19]. A simple procedure to prevent *in vivo* deiodination of the labeled peptide is very attractive. In a previous study[20], we found that after N-termination with t-BOC-tyrosine, CNGRC became quite resistant to *in vivo* deiodination, leading to negligible radioactivity accumulation in both thyroid and stomach, and such a *t*-BOC protected peptide was stable in human serum even after 24 h. Moreover, it has been confirmed that the bioactive center of NGR is the Asn-Gly-Arg motif and other conjugated groups to the motif affect little on its efficacy to binding to CD13/APN [4, 5, 7–9].

In this paper, the cyclic peptide YGGGGGC<u>NGRC</u> (YG5) and t-butyloxycarbonyl (Boc)-modified analog (Boc-YG5), shown in Fig.1, are labeled with radioiodine. Their biodistributions in tumor-bearing mice are evaluated.

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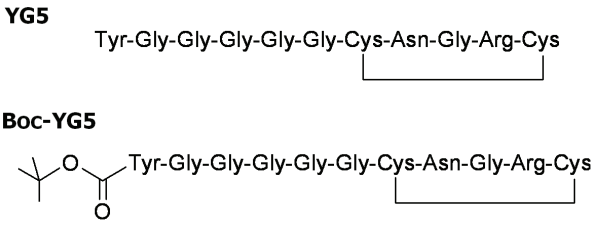

Fig.1 Structure of Boc-YG5 and YG5.

2 Materials and methods

All materials were of reagent grade and used without further purification. The cyclic peptide was purchased from GL Biochem Ltd. (Shanghai, China). A Symmetry C18 column (5 μm, 3.9×150 mm, Waters) was used for sample analysis and separation. All RP-HPLC analyses were performed by using a Waters 600E multisolvent delivery system. The elution was monitored with a Packard 500 TR flow scintillation radioactivity detector (Canberra Industries) in addition to the UV detector. A Packard Cobra II Series Counting System was used to count the radioactivity of tissue samples. A Waters C18 Sep-Pak cartridge was employed for desalination. $Na^{131}I$ was purchased from China Institute of Atomic Energy. The peptides were examined with an MALDI-TOF-MS (matrix-assisted laser desorption ionization-time of flight mass spectrometer) at Mass Spectrometry Center, Chinese Academy of Sciences. BALB/C nude mice (weighing 20–25 g) with MCF7 human breast cancer were purchased from the Cancer Hospital, Chinese Academy of Medical Sciences, Beijing.

2.1 Synthesis and radioiodine labeling of Boc-YG5 and YG5

Boc-YG5 and YG5 were synthesized and purified by our laboratory[20]. The m/z ratios, calculated ($[M+H]^+$) as 1097.4 for Boc-YG5 and 998.4 for YG5, were 1098.3 and 998.3, respectively, by MALDI-TOF-MS.

The peptides were labeled with ^{131}I by using the Iodogen method described in our previous paper[20]. The crude product was desalinated and purified by C18 Sep-Pak column. Radiochemical purity of the radiolabeled product was determined with RP-HPLC. The sample was eluted isocratically with a mobile solution composed of 90% (*V*/*V*) of solvent A (0.1% of TFA in water) and 10% of solvent B (0.1% of TFA in acetonitrile) for 5 min, and followed by gradient elution from 90% to 40% of solvent A over a period of 15 min at a flow rate of 1 mL/min. The retention time was 13.30 min for ^{131}I-Boc-YG5 and 9.60 min for ^{131}I-YG5, while it was 1.90 min for ^{131}I. ^{131}I-Boc-YG5, usually in the purity of over 95%, would not need further purification. However, the radiochemical purity of ^{131}I-YG5 was only about 70%, and a further purification by RP-HPLC was performed. The effluent of radiolabeled peptides was vacuum-dried to remove organic solvent before injection into animals. The radiochemical purity of ^{131}I-Boc-YG5 or ^{131}I-YG5 used in biodistribution was almost 100%, determined by RP-HPLC.

2.2 Biodistribution

Each BALB/C nude mouse bearing MCF7 tumor received a 0.1 mL (5.6×10^4 Bq, 25 nmol/kg) dose of ^{131}I-Boc-YG5 or ^{131}I-YG5 by tail vein injection. The mice were sacrificed by cervical dislocation in groups of three at 1 and 2 h post-injection. The organs or tissues of interest were removed, washed, and weighed prior to radioactivity counting. Such injection solutions (0.1 mL) were taken as standard for calculating the percent injected dose per gram of tissue, i.e., %ID/g. The final results were expressed as mean ± standard deviation (SD). Tumor-to-tissue ratios were calculated from %ID/g of the tumor and relevant organs. All experiments were carried out following the principles of laboratory animal care and the China's law on the protection of animals.

