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Positron Emission Tomography Imaging of Angiogenesis in a Murine Hindlimb Ischemia Model with ⁶⁴Cu-Labeled TRC105

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Abstract

The goal of this study was to assess ischemia-induced angiogenesis with ⁶⁴Cu-NOTA-TRC105 positron emission tomography (PET) in a murine hindlimb ischemia model of peripheral artery disease (PAD). CD105 binding affinity/specificity of NOTA-conjugated TRC105 (an anti-CD105 antibody) was evaluated by flow cytometry, which exhibited no difference from unconjugated TRC105. BALB/c mice were anesthetized and the right femoral artery was ligated to induce hindlimb ischemia, with the left hindlimb serving as an internal control. Laser Doppler imaging showed that perfusion in the ischemic hindlimb plummeted to ~20% of the normal level after surgery, and gradually recovered to near normal level on day 24. Ischemia-induced angiogenesis was non-invasively monitored and quantified with ⁶⁴Cu-NOTA-TRC105 PET on postoperative days 1, 3, 10, 17, & 24. ⁶⁴Cu-NOTA-TRC105 uptake in the ischemic hindlimb increased significantly from the control level of 1.6±0.2 %ID/g to 14.1±1.9 %ID/g at day 3 (n=3), and gradually decreased with time (3.4±1.9 %ID/g at day 24), which correlated well with biodistribution studies performed on days 3 & 24. Blocking studies confirmed the CD105 specificity of tracer uptake in the ischemic hindlimb. Increased CD105 expression on days 3 and 10 following ischemia was confirmed by histology and RT-PCR. This is the first report of PET imaging of CD105 expression during ischemia-induced angiogenesis. ⁶⁴Cu-NOTA-TRC105 PET may play multiple roles in future PAD-related research and improve PAD patient management by identifying the optimal timing of treatment and monitoring the efficacy of therapy.

Keywords

angiogenesis; ischemia; positron emission tomography (PET); peripheral artery disease (PAD); imaging; CD105 (endoglin)

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DISCLOSURES

CPT is an employee of TRACON Pharmaceuticals, Inc. The other authors declare no conflicts of interest.

INTRODUCTION

Peripheral artery disease (PAD) is a consequence of systemic atherosclerosis that results in progressive narrowing of the arteries of the lower extremities.¹ PAD is most commonly seen in the elderly population and its prevalence is correlated with increasing age.¹ Angiogenesis plays an important role in the pathogenesis of PAD, as arterial narrowing and resulting ischemia/hypoxia can initiate the process of angiogenesis. Under the condition of ischemia, tissues release paracrine angiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), etc. that subsequently induce angiogenesis in an attempt to restore the blood circulation to the ischemic tissue.² Different pro-angiogenic treatments, mainly growth factors, have been proposed to enhance tissue revascularization.^{2, 3} However, assessing the efficacy of pro-angiogenic treatment has been challenging due to the lack of reliable, non-invasive imaging methods.^{4, 5}

Various imaging techniques can detect changes in extremity blood circulation.^{5–7} Non-invasive molecular imaging methods can provide information on the response of the ischemic tissues to the treatment, as well as detect molecular abnormalities that contribute to the early pathogenesis, thereby permitting early intervention. Positron emission tomography (PET) is a non-invasive, sensitive, and quantitative radionuclide-based imaging modality,^{8, 9} which can be used to detect molecular changes during ischemia.^{5, 6} CD105 (also called endoglin) is a 180 kDa disulfide-linked homodimeric transmembrane protein that has been employed as the target molecule for PET imaging of angiogenesis,^{10–15} since it is overexpressed on newly formed blood vessels and serves as a reliable marker of angiogenesis.¹⁶

TRC105, a chimeric monoclonal antibody that binds with high affinity to human and murine CD105, has completed a Phase I study and is currently in multiple Phase 2 trials in oncology patients.¹⁷ In this work, we report the use of ⁶⁴Cu-NOTA-TRC105 (NOTA denotes 1,4,7-triazacyclononane-1,4,7-triacetic acid) for non-invasive, tomographic, temporal, and quantitative monitoring of angiogenesis in a murine hindlimb ischemia model of PAD by PET. CD105 expression levels in mouse ischemic hindlimb were non-invasively assessed by ⁶⁴Cu-NOTA-TRC105 PET over a 4-week period after surgery, and the PET results were validated by various in vivo and ex vivo experiments (e.g. laser Doppler, blocking with excess of TRC105, biodistribution study, histology, RT-PCR, etc.).

