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Biodistribution, pharmacokinetics, and nuclear imaging studies of ¹¹¹In-labeled rGel/BLyS fusion toxin in SCID mice bearing B-cell lymphoma

Xiaoxia Wen¹, Mi-Ae Lyu², Rui Zhang¹, Wei Lu¹, Qian Huang¹, Dong Liang³, Michael G. Rosenblum², and Chun Li^{1,*}

¹Department of Experimental Diagnostic Imaging, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

²Department of Experimental Therapeutics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

³College of Pharmacy, Texas Southern University, Houston, Texas

Abstract

Purpose: We examine the biodistribution and pharmacokinetics of ¹¹¹In-labeled rGel/BLyS, a gelonin toxin (rGel)-B lymphocyte stimulator (BLyS) fusion protein.

Materials and Methods: rGel/BLyS was labeled with In-111 through DTPA with a labeling efficiency >95%. Biodistribution/imaging studies were obtained in SCID mice bearing diffuse large B-cell lymphoma OCI-Ly10. Pharmacokinetic studies were performed in BALB/c mice.

Results: *In vitro*, DTPA-conjugated rGel/BLyS displayed selective cytotoxicity against OCI-Ly10 cells and mantle cell lymphoma JeKo cells. *In vivo*, rGel/BLyS exhibited a tri-exponential disposition with a rapid initial mean distribution followed by an extensive mean distribution and a long terminal elimination phase. At 48 h after injection, uptake of the radiotracer in tumors was 1.25% ID/g, with a tumor-to-blood ratio of 13. Tumors were clearly visualized at 24–72 h post-injection. μ SPECT-CT images and *ex vivo* analyses confirmed the accumulation of rGel/BLyS in OCI-Ly10 tumors and association of rGel/BLyS with BAFF-R receptor.

Conclusions: ¹¹¹In-DTPA-rGel/BLyS targeted the BLyS receptors in tumors. Preclinical antitumor studies using rGel/BLyS should use a twice per week treatment schedule.

Keywords

B-cell lymphoma; Biodistribution; Pharmacokinetics; Gamma Imaging; Cytokine B lymphocyte stimulator; Gelonin

*Corresponding author: Department of Experimental Diagnostic Imaging, Unit 59, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030. Phone: (713) 792-5182. Fax: (713) 794-5456. cli@mdanderson.org.

INTRODUCTION

B lymphocyte stimulator (BLyS) is a member of the tumor necrosis factor (TNF) superfamily of cytokines that binds only to B cells. BLyS is known to promote B cell proliferation, activation, and differentiation, enhance B lymphocyte survival, and stimulate immunoglobulin production both *in vitro* and *in vivo* [1–6]. The biological functions of BLyS are mediated by three cell surface receptors: transmembrane activator and calcium-modulating cyclophilin ligand interactant (TACI), B cell maturation antigen (BCMA), and BAFF-receptor, or BLyS receptor 3 (BAFF-R) [7–9]. BAFF-R has been identified as the main BLyS receptor responsible for peripheral B cell homeostasis and binds only to BLyS, whereas TACI and BCMA also bind to a proliferation-inducing ligand (APRIL) [10; 11]. BLyS receptors are expressed on a broad range of tumor cells, including multiple myeloma, large B cell lymphoma, follicular B cell lymphoma, chronic lymphocytic leukemia, and Burkitt's lymphoma cells [12]. In a phase I clinical trial in relapsed/refractory follicular non-Hodgkin's lymphoma, a radiolabeled recombinant BLyS protein, LR131, was shown to specifically target the tumor site and was rapidly cleared from the blood and normal organs [13]. Taken together, these studies show that BLyS is a promising targeting protein for the selective delivery of chemotherapeutic agents and toxins to B-cell malignancies.

The recombinant gelonin (rGel) toxin is a single-chain glycosidase similar in action to ricin A chain [14; 15]. The rGel toxin lacks a cell membrane binding domain and is, therefore, relatively nontoxic to intact cells. The toxin requires a carrier to cross the cell membrane and, once internalized, is lethal to mammalian cells within 72 to 96 hours. Fusion constructs containing rGel and targeting proteins or antibodies can specifically bind to tumor cells, internalize through endocytosis, and translocate to the cytosol, where rGel inhibits protein synthesis by inactivating ribosomes [16; 17]. Constructs containing rGel have shown impressive cytotoxic effects when delivered to cells using growth factor ligands, as chemical conjugates with monoclonal antibodies, or fused to single-chain antibodies [16; 18–21]. A chemical conjugate of rGel with an antibody targeting CD33 is currently in phase I development at The University of Texas M. D. Anderson Cancer Center [21].

