TARGETING $\alpha_v\beta_3$ INTEGRIN EXPRESSION ON INTRAPERITONEALLY GROWING TUMORS WITH A RADIOLABELED RGD PEPTIDE

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Introduction: Because of the restricted expression in normal tissues and its abundant expression in various types of tumors, $\alpha_v\beta_3$ integrin is considered a suitable receptor for tumor-targeting. RGD peptides contain the Arg-Gly-Asp sequence and preferentially bind to the $\alpha_v\beta_3$ integrin receptor. In previous studies, we have shown that radiolabeled cyclic RGD peptides specifically accumulated in subcutaneously growing tumors in nude mice. Here we studied the tumor-targeting potential of an ^{111}In-labeled cyclic DOTA-E-c[RGDfK] in athymic mice with intraperitoneally (ip) growing ovarian carcinoma tumors. Furthermore, the therapeutic potential of the ^{177}Lu-labeled RGD peptide was investigated.

Experimental: DOTA-E-c(RGDfK) was labeled with ^{111}In at a specific activity of 10 GBq/μmol. Specific activity with Lu-177 was 162 GBq/μmol. Tumor targeting of the ^{111}In-labeled compound was studied in athymic mice with i.p. growing NIH:OVCAR-3 xenografts. The optimal peptide dose of ^{111}In-DOTA-E-c(RGDfK) in this model was determined. In addition, the biodistribution at optimal dose was determined at various time points. The effect of the route of administration was studied (ip vs iv). The therapeutic potential was investigated, one group of mice (n=7) with ip OVCAR-3 tumors received 37 MBq/mouse ^{177}Lu-DOTA-E-c(RGDfK), while a control group did not receive any treatment.

Results and Discussion: Optimal tumor uptake of ^{111}In-DOTA-E-c(RGDfK) was observed at peptide doses ranging from 0.03 μg to 0.1 μg (20.6 ± 9.7% ID/g and 18.5 ± 5.9% ID/g, respectively). At 2 h pi, the tumor-to-blood ratio at a peptide dose of 0.1 μg was 133 ± 46. At higher peptide doses the uptake in the tumor was significantly lower, indicating receptor saturation. At a peptide dose of 0.1 μg, tumor uptake peaked at 4 h pi (38.8 ± 2.7% ID/g) and gradually decreased with time. Blood levels were 0.98 ± 0.20% ID/g at 0.5 h pi and rapidly decreased to 0.006 ± 0.001% ID/g at 72 hr pi, resulting in extremely high tumor-to-blood ratios (3216 ± 120). At 2 h pi, tumor uptake after ip injection was 35.2 ± 3.8% ID/g whereas after iv injection, the tumor uptake was only 0.98 ± 0.26% ID/g. Mice that received 37 MBq ^{177}Lu-DOTA-E-c(RGDfK) ip showed a significant longer survival than the mice that received no treatment (p = 0.017). Median survival of mice that were treated with the ^{177}Lu labeled RGD peptide was 21 wks, whereas that for the untreated mice was 5 wks.

Conclusion: We showed that ^{111}In-DOTA-E-c(RGDfK) has high and specific uptake in mice with i.p. growing OVCAR-3 tumors. PRRT experiments in this model of ovarian cancer indicated that ip tumor growth can be inhibited significantly by a therapeutic dose of ^{177}Lu-DOTA-E-c(RGDfK).

Keywords: Avb3 Integrin, RGD Peptide Targeting, Peptide Receptor Radiotherapy (PRRT), Ovarian Cancer

