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Synthesis and biodistribution studies of iodine-131 D-amino acid YYK peptide as a potential therapeutic agent for labeling an anti-CD20 antibody

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A major drawback of conventionally radioiodinated monoclonal antibodies for radioimmunotherapy is *in vivo* dehalogenation of iodine as a result of deiodinase recognition. To solve this problem we have synthesized a YYK tri-peptide consisting of non-metabolizable D-amino acids modified with the *N*-succinimidyl (*N*-Succ) function. The chemical purity of the synthesized peptide as assessed by analytical high performance liquid chromatography was 95%. Labeling of the Fmoc-D-Tyr($_t$Bu)-D-Tyr($_t$Bu)-D-Lys(Boc)-*N*-Succ was performed using the chloramine-T method and the conventional extraction, resulting in a radiochemical yield of 50–71% and a radiochemical purity of >95%. Radioiodination of the peptide was followed by conjugation to anti-CD20 antibody with 65–75% labeling efficiency and 90% radiochemical purity. The effect of radioiodinated peptide on the biological behavior of the conjugate was evaluated through biodistribution studies in normal Lewis rats. Thyroid and stomach levels from Rituximab labeled with [^{131}I]-YYK-peptide were two- to four-fold less than those with directly labeled [^{131}I]-Rituximab, suggesting low recognition of its D-iodotyrosine residue by endogenous deiodinases. The favorable *in vitro/in vivo* stability and biodistribution profiles suggest that this radioiodine-labeled YYK peptide is a good candidate for further exploration of its potential clinical application.

Keywords: radioiodination; radioimmunotherapy; dehalogenation; D-amino acid

Introduction

Non-Hodgkin lymphoma (NHL) involves a heterogeneous group of malignancies derived from lymphoid tissues, which have the ability to spread to other organs, with differences in appearance behavior and response to therapy.[1] Studies indicated that NHL can be treated effectively by radioimmunotherapy (RIT) for several reasons, including the inherent radiosensitivity of lymphocyte, the vascular accessibility of these malignancies and the large number of target antigens on the surface of lymphocytes.[2]

The integral membrane protein CD20 has been identified as an important therapeutic target in the treatment of NHL.[3] CD20 is a 35 kD hydrophobic transmembrane protein expressed on pre-B-cells and mature B-lymphocyte. It is expressed on more than 90% of B-cell NHL.[4] The absence of antigen expression on stem cells allows for the recovery of normal B-cell following RIT, which leads to the destruction of both malignant and normal B-cells.[5]

The first antibody to be approved in 1997 for treating NHL was Rituximab, a chimeric anti-CD20 immunoglobulin.[6] Rituximab acts via various mechanisms to kill tumor cells, including complement-dependent cytotoxicity, antibody-dependent cell cytotoxicity and induction of apoptosis. The next agent to be approved was ^{90}Y-ibritumomab tiuxetan (Zevalin).[7] This was followed approximately 1.5 years later by ^{131}I-tositumomab (Bexxar).[8]

Iodine-131 is a relatively inexpensive and readily available nuclide for labeling of radiopharmaceuticals. It has a long half-life of 8.1 days with beta emission of 0.69 MeV for therapy, and a gamma emission for imaging, which is useful for making dosimetry estimates.[5] The widespread use of iodine-131 for the labeling of tumor-associated antibodies has shown that this nuclide suffers substantially from undesirable physical and biological properties, principally the rapid and persistent *in vivo* dehalogenation of the radiolabeled antibody.[9] Such *in vivo* removal of radioiodine from target cells within the first 24–120 h postinjection of the labeled antibodies reduces tumor to non-tumor ratio, which is important for radiodiagnosis. In addition, it reduces the residence time of radioiodine in target cells, which significantly affects radiotherapy effectiveness.[10]

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There has been an ongoing effort to develop new methods of radioiodination designed to trap radioiodine inside tumor cells following delivery by a labeled antibody. Early work was based on the use of non-metabolizable carbohydrates as linking agents. Dilactitol-tyramine[11] and tyramine cellobiose[12] are two substrates studied for this purpose. Another technique involves the use of non-metabolizable peptide adduct.[13,14]

In this research, we describe the use of YYK-peptide-*N*-Succ containing D-amino acids as an improved radioiodine labeling method for metabolically stable attachment of radioiodine. This study is based on our knowledge about non-metabolizable peptides, which are not a natural substrate for endogenous enzyme[15] including those present in plasma.

