ELEVATION OF 3-[^{125}I]IODO-α-METHYL-L-TYROSINE TISSUE ACCUMULATION BY REGULATION OF RENAL EXCRETION WITH OAT INHIBITORS

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From the view point of improvement of drug accumulation in target organs, to keep its blood concentration high, which is followed by transient competitive inhibition of renal excretion, may induce its higher uptake to target tissues. Tubular secretion mainly contributes to renal excretion of drugs and many drugs are excreted in urine *via* various transporters on basolateral membrane of tubular epithelial cells. 3-Iodo-α-methyl-L-tyrosine (I-AMT) is a clinically promising tumor-seeking amino acid agent and we reported it accumulates in the brain and the pancreas by L-type amino acid transporters [1-3]. Our recent basic experiments also revealed the possibility of the suppression on its renal excretion by transient inhibition of organic anion transporters (OAT) [4,5]. In present study, we investigated the effects of OAT inhibitors on I-AMT tissue accumulation in *in-vivo*. Probenecid, *N*-benzoyl-β-alanine and cefazolin were used as OAT inhibitors whose are clinically available in safety. OAT inhibitors were injected intravenously in mice respectively. Five minutes after injection of them, ^{125}I-AMT was injected intravenously in mice and biodistribution study was conducted. Examination with ^{99m}Tc-MAG_3, which showed high affinity for renal OAT [6], was also performed as a comparison.

Inhibition of renal excretion by OAT inhibitors drastically affected biodistribution pattern of ^{125}I-AMT in early phase. In mice with probenecid loading, accumulation of ^{125}I-AMT in kidneys was decreased and that in blood was remarkably increased (1.1-2.4 times) compared with unloaded mice. In addition, brain and pancreas accumulation of ^{125}I-AMT was achieved up to 1.8 times and 3.4 times compared with unloaded mice, respectively. ^{99m}Tc-MAG_3 also showed the slow clearance in OAT inhibitors loaded mice as compared with unloaded mice. Radioactivity in blood of ^{99m}Tc-MAG_3 was 3.5 times higher in probenecid loaded mice than in unloaded mice.

As our expected hypothesis, regulation of renal excretion by OAT inhibitors was able to achieve the elevation of ^{125}I-AMT accumulation in brain and pancreas. Furthermore, since effects of these inhibitors are transient and safety, the regulation technique of renal excretion may be clinically applicable to control tissue distribution of radiopharmaceuticals.

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Keywords: 3-Iodo-alpha-Methyl-L-Tyrosine, Renal Excretion, OAT Inhibition

Ho-166 LABELED DTPA DERIVATIVE FOR INTRAVASCULAR RADIATION THERAPY WITH LIQUID BALLOONS: DUAL-FUNCTIONAL COMPLEX FOR RESTENOSIS PREVENTION AND CT IMAGING

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ABSTRACT SUMMARY

To develop a new liquid radiation source for the prevention of restenosis having the characteristic of CT imaging, DTPA bisamide derivative, DTPA-BTIPA (3-amino-2,4,6-triiodoisophthalic acid) was synthesized by the reaction of DTPA dianhydride with 3-amino-2,4,6-triiodoisophthalic acid. The compound can be utilized as an iodinated X-ray contrast agent; the content of iodine in the molecule was 57 %. The optimum condition of radiolabeling of DTPA-BTIPA with Ho-166 was achieved by varying different reaction parameters. Both radiochemical and biological studies revealed that ^{166}Ho labeled DTPA-BTIPA can be further explored as a potential agent for vascular brachytherapy having the characteristic of CT contrast agent. The use of the ^{166}Ho-DTPA-BTIPA is a good alternative to see if the balloon has close contact with the blood vessel wall for the delivery of a sufficient radiation dose to the stenotic artery.

