COMBINED THERAPY WITH AN ^{111}In-LABELED EXENDIN-4 CONJUGATE ALONG WITH A VEGF-RECEPTOR INHIBITOR IN A TRANSGENIC MOUSE MODEL

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Introduction: The Glucagon-like-Peptide-1 (GLP-1) receptor is an interesting target in tumor diagnosis and therapy with Exendin-based radiopeptides as it is overexpressed on human insulinomas and gastrinomas. We have recently shown high therapeutic efficacy using a radiopeptide labeled with the Auger-emitter ^{111}In in a transgenic mouse model developing insulinoma-like tumors originating from pancreatic islet cells. Because of high kidney uptake kidney toxicity was found in the animals. The aim of this work was to study efficacy and kidney toxicity of a combination of the angiogenesis inhibitor PTK 787 with ^{111}In-Exendin-4.

Experimental: Rip1Tag2 transgenic mice develop insulinoma-like tumors in a reproducible manner. We performed oral treatment with PTK 787 daily for 1 week and studied 4 h-biodistribution using ^{111}In-Exendin-4 0, 3, 5 and 7 days after the initiation of PTK 787 treatment. To assess the efficacy of combined antiangiogenesis treatment and targeted radionuclide therapy, Rip1Tag2 mice were injected with 1.1 MBq ^{111}In-Exendin-4 shortly after initiating PTK 787 treatment. After 7 days mice were sacrificed and tumor volume, histology (H&E) and cell proliferation studied. In addition, monotherapies with either 1.1 MBq, 28 MBq ^{111}In-Exendin-4 or PTK were performed. Long term toxicity was performed in C57BI/6J mice 6 months after combined and monotherapy.

Results and Discussion: Due to high GLP-1 receptor expression in the tumor of Rip1-Tag2 mice, the tumor uptake of the radiopeptide was 210±52% IA/g 4h after start of combination therapy with a tumor-to-kidney ratio of 0.7. Five and 7 days after initiating PTK treatment this value decreased to <90% IA/g. Therapy efficacy of combination treatment and different monotherapies is shown in Table 1. Combined therapy shows very similar efficacy as high dose radionuclide therapy. Whereas the latter showed tubular necrosis and glomerular sclerosis 6 months after initiation, the combined therapy did not reveal any differences in the kidney morphology between control and treated mice.

Table 1. Tumor volumes 7 days after different therapies

	Median, mm^3
Control n=11	18.6 (100%)
1.1 MBq n=7	3.7 (20%)
PTK n=11	4.6 (25%)
28 MBq n=11	0.83 (4.5%)
1.1 MBq+PTK n=10	0.53 (2.8%)

Conclusion: The present study indicates that a combination of antiangiogenesis treatment and low dose targeted radionuclide therapy can massively reduce tumor volume (>97%) and cell proliferation of small tumors without any organ toxicity. As VEGF-mediated angiogenesis and GLP-1 receptor density in the Rip1Tag2 mouse tumor model are comparable in human insulinomas, the results might be transferable to human tumors with high GLP-1 receptor density and concomitant VEGF-driven angiogenesis.

Keywords: Targeted Radionuclide Therapy, ^{111}In-Exendin-4, GLP-1 Receptor, PTK 787, Antiangiogenesis Treatment

POTENTIAL ROLE OF TUMOR SUPPRESSOR PROTEIN p53 IN THE TRAFFICKING OF COPPER TO THE NUCLEI OF TUMOR CELLS

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Introduction: Copper-64 ($T_{1/2}$ = 12.7 h) emits β^+ and β^- particles for applications in both PET imaging and cancer therapy. In radioimmunotherapy studies, we have shown that ^{64}Cu-labeled mAb 1A3 caused complete tumor growth inhibition in a well-established animal model at dramatically lower absorbed doses compared to doses reported for both ^{131}I- and ^{90}Y-labeled mAbs in the same animal model. More recently, we reported that ^{64}Cu from ^{64}Cu-TETA-octreotide localized to the nuclei of somatostatin-receptor positive tumor cells. The mechanism(s) of copper trafficking to the cell nucleus, and its effects on tumor cell killing are not well understood. We are investigating the potential role of the tumor suppressor protein p53 in this process. Two EGFR-expressing human colorectal HCT116 cell lines, that are positive or negative for p53, were used to compare internalization and nuclear localization of ^{64}Cu-acetate **(1)** and the EGFR antibody ^{64}Cu-DOTA-cetuximab **(2)**.