EXPERIMENTAL SECTION

⁶⁴Cu-labeling of TRC105

TRC105 was provided by TRACON Pharmaceuticals (San Diego, CA) and S-2-(4-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (p-SCN-Bn-NOTA) was purchased from Macrocyclics, Inc. (Dallas, TX). ⁶⁴Cu was produced with a CTI RDS 112 cyclotron using the ⁶⁴Ni(p,n)⁶⁴Cu reaction, which has specific activity of > 5 Ci/μmol at the end-of-bombardment.

⁶⁴Cu-NOTA-TRC105 was synthesized as described previously.¹⁵ In brief, NOTA conjugation was carried out at pH 9.0, with the ratio of p-SCN-Bn-NOTA:TRC105 being 25:1. NOTA-TRC105 was purified using PD-10 columns (GE Healthcare, Buckinghamshire, UK) with phosphate-buffered saline (PBS, HyClone laboratories, Logan, UT) as the mobile phase. For radiolabeling, ⁶⁴CuCl₂ (74 MBq) was diluted in 300 μL of 0.1 M sodium acetate buffer (pH 6.5) and added to 50 μg of NOTA-TRC105. The reaction mixture was incubated for 30 min at 37 °C with constant shaking. ⁶⁴Cu-NOTA-TRC105 was purified using PD-10 columns with PBS as the mobile phase. The radioactive fractions

containing ^{64}Cu -NOTA-TRC105 were collected and passed through a 0.2 μm syringe filter for in vivo experiments.

Flow cytometry

The immunoreactivity of TRC105 and NOTA-TRC105 to human umbilical vein endothelial cells (HUVECs, high CD105 expression^{10, 18}) was evaluated by fluorescence activated cell sorting (FACS). Briefly, cells were harvested and suspended in cold PBS with 2% bovine serum albumin at a concentration of 5×10^6 cells/mL. The cells were incubated with TRC105 or NOTA-TRC105 (1 or 5 $\mu\text{g/mL}$) for 30 min at room temperature (RT), washed three times with cold PBS, and centrifuged at 1,000 rpm for 5 min. The cells were then incubated with AlexaFluor488-labeled goat anti-human IgG for 30 min at RT. Afterwards, the cells were washed and analyzed by FACS using a BD FACS Calibur four-color analysis cytometer, which is equipped with 488 nm and 633 nm lasers (Becton-Dickinson, San Jose, CA) and FlowJo analysis software (Tree Star, Ashland, OR).

Mouse model of hindlimb ischemia

All animal studies were conducted under a protocol approved by the University of Wisconsin Institutional Animal Care and Use Committee. Unilateral hindlimb ischemia in the right leg was induced in six-week-old female BALB/c mice (Harlan, Indianapolis, IN) by ligation and excision of the right femoral artery distal to the inguinal ligament, upon anesthesia with 2% isoflurane delivered in O_2/N_2 mixture. A sham procedure was performed on the contralateral hindlimb to serve as the internal control. Of note, the incision was placed on the mid-abdominal level to eliminate the possibility of superposition of radioactivity signals from surgical wound (wound healing process is highly angiogenic) and the ischemic muscle tissue.

Laser Doppler imaging of hindlimb perfusion

Laser Doppler images were obtained before and soon after surgery, as well as on postoperative days 10 and 24, with a laser Doppler imaging system (moorLDI2-HR, Moor instruments, DE, USA). The mice were kept at 37 °C throughout the procedure. Average hindlimb perfusion was expressed as the ratio of ischemic to non-ischemic hindlimb by region-of-interest (ROI) analysis of both hindlimb.