INTRODUCTION

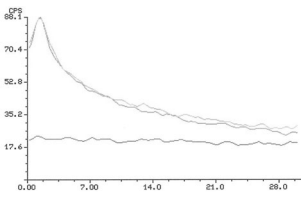
Brachytherapy is one of the effective treatment for in-stent restenosis. Filling the dilatation catheter balloon with radioactive solutions has advantages on accurate source positioning and uniform dose delivery to the vessel walls. In addition, it can be used easily with existing catheter. Moreover, solution based beta ray source allow the treatment of large vessels. Of the variety radioisotopes prepared in a soluble form, Ho-166 is a good radioisotope, because can be readily produced by irradiating natural Ho target. For X-ray imaging, various iodinated X-ray contrast agents having the 1,3,5-triiodobenzoic acid platform are used mainly computed tomography (CT) and angiographic applications. In the present study, we prepared a new DTPA derivative containing iodine in the structure. To estimate the ^{166}Ho-complex as a liquid radiation source for the potential application of IVRT, which readily excreted through urinary system in the event of balloon rupture, dynamic imaging was acquired.

EXPERIMENTAND RESULTS

^{166}Ho-(DTPA-BTIPA) was prepared by simple mixing at room temperature. High radiochemical stability (>98%) was maintained over a period of 6 hours at room temperature. The radioactivity curve in kidneys of rabbit administered with ^{166}Ho-(DTPA-BTIPA) via ear vein showed that the ^{166}Ho-(DTPA- BTIPA) was rapidly cleared through the kidneys. The average of T_{max} and $T_{1/2}$ of ^{166}Ho-(DTPA-BTIPA) in the kidneys were 2.26 ± 0.78 min and 7.80 ± 1.16 min, respectively (Fig.1). The serial static image scans of rabbit administered with ^{166}Ho-complex revealed that none of the tissues except the urinary system has radioactivity concentrations.

DISCUSSION AND CONCLUSION

The prepared complex of ^{166}Ho-(DTPA-BTIPA) was reveled that the rapid renal clearance, adequate *in vivo* stability and low uptake in vital organs to take care of any accidental release of activity inside the body due to rupture of the balloon. ^{166}Ho-(DTPA-BTIPA) has beneficiary effects such as visualization of both the position and shape of the balloon are possible and most importantly, whether or not there is a formation of a void volume of liquid inside the balloon as well as the detection of radiation leakage on a real-time basis, on site during the angioplasty. The DTPA-BTIPA can be applicable for the preparation of ^{188}Re-complex as a radiation source for the prevention of in-stent restenosis.



Keywords: Vascular Radiation Brachytherapy, Restenosis, Holmium-166

TUMOR TARGETING USING ^{177}Lu-CHX-A″-DTPA-PERTUZUMAB

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Purpose

Previous efforts have shown that antibodies labeled with radioactivity can be effective in cases where resistance to antibody treatment has arisen. The antibody pertuzumab (Omnitarg®, Genentech), which targets the dimerization subdomain of HER-2 is currently being evaluated for effect on tumors overexpressing this receptor. The purpose of these experiments was to ascertain whether pertuzumab retains targeting capacity after labeling with the low energy beta emitter ^{177}Lu as well as make an initial characterization of this putative therapeutic agent.

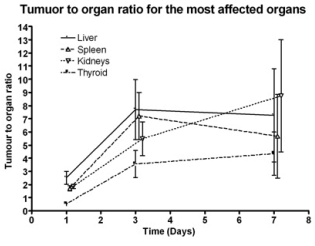
Methods

Pertuzumab was conjugated with isothiocyanate-benzyl-CHX-A"-DTPA and then chelated to ^{177}Lu. Immunoreactive fraction was tested using SKOV-3 at a molar excess of 100 receptors per antibody. The affinity of the coupled product was measured using both Biacore and a saturation analysis on SKOV-3 cells.

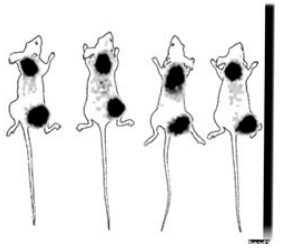
Cellular retention was measured after a 4.5 hour long accumulation. *In vivo* biodistribution, targeting and specificity was assessed using Balb/c (nu/nu) mice with SKOV-3 xenografts. [^{177}Lu]pertuzumab was administered by subcutaneous injection in the neck with and without a 100-fold molar excess of non-labeled antibody. Dissections were made at 1, 3 and 7 days and the activity of the organs was determined in a well crystal scintillator.

Results

Pertuzumab was successfully labeled with ^{177}Lu using CHX-A"-DTPA. The stability when challenged overnight with 200:1 molar excess of EDTA was above 78 % and the immunoreactive fraction was above 85 %. The affinity of conjugated pertuzumab was determined to 2 nM, and that of [^{177}Lu]pertuzumab to 4 nM.



The cellular retention of ^{177}Lu was 90 % after 20 hours. The targeting was found to be specific *in vivo* since uptake of labeled antibody could be inhibited ($p<0.01$). The biodistribution of [^{177}Lu]pertuzumab was dominated by a large tumor uptake. The organs that had the highest relative activity versus tumor were thyroid, kidneys, spleen and liver as shown in figure 1. Gamma camera images of the three day mice showed significant hotspots in the tumor, situated on the right anterior leg, and injection site as can be seen in figure 2.



Discussion

We were able to label pertuzumab with high efficiency and stability. The labeled antibody was shown to be specific and have good intracellular retention. We were also able to confirm the specificity *in vivo*.

Although the antibody's injection site is evident in the gamma camera images and obscures some of the organs the biodistribution is very promising. Should the injection be done intravenously the injection site hotspot would most probably disappear. Although pertuzumab gives nice images we believe that the main application of the antibody will be treatment while smaller and faster molecules are used for imaging. The biodistribution as well as gamma camera images of labeled pertuzumab show promise for treatment using low energy beta emitters or possibly alpha emitters.

Keywords: HER-2, Pertuzumab, Lutetium-177

PREPARATION AND EVALUATION OF ^{177}Lu-CHX-A″-DTPA-ABD-($Z_{HER2:342}$)$_2$ AFFIBODY MOLECULE FOR RADIONUCLIDE THERAPY OF DISSEMINATED HER2-EXPRESSING TUMORS

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Eradication of single spread malignant cell and micrometastases in the case of disseminated cancer is a big challenge, since they can not be detected by usual means. The use of molecular recognition for selective delivery of cytotoxic radionuclides might be a way to destroy such cells while sparing healthy tissues. A possible target isHER2/neu antigen, which is expressed in, e.g. 25-30 % of breast and ovarian carcinomas. It has been shown, that in these tumors HER2 has high and homogenous expression, which is preserved in metastases.

Recently, a new class of phage-display-derived proteins, Affibody molecules, has been reported. Being derived from protein A, Affibody molecules have a robust structure, and preserve their binding capacity after radiolabeling. The small size of Affibody molecules, about 7 kDa enables good extravasation and tumor-penetration properties. An Affibody molecule $Z_{HER2:342}$ binds HER2 with affinity of 20 pM. The goal of this study was to develop an anti-HER2 therapeutic conjugate based on $Z_{HER2:342}$.

In order to prolong blood residence time, a $Z_{HER2:342}$ dimer ($Z_{HER2:342}$)$_2$ was fused with albumin-binding domain ABD. The resulting fusion protein ABD-($Z_{HER2:342}$)$_2$ (MW 19.1 kDa) was conjugated to isothiocyanate-benzyl- CHX-A"-DTPA in alkaline media under elevated temperature. Free chelator was separated and buffer was changed to 1 M amminium acetate, pH 5.5 by size-exclusion chromatography.

Labeling with ^{177}Lu was performed in metal-free 1M acetate buffer, pH 5.5 in the presence of ascorbic acid. Typically, the yield was more that 95% after 1 h long incubation. Additional purification using a disposable size-exclusion column gave a product in phosphate-buffered saline with radiochemical purity exceeding 98%. Tests on HER2-expressing SK-OV-3 ovarian carcinoma cells demonstrated that labeled ^{177}Lu-CHX-A″-DTPA-ABD-($Z_{HER2:342}$)$_2$ retained immunoreactivity. The cell tests indicated that the conjugate was internalized, and residualising radiometal label provided good cellular retention.

Biodistribution studies demonstrated efficient targeting of HER2-expressing SK-OV-3 xenografts in Balb C nu/nu mice. A tumor uptake of 25.5 ± 4.8 % IA/g was reached 48 h pi. Comparison with tumor uptake after pre-injection of large amount of non-labeled conjugate and uptake of size-matched non-specific Affibody conjugate demonstrated that tumor uptake was highly specific. The use of binding to albumin enabled to prolong blood circulation. At the same tome, kidney uptake was reduced in comparison with analogs, which were not fused with ABD. Result of dosimetric evaluation predicted doses of 1.8 Gy/MBq for tumors, 1.3 Gy/MBq for kidney and 0.2 Gy/MBq for bones, which make this conjugate promising for radionuclide therapy of disseminated HER2-expressing tumors.

Acknowledgement: This work was partially supported by grants from Swedish Cancer Society (Cancerfonden)

Keywords: Lutetium-177, Affibody Molecule, HER2/neu

RAPID RENAL EXCRETION OF NON-INCORPORATED RADIOACTIVITY IN SOLUTIONS CONTAINING RADIOLABELED DOTA-PEPTIDES

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Objectives: A new field of interest is the application of radiolabeled DOTA-peptides for Peptide Receptor Radionuclide Therapy (PRRT) and Scintigraphy (PRRS). Large amounts of radionuclides are herefore administered, and, although incorporation of the radionuclide is almost 100 %, there is always a free ionic fraction of the radionuclide (67/68Ga3+, 90Y3+, 111In3+, and 177Lu3+) accumulating in bone and/or bone marrow. The calculated radiation doses from these free ionic radionuclides in human bone marrow are: 0.11 (67Ga3+); 0.04 (68Ga3+); 3.39 (Y3+); 0.53 (111In3+); and 1.1 (177Lu3+) mGy/MBq. In vivo these radionuclides - chelated with EDTA or DTPA - are rapidly excreted via the urine. Therefore, such chelator was added to the radiolabeled DOTA-Y3-octreotide (DOTATOC) and DOTA-Y3-octreotate (DOTA-tate) injection fluid post radiolabeling and prior to the administration in order to complex the free ionic form of the radionuclide. Moreover, for analytical purposes the addition of chelator is essential to avoid false-positive results on the incorporation of the radionuclide.

The biodistribution of all 4 radionuclides in various forms was investigated in rats.

Methods: Biodistribution of 68Ga3+, 90Y3+, 111In3+ and 177Lu3+,

1) all as chloride.

2) complexed with DTPA or EDTA (mol/mol ratios of 30 over DOTA).

3) all 4 radionuclides labeled with DOTATOC or DOTA-tate at 100 % incorporation.

4) all 4 radionuclides labeled with DOTATOC or DOTA-tate at 90 % incorporation (thus 10 % free ionic radionuclide).

5) as in 3). with the addition of excess of chelator.

6) as in 4). with the addition of excess of chelator.

Results: 1) Formation of 90Y-, 111In-, and 177Lu-DTPA in vitro was rapid and complete. The formation of 68Ga-DTPA was slow and incomplete, while formation of 68Ga-EDTA was rapid and complete; therefore we continued our 68Ga-studies with EDTA.

2) Radiolabeled DOTATOC and DOTA-tate remained stable with <1-% transchelation up to 24 h in the presence of 4 mM DTPA or EDTA.

3) All 4 radiochlorides showed high accumulation in skeleton and liver and slow blood clearance.

4) These values in skeleton, liver and blood could be strongly reduced by the in vitro addition of DTPA or EDTA, and with rapid renal clearance: TB<5 % ID at 24 h pi.

5) Biodistribution of radiolabeled DOTATOC and DOTA-tate showed high uptake in somatostatin receptor-positive tissues, with no significant differences in these tissues between 100 and 90 % incorporation of the radionuclide.

6) Rats injected with DOTATOC and DOTA-tate with 90 % incorporation indeed showed higher retention in blood, and accumulation in liver and skeleton.

7) The retention in blood and accumulation in liver and skeleton could be prevented by previous addition of chelator.

Conclusions: 1. Free ionic radionuclide in injection fluids with radiolabeled DOTA-peptides can effectively be complexed by the addition of chelator before the injection, and therefore can be rerouted in vivo.

2. Since free ionic radionuclide in radiolabeled DOTA-peptides can be complexed and rerouted effectively the specification for the % of incorporation in clinical trials can be lowered.

Keywords: Reduction Bone Marrow Dose, Rerouting Free Ionic Radionuclide In Vivo, Accumulation of Free Ionic Radionuclide in Bone

TUMOR RESPONSE AND SIDE EFFECTS AFTER [^{177}Lu-DOTA,Tyr^3]OCTREOTATE THERAPY IN RATS

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Introduction: A new and promising cancer treatment modality is peptide receptor radionuclide therapy with ^{177}Lu-labeled somatostatin analogs.

Methods and Results: We evaluated [DOTA,Tyr^3]octreotate, with high affinity binding to somatostatin receptor subtype 2 (sst_2). [^{177}Lu-DOTA,Tyr^3]octreotate showed high tumor uptake in sst_2-positive tumors in patients and rats. Radionuclide therapy in rats bearing sst_2-positive CA20948 or AR42J tumors resulted in up to 100% cure after 555 MBq (max tumor dose 140 Gy) [^{177}Lu-DOTA,Tyr^{3}octreotate. Tumor response was dependent on tumor size; [^{177}Lu-DOTA,Tyr^3]octreotate appeared optimal for eradication of small tumors. Tumor doses > 100 Gy also cured rats with bigger tumors. Because of the excellent tumor responses after [^{177}Lu-DOTA,Tyr^3]octreotate, knowledge about side effects becomes most relevant.

Mortality because of radiotoxicity was not observed. Dose-limiting organs in the rat are the radiosensitive kidneys. Radiation dose was 50 and 100 Gy (without kidney protection using amino acids) after 278 and 555 MBq [^{177}Lu-DOTA,Tyr^3]octreotate, respectively. In these treated groups a dose dependent, transient rise in urinary protein was found, starting 70 days post therapy. Kidney histology showed time and dose dependent, irreversible damage, ranging from mild (50 Gy) to severe (100 Gy). Serum creatinine increased after 50 and 100 Gy, dependent on dose, time post therapy and length of interval between repeated doses. I.v. D-lysine (400 mg/kg) normalized these values.

Bone marrow toxicity: a dose and time dependent drop in white blood cells and platelets was found. No change in red blood cells and Hb was seen. In somatostatin receptor-positive pancreas and receptor-negative liver no long term toxicity was found.

Conclusion: Radionuclide therapy using [^{177}Lu-DOTA,Tyr^3]octreotate is most promising. It can cause long term damage, especially in kidneys after high radiation doses. Amino acids such as lysine can reduce renal uptake, radiation dose and toxicity.

Keywords: Radionuclide Therapy, Somatostatin Analog, Lutetium-177

DOTA-OLIGOMERS CONJUGATED TO OCTREOTATE: THE INFLUENCE OF THE NUMBER OF CHELATES ON RECEPTOR BINDING AND BIODISTRIBUTION

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The clinical impact of peptides that accumulate in tumors is determined by the number of particle-emitting isotopes attached. We have recently shown that peptide conjugates of antisense oligonucleotides can be successfully targeted to SSTR expressing tumors. This demonstrates an astonishing cargo capacity of SSTR affine peptides without loss of binding affinity. In order to exploit this carrying capacity further, a solid phase synthesis strategy was developed to produce a series of octreotate conjugates with multiple DOTA chelates per molecule octreotate. We describe a general synthetic route to conjugates that allows insertion of multiple DOTA moieties (n = 3, 6 or 9) at the N-terminal end of Tyr3-octreotate (1). These conjugates were further charcterized with respect to labeling efficeiency and in vitro and in vivo behaviour.

The peptide moiety was assembled by Fmoc solid phase synthesis and oxidized to form the cyclic disulphide. Subsequently the required number of DOTA-tris tert-butyl ester chelating units were attached to the side chains of lysines. The conjugates were purified and thoroughly studied by RP-HPLC, size exclusion HPLC, and mass spectrometry. The ease of labeling of the novel conjugates and of DOTA-Tyr3-octreotate (DOTATATE) was exemplified for 90-Y and 111-In. Receptor binding affinities were determined by competition experiments with rat cortex membranes. Biodistribution experiments were performed in Lewis rats bearing the SSTR positive CA20948 tumors.

The relative ease of labeling of compounds 1, 2, 3 and DOTATATE was examined by labeling with 90-Y and 111-In. These compounds were easily labeled with both radionuclides. In vitro the conjugates were stable in human serum for up to 24 h. Receptor binding studies revealed a slight decrease in receptor affinity when increasing the number of DOTA chelates. However, even for the 9-mer conjugate a high affinity (IC50 = 7.41 +- 1.31) was retained.

The stepwise synthesis strategy followed in this study allows the defined synthesis of Tyr3-octreotate conjugated to a high number of DOTA chelates. These compounds will facilitate the synthesis of labeled peptides with matchless specific activity and therefore hold promise for further development and application of radiopeptide therapy. The methodology described allows the versatile introduction of multiple DOTA chelates into a peptide sequence, yielding compounds with high SSTR affinity.

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Keywords: Somatostatin Receptors, Peptide, Chelating Agents

IMPROVEMENT OF *N*-ISOPROPYL-*p*-[^{123}I]IODOAMPHETAMINE CEREBRAL ACCUMULATION BY COMPETITIVE DISPLACEMENT OF SERUM PROTEIN BINDING WITH AMINO-ACID INFUSION

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It is an important factor that increasing the accumulation of radiopharmaceuticals in target tissue leads to the improvement of image contrast in nuclear medicine. We have identified using ^{99m}Tc-MAG$_3$ that concurrent administration of drugs with high protein binding affinity produced competitive displacement at the specific binding sites and enhanced total clearance and tissue distribution of this tracer [1-2]. Otherwise, the displacement effects and mechanism of amino-acid fluids on serum protein binding were found and reported in recent our paper [3]. In this study, we aimed that cerebral accumulation of ^{123}I-*N*-isopropyl-*p*-iodoamphetamine (^{123}I-IMP) was increased using the displacement method with amino-acid fluid, which is essential nutrient and applied well clinically.

The effects of amino-acid infusion with radiopharmaceuticals were evaluated in *in-vitro* human serum. As amino-acid fluids, Proteamine 12X (PTA, Tanabe Seiyaku) and Kidomin (Otsuka Pharmaceutical) were selected. For confirmation of serum protein binding sites of amino-acid fluids, binding site markers, such as ^{14}C-warfarin, ^{14}C-diazepam for binding site I, II of human serum albumin (HSA) and ^{3}H-propranolol for α_1-acid glycoprotein (AGP) were used, respectively. Subsequently, displacement of ^{123}I-IMP serum protein binding with amino-acid fluid, PTA has been attempted in monkeys.

As a result of the *in-vitro* binding assay, since the free fraction rates of ^{14}C-diazepam and ^{3}H-propranolol loaded with amino-acid fluids were extremely elevated, amino-acid fluids were noted as suitable displacers of binding site II on HSA and also AGP. The free fraction of ^{123}I-IMP, bound to HSA site II and AGP [2,4], with PTA loading was significantly increased. Therefore, ^{123}I-IMP scintigraphies were performed in two monkeys