Result and discussion

Fmoc-D-Tyr($_t$Bu)-D-Tyr($_t$Bu)-D-Lys(Boc)-*N*-Succ was selected as the linking agent between Rituximab and iodine-131 and the effectiveness parameters for labeling and its characters were studied. These amino acids were connected via non-metabolizable D-amino acid bonds. This peptide promotes the stability of radioiodine at the tumor site following Rituximab attachment to the cell surface antigen (CD20), matching the fact that mono iodo-D-tyrosine is more stable to deiodination *in vivo* than its L enantiomer.[16]

The YYK peptide was synthesized by standard Fmoc solid-phase synthesis on trityl chloride resin (substitution, 0.8 mmol/g) on a semiautomatic peptide synthesizer.[17] The Fmoc-protected first amino acid D-lys-Boc **I** was attached via its carboxyl group to the resin. Then the peptide was enlarged by the sequential addition of Fmoc-D-Tyr-$_t$Bu (Figure 1). After coupling the last D-tyrosine **III**, the peptide was cleaved from the resin and collected by filtration of the reaction mixture, and solvent removal furnished the protected peptide **IV** as a gummy material, which was essentially pure as determined by analytical high performance liquid chromatography (HPLC) (>95%) with a yield of 90%.

N-hydroxysuccinimide was coupled to the C-terminal of YYK peptide **IV** using a solution of dicyclohexylcarbodiimide (DCC) in *N*,*N*-dimethylformamide (DMF). Molarity was the same for all materials.[18] It gave a brown residue with a yield of 60% and a retention time of 32 min on analytical HPLC. YYK-peptide-*N*-Succ was dissolved in anhydrous DMSO and frozen in aliquots for the labeling process.