Small animal PET imaging and biodistribution studies

Serial PET imaging after surgery was performed on an Inveon microPET/microCT rodent model scanner (Siemens Medical Solutions USA, Inc.).^{19, 20} Each mouse was intravenously injected with 5–10 MBq of ^{64}Cu -NOTA-TRC105. Five- to ten-minute static PET scans were performed at various time points post-injection (p.i.) with the animals maintained under 2% isoflurane anesthesia. The images were reconstructed using a maximum a posteriori (MAP) algorithm, with no attenuation or scatter correction. ROI analysis of each PET scan was performed using vendor software (Inveon Research Workplace [IRW]) on decay-corrected whole-body images as described previously,^{19, 20} to calculate the percentage injected dose per gram of tissue (%ID/g) values for ischemic and non-ischemic muscles of both hindlimb. A cohort of three mice was each injected with 2 mg of TRC105 at 2 h before ^{64}Cu -NOTA-TRC105 administration on days 3 and 10 after surgery to evaluate CD105 specificity of ^{64}Cu -NOTA-TRC105 in vivo (i.e., blocking experiment).

Immediately after the last PET scan, biodistribution studies were carried out to confirm that the quantitative tracer uptake values based on PET imaging and ROI analysis accurately represented the radioactivity distribution. In addition, a separate group of three mice was each injected with ^{64}Cu -NOTA-TRC105 three days after surgery and euthanized at 48 h p.i.

for biodistribution studies. Blood, hindlimb muscle tissue, and major organs and tissues were harvested and wet-weighted. The radioactivity in the tissue was measured using a Cobra II γ -counter (Perkin-Elmer) and presented as %ID/g.

Histology

Frozen tissue slices of 5 μ m thickness were fixed with cold acetone for 10 min. After rinsing with PBS and blocking with 10% donkey serum for 30 min at RT, the slices were incubated with a mixture of TRC105 (5 μ g/mL) and rat anti-mouse CD31 antibody (BD biosciences, San Jose, CA) for 1 h at RT, and visualized using AlexaFluor488-labeled goat anti-human IgG (Invitrogen, Eugene, OR) and Cy3-labeled donkey anti-rat IgG (Jackson laboratories, West Grove, PA) respectively. All images were acquired with a Nikon Eclipse Ti microscope.

Quantitative real time RT-PCR

The total RNA was isolated from hindlimb muscle using Qiagen RNeasy Minikit (Qiagen Inc., Valencia, CA), reversely transcribed and amplified using Qiagen OneStep RT-PCR kit (Qiagen Inc., Valencia, CA). Real time PCR was performed using TaqMan primers for CD105 (Mm00468256_m1 Eng) and GAPDH (Mm99999915_g1 Gapdh) (Applied Biosystems, Carlsbad, CA). The PCR product was run on 0.5% agarose gel under 100 V for 1 h.

Statistical analysis

Data were presented as mean $\pm$ SD. Differences between multiple groups were assessed with 1-way ANOVA followed by the Tukey's post hoc test for multiple comparisons. All *P* values were calculated with 2-tailed statistical tests. Differences were considered significant when *P* < 0.05. Data were analyzed with IBM SPSS Statistics 16.0.

RESULTS

Flow cytometry

HUVECs display high density of membrane bound CD105.¹⁸ Analysis of HUVEC binding by FACS indicated no detectable differences in specificity and affinity between TRC105 and NOTA-TRC105 at 1 μ g/mL or 5 μ g/mL concentrations (Figure 1A). NOTA-TRC105 was stable in complete mouse serum at 37°C for up to a week, with no significant changes in HUVEC binding affinity or specificity based on FACS analysis.

⁶⁴Cu-labeling of TRC105

⁶⁴Cu-labeling including final purification using size exclusion column chromatography took 80 $\pm$ 10 min (*n* = 5). The decay-corrected radiochemical yield was > 85% based on 25 μ g of NOTA-TRC105 per 37 MBq of ⁶⁴Cu, and the radiochemical purity was > 98% (Figure 1B). The specific activity of ⁶⁴Cu-NOTA-TRC105 was about 3.0 GBq/mg of protein, assuming complete recovery of NOTA-TRC105 after size exclusion column chromatography.

Laser Doppler imaging of hindlimb perfusion

Induction of tissue ischemia following femoral artery ligation was confirmed by laser Doppler imaging. Soon after ligation, the average hindlimb tissue perfusion ratio decreased from 0.896 $\pm$ 0.098 to 0.218 $\pm$ 0.077 (*n* = 3; *P* < 0.01) (Figure 2A,B). Hindlimb tissue perfusion ratio on days 10 and 24 was 0.581 $\pm$ 0.222 and 0.914 $\pm$ 0.250, respectively. PET imaging of ischemic mice on postoperative day 1, at 15 minutes p.i. of ⁶⁴Cu-NOTA-TRC105 (when most of the radioactivity is in the blood thereby indicating blood perfusion

within the mouse body), also indicated very low blood flow in the ischemic hindlimb and confirmed the success of surgical ligation (Figure 2C).