3 Result and discussion

It has been shown that NGR can bind to vessels associated with metastatic breast tumor[21], and it is also used as a model for evaluating effects of new agents on angiogenic chemotherapy[5]. The tissue distribution data of ^{131}I-YG5 and ^{131}I-Boc-YG5 in mice bearing MCF7 human breast tumor are presented in Table 1.

Table 1 Bio-distribution of ^{131}I-YG5 and ^{131}I-Boc-YG5 in tumor-bearing mice (%ID/g).

Tissue	^{131}I-YG5		^{131}I-Boc-YG5	
	1 h	2 h	1 h	2 h
Liver	0.99±0.46	0.83±0.31	0.87±0.36	1.23±0.65
Heart	0.90±0.39	0.64±0.29	0.22±0.05	0.22±0.08
Kidney	2.08±1.00	1.77±0.46	1.64±0.38	1.59±0.27
Lung	2.41±0.16	1.40±0.36	0.53±0.11	0.37±0.06
Spleen	1.30±1.17	0.77±0.24	0.31±0.05	0.25±0.06
Stomach	8.08±4.00	12.29±9.02	1.43±1.06	0.87±0.40
Small intestine	1.44±1.01	1.67±0.96	19.61±4.37	6.67±7.46
Large intestine	1.04±0.17	1.50±0.72	3.10±2.63	23.10±13.19
Muscle	0.62±0.26	0.90±0.34	0.19±0.05	0.38±0.13
Blood	1.58±0.98	1.69±0.64	0.53±0.19	0.46±0.01
Tumor	1.92±0.19	1.28±0.36	0.90±0.15	0.49±0.08

An important parameter to assess the efficacy of tumor imaging agent is the clearance speed of radioactivity from normal tissue. It can be seen that ^{131}I-Boc-YG5 had significantly faster clearance rate than ^{131}I-YG5 from the most examined organs or tissues.

As shown in Fig.2, the thyroid uptake results of ^{131}I-Boc-YG5 and ^{131}I-YG5 were the same as found in normal mice[20]. The uptake of ^{131}I-Boc-YG5 was 0.20±0.07 at 1 h post injection, while the uptake of ^{131}I-YG5 was 4.76±0.66. The difference in the thyroid uptakes of ^{131}I-Boc-YG5 and ^{131}I-YG5 at 2 h post injection was very significant. Therefore, we postulate that it was the Boc group that protected the labeled radioiodine from deiodination. Generally, the radio-iodinated tyrosine can be recognized by deiodinases due to its structure similar to thyroid hormones. After the tyrosine was modified by Boc group, the structure was different from thyroid hormones, *in vivo* deiodination was avoidable[20].

For both ^{131}I-Boc-YG5 and ^{131}I-YG5, the tumor uptake at 1 h was higher (0.90 and 1.92 %ID/g, respectively) than that at 2 h (0.49 and 1.28 %ID/g, respectively). Although the tumor uptake of ^{131}I-Boc-YG5 was lower than that of ^{131}I-YG5, the tumor-to-non tumor uptake ratios (T/NT) increased remarkably for the blood, muscle, heart and lung (Fig.3), with the T/NT at 1 h post injection being 1.70, 4.73, 4.09, and 1.70, respectively, compared to 1.22, 3.10, 2.13, and 0.80 respectively for the liver, large intestine and small intestine. Except for the blood, the differences are significant ($p<0.05$) or very significant ($p<0.01$). It was reported that the modification by linking a prosthetic group to an imaging agent could prevent deiodination[10–18, 22], but this did not always improve the imaging due to the rapid clearance from tumor[18]. In the present study, the introduction of Boc group to the peptide YG5 makes both deiodination and tumor selectivity improved, except the kidneys, liver and gastro-vascular system through which the administered radioiodine is excreted.

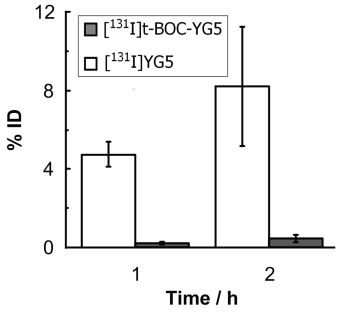

Fig.2 Thyroid uptake of [^{131}I]YG5 and [^{131}I]t-BOC-YG5 in tumor-bearing mice, expressed as %ID per organ.