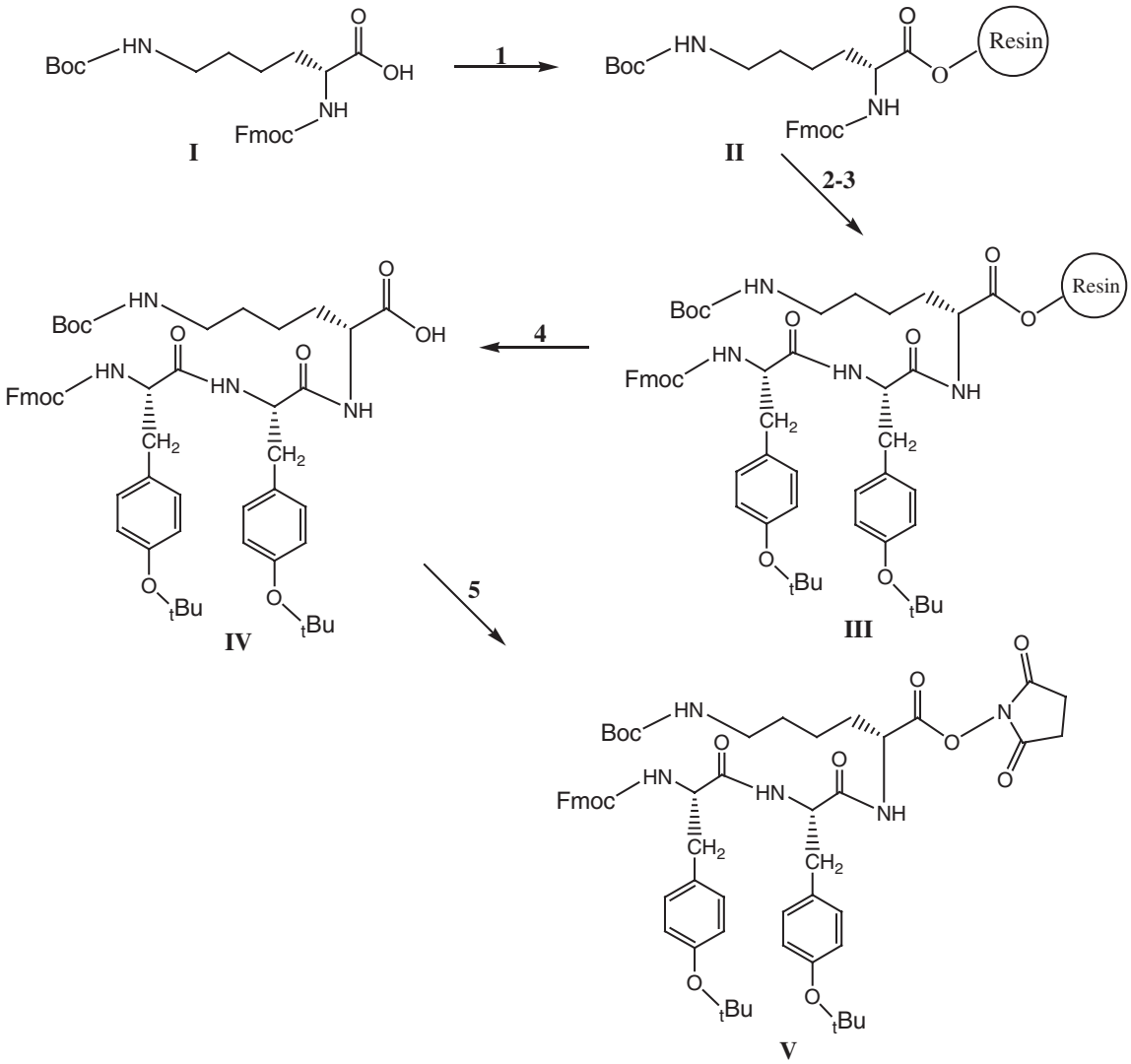

Figure 1. Synthesis of YYK-peptide (**IV**) on trityl chloride resin and its coupling with NHS: (1) 2-chlorotrityl chloride resin, DIPEA, DCM, 18 h; (2) 20% piperidine/DMF; (3) Fmoc-D-Tyr($_t$Bu), activated with HOBT and DIC, DIPEA, 18 h, steps 2 and 3; (4) 20% CH_3COOH/DMF; and (5) NHS, DCC/DMF, 18 h.

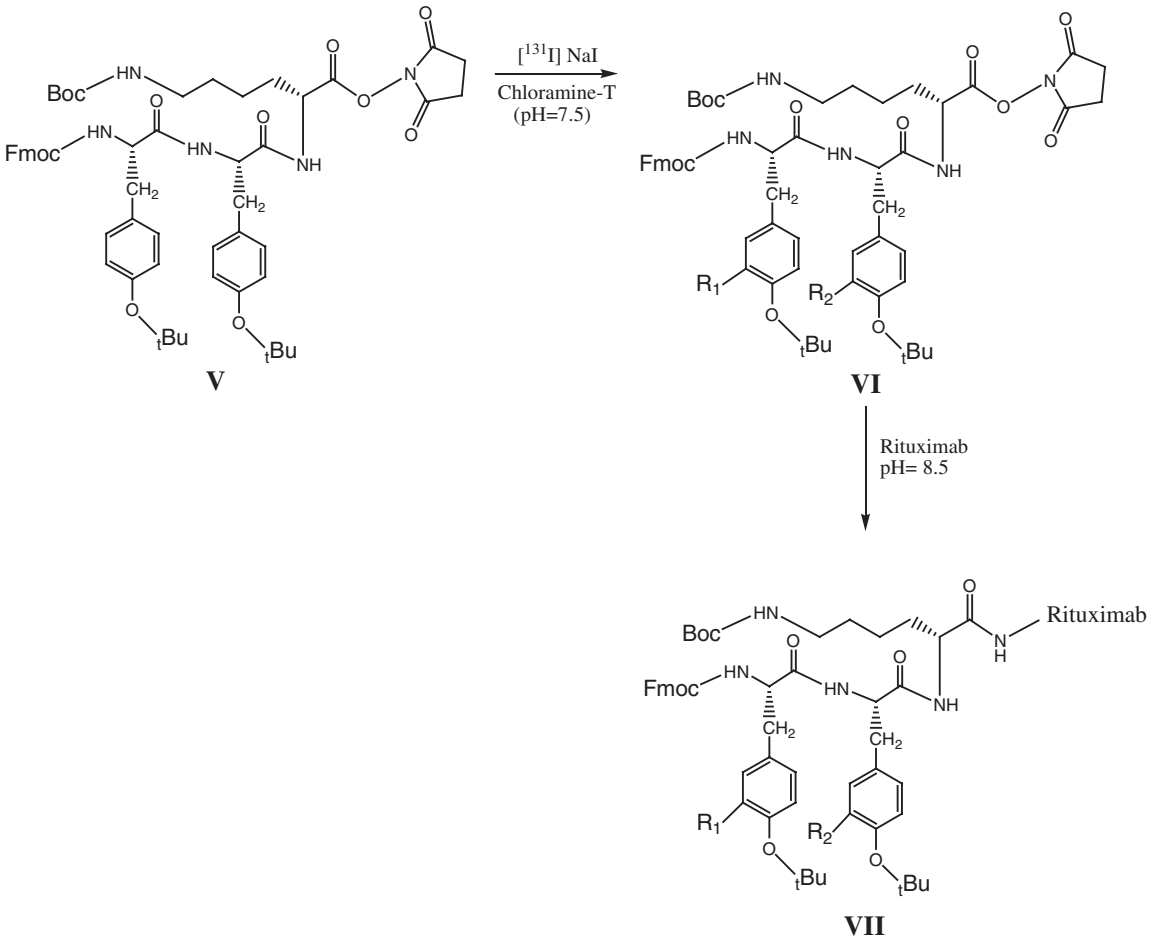

Figure 2. Preparation of [^{131}I]-YYK-peptide-*N*-Succ and labeling with Rituximab (R_1 or $R_2 = {}^{131}$I).