We have developed a fusion toxin, rGel/BLyS, in which recombinant gelonin is linked to the BLyS protein at its NH₂ terminus, for specific delivery of the toxin to B cells expressing BLyS receptors. Our previous studies showed that rGel/BLyS was highly cytotoxic against mantle cell lymphoma (MCL) and diffuse large B-cell lymphoma (DLBCL) cell lines that express all three BLyS receptors. The fusion toxin specifically bound to cells expressing BLyS receptors and was rapidly internalized into target cells. The cytotoxic effects of rGel/BLyS were inhibited by pretreatment with free BLyS or with soluble BAFF-R, TACI, and BCMA receptors, suggesting that the cytotoxic effects were mediated through BLyS receptors. rGel/BLyS inhibited cell growth through induction of apoptosis associated with caspase-3 activation and poly (ADP-ribose) polymerase cleavage [22]. Studies in B-cell chronic lymphocytic leukemia (B-CLL) also showed the specific targeting of rGel/BLyS to BAFF-R-expressing B-CLL cells. The fusion construct was internalized by B-CLL lymphocytes through BAFF-R and caused selective cell death at nanomolar concentrations. In contrast, rGel alone was not internalized by these lymphocytes. The rGel/BLyS construct inhibited protein synthesis, with an IC₅₀ of less than 3 nM, compared with more than 5000

nM for rGel alone. The cytotoxic effect was associated with a decline in cellular protein and DNA synthesis, resulting in a reduction in proteins that provide a survival mechanism to CLL cells [23]. All these data suggest that rGel/BLyS is an excellent candidate for the treatment of B-cell malignancies.

The purpose of this study was to investigate the *in vivo* tumor-targeting effects through the pharmacokinetic and biodistribution studies of radiolabeled rGel/BLyS using mice as an animal model, and to evaluate imaging properties of radiolabeled rGel/BLyS in B-cell tumors.

MATERIALS AND METHODS

Materials.

2-(4-Isothiocyanatobenzyl)-dimethylenetriaminepentaacetic acid (SCN-Bn-DTPA) was purchased from Macrocyclics (Dallas, TX). PD-10 columns were obtained from GE Healthcare (Piscataway, New Jersey). Centricon centrifuge devices were purchased from Millipore (Billerica, MA). $^{111}\text{InCl}_3$ was obtained from Perkin Elmer (Waltham, MA). Instant thin-layer chromatography (ITLC) silicone gel-impregnated glass fiber sheets were purchased from Pall Corporation (East Hill, NY). The fluorescein isothiocyanate-conjugated anti-goat antibody or anti-rabbit antibody and all the other chemicals and reagents were purchased from Sigma (St. Louis, MO). The anti-goat BAFF-R antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Cell proliferation kit II (XTT) and *in situ* cell death detection kit were purchased from Roche (Mannheim, Germany). The rGel/BLyS molecule was expressed in *E. coli* and purified to homogeneity as previously described [22].

DTPA modification and ^{111}In -labeling of rGel/BLyS.

SCN-Bn-DTPA was conjugated to rGel/BLyS at a molar ratio of DTPA to rGel/BLyS of 7 to 2. Briefly, 70 μg of SCN-Bn-DTPA in dimethylformamide was added into 1.35 mg of rGel/BLyS in 20 mM sodium bicarbonate buffer, pH 8.5. After 2 h incubation at 4°C, DTPA-rGel/BLyS was purified using a PD-10 column eluted with phosphate-buffered saline (PBS), pH 7.4, and concentrated on a Centricon centrifuge device. For radiolabeling, 300 μg of DTPA-rGel/BLyS in 100 μL PBS was incubated with 110 MBq (3.0 mCi) of $^{111}\text{InCl}_3$ in 10 mM sodium acetate, pH 5.2, at room temperature for 30 minutes. Free ^{111}In was removed by using the PD-10 column. The purity and labeling efficiency of ^{111}In -DTPA-rGel/BLyS were analyzed by ITLC. For ITLC, aliquots (1.0 μL) of the purified solution were spotted on ITLC-gel sheets and developed in PBS containing 4 mM ethylenediaminetriacetic acid (EDTA). Radio-TLC analysis was performed on a Bioscan AR-2000 radio-TLC scanner (Washington, DC).