PREPARATION OF ^{68}Ga-NOTA-RGDyK AND FEASIBILITY TEST FOR ANGIOGENESIS IMAGING

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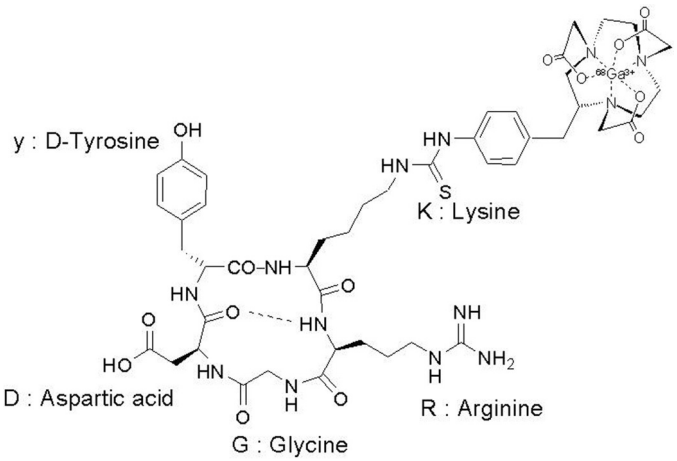
Introduction: RGD (Arg-Gly-Asp) derivatives have been labeled with various radioisotopes for imaging angiogenesis of ischemic tissue in which $\alpha_v\beta_3$ integrin plays an important role (1, 2). In this study, cRGDyK (cyclic Arg-Gly-Asp-D-Tyr-Lys) was conjugated with 2-(*p*-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid and labeled with ^{68}Ga, and then the labeled RGD was tested for in vitro binding and in vivo biodistribution.

Experimental: 2-(p-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid was coupled with lysine fuctional group of cRGDyK by thiourea formation. The conjugate was purified by semi-prep. HPLC. Purified NOTA-cRGDyK was labeled with ^{68}Ga from ^{68}Ge/^{68}Ga-generator and purified with semi-prep HPLC. Competative binding assay of cRGDyK and NOTA-cRGDyK was performed using ^{125}I-cRGDyK as a radioligand and a_vb_3 integrin coated plates as solid phase. ^{68}Ga -NOTA-cRGDyK (6 μCi/100 μL) was injected into hindlimb ischemic ICR mice model (n = 4) through the tail vein. For blocking study, cold RGD (3 mg/Kg) was added to the injectates. Mice were sacrificed at 1 h and 2 h and the activities of organs were counted for biodistribution study.

Results and Discussion: Labeling of ^{68}Ga-NOTA-cRGDyK was quantitative. Ki values of cRGDyK and NOTA-cRGDyK were 1.18 nM and 1.89 nM, respectively. In biodistribution study, uptake of ^{68}Ga-NOTA-cRGDyK in ischemic muscles was 1.59±0.23 ID/g. Uptake of ^{68}Ga-NOTA-cRGDyK in ischemic muscles was blocked with cold cRGDyK. The ratio of ischemic muscles to blood was 2.35 whereas the ratio decreased to 1.02 after blocking.



Conclusion: ^{68}Ga -NOTA-cRGDyK was obtained with high a yield, showed a high affinity to a_vb_3 integrin and showed specific uptake to angiogenic muscle in vivo. ^{68}Ga-NOTA-cRGDyK is a promising radioligand for imaging angiogenesis.

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Keywords: NOTA, RGD, Angiogenesis, Ga-68, αvβ3

LABELING OF AN ANTI-VEGF MONOCLONAL ANTIBODY WITH RADIOACTIVE ARSENIC ISOTOPES

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Introduction: The inhibition of tumor-induced angiogenesis is an emerging therapeutic strategy in clinical oncology aiming at halting cancer progression by suppressing tumor blood supply. One of the better-defined factors, involved in the angiogenesis process, is vascular endothelial growth factor (VEGF). Tumor-derived VEGF is a new target in the design of anticancer medicines, since blocking VEGF with the adequate monoclonal antibody may block tumor development. VG76e, an anti-VEGF monoclonal antibody, has been labeled with ^{124}I, ^{99m}Tc, ^{153}Sm and ^{177}Lu for tumor detection using SPECT/PET imaging [1], with encouraging results which warrant the need for further investigation using other radionuclides. Since the enrichment of antibodies in tumor tissue is a slow process, covering several days, radionuclides with a long physical half-life are necessary to assess their pharmacokinetics. Recently, ^{72}As and ^{74}As have been identified as positron emitting radionuclides with long physical half-lives of 26 h and 17.4 d, respectively [2].

Experimental: The labeling of proteins with radioactive arsenic isotopes is based on their high affinity to free –SH groups. As a direct method, the reduction of disulfides of the antibody was performed via TCEP*HCl (tris(2-carboxyethyl)phosphine hydrochloride). The number of created –SH groups was estimated before each labeling experiment. The modified antibody VG76e was directly incubated with an ethanolic solution of nca [$^{72/74/77}$As]AsI_3 at 37°C for 30 minutes. The labeling of VG76e was optimized with reactor produced nca ^{77}As. The labeling yields were determined by SEC-HPLC. Purification of VG76e was performed by gel filtration.