Table 1. Labeling yield of [^{131}I]-YYK-peptide-*N*-Succ after extraction into benzene/DMF at different time points and CAT concentrations ($n = 3$)

Time (s)	Chloramine-T (2 mg/ml)	Chloramine-T (3 mg/ml)	Chloramine-T (4 mg/ml)
15	46.62% ± 0.74	52.25% ± 0.19	61.96% ± 1.21
30	49.39% ± 0.51	66.44% ± 0.83	56.41% ± 0.87
45	55.86% ± 1.70	65.31% ± 0.61	56.28% ± 1.43
60	58.40% ± 0.22	71.84% ± 1.51	63.46% ± 0.59

Labeling of this peptide **V** (Figure 2) was performed by the electrophilic substitution of iodine-131 in the D-tyrosine ring, using chloramine-T as the oxidant. Since the NHS group in the YYK peptide is sensitive to hydrolysis, the radioiodination was terminated after 60 s by the conventional extraction of labeled peptide into benzene/DMF and evaporation to dryness with a stream of nitrogen.

Labeling conditions such as chloramine-T concentration and extraction time were optimized to obtain the maximum labeling yield. The isolated radiochemical yield was between 50 and 71% with a maximum yield after 60-s incubation time (Table 1). TLC analysis showed a radiochemical purity of >95%.

To provide a stable conjugate without affecting the affinity and specificity of the antibody for CD20 antigen, Rituximab was added to the labeled peptide under mild conditions (borate buffer, 50 mM, pH = 8.5) and incubated for 2 h at room temperature. Differential protections of amines in D-lysine side chain and also the peptide's *N*-terminus with Fmoc and Boc protecting groups[19] enabled the specific conjugation of activated [^{131}I]-YYK-peptide-*N*-Succ **VI** to NH_2-lysine antibody. The labeled Rituximab **VII** was purified using a 1 × 20-cm Sephadex G-50 column. At a Rituximab concentration of 10 mg/ml, coupling efficiencies of 65–75% were obtained with a radiochemical purity of 90% and specific radioactivity of 185 MBq/mg (5 mCi/mg).

Table 2. Stability of [^{131}I]-YYK-peptide-Rituximab at 37°C up to 48 h ($n = 3$)

Time postlabeling (h)	PBS	Serum media
4	87.20% ± 1.51	85.54% ± 2.21
24	78.42% ± 0.53	75.74% ± 1.25
48	73.62% ± 2.18	71.25% ± 1.39

In vitro stability of the rodioiodinated antibody under physiological conditions is an important parameter in the evaluation of *in vivo* dehalogenation. Such *in vivo* removal of free iodide causes radiation dose exposure of non-target tissues limiting the clinical efficacy. Thus, the stability of the labeled antibody was assessed in the presence of PBS solution and human serum for up to 48 h at 37°C (Table 2). Studies have demonstrated that the labeled antibody was radiochemically

Table 3. Stability of [^{131}I]-Rituximab at 37°C up to 48 h ($n = 3$)

Time postlabeling (h)	PBS	Serum media
4	92.90% ± 0.13	79.85% ± 0.83
24	82.83% ± 0.71	65.29% ± 1.13
48	79.29% ± 1.13	58.10% ± 2.21

stable in both PBS and human serum for 2 days and TLC analysis showed comparable decrease in both media, which was most likely because of beta emission radiolysis by iodine-131.[20,21] For Rituximab labeled with direct method, a considerable decrease of stability in human serum media has been obtained (Table 3). Immunoreactivity was measured comparatively for two labeling methods using direct binding on fresh Raji cells according to the method of Lindmo *et al.*[22] The results showed a statistically significant higher binding for ^{131}I-labeled YYK-peptide-Rituximab than for [^{131}I]-Rituximab (86.1% ± 1.4 and 76.3% ± 1.8, respectively; $P < 0.05$).

As previous studies have shown, a potential concern with labeling antibodies by most conventional methods is that these tracers are rapidly dehalogenated *in vivo*, as reflected by high radioactivity levels in thyroid and stomach.[23] Distribution and radioactivity uptake of ^{131}I-labeled YYK-peptide-Rituximab in tissues of normal rat are shown in Figure 3 and compared with the distribution of [^{131}I]-Rituximab labeled via the direct method (Figure 4). Differences in normal tissue uptake of radioiodine activity were observed between [^{131}I]-YYK-peptide labeling and the direct method.