PET imaging and biodistribution studies

^{64}Cu -NOTA-TRC105 PET was carried out at days 1, 3, 10, 17, 24 after surgery to monitor CD105 expression with non-invasive imaging. On the basis of our previous experience with *in vivo* PET imaging using TRC105-based agents to image proliferating tumor vasculature,^{10, 15} the time points of 4, 24, and 48 h p.i. were chosen for serial PET scans (Figure 3A). At 4 h p.i., there was a relatively high level of blood radioactivity and background signal because of the long circulation half-life of the radiolabeled antibody. ^{64}Cu -NOTA-TRC105 uptake in the tissue of interest (e.g. tumor and ischemic tissue) typically plateaued between 24 and 48 h p.i.^{10, 15}

The peak of ^{64}Cu -NOTA-TRC105 uptake in ischemic hindlimb was observed at 48 h p.i., which was 9.0 ± 2.2 , 14.1 ± 1.9 , 11.4 ± 1.5 , 6.2 ± 1.5 , 3.4 ± 1.9 %ID/g on days 1, 3, 10, 17, and 24 after surgery, respectively ($n = 3$; Figure 3B). These values were significantly higher ($P < 0.05$ in all cases) than that of the non-ischemic contralateral hindlimb (2.0 ± 0.4 , 1.6 ± 0.2 , 2.5 ± 0.5 , 0.4 ± 0.3 , 0.7 ± 0.3 %ID/g on days 1, 3, 10, 17 and 24 respectively; $n = 3$; Figure 3C). ^{64}Cu -NOTA-TRC105 uptake at 48 h p.i. in the ischemic hindlimb on days 3 and 10 was significantly higher than all other days examined ($P < 0.05$; Figure 3C).

Administering a blocking dose of TRC105 at 2 h before ^{64}Cu -NOTA-TRC105 injection significantly reduced tracer uptake in the ischemic hindlimb at 48 h p.i. to 7.5 ± 0.7 and 5.9 ± 0.4 %ID/g on days 3 and 10 after surgery, respectively ($n = 3$; Figure 3A,B), which confirmed CD105 specificity of the tracer *in vivo*. *Ex vivo* biodistribution data of ^{64}Cu -NOTA-TRC105 in blood, major organs, and tissues after the last PET scans at 48 h p.i. are summarized in Figure 4, which corroborated the findings from non-invasive PET scans and confirmed that PET imaging enabled accurate quantification of ^{64}Cu -NOTA-TRC105 uptake in mice.

Histology

Representative CD31/CD105 co-staining images of the ischemic hindlimb muscle are shown in Figure 5. The highest density of CD105 positive vessels was observed on postoperative day 3 (358 ± 82 vessels/mm²; $n = 5$), followed by day 10 (279 ± 191 vessels/mm²; $n = 5$). The average number of CD105 positive vessels dropped to 67 ± 85 vessels/mm² ($n = 5$) on day 17. The difference in CD105 positive vessel density between postoperative days 3 and 10 was not statistically significant, while the difference between day 17 and day 3 (or day 10) was statistically significant ($P < 0.05$). No significant CD105 staining was observed on the other days.

Quantitative real time RT-PCR

The expression level of CD105 in ischemic hindlimb muscle was the highest on day 3 ($C_T = 21.1 \pm 0.5$, where C_T denotes threshold cycle; $n = 3$; Figure 6), which corroborated *in vivo* PET findings. The C_T values were 29.6 ± 0.6 , 24.4 ± 0.8 , 25.2 ± 1.4 , 26.8 ± 0.6 for postoperative days 1, 10, 17, and 24 respectively ($n = 3$). The C_T for GAPDH was 17.4 ± 0.4 , whereas the C_T for the control group was 32.3 ± 0.7 ($n = 3$). DNA gel electrophoresis confirmed real time RT-PCR results.