Experimental: DOTA was conjugated to cetuximab, and radiolabeling with ^{64}Cu was accomplished using literature methods. Receptor binding of **2** to HCT116 cell membranes was performed. Internalization and nuclear localization of **1** or **2** by HCT116 cells were measured during a 24h time course.

Results and Discussion: The K_d and B_{max} of **2** were similar for the two cell lines (HCT116 +/+: K_d = 0.86 nM ± 0.24 nM, B_{max} = 1854 fmol/mg ± 172 fmol/mg; HCT116 -/-: K_d = 0.95 nM ± 0.18 nM, B_{max} = 1989 fmol/mg ± 124 fmol/mg). Internalization of **1** and **2** in both HCT116 cell lines increased over time. At earlier time points (1h, 4h) uptake of **1** was independent of p53 status; however, at later time points (18h, 24h) there was a significantly higher uptake in the HCT116 +/+ cell line ($P<0.05$). At 24h the uptake of **1** in the nuclei of HCT116 +/+ cells was significantly higher (29.4% ± 2.0% of cell-associated activity) than in HCT116 -/- cells (13.8% ± 0.34%) ($P<0.0001$). Similar to **1**, there was only significantly increased uptake of **2** in HCT116 +/+ cells at 24h ($P<0.05$). Nuclear localization of **2** showed increased uptake in the nuclei of p53 positive cells at 1h (15.6% ± 0.65% of cell-associated activity) compared to the p53 negative cells (10.9% ± 0.57%) ($P<0.01$).

Conclusion: These data demonstrate that ^{64}Cu is delivered to tumor cell nuclei of p53 positive cells in significantly greater amounts than p53 negative cells by both non-specific and receptor-mediated uptake mechanisms. The data suggest that ^{64}Cu radiopharmaceuticals will have improved tumor cell killing properties in p53 positive tumors, in part due to the delivery of a higher radiation dose to the cell nucleus.

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Keywords: Copper-64, Targeted Radiotherapy, p53

LOCOREGIONAL RADIOIMMUNOTHERAPY OF GLIOMA WITH A Re-188 TENASCIN ANTIBODY: PRE-CLINICAL EVALUATION AND FIRST CLINICAL STUDY

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Introduction: A lot of parameters still have to be optimized in the locoregional radioimmunotherapy of gliomas with monoclonal Tenascin antibodies (TN-mAb). In respect to the application route and singular vs. fractionated application also the choice of radionuclide seems to be important. Over 90% of all recurrent tumors are localized in a 2 cm surrounding of the primary tumor cavity, so the present study was designed to perform the pre-clinical evaluation of a Re-188 labelled TN-mAb and a first study in patients.

Experimental: For direct radiolabelling of the TN-mAb with Re-188 three different reduction methods were compared. The labeled proteins were investigated for their in vitro stability (0,9% PBS, cysteine, plasma) and immunoreactivity (gel-shift-analysis with human TN before and after incubation in serum). In an intracranial C6 glioma model in Wistar rats the biodistribution of the Re-188 TN-mAb was determined and the data were compared to I-131 labelled IgG. After systemic application 3-4 animals were euthanasized at different time points up to 98h p.i. and the T/NT-ratios were calculated. In a first dose escalating patient study with Re-188 mAb (370 to 1110 MBq) the pharmacokinetics (imaging, urine, plasma) and side effects are investigated.

Results and Discussion: The best results in direct radiolabelling of TN-mAb's were obtained with Tris-(2-carboxyethyl)phosphine (TCEP) as reductant, because with this method the highest in vitro stability (> 80% after 20 hrs in different media) and the best results in the immunoreactivity test with the gel-shift-analysis (75% TN-bound immediately after labelling, about 60% after 20 hrs in plasma) were achieved. In the biodistribution study the tumor uptake after i.v. application of Re-188-mAb was higher than the uptake of I-131 mAb and the T/NT-ratios were significant better (5 to 17:1 vs. 2 to 8:1 for the tumor/brain-ratio). In the first patient study the Re-188-mAb showed a good binding in the tumor cavity (biological half life about 10 hr) and a fast renal clearance of systematically observed radioactivity (<10% of I.D.). Analysis of urine and plasma showed only compounds of low molecular weights. At the third of four dose escalating levels no local or systemic side effects occured.

Conclusion: The results of the animal and the patient studies demonstrate that Re-188-mAb showed a good accumulation in tumor tissue and the pharmacokinetic behaviour is comparable to the I-131 labelled IgG. Further studies have to demonstrate that the longe range radionuclide Re-188 has a significant influence on the survival time of glioma patients.

Keywords: Radioimmunotherapy, Re-188, Antibody, Tenascin, Glioma

AN INTERNALIZING ANTI-EGFRvIII MONOCLONAL ANTIBODY LABELED WITH ^{177}Lu: EFFECT OF CHELATING AGENT ON INTRACELLULAR RETENTION

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Introduction: Monoclonal antibodies (mAbs) reactive to mutant epidermal growth factor receptor variant III (EGFRvIII) undergo extensive internalization after receptor binding. Compared with directly radioiodinated mAbs, the radioactivity from radiometal-labeled mAbs is generally retained better in tumor cells after internalization. The present study was performed to evaluate the potential utility of different chelates labeling L8A4, an anti-EGFRvIII mAb, with ^{177}Lu. Intracellular trapping of the ^{177}Lu-labeled L8A4 conjugates was compared to that of L8A4 radioiodinated directly, and using the residualizing label SGMIB[1].

Experimental: L8A4 was conjugated with A) CHX-A'-DTPA, B) p-SCN-Bn-DTPA, C) 2-(4-isothiocyanatobenzyl)-6-methyl-DTPA (1B4M-DTPA or MX-DTPA), D) NHS-DOTA, E) C-DOTA, and F) MeO-DOTA, and then labeled with ^{177}Lu following a literature[2] protocol. L8A4 was labeled with ^{125}I directly using Iodogen or with SGMIB as described[1]. Internalization assays[1] were performed, in single- or paired-label format, using EGFRvIII-expressing U87 delta-EGFR cells.

Results and Discussion: Intracellularly trapped radioactivity (% of initially bound) of ^{177}Lu-labeled mAbs increased with time over 24 h, while directly radioiodinated mAb decreased (Table 1). L8A4 labeled with acyclic C and cyclic F was retained best (~35%) at 24 h. The internalized radioactivity from SGMIB-labeled mAb was higher than that from ^{177}Lu-labeled mAb up to 8 h; however, at 24 h, the ^{177}Lu-labeled mAb demonstrated a 1.5- to 2.5-fold advantage. These results demonstrate that that there are chelator-dependent differences in the trapping of ^{177}Lu after mAb internalization, with no clear advantage for acyclic vs. cyclic ligands. Furthermore, at earlier time points, SGMIB provides as good results as any of the metal-chelate conjugates.

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	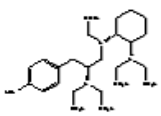		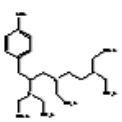		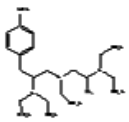		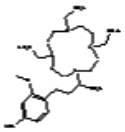		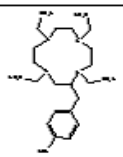		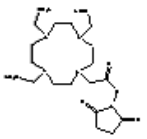	
Time [h]	^{177}Lu	^{125}I*	^{177}Lu	^{125}I	^{177}Lu	^{125}I	^{177}Lu	^{125}I	^{177}Lu	^{125}I*	^{177}Lu	^{125}I
1	9.3±0.6	14.5±1.6	10.2±0.3	16.0±0.6	10.7±0.1	11.0±0.4	8.7±1.2	12.0±1.4	5.8±1.7	12.8±0.25	16.7±0.6	19.4±1.9
8	12.1±0.9	15.6±0.5	12.9±1.4	14.2±1.5	17.5±1.5	9.0±0.2	16.5±1.0	9.3±0.6	16.4±0.3	17.5±0.7	15.8±0.8	7.3±1.5
24	24.8±1.4	9.7±0.6	27.7±2.4	10.7±0.9	35.1±2.3	2.9±0.4	30.5±1.7	2.6±0.2	34.4±1.0	20.7±0.1	30.8±0.3	1.7±0.1

**Conjugates were radioiodinated using SGMIB.*

Table 1. Percent internalized activity for ^{177}Lu- and ^{125}I-labeled L8A4.

Conclusion: We conclude that better labeling methods are still needed for maximizing the intracellular retention after internalization of both radiometal and radiohalogen labeled mAbs.

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Keywords: Radioimmunotherapy, Monoclonal Antibodies, Lutetium-177, Internalization

ANTIBODY DERIVATISATION WITH DENDRITIC CHELATOR-STRUCTURES

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Introduction: Antibodies are efficient targeting vectors for diagnosis and endoradiotherapy.

For this purpose radionuclides are conjugated to the antibody carrier molecule. In general the cargo capacity of the antibodies is not completely exploited. This limits their diagnostic potential and therapeutic success.

A polyconjugation of chelating agents to the antibody molecule could improve their therapeutic potential by enhancing the cytotoxic effect exerted per antibody. The polyconjugation could be performed by a random introduction of several single chelators or by conjugation of dendritic structures containing chelators leading to a less altered structure and thus to a reduced influence on the binding affinity to the antigen. Here we report the synthesis of dendritic structures containing a defined number of chelators and their introduction into antibodies.

Experimental: PAMAM dendrimers of different size were synthesised by convergent synthesis on a polyethylene glycol core. DOTA was introduced into the dendritic molecules by first reacting the free amines of the dendrimer with a heterobifunctional crosslinker containing a maleiimido function to which DOTA, derivatised with a thiol, could be linked. These scaffolds allow the introduction of a defined number of DOTA moieties into the carrier molecule. The dendritic DOTA containing structures were then coupled to an anti-EGFR-antibody by reacting it with Sulfo-SMCC and subsequent coupling of the dendritic structures that contain a free thiol under physiological conditions.

Results and Discussion: The PAMAM synthesis on w-aminoethoxy-pentaethylenglycol-ethanthiol according to literature methods was found to yield heterogeneous products; extended reaction times at low temperatures led to substantially improved dispersity of the dendrimers. Six different PAMAM-conjugates containing 1 to 36 DOTA moieties were synthesized in good yield. Purification of the dendritic structures and the DOTA conjugates was performed using HPLC. The number of functional chelators introduced into the dendritic structures was determined by saturative complexation with $^{111}In/^{115}In$ mixtures. The influence of the dendrimer size and number on the properties of the derivatized antibody was investigated to determine the optimal derivatisation with respect to binding affinity and cytotoxic effect.

Conclusion: DOTA containing dendrimers of different size were synthesised and coupled in a defined number into antibody molecules. The influence of the dendrimer size and number on the binding affinity of the antibodies was investigated. The derivatised antibodies allow an improvement of endoradiotherapy when compared to antibodies containing several randomly distributed single chelators.

Keywords: Antibodies, Dendrimer, DOTA, Endoradiotherapy, Diagnosis