With ^{131}I-labeled YYK-peptide-Rituximab, liver levels were significantly higher than those for [^{131}I]-Rituximab (5.33 ± 0.86 and 2.66 ± 0.63 %ID/g at 24 h, respectively; $P < 0.05$), whereas thyroid and stomach were significantly lower ($P < 0.05$), suggesting the fact that most radioiodine activity eliminated through liver in the form of [^{131}I]-YYK-peptide-Rituximab and less free iodine was observed. Thyroid and stomach levels from Rituximab labeled with [^{131}I]-YYK-peptide (1.1 ± 0.15 and 3.67 ± 0.31 %ID/g at 24 h, respectively) were two- to four-fold less than the levels for directly labeled [^{131}I]-Rituximab (2.8 ± 0.39 and 6.7 ± 0.85 %ID/g at 24 h, respectively), suggesting low recognition of its D-iodotyrosine residue by endogenous deiodinases.[15] Thyroid levels for [^{131}I]-YYK-peptide-Rituximab were 57, 39 and 26% of the levels for the directly labeled [^{131}I]-Rituximab at 4, 24 and 48 h, respectively.

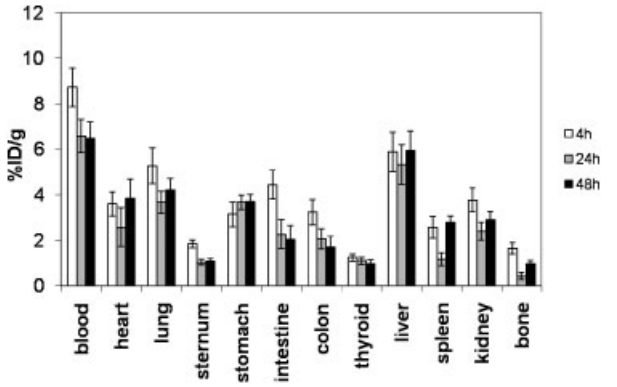

Figure 3. Biodistribution studies in normal Lewis rats, expressed as %ID/g organs for [^{131}I]-YYK-peptide-Rituximab at 4, 24 and 48 h after **IV** administration (mean ± SD).

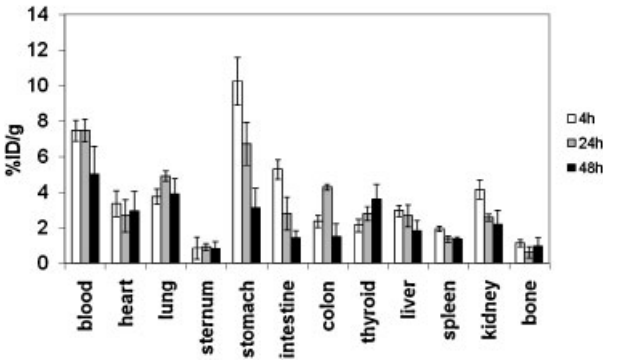

Figure 4. Biodistribution studies in normal Lewis rats, expressed as %ID/g organ for [^{131}I]-Rituximab at 4, 24 and 48 h after **IV** administration (mean ± SD).

Experimental

All chemicals were obtained from known commercial sources and used without further purification. Tritylchloride resin and 9-fluorenylmethoxy carbonyl (Fmoc)-protected amino acids were purchased from Nova Biochem (Germany). The reactive side chains of the amino acids were masked with one of the following groups: lysine, *t*-butoxycarbonyl (Lys-Boc), tyrosine, *t*-butyl (Tyr-$_t$Bu). 1-Hydroxybenzotriazole (HOBT), diisopropylcarbodiimide (DIC), *N*-methylpyrrolidone (NMP), piperidine, DMF, dichloromethane (DCM) and DCC were purchased from Fluka. Rituximab, a mouse–human chimeric anti-CD20 antibody, was commercially obtained from Roche (Mabthera, Switzerland, 100 mg/10 ml).

^{1}H NMR spectra were obtained with BRUKER (500 MHz) instrument using $CDCl_3$ as the solvent. High-resolution fast bombardment mass spectroscopy was performed using Finnigan Mat-TSQ 70, USA. A well counter EG & G, ORTEC, Model 4001M was used. Analytical reverse-phase HPLC was carried out on a JASCO 880-PU intelligent pump HPLC system (Tokyo, Japan). CC 250/4.6 Nucleosil 120-3 C18 columns from Macherey-Nagel (Germany) were used for analytical HPLC. The gradient systems consisted of 0.1% trifluoroacetic acid/water (solvent A) and acetonitrile (solvent B). For analytical HPLC, this gradient was used: 0 min 95%A (5%B), 5 min 95%A (5%B), 30 min 0%A (100%B), 35 min 0%A (100%B), 40 min 95%A (5%B), flow: 1 ml/min, $\lambda = 280$ nm.