DISCUSSION

As the importance of preventive medicine has been recognized, the focus of cardiovascular medicine is gradually shifting to the early, sensitive, and specific detection of diseases at the

molecular level.^{6, 9, 21} Molecular imaging techniques have the potential to detect early changes during the course of a disease prior to the development of clinical symptoms. Moreover, non-invasive, targeted, radionuclide-based imaging techniques such as PET may also be used to monitor disease progression and response to therapy.^{5, 6, 9}

In this study we demonstrated, for the first time, that CD105 expression levels in murine hindlimb ischemia can be monitored by non-invasive PET imaging with ⁶⁴Cu-NOTA-TRC105. ⁶⁴Cu-NOTA-TRC105 uptake increased substantially in the ischemic hindlimb compared with the contralateral hindlimb and peaked at 3 days following surgery, which correlated well with CD105 expression levels as documented by histology and quantitative real time RT-PCR. The mouse hindlimb ischemia model of PAD is a well-established model, easy to perform, and is less expensive than several other PAD models.²² One limitation of this model is that hindlimb ischemia is induced acutely and unilaterally, whereas human disease is usually bilateral and exhibits a much more complex and chronic progression. In addition, human PAD is often associated with other diseases (e.g. diabetes, hypertension, hypercholesterolemia) which are usually absent in animal models.^{23–25}

Molecular imaging has significant advantages over traditional methods of vascular imaging such as contrast angiography, magnetic resonance (MR) angiography, and computed tomography (CT) angiography.²⁶ Contrast angiography is an invasive technique that requires significant skills and expertise. High resolution MR angiography and spectroscopy, as well as CT angiography, are newer vascular imaging strategies that are less invasive than the conventional techniques, however they cannot detect molecular changes occurring early in the disease process. Similarly, PET imaging with small molecules such as ¹³N-NH₃ or ¹⁵O-H₂O to measure extremity blood flow does not assess molecular changes either.^{23, 27–29} Laser Doppler perfusion imaging is frequently used to examine the hindlimb perfusion. This technique, however, is limited to measurement of superficial blood flow and is unlikely to provide reliable information about the subtle changes in global blood flow. In this study, relatively low blood flow values were obtained with laser Doppler imaging on postoperative day 10 (0.581 ± 0.222 of the contralateral hindlimb), whereas PET imaging (validated by histology and RT-PCR) indicated high levels of physiological angiogenesis between days 3 and 10, thereby offering invaluable biological insights underlying low blood perfusion. Dedicated small animal PET scanners have become increasingly popular over the last decade, which can facilitate clinical translation of validated PET tracers and speed up the development and real time assessment of new therapeutic agents.

Literature reports on PET imaging of PAD have addressed the potential roles of different radionuclides (e.g., ⁶⁴Cu, ⁶⁸Ga, ⁷⁶Br) and tracers for visualization and quantification of various molecular markers of angiogenesis under ischemic conditions.^{27, 30–32} ⁶⁴Cu was chosen for the current work due to its wide availability, low cost, and versatile chemistry.^{33, 34} The $E_{\max}$ of ⁶⁴Cu (656 keV) for its positron emission is comparable to ¹⁸F, which can give PET images with good quality and spatial resolution. Integrin $\alpha_v\beta_3$ and VEGF receptors are among the most commonly studied markers of angiogenesis for PET imaging.^{35, 36} CD105 is selectively expressed on proliferating endothelial cells of newly formed vessels, and is expressed at significantly higher level than other angiogenesis-related targets including the VEGF receptors.³⁷ Our group has recently conducted non-invasive imaging studies in multiple tumor types using radiolabeled TRC105,^{10–15} and in this study we have demonstrated that TRC105-based PET tracers could also be invaluable tools in cardiovascular diseases such as PAD. When compared with previous literature reports using other tracers which typically had uptake of < 2%ID/g in the ischemic hindlimb muscle,^{27, 30–32} tracer uptake of ⁶⁴Cu-NOTA-TRC105 is several fold higher (~15%ID/g). Importantly, the signaling pathways of VEGF and CD105 are distinct. VEGF receptors signal through receptor tyrosine kinases (e.g. PI3K), while CD105 modulates TGF- β

receptor signaling through serine/threonine kinases, mainly modifying the phosphorylation of Smad proteins.¹⁶ Therefore, PET imaging with tracers that are specific for each of these pathways is expected to provide complementary information in future studies.