Results and Discussion: The direct method of endogenous disulfide reduction with TCEP*HCl was optimized. The resulting number of –SH groups was 4 per antibody for the direct method. Labeling was quantitative at 37°C and 30 min. The stability of a purified antibody fraction was monitored over 100 h in PBS buffer and BSA containing solution and showed no loss of activity. The immunoreactivity has not yet been tested.

Conclusion: A method for the labeling of VG76e with arsenic isotopes has been optimized with nca ^{77}As to give quantitative yields after 30 minutes reaction time at 37°C. The label is stable in vitro for more than 100 h. The in vivo evaluation of VG76e will be performed with ^{72}As or ^{74}As labeled antibody via small animal PET.

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Keywords: Radioactive Arsenic Isotopes, Labeling of Antibodies, TCEP, VEGF

SMALL ANIMAL PET IMAGING OF TUMOR VASCULATURE USING A ^{76}Br-LABELED HUMAN RECOMBINANT ANTI-ED-B FIBRONECTIN ANTIBODY FRAGMENT

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Introduction: Angiogenesis is fundamental for oxygen and nutrient supply to solid tumors and is related to tumor aggressiveness, chance of metastasis and poor prognosis. Recently, selective targeting of tumor neo-vasculature was obtained by using fragments of a human recombinant antibody (L19) specific for the extra domain B (ED-B) of fibronectin (1-4). Imaging this angiogenesis biomarker with PET in primary and metastatic tumors could be an important step for an early evaluation of the clinical outcome of antiangiogenic therapies, as PET allows the quantification of radiotracer uptake. Therefore, we labeled a L19 small immunoprotein (SIP) with the positron-emitter ^{76}Br and we carried out small animal PET imaging and biodistribution studies in F9 tumor-bearing mice.

Experimental: The ^{76}Br-bromination of L19-SIP was achieved by using bromoperoxidase/H_2O_2. ^{76}Br-L19-SIP (RCP> 95%, 83-84% immunoreactivity) was injected i.v. in 129/sv mice bearing F9 tumors. MicroPET imaging (n=2) and biodistribution (n=3-4) evaluation were carried out at 5, 24, and 48h p.i.

Results and Discussion: The microPET imaging studies showed high specific uptake of radioactivity in the tumors expressing the ED-B fibronectin target which were lit up at each considered time point, with low background activity. The biodistribution data confirmed tumor uptakes similar to those of ^{125}I- and ^{111}In-labeled L19-SIP (1, 3) and higher than those of a ^{99m}Tc-labeled scFv dimer L19 fragment (4) (18.1±7.6, 9.3±3.5, and 14.3±1.6%ID/g at 5, 24 and 48h p.i., respectively). However, residual radioactivity in blood and other non target organs led to significantly lower T/NT ratios compared to the published data. This is probably due to partial in vivo debromination of ^{76}Br-L19-SIP. To confirm this hypothesis, the evaluation in vivo ^{76}Br-L19-SIP metabolism is in progress.

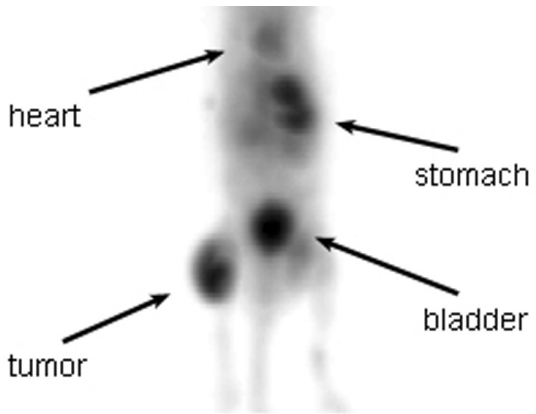

Fig. 1. MicroPET projection image of a tumor bearing mouse 48 h after ^{76}Br-L19-SIP administration.

Conclusion: Our data confirm the feasibility of PET imaging of the ED-B fibronectin target in solid tumors. This provides a novel approach to the early evaluation of antiangiogenic agents for a better therapy management.

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Keywords: Angiogenesis, ED-B Fibronectin, Bromine-76, MicroPET, Biodistribution