Iodine-131 was prepared by the radioisotope division (Atomic Energy Organization of Iran (AEOI)).

Synthesis of YYK tri-peptide

Fmoc-D-Lys-Boc (0.423 g; 0.903 mmol) **I** (Figure 1) was dissolved in anhydrous DCM (3 ml) and mixed with DIPEA (0.617 ml). The solution was stirred with 0.215 g of 2-chlorotrityl chloride resin in a 10 ml filter cartridge and the content shaken vigorously for 18 h. The combined resin **II** was filtered and the resin was washed with solvents: 3 × 2 ml of DCM, 3 × 2 ml of DCM/MeOH (17/3 v/v) and 3 × 2 ml DMF to remove unbound amino acids. The reaction was monitored by the ninhydrin test and the Fmoc groups were removed by adding 2 ml of 20% piperidine in DMF for 5 min, draining the solution off and continuing cleavage with 20% piperidine in DMF for 15 min. This was followed by a wash with 6 × 2 ml DMF. The peptide was prepared using activated

Fmoc-D-Tyr-$_t$Bu. The activation was carried out using 0.414 g (0.9 mmol) of Fmoc-D-Tyr-$_t$Bu, 0.138 g of HOBT in 2 ml of NMP, by adding to the clear solution of 0.14 ml of DIC and keeping at room temperature for 30 min. After this period, 0.9 mmol (0.154 ml) of DIPEA was added, and the mixture was shaken for 18 h. The wash sequence following Fmoc cleavage and subsequent wash sequences were done as described above. A second coupling using activated Fmoc-D-Tyr-$_t$Bu 0.9 mmol was carried out in the same manner. After coupling the last amino acid **III**, the peptide chain with all protecting groups **IV** was cleaved from the resin by 2-h treatment in a solution of 20% acetic acid in DMF. For further exploration, removal of lateral protecting groups was performed by treatment with a solution of trifluoroacetic acid (90%), water (5%) and thioanisole (5%) at room temperature for 10 min. The solution was then concentrated to a small volume and the peptide was precipitated by the addition of ether. The side product with formula of $C_{24}H_{32}N_4O_6$ and calculated Mw of 472.53 exhibited a single peak with a retention time of 14 min on analytical reverse-phase HPLC with a yield of 90%. The electrospray mass spectrum showed an $[M+H]^+$ peak at *m/e* 473.2 and an $[M-H]^-$ at *m/e* 471.1. ^{1}H NMR: (D_2O) δ: 0.711 (tt, 2H, $J=11$ Hz); 1.358 (tt, 2H, $J=14.5$ Hz); 1.436 (tt, 2H, $J=8.5$ Hz); 2.755 (t, 2H, $J=7.5$ Hz); 2.979 (m, 4H, $J=6.5$ Hz); 4.023 (t, 1H, $J=4.5$ Hz); 4.065 (t, 1H, $J=6.5$ Hz); 4.475 (t, 1H, $J=5$ Hz); 6.697 (2d, 4H, $J=7.75$ Hz); 6.972 (2d, 4H, $J=13.25$ Hz).

Synthesis of YYK tri-peptide-*N*-Succ (V)

The protected peptide cleaved from the resin **IV** was derivatized with *N*-succinimidyl function. The solution of NHS (0.045 g, 0.39 mmol) and DCC (0.08 g, 0.39 mmol) in DMF (10 ml) was added to the precipitated peptide (0.355 mg, 0.39 mmol). The reaction mixture was stirred for 18 h at room temperature. The reaction mixture was filtered and the filtrate was concentrated to dryness to give a brown residue. The product with formula of $C_{56}H_{69}N_5O_{12}$ and calculated Mw of 1004.17 exhibited a peak with a retention time of 32 min on analytical HPLC with a yield of 60%; m.p. 155–158°C; electrospray mass spectrum shows an $[M+H]^+$ peak at *m/e* 1005.2 and an $[M-H]^-$ peak at *m/e* 1003.4. ^{1}H NMR: ($CHCl_3$) δ: 1.31 (t, 2H, $J=4$ Hz); 1.39 (s, 9H); 1.431 (s, 18H); 1.596 (t, 2H, $J=3.5$ Hz); 1.725 (t, 2H, $J=3.5$ Hz); 2.705 (s, 4H); 2.931 (t, 2 H, $J=6$ Hz); 3.051 (m, 4H, $J=10.5$ Hz); 4.149 (m, 1H); 4.163 (t, 1H); 4.265 (m, 2H); 4.314 (t, 1H); 4.328 (t, 1H), 6.912 (dd, 4H, $J=17.5$ Hz); 7.032 (dd, 4H, $J=10$ Hz); 7.295–7.544 (m, 6H).