The ideal targeting molecule for PET imaging should be a multivalent, high affinity compound that is small enough to reach the target across biological barriers to allow prominent uptake and sustained retention in the tissues of interest.^{8, 38, 39} The main obstacles that need to be overcome for a successful molecular imaging agent include rapid metabolism/excretion, non-specific binding, difficulty in crossing biological barriers, etc. Since radiolabeled intact antibodies typically have long circulation lifetime, the use of Fab or F(ab')₂ fragments of TRC105 may exhibit improved image contrast in PAD in future investigation.^{40, 41} Lastly, recent development in nanotechnology may help to improve the delivery of therapeutic agents to PAD.^{42, 43} Imaging component can be integrated into these multifunctional nanoparticles, which can also be designed to isolate and protect the radioisotope (e.g. ⁶⁴Cu) from being rapidly recognized and metabolized, thereby allowing more accurate readout of the nanoparticle biodistribution in vivo.

CONCLUSION

In this study, ⁶⁴Cu-NOTA-TRC105 was investigated for serial PET imaging of CD105 expression in a mouse model of hindlimb ischemia. Persistent and CD105-specific uptake of ⁶⁴Cu-NOTA-TRC105 in the ischemic hindlimb was observed, which was validated by various *in vivo* and *ex vivo* experiments. PET is a suitable method for molecular imaging of angiogenesis in hindlimb ischemia since it is very sensitive, tomographic, quantitative, and clinically translatable. ⁶⁴Cu-NOTA-TRC105 PET may play multiple roles in future PAD-related research and improve PAD patient management by identifying the optimal timing for treatment and monitoring the therapeutic efficacy. Furthermore, this tracer may also be applicable in other cardiovascular diseases where CD105 expression is upregulated or modulated.

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Figure 1.

A. Flow cytometry analysis indicated no detectable difference in CD105 binding affinity or specificity between TRC105 and NOTA-TRC105 at various concentrations. **B.** Representative size exclusion column chromatography profile indicating baseline separation of 64 Cu-NOTA-TRC105 (elute at 3–4 mL) from 64 Cu (elute after 6 mL).

Figure 2.

A. Laser Doppler imaging shows the gradual recovery of blood perfusion in the ischemic hindlimb, following an acute decrease in blood flow after surgical ligation of the femoral artery. **B.** A graph showing the blood flow in the ischemic hindlimb as percentages of the blood flow in the contralateral hindlimb (n = 3). **C.** A representative PET image of ischemic mice on postoperative day 1 at 15 minutes after injection of ^{64}Cu -NOTA-TRC105, indicating very low blood flow in the ischemic hindlimb.

Figure 3.

A. Representative PET images at 4, 24, and 48 h post-injection of ^{64}Cu -NOTA-TRC105 on days 1, 3, 10, 17, and 24 days after surgical creation of hindlimb ischemia (arrowheads). Corresponding PET images of mice in the blocking group on days 3 and 10 after surgery are also shown. **B.** %ID/g values of ^{64}Cu -NOTA-TRC105 uptake in the ischemic hindlimb, indicating that tracer uptake plateaued between 24 and 48 h post-injection. Significantly lower tracer uptake was observed in the blocking group. **C.** The differences between ^{64}Cu -NOTA-TRC105 uptake in the ischemic and non-ischemic hindlimb were statistically significant at all time points examined. The highest uptake of ^{64}Cu -NOTA-TRC105 in the ischemic hindlimb was on postoperative days 3 and 10, which were significantly higher than tracer uptake on the other days. $n = 3$. *: $P < 0.05$. H: heart.

Figure 4.

Biodistribution studies at 48 h post-injection showed that ^{64}Cu -NOTA-TRC105 uptake in the ischemic hindlimb on postoperative day 3 was much higher than all major organs. Tracer uptake in the ischemic hindlimb at day 24 was at the background level. A pre-injected blocking dose of 2 mg of TRC105 per mouse significantly reduced tracer uptake in the ischemic hindlimb.

Figure 5. Immunofluorescence staining confirmed the increased expression of CD105 in the ischemic hindlimb on postoperative days 3 and 10, significantly higher than the other days and the control hindlimb. Green: CD105; red: CD31; blue: DAPI. Scale bar: 100 μ m.

Figure 6.

Quantitative real time RT-PCR yielded similar results as that of PET imaging and histology. The highest expression of CD105 in the ischemic hindlimb was on postoperative day 3 (i.e., the C_T value was the lowest). The result of agarose gel electrophoresis of the PCR product is also shown. Data represent triplicate samples.