Cell lines and cell culture.

The JeKo-1 mantle cell lymphoma cell line was grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin. The OCI-Ly10 diffuse large B cell lymphoma cell line was grown in 20% fetal bovine serum. The JeKo-1 cell line was kindly provided by Dr. Hesham Amin (M. D.

Anderson Cancer Center, Houston, TX). The OCI-Ly10 cell line was kindly provided by Dr. Ricardo Aguiar (UT Health Science Center, San Antonio, TX).

Confirmation of the biological activity of DTPA-rGel/BLyS conjugate.

To examine the biological activity of DTPA-rGel/BLyS conjugate, OCI-Ly10 or JeKo-1 cells were seeded (1×10^4 cells/well) in flat-bottom 96-well microtiter plates, and various concentrations of DTPA-rGel/BLyS, rGel/BLyS, or rGel were added in quadruplicate wells. After 4 days, cell viability was assessed using the XTT assay, as described previously [22]. Absorbance was measured at 450 nm using an ELISA reader (Bio-Tek Instruments, Winooski, VT).

Animals.

Animal procedures were performed according to a protocol approved by M. D. Anderson. Pharmacokinetic studies were performed with female BALB/c mice (Charles River Laboratories, Wilmington, MA). Male severe combined immunodeficiency (SCID) mice (CB.17 SCID) were supplied by Taconic Farms (Germantown, NY) at 3-4 weeks of age. The OCI-Ly10 tumor model was generated by subcutaneous injection of 1×10^7 cells in 50 μ L PBS into the hind legs of the CB17.SCID mice. The mice were subjected to biodistribution and imaging experiments when the tumor volume reached 1.0-1.2 cm in diameter. Mice were euthanized by the end of the imaging session with CO₂ overexposure.

Pharmacokinetics.

Seven female BALB/c mice (4-6 weeks of age) received intravenous injection of ¹¹¹In-DTPA-rGel/BLyS through the tail vein at a dose of 12 μ g, 3.7 MBq (100 μ Ci) per mouse. Ten μ L of blood was collected via the tail vein of each mouse at the designated times after injection (5 min, 30 min, 1, 2, 4, 7, 24, 48, 96, 120, and 168 h). The radioactivity of each blood sample was measured by a Cobra gamma counter (Packard, Downers Grove, IL). The blood pharmacokinetic parameters for the radiotracer were analyzed with a three-compartmental open model using WinNonlin™ 5.0.1 software (Pharsight Corp., Palo Alto, CA).

Biodistribution.

Five SCID mice bearing OCI-Ly10 tumors received intravenous injection of 6 μ g, 1.85 MBq (50 μ Ci) ¹¹¹In-DTPA-rGel/BLyS per mouse. At 48 h after injection, the mice were sacrificed and dissected. Blood and organ tissues, including heart, liver, spleen, kidney, lung, stomach, intestine, muscle, bone, brain, and tumor, were removed from each mouse. The tissues were weighed and radioactivity of each tissue was measured by a Cobra auto-gamma counter (Packard). The percentage of the injected dose per gram of tissue (ID%/g) was calculated for each sample.

Gamma imaging.

Two SCID mice with OCI-Ly10 tumors were intravenously administered 48 μ g, 14.8 MBq (400 μ Ci) ¹¹¹In-DTPA-rGel/BLyS per mouse. An M.CAM gamma camera (Siemens, Malvern, PA), equipped with a medium-energy high-sensitivity collimator and ICON

software (Siemens) for data processing, was used for the gamma imaging. The mice were placed prone on the collimator, and gamma images were acquired immediately after injection and at 1, 2, 4, 24, 48, and 72 h after injection of the radiotracer. Regions of interest (ROIs) were drawn around the tumors and the standard on the images at the specified time points, and the radioactivity counts per pixel in the tumors were measured by using Image J software (National Institutes of Health). The percentage of injected total radioactivity in each tumor was calculated and plotted against the time interval after injection.

SPECT-CT imaging.