Preparation of [^{131}I]-YYK-peptide-*N*-Succ (VI)

YYK-peptide-*N*-Succ (20 mg, 0.024 mmol) was dissolved in DMSO (2 ml) and frozen in aliquots. Two microliters of YYK-peptide-*N*-Succ (20 μg, 2.2×10^{-5} mmol) **V** (Figure 2) was added to a solution of 200 μCi $Na^{131}I$ (10 μl, 7.4 MBq in 0.1 N NaOH) in buffer PBS (50 μl, 0.5 M, pH = 7.5), followed by 25 μl chloramine-T (4 mg/ml PBS 50 mM, pH = 7.5). The component was mixed by a pipette for 1 min and the reaction was terminated by extracting the iodine-131-labeled YYK-peptide-*N*-Succ by adding 100 μl extraction solvent: benzene/DMF (100/5 v/v). After mixing with a pipette, the organic phase was removed to a glass vial and dried with a gentle stream of nitrogen. The extraction of the aqueous phase was repeated twice following the steps described above. The extract fraction of [^{131}I]-YYK-peptide-*N*-Succ was analyzed by TLC using silica gel (Silica gel 60; Merck) and acetate–methanol (9/1 v/v) as the mobile phase ([^{131}I]-YYK-peptide-*N*-Succ: $R_f=0.82$, YYK-peptide-*N*-Succ: $R_f=0.16$).

Labeling Rituximab with [^{131}I]-YYK-peptide-*N*-Succ and $Na^{131}I$

The solvent of [^{131}I]-YYK-peptide-*N*-Succ **VI** extract was completely removed with a gentle stream of nitrogen. One microliter of Rituximab (10 μg, 6.6×10^{-5} μmol) was mixed with 100 μl of borate buffer (50 mM, pH = 8.5) and incubated with [^{131}I]-YYK-peptide-*N*-Succ (5 μg, 0.55×10^{-5} mmol) at room temperature for 2 h. The labeled antibody was isolated by gel filtration column (Sephadex G-50 Pharmacia). The column was eluted with PBS buffer (0.2 M, pH = 7.5) in 20 aliquots of 1 ml. Radioactivity in each fraction was determined by well counter, and the fraction containing the labeled antibody **VII** was separated.

In the direct labeling method, 1 μl of Rituximab (10 μg, 6.6×10^{-5} μmol) was added to 50 μl of sodium phosphate buffer (0.5 M, pH = 7.5), followed by 200 μCi $Na^{131}I$ (10 μl, 7.4 MBq in 0.1 N NaOH) and 25 μl of chloramine-T (4 mg/ml PBS 50 mM, pH = 7.5). The mixture was stirred for 1 min and 50 μl of sodium metabisulfite (4 mg/ml PBS 50 mM, pH = 7.5) was added followed by 100 μl of KI (10 mg/ml). The reaction mixture was immediately applied to a gel filtration column (Sephadex G-50 Pharmacia) and the fraction containing [^{131}I]-Rituximab was isolated as described above.

Biodistribution studies of Rituximab labeled with [^{131}I]-YYK-peptide-*N*-Succ and $Na^{131}I$

Animal experiment was performed in compliance with the regulation of our institution and with generally accepted guidelines governing such work. Eighteen normal male Lewis rats (150–200 g) were administered with 0.37 MBq (2 μg, 1.3×10^{-5} μmol) of [^{131}I]-YYK-peptide-Rituximab and 0.37 MBq (2 μg, 1.3×10^{-5} μmol) of [^{131}I]-Rituximab in 0.2 ml via lateral tail vein. Animals were divided into three groups of three for each time studied, 4, 24 and 48 h, respectively. The rats were sacrificed, the blood was collected and organs of interest were dissected, weighed and counted for ^{131}I activity in well gamma counter. The percent injected dose per gram was calculated (%ID/g). All mean values are given as mean ± SD. Statistical analysis was performed using the *t*-test. The level of significance was set at $P<0.05$.

Conclusion

The favorable biodistribution data obtained with [^{131}I]-YYK-peptide-Rituximab suggest further investigation of this labeled antibody for RIT of NHL. We do not know at this time whether improved intracellular retention of radioiodine activity would also be obtained with this D-amino acid peptide. In near future we are planning to study internalization of this peptide with other antibodies.