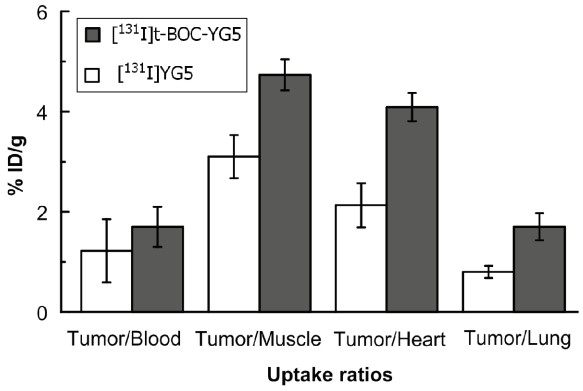

Fig.3 The tumor-to-non tumor uptake ratios (T/NT) of [^{131}I]YG5 and [^{131}I]t-BOC-YG5 in tumor-bearing mice at 1 h post injection, expressed as %ID/g.

Although NGR has been reported more potent than RGD-4C in targeting tumor cells[2], our radioiodine-labeling experiment showed its less effectiveness in tumor imaging, partly because of deiodination *in vivo*, partly due to relatively poor accumulation in tumor. After N-terminal was modified by Boc-group, a very ordinary agent, deiodination almost disappeared and non-targeted radiotracer can be eliminated from the system rapidly. Coupling peptide to a labeled prosthetic group doesn't always bring on higher tumor-to-tissue ratio due to faster elimination from tumor[18]. However, in our case, tumor-to-tissue ratios were also improved.

Conclusively, we demonstrated that Boc-group is an effective prosthetic one to not only prevent deiodination *in vivo* but also improve tumor imaging for NGR.

References

1 Koivunen E, Gay D A, Ruoslahti E. J Biol Chem, 1993, **268**: 20205 – 20210.

2 Koivunen E, Wang B C, Ruoslahti E. J Cell Biol, 1994, **124**: 373 – 380.

3 Healy J M, Murayama O, Maeda T, *et al*. Biochemistry, 1995, **34**: 3948 – 3955.

4 Pasqualini R, Koivunen E, Kain R, *et al*. Cancer Res, 2000, **60**: 722 – 727.

5 Arap W, Pasqualini R, Ruoslahti E. Science 1998, **279**: 377 – 380.

6 Chen X Y, Park R, Shahinian A H, *et al*. Nucl. Med. Biol, 2004, **31**: 11 – 19.

7 Ellerby H M, Arap W, Ellerby L M, *et al*. Nat Med, 1999, **5**: 1032 – 1038.

8 Curnis F, Sacchi A, Borgna L, *et al*. Nat Biotechnol, 2000, **18**: 1185 – 1190.

9 Sacchi A, Gasparri A, Curnism F, *et al*. Cancer Res, 2004, **64**: 7150 – 7155.

10 Wikstrand C J, Hale L P, Batra S K, *et al*. Cancer Res, 1995, **55**: 3140 – 3148.

11 Geissler F, Anderson S K, Venkatesan P, *et al*. Cancer Res, 1992, **52**: 2907 – 2915.

12 Hart S L, Knight A M, Harbottle R P, *et al*. J Biol Chem, 1994, **269**: 12468 – 12474.

13 Stein R, Govindan S V, Mattes M J, *et al*. Cancer Res, 2003, **63**: 111 – 118.

14 Govindan SV, Mattes MJ, Stein R, *et al*. Bioconjugate Chem, 1999, **10**: 231 – 240.

15 Reist CJ, Garg P K, Alston K L, *et al*. Cancer Res, 1996, **56**: 4970 – 4977.

16 Foulon C F, Reist C J, Bigner D D, *et al*. Cancer Res, 2000, **60**: 4453 – 4460.

17 Foulon C F, Welsh PC, Bigner D D, *et al*. Nucl Med Biol, 2001, **28**: 769 – 777.

18 Reist C J, Archer GE, Wikstrand C J, *et al*. Cancer Res, 1997, **57**: 1510 – 1515.

19 Shankar S, Vaidyanathan G, Affleck D J, *et al*. Nucl Med Biol, 2004, **31**: 909 – 919.

20 Sun X, Chu T W, Liu X Q, *et al*. Appl Radiat Isot, 2006, **64**: 645 – 650.

21 Curnis F, Arrigoni G, Sacchi A, *et al*. Cancer Res, 2002, **62**: 867 – 874.

22 Amartey J K, Esguerra C, Ai-Otaibi B, *et al*. Appl Radiat Isot, 2005, **62**: 39 – 47.