The same mice used for gamma imaging were also scanned with a FLEX X-O.X μ SPECT imaging system (Gamma Medica-IDEAS, Northridge, CA). The mouse was placed prone in a cradle, and μ SPECT and CT images were acquired at 2, 24, 48, and 72 h after ^{111}In -DTPA- rGel/BLyS injection. The μ SPECT and CT images were reconstructed and merged by using X SPECT Fusion 2 software (Gamma Medica-IDEAS).

Autoradiography.

The frozen blocks of the tumor tissue removed at 72 h after injection of the radiotracer were cut into 10- μm sections and transferred onto glass slides. The slides were dried and exposed to a previously erased imaging plate (Fuji Film, Stamford, CT). After overnight exposure, the plate was read on the radioisotopic detector of a FLA-5100 imager (Fuji Film). The same slides were also stained with hematoxylin and eosin (H&E) for histological examination.

Immunofluorescence staining.

To examine the presence of BAFF-R and rGel, OCI-Ly10 tumor frozen tissue sections were dried, fixed in 3.7% formaldehyde (Sigma) for 20 min at room temperature, and briefly rinsed with PBS. Cells were then permeabilized for 10 min in PBS containing 0.2% Triton X-100, washed three times with PBS, and blocked with PBS containing 3% bovine serum albumin for 1 h at room temperature. Fixed cells were incubated with anti-rabbit rGel antibody or anti-goat BAFF-R antibody for 2 h at room temperature. The slides were washed three times with PBS and then incubated with anti-rabbit IgG-FITC-conjugated antibody or swine anti-goat IgG-FITC-conjugated antibody for 1 h at room temperature. After a final wash step, the slides were mounted in mounting medium and analyzed under a Zeiss Axio Observer.Z1 fluorescence microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany).

Detection of apoptosis.

Apoptosis was detected by the TdT-mediated dUTP nick end-labeling (TUNEL) assay. Frozen tumor sections were dried, fixed in 3.7% formaldehyde (Sigma) for 20 min at room temperature, and briefly rinsed with PBS. The slides were then permeabilized for 10 min in PBS containing 0.2% Triton X-100 and 0.1% sodium citrate, washed three times with PBS, and blocked with PBS containing 3% bovine serum albumin for 1 h at room temperature. Fixed cells were stained using an *in situ* cell death detection kit (Roche). After a final wash step, the slides were mounted in mounting medium and analyzed under a Zeiss Axio Observer.Z1 fluorescence microscope.

RESULTS

In vitro biological function of DTPA-rGel/BLyS and radiolabeling.

When the molar ratio of DTPA to rGel/BLyS was greater than 5, the fusion protein lost most of its cytotoxic activity, suggesting deterioration of rGel's cell killing effect and/or BLyS's binding affinity towards its targets. On the other hand, when the molar ratio of DTPA to rGel/BLyS was less than 2, the radiolabeling efficiency was only 30% and the specific activity only 0.03 MBq/ μ g. The optimal ratio was found to be 3.5. Under such a condition, DTPA-rGel/BLyS retained its cytotoxic activities against both JeKo-1 and OCI-Ly10 cells (Figure 1), while the specific radioactivity of ^{111}In -labeled DTPA-rGel/BLyS was as high as 0.3 MBq/ μ g (8.1 $\mu\text{Ci}/\mu\text{g}$) with radiochemical purity over 95% after purification with gel permeation chromatography (Figure 2).

Pharmacokinetics.