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References

[1] J. O. Armitage, *N. Engl. J. Med.* **1993**, *328*, 1023–1030.
[2] M. S. Krieger, P. L. Weiden, H. B. Breitz, *Radioimmunotherapy in the Treatment of Non-Hodgkin's Lymphoma*, Marcel Dekker, New York, **2000**, pp. 245–263.
[3] A. E. Ernst, H. Li, H. S. Kim, G. R. Nakamura, D. G. Yansura, R. L. Vandlen, *Biochemistry* **2005**, *44*, 15150–15158.
[4] M. G. Hernandez, S. J. Knox, *Int. J. Radiat. Oncol. Biol. Phys.* **2004**, *59*, 1274–1287.
[5] P. Chinn, G. Braslawsky, C. White, N. Hanna, *Cancer Immunol. Immunother.* **2003**, *52*, 257–280.
[6] R. M. Sharkey, J. Burton, D. M. Goldenberg, *Expert Rev. Clin. Immunol.* **2005**, *1*, 47–62.
[7] T. E. Witzig, L. I. Gordon, F. Cabanillas, M. S. Czuczma, C. Emmanouilides, R. Joyce, B. L. Pohlman, N. L. Bartlell, G. A. Wiseman, N. Parde, A. J. Lopez, P. Multani, C. A. White, *J. Clin. Oncol.* **2002**, *20*, 2453–2463.
[8] M. S. Kaminski, A. D. Zelenetz, O. W. Press, M. Saleh, J. Leonard, L. Fehrenbacher, T. A. Lister, R. G. Stagg, G. F. Tidmarsh, S. Kroll, R. L. Wahl, S. J. Knox, *J. Clin. Oncol.* **2001**, *19*, 3918–3928.
[9] I. E. Normando, *Nucl. Med. Biol.* **1998**, *25*, 441–447.
[10] R. Stein, S. V. Govindan, M. J. Mattes, S. Chen, L. Reed, G. Newsome, B. J. McBride, G. L. Griffiths, H. J. Hansen, D. M. Goldenberg, *Cancer Res.* **2003**, *63*, 111–118.
[11] R. Stein, D. M. Goldenberg, S. R. Thorpe, M. J. Mattes, *Cancer Res.* **1995**, *55*, 3132–3139.
[12] S. A. Ali, S. D. Warren, K. Y. Richter, C. C. Badger, J. F. Ery, O. W. Press, K. A. Krohn, I. D. Bernstein, W. B. Nelp, *Cancer Res.* **1990**, *50*, 783–788.
[13] C. F. Foulon, P. C. Welsh, D. D. Bigner, M. R. Zalutsky, *Nucl. Med. Biol.* **2001**, *28*, 769–777.
[14] S. V. Govindan, M. J. Mattes, R. Stein, B. J. McBride, H. Karacay, D. M. Goldenberg, H. J. Hansen, G. L. Griffiths, *Bioconjugate Chem.* **1999**, *10*, 231–240.
[15] R. C. Milton, S. C. Milton, S. B. Kent, *Science* **1992**, *256*, 1403–1404.
[16] K. Kawai, Y. Fujibayashi, H. Saji, J. Konishi, A. Kubodera, A. Yokoyama, *Nucl. Med. Biol.* **1990**, *17*, 369–376.
[17] E. Atherton, R. Sheppard, *Solid Phase Peptide Synthesis*: *A Practical Approach*, Oxford Information Press, Oxford, **1989**.
[18] M. J. Abrams, M. Juweid, C. I. Tenkate, *J. Nucl. Med.* **1990**, *31*, 2022–2028.
[19] a) X. Sun, T. Chu, X. Liu, X. Wang, *Appl. Radiat. Isot.* **2006**, *64*, 645–650; b) T. Lindmo, E. Boven, F. Cuttitta, J. Fedorko, P. A. Bunn, *J. Immunol. Methods* **1984**, *72*, 77–89.
[20] R. Kishore, J. F. Eary, K. A. Krohn, W. B. Nelp, T. W. Menard, P. L. Beaumier, K. E. Hellstrom, I. Hellstrom, *Int. J. Radiat. Appl. Instrum. B* **1986**, *13*, 457–459.
[21] G. L. Denardo, S. J. Denardo, B. W. Wessels, D. L. Kukis, N. Miyao, A. Yuan, *Cancer Biother. Radiopharm.* **2000**, *15*, 547–560.
[22] T. Lindmo, E. Boven, F. Cuttitta, J. Fedorko, P. A. Bunn, *J. Immunol. Methods* **1984**, *72*, 77–89.
[23] P. K. Garg, K. L. Alston, P. C. Welsh, M. R. Zalutsky, *Bioconjugate Chem.* **1996**, *7*, 233–239.