Radiolabeling of genetically engineered dimeric antibody mimetic with Tc-99m and biodistribution study for evaluation of its prolonged blood circulation

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1. Introduction

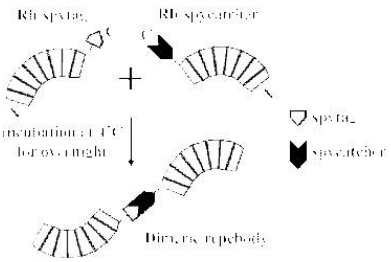
Pharmacokinetic study, particularly biodistribution, provides crucial quantitative and tracking information in all major tissues after administration of biological active molecule. It is necessary to provide preclinical safety evaluation and development of improved therapy strategy of medicines. Technetium-99m is a commonly used radionuclide to evaluate biodistribution of molecules in the field of nuclear medicine. Its short half-life (6 h), favorable γ photon emission of 140 keV (nearly ideal for SPECT imaging), low radiation absorbed dose burden to patients, low price and excellent availability of $^{99}Mo/^{99m}Tc$ generators makes it the most suitable radioisotope for initial studies.

Small-sized non-antibody scaffolds have attracted considerable interest as alternatives to antibody. Repebody is antibody-like scaffold that consist of highly diverse leucine-rich repeat (LRR) modules, and it has been constructed to bind to interleukin-6 for cancer therapy. However, their short half-life is considered a drawback in the development of therapeutic agents. Herein we demonstrate that a homo-dimeric form of a repebody enhances the anti-tumor activity than a monomeric form through prolonged blood circulation. Representative results of extended blood circulation and biodistribution are presented.

2. Methods and Results

2.1 Construction of a homo-dimeric from of a repebody

Spytag and spycatcher were genetically fused to the C-terminus of a respective human IL-6-specific repebody, and the resulting two repebody constructs were mixed at an equimolar ratio to produce a homo-dimeric form through interaction between spytag and spycatcher.



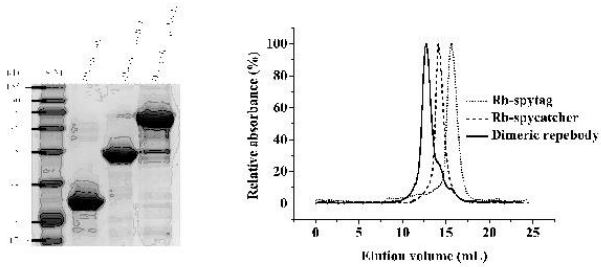


Fig. 1. Construction of a homo-dimeric repebody via spytag and spycatcher

2.2 Radiolabeling of a dimeric repebody with $[^{99m}Tc(OH_2)_3(CO)_3]^+$

To investigate the biodistribution and blood clearance, genetically engineered antibody-like scaffold protein, repebody, was successfully radiolabeled with the $[^{99m}Tc(OH_2)_3(CO)_3]^+$ (^{99m}Tc-tricarbonyl) by using a site-specific direct labeling method via hexahistidine-tag, which is a widely used for general purification of proteins with His-affinity chromatograpy.

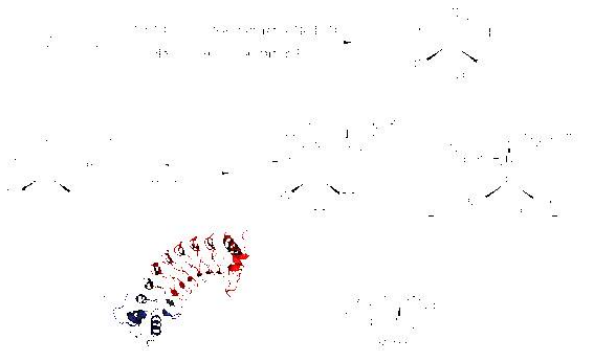


Fig. 2. Schematic illustration for radiolabeling of a dimeric repebody with $[^{99m}Tc(OH_2)_3(CO)_3]^+$

2.3 Evaluation of the blood clearance and bio-distribution of a dimeric repebody

The pharmacokinetic profiles were determined by measurement of serum concentration derived from radioactivity in H1650 tumor bearing mice (n=5 per time point) following a single intravenously injected dose (15 μg, 18 μCi) in a volume of 100 μL. For

comparison, the first value of serum concentration at 3 min was set to 100%. Blood samples were taken from the tumor-bearing mice at intervals of 0.05 h, 0.5 h, 1 h, 3 h, 6 h, and 24 h after injection. Serum concentrations of the dimeric or monomeric repebody were determined by measuring the radioactivity of the serum sample (100 μL) using the gamma counter. Serum half-life of a monomeric and dimeric repebody was calculated with Origin program. For organ distribution, Major visceral organs, including liver, spleen, stomach, intestine, kidneys, heart, lung and tumor, were also collected from the mice for measuring the radioactivity using a gamma counter. The weight of the collected samples was measured, and the radioactivity in the samples was expressed as the percentage of injected radioactive dose per gram of tissue (% ID/g).

Table I. Organ distributions of a ^{99m}Tc-labeled (a) monomeric and (b) dimeric repebody in H1650 tumor bearing mice (n = 5)

(a)

Organ	0.5 h	1 h	3 h	6 h	24 h
Blood	7.88±0.56	3.05±1.57	1.07±0.13	0.84±0.26	0.22±0.07
Liver	45.14±9.78	59.33±7.77	52.53±7.47	49.68±2.63	22.91±4.28
Spleen	14.04±2.77	14.20±1.58	14.41±4.87	10.50±2.11	6.68±0.94
Stomach	3.44±1.79	2.39±0.81	2.67±0.77	1.27±0.02	0.44±0.11
Small Intestine	3.26±1.10	4.15±1.01	4.05±0.87	1.40±0.01	0.75±0.21
Large Intestine	0.89±0.25	0.91±0.27	4.36±0.66	1.60±0.04	1.33±0.43
Kidney	62.62±8.79	67.88±15.72	68.81±18.64	58.77±2.94	32.59±9.93
Heart	2.21±0.25	1.98±0.32	1.81±0.28	1.27±0.11	0.65±0.15
Lung	4.27±1.10	2.95±1.20	3.91±2.45	1.57±0.15	1.28±0.72
Tumor	2.84±2.43	1.76±1.22	0.57±0.22	1.12±0.05	0.34±0.12

(b)

Organ	0.5 h	1 h	3 h	6 h	24 h
Blood	1.43±0.40	0.77±0.31	0.69±0.15	0.28±0.05	0.17±0.03
Liver	16.22±0.37	12.99±4.06	14.27±4.60	15.91±2.19	8.94±1.93
Spleen	4.61±0.49	1.98±0.15	3.70±1.99	6.04±0.87	4.01±1.21
Stomach	3.35±0.80	2.20±0.44	2.41±0.62	1.38±0.25	0.76±0.09
Small Intestine	2.07±0.21	1.67±0.34	1.81±0.32	1.39±0.16	0.94±0.15
Large Intestine	1.32±0.16	1.14±0.28	2.42±0.88	1.65±0.19	0.94±0.15
Kidney	84.23±15.63	75.08±4.20	74.96±21.65	67.26±18.47	52.68±7.61
Heart	3.04±1.45	1.19±0.35	1.53±0.56	1.84±0.26	0.87±0.13
Lung	4.67±4.43	2.63±1.82	2.60±1.70	3.12±1.65	0.94±0.17
Tumor	1.38±0.37	0.88±0.08	1.98±1.75	0.68±0.30	0.73±0.28

Strong radioactivity was observed in the kidneys of respective mice injected with a monomeric or a dimeric repebody. The mice injected with a monomeric repebody showed a rapid decrease in the radioactivity in the kidneys from 6 h post-injection. Considering the result of the serum radioactivity at 3 h post-injection (< 0.4% ID), a sharp decrease in the renal radioactivity at 6 h post-injection might be due to fast renal excretion of the monomeric repebody before 3 h postinjection. In contrast, in the case of the dimeric repebody, high radioactivity was detected in the kidneys even at 6 h post-injection, suggesting a long blood circulation of a dimeric repebody compared to a monomeric repebody. The result of organ distribution also supports that the 99mTc-repebody was excreted through the kidneys from the beginning of intravenous injection, and the dimeric repebody had a prolonged blood circulation. The radioactivity in the kidneys was relatively high in respective mice injected with a monomeric or a dimeric repebody over entire period of the experiment (Fig. 4C and D), which is consistent with the result of the SPECT/CT imaging.

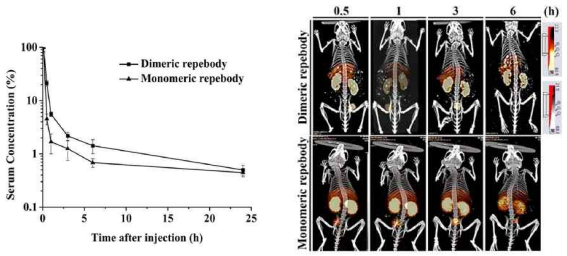


Fig. 3. Blood circulation profile and biodistribution of the dimeric repebody in mice.

The serum radioactivity of the mice injected with a monomeric repebody sharply dropped to a level of 1.43 ± 0.40% ID/g at 0.5 h post-injection. This rapid clearance seems to be mainly owing to fast excretion of the small sized repebody (31 kDa) to urine. In contrast, the mice injected with a dimeric repebody showed a much slower decrease in the serum radioactivity. At 0.5 h post injection, serum radioactivity was estimated to be 7.88 ± 0.56% ID/g, which corresponds to about 5.5 times higher than the monomeric repebody, and this was maintained until 6 h post injection. The initial and terminal serum half-lives of the dimeric repebody were estimated to be 0.12 h and 3.2 h, respectively, showing longer values than the monomeric one of 0.06 h and 1.9 h.

Table II. Pharmacokinetic parameters of ^{99m}Tc-labeled dimeric and monomeric repebody

	$T_{1/2\alpha}$	k_{el}	$T_{1/2\beta}$	k_{el}
Dimer	0.12	4.07	3.2	0.15
Monomer	0.06	8.29	1.9	0.25

2.4 In vivo anti-tumor activity of the dimeric repebody

From the improved pharmacokinetic property, the dimeric repebody is expected to show increased bioavailability and enhanced therapeutic effect. We examined the anti-tumor activity of the homodimeric repebody in xenograft mice with H1650 cells. When the tumor volume reached around 100mm3, the dimeric repebody (10 mg/kg) was intravenously injected into the tumor bearing mice every three days. A monomeric repebody (10 mg/kg) and PBS were also tested as a control. As a result, the dimeric repebody showed a much higher tumor suppression effect than the monomeric repebody. The dimeric repebody has an almost 2.5-fold higher molecular weight (78 kDa) than the monomeric repebody (31 kDa). It is thus noteworthy that even though the molar concentration of the dimeric repebody was about 2.7-fold lower than the monomeric

repebody, the dimeric repebody resulted in more than a 2-fold higher tumor suppression effect.

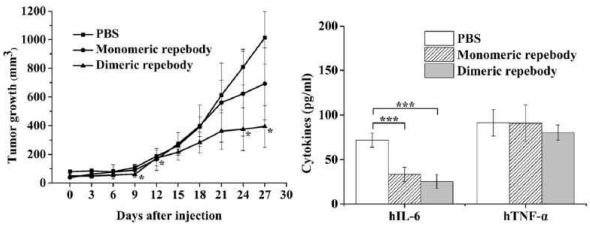


Fig. 4. Anti-tumor activity of the dimeric repebody in xenograft mice with H1650 cells.

3. Conclusions

We have demonstrated a simple construction of a homo-dimeric repebody (78 kDa) specific for human IL-6 using the spytag/spycatcher chemistry. The use of spytag and spycatcher chemistry was shown to be effective for constructing a homo-dimeric repebody with high stability and homogeneity. The blood concentration level of a dimeric repebody was estimated to be about 5-fold higher in tumor bearing mice compared to a monomeric repebody when injected intravenously, leading to a much higher tumor suppression effect in xenograft mice. It is interesting to note that the dimeric repebody exhibited a higher anti-tumor effect in xenograft mice even at about a 2.5-fold lower molar concentration than a monomeric repebody. This can be attributed to the reduced renal clearance owing to an increased hydrodynamic radius and rapid elimination of human IL-6 through an antagonistic effect. Taken together, it is likely that the present approach can be effectively used to enhance the pharmacokinetic property of small-sized protein scaffolds, and consequently, the therapeutic efficacy.

REFERENCES

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