Figure 3 plots mean blood radioactivity, expressed as a percentage of injected dose per mL blood (%ID/mL), versus time after intravenous injection of ^{111}In -DTPA-rGel/BLyS into healthy female BALB/c mice. The pharmacokinetic parameters are summarized in Table 1. The blood showed a tri-exponential disposition of ^{111}In -DTPA-rGel/BLyS. A three-compartment model was used to describe the blood pharmacokinetics of the agent. Following intravenous administration, ^{111}In -DTPA-rGel/BLyS had a rapid initial distribution to a highly perfused central compartment (mean $t_{1/2\alpha} = 0.65$ h), followed by an extensive distribution to a second or tissue compartment (mean $t_{1/2\beta} = 7.6$ h), during which the radio-conjugate spread from the central blood compartment to the extravascular space of the solid tissues, and a very slow distribution to the third or deep tissue compartment with a prolonged elimination phase (mean $t_{1/2\gamma} = 138$ h) for the clearance of the radio-conjugate. It was noted that there were large variations on the terminal elimination half-lives among the mice. Despite excellent fittings of terminal elimination phases for all mice, two out of the seven mice exhibited significantly longer half-lives of 237 and 454 h, respectively. Whereas the other 5 mice have a very tight mean terminal elimination half-life of 54.6 ± 13.1 h. The mean volume of distribution at steady state (V_{ss}) for ^{111}In -DTPA-rGel/BLyS was 13.5 mL, which was over 3 times higher than V_d , suggesting high tissue (i.e. spleen and tumor) affinity of the radiolabeled fusion toxin. The mean initial drug concentration in blood was estimated to be 28.09 %ID/mL. The mean systemic blood clearance (CL) of the drug was 0.55 mL/h in mice.

Biodistribution.

The %ID/g of ^{111}In -DTPA-rGel/BLyS in organs and tissues dissected at 48 h after radiotracer injection is summarized in Figure 4. The spleen showed the highest uptake among the tissues studied ($16.2\% \pm 3.3\%$). This result agrees with the biodistribution of ^{125}I -labeled BLyS, which also showed highest uptake in the spleen [24]. High uptakes of ^{111}In -DTPA-rGel/BLyS were also found in the liver ($5.4\% \pm 1.0\%$), the kidney ($4.0\% \pm 0.3\%$), and the bone ($1.31\% \pm 0.4\%$). There was still $1.25\% \pm 0.4\%$ ID/g in the tumor 2 days after injection of ^{111}In -DTPA-rGel/BLyS. The tumor-to-blood and tumor-to-muscle ratios were 13.3 and 4.9, respectively, at 2 days after radiotracer injection.

Nuclear imaging.

Figure 5A shows representative μ SPECT-CT images obtained from a mouse injected with ^{111}In -DTPA-rGel/BLyS at a dose of 48 μg , 14.8 MBq per mouse. The radiotracer primarily accumulated in spleen, liver, and kidney. Uptake in the tumor is clearly visualized at 24, 48, and 72 h post-injection. Tumor uptake at different times after ^{111}In -DTPA-rGel/BLyS injection was quantified on the basis of gamma images acquired from the same mice used for the μ SPECT-CT imaging study. The accumulation of ^{111}In -DTPA-rGel/BLyS in tumor increased steadily over time, reaching the highest level at 24 h post-injection (Figure 5B).

Intratumor distribution of rGel/BLyS.

Autoradiographic analysis showed that at 72 h after injection, ^{111}In -DTPA-rGel/BLyS accumulated heterogeneously in the tumor, with most activity concentrated in areas with extensive apoptotic response (compare TUNEL staining in regions 2 and 3 in Figure 6C with regions 2 and 3 in Figure 6A). Immunohistochemical staining of the adjacent sections confirmed the expression of BAFF-R and the presence of rGel in the “hot” areas (regions 2 and 3) but not in the area without radiotracer uptake (region 1).

DISCUSSION

Our results suggest that rGel/BLyS effectively targets B-cell tumors *in vivo*, and that preclinical antitumor studies should use a twice per week treatment schedule based on pharmacokinetic and imaging data. However, rGel/BLyS is distributed in the tumor in a heterogeneous fashion.

Radiolabeling of drugs is an investigative tool widely used to carry out absorption, distribution, metabolism, and excretion studies during drug discovery and development. Improvements in radiochemistry technology have enabled the use of radiolabeled materials earlier in pre-clinical and clinical development to address pharmacokinetic and mechanistic issues. In *in vitro* studies, radiolabeled probes are utilized to evaluate the binding affinity with various targeting molecules. In *in vivo* studies, radiolabeled drugs are employed to investigate the extent of absorption, bioavailability, tissue distribution, routes of excretion, and pre-systemic metabolism [25]. In those situations in which the drug can be radiolabeled, the time course of tissue delivery can be determined noninvasively *in vivo*. Such information should be useful for determining dosing schedules, establishing efficacy, and predicting possible toxicity. In our studies, DTPA-rGel/BLyS obtained with a DTPA-to-rGel/BLyS ratio of 3.5 retained its cytotoxic activities against both JeKo-1 and OCI-Ly10 cells while the specific radioactivity of ^{111}In -labeled DTPA-rGel/BLyS was as high as 0.3 MBq/ μg (8.1 $\mu\text{Ci}/\mu\text{g}$). This level of specific radioactivity is suitable for receptor imaging with radiolabeled antibodies and proteins [24; 26].

Recombinant human BLyS is a soluble protein of 17 KDa. It assembles into a trimer in solution, similar to the trimeric structure of other TNF family members. The trimeric nature and the COOH terminus of the BLyS molecule are critical for receptor recognition [27]. Therefore, in this study the rGel component was fused to the NH2 terminus of the BLyS molecule. The purified rGel/BLyS construct migrated on SDS-PAGE as a 45.5 KDa

monomer under reducing conditions. Molecular weight analysis using gel filtration chromatography indicated that the fusion protein has a molecular weight of 136.5 KDa, which is consistent with a trimeric structure [22].

The pharmacokinetic data for ^{111}In -DTPA-rGel/BLyS were best characterized by a tri-exponential disposition with a rapid initial and secondary disposition in the blood pool. The pharmacokinetic parameters such as AUC, CL, and Vc were very consistent with a mean coefficient of variant (CV%) of less than 12% ($\text{CV\%}=\text{SD}/\text{Mean}$). However, the terminal elimination half life ($t_{1/2\gamma}$) was much more variable among the animals than expected. Two mice had more than 4 times higher $t_{1/2\gamma}$ than the mean of the other 5 mice, which was 54.6 h. In general, at least 3-5 $t_{1/2\gamma}$ of blood sampling is required in order to characterize the true pharmacokinetic profiles of the drug. Our study was sampled up to 168 hours post dose, which might not be sufficient in evaluating the terminal elimination half-lives of the mice data. Thus, additional mice pharmacokinetic studies with longer sampling time are warranted for better evaluation of the drug's pharmacokinetic behavior *in vivo*. Nonetheless, the prolonged disposition of ^{111}In -DTPA-rGel/BLyS in mice may be attributable to the size of the fusion protein. In contrast, BLyS with a molecular weight of 52 KDa cleared much faster ($t_{1/2\beta}=2.7$ h) in mice [24]. Based on the pharmacokinetic parameters obtained from this single-dose study, it was estimated that the mean steady-state blood concentrations of ^{111}In -DTPA-rGel/BLyS would be 2.19 %ID/mL following a twice weekly dosing schedule.

The prolonged circulation of radiolabeled rGel/BLyS in blood allowed for accumulation and retention of the fusion toxin in the tumor over time, as shown in the $\mu\text{SPECT-CT}$ imaging study in mice bearing OCI-Ly10 tumors. The tumors were clearly visualized up to 3 days after radiotracer injection (Figure 5A). The uptake of rGel/BLyS in the tumor increased over time and reached the highest level at 24 h post-injection of the radiotracer (Figure 5B). Similarly, the Cu-64-labeled rGel/VEGF₁₂₁ fusion construct, an 84-KDa homodimer, exhibited tumor accumulation and retention from 1 to 48 h after injection in mice with glioblastoma xenografts, with the tumor uptake steadily increasing and peaking at 18 h after injection [28]. Tumor accumulation of rGel/BLyS is attributable to the enhanced permeability and retention effect of macromolecules, as well as receptor-mediated binding and cellular internalization.

On the basis of SPECT/CT imaging data, tumor accumulation of ^{111}In -labeled rGel/BLyS peaked at 24-48 h postinjection. To further quantify the percentage of injected dose in major organs, tissue distribution study was performed by cut-and-count method at 48h time point. The tissue distribution of ^{111}In -labeled rGel/BLyS in SCID mice bearing OCI-Ly10 tumors was measured by direct counting of radioactivity in dissected tissues. The spleen, which has a high proportion of B cells, showed relatively high uptake, indicating the specificity of rGel/BLyS for B cells. The result is consistent with observations of ^{125}I -labeled BLyS, which also had the highest uptake in the spleens of mice bearing J558 tumors [24]. High levels of radioactivity were also observed in the liver and the kidney, probably due to the relatively large size of rGel/BLyS molecules and/or its binding to normal organs. The relatively high activity in the bone suggest that rGel/BLyS as a large molecular weight protein could be readily taken up by the cells of the reticuloendothelial system.

The tumor-to-background ratio is a very important parameter for efficient delivery of therapeutic agents to the tumor site with minimum systemic toxicity. Buchsbaum et al. [29] studied the biodistribution of radioiodinated anti-J5 antibody, an anti-B cell antibody, in athymic mice bearing human B cell Namalwa tumors. The tumor-to-blood ratio ranged from 0.7 to 1.3 over a 7-day period, with the maximum ratio of 1.3 reached at 4 days after injection. The plasma half-life ($t_{1/2, \beta}$) of the antibody was 40 h. In another study, a single-chain anti-gp240 antibody (MEL sFv) was fused into rGel, and the fusion protein demonstrated dramatically higher (~250-fold) cytotoxicity than free rGel. Biodistribution studies of ^{125}I -labeled MEL sFv-rGel in nude mice with human melanoma tumor xenografts showed tumor-to-blood ratios of 0.8 to 2 over a period of 24 to 72 h post-injection [18]. In comparison, at 48 h after injection of ^{111}In -labeled rGel/BLyS, the tumor-to-blood ratio in the OCI-Ly10 tumors was 13, remarkably higher than that of the anti-B cell antibody and the other targeted rGel fusion construct. The higher tumor-to-blood and tumor-to-muscle ratios (4.9) with rGel/BLyS were consistent with our imaging finding that tumors could be visualized with good contrast (Fig. 5A).

The biological effects of BLyS are mediated by three cell surface receptors designated BAFF-R, TACI, and BCMA. Pretreatment of JeKo-1 cells with BLyS blocked the binding of the rGel/BLyS fusion toxin to the BLyS receptors, and pretreatment with BAFF-R:Fc, TACI:Fc, or BCMA:Fc decoy receptors inhibited the rGel/BLyS-mediated cytotoxicity in JeKo-1 cells [22]. In that study, the cytotoxic activity of rGel/BLyS was associated with an apoptotic mechanism; rGel/BLyS-treated JeKo-1 cells had an apoptotic cell death rate of 34%, whereas treatment with rGel showed no induction of cellular apoptosis. To investigate whether the presence of rGel/BLyS was directly associated with apoptotic response, we performed an autoradiographic study of OCI-Ly10 tumors from mice injected with ^{111}In -DTPA-rGel/BLyS. Our data showed that ^{111}In -DTPA-rGel/BLyS localized in the apoptotic area of the tumor, suggesting that distribution of rGel/BLyS to the tumor was the direct cause of tumor apoptotic response. Moreover, the intratumoral distribution of the radioactive signals matched that of fluorescent signals from anti-rGel antibody and anti-BAFF-R antibody, suggesting that the ^{111}In radionuclide was associated with the rGel/BLyS construct *in vivo* and that the tumor uptake of the fusion protein was likely mediated by the BAFF-R receptor. The observed heterogenic radiotracer distribution pattern may be caused by receptor binding, but may also be attributed to the heterogenic pattern of the tumor vasculature. Further studies are needed to address the specificity of the uptake of rGel/BLyS to tumors.

Our preliminary *in vivo* antitumor efficacy studies confirmed that rGel/BLyS significantly inhibited tumor growth in OCI-Ly10 xenograft model using a twice per week schedule [30]. Therefore, although rGel/BLyS displayed a relatively low tumor uptake (1.25% ID/g at 48 h post injection), sufficient antitumor efficacy could be achieved when dose and schedule are optimized. Nevertheless, the uptake of the rGel/BLyS fusion construct into normal tissues, in particular the spleen, is of concern, and toxicology studies examining the systemic effects of rGel/BLyS are ongoing to address this issue. Maximal tolerated dose (MTD), toxicity assessment, and efficacy studies in xenograft models are all ongoing.

In conclusion, ^{111}In -labeled rGel/BLyS allowed us to study the pharmacokinetics and biodistribution of this toxin construct targeted to B-cell lymphoma. We demonstrated that ^{111}In -labeled rGel/BLyS was able to distribute to B-cell tumors *in vivo* and that preclinical antitumor studies with rGel/BLyS should use twice-a-week treatment schedule. The ability to noninvasively image tumor uptake of the fusion protein may guide further clinical development of this novel anticancer therapeutic agent. When incorporated into early clinical trial studies, imaging studies using ^{111}In -labeled rGel/BLyS may be employed to select patients who are most likely to respond to treatment with rGel/BLyS, since these studies suggest that tumor apoptosis is directly related to the tumor uptake and intratumoral distribution of this agent. Because of the heterogeneous intratumoral distribution pattern noted for the rGel/BLyS construct, complete eradication of solid tumors with rGel/BLyS alone may be difficult. Thus, combining rGel/BLyS with other treatment modalities should be considered when designing future preclinical and clinical antitumor efficacy studies.

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Fig. 1.
Cytotoxicities of DTPA-rGel/BLyS, rGel/BLyS, and rGel after 96-h incubation in Jeko-1 and OCI-Ly10 cells. No significant difference in inhibition of cell growth was observed after DTPA modification of rGel/BLyS compared to the unmodified rGel/BLyS.

Fig. 2. Radio-instant thin layer chromatography revealed that the radiochemical purity of ^{111}In -DTPA-rGel/BLyS was greater than 95% after purification with a PD-10 column. The specific activity of the radio-labeled conjugate was 0.3 MBq/ μg (8.1 $\mu\text{Ci}/\mu\text{g}$).

Fig. 3.
The mean blood concentration-time profile of ^{111}In -DTPA-rGel/BLyS generated using WinNonlin software. The data showed a tri-exponential disposition of the drug following intravenous administration.

Fig. 4. Biodistribution of ^{111}In -DTPA-rGel/BLyS at 48 h after intravenous injection of the radiotracer in SCID mice bearing OCI-Ly10 tumors. Data are expressed as percentage of injected dose per gram of tissue (%ID/g), represented as mean $\pm$ standard deviation ($n = 5$).

A

Fig. 5.

(A) Representative μ SPECT-CT images of a mouse bearing an OCI-Ly10 tumor after intravenous injection of ^{111}In -DTPA-rGel/BLyS. The radiotracer primarily accumulated in the spleen, liver, and kidney. Uptake in the tumor is clearly visualized at 24, 48, and 72 h post-injection. (B) Accumulation of ^{111}In -DTPA-rGel/BLyS in OCI-Ly10 tumor at different times after intravenous administration of ^{111}In -DTPA-rGel/BLyS at a dose of 48 μg per mouse. The percentage of injected radioactivity in each tumor was calculated on the basis of gamma images and quantitative analysis of the counts per pixel in the tumors. The tumor uptake of ^{111}In -DTPA-rGel/BLyS steadily increased over time and peaked at 24 h post-injection.

Fig. 6. Representative autoradiogram and histological analysis of an OCI-Ly10 tumor from a mouse that received intravenous administration of ^{111}In -DTPA-rGel/BLyS at 72 h before tumor removal. **(A)** Autoradiographic image depicts the intratumor distribution pattern of the radiolabeled rGel/BLyS, which was confirmed by H&E staining **(B)** of the same section. **(C)** Immunohistochemical staining of regions 1, 2, and 3 in **(A)** at higher magnification. The slides adjacent to the slide used for autoradiographic study were stained with TUNEL for apoptotic cells, BAFF-R antibody and anti-rGel antibody, respectively. Each marker was pseudo-colored green.

Table 1

Summary of pharmacokinetic parameters of ^{111}In -DTPA-rGel/BLyS after intravenous injection in BALB/c mice.

Parameter	Mean	SD
$T_{1/2\alpha}$ (h)	0.65	0.63
$T_{1/2\beta}$ (h)	7.57	1.65
$T_{1/2\gamma}$ (h)	137.70	155.49
C_0 (%ID/mL)	28.09	6.28
V_c (mL)	3.70	0.73
V_{ss} (mL)	13.53	10.75
AUC (%ID h/mL)	183.93	21.42
CL (mL/h)	0.55	0.06
MRT (h)	26.53	25.49

The pharmacokinetic parameters were obtained from 7 mice. $T_{1/2\alpha}$, $T_{1/2\beta}$, and $T_{1/2\gamma}$ are half-lives in initial distribution phase, extensive distribution phase, and terminal elimination phase, respectively; C_0 is the estimated initial drug concentration in the blood; V_c is volume of distribution of central compartment; V_{ss} is volume of distribution at steady-state; AUC is area under the %ID/mL blood-time curve; CL is total body clearance; and MRT is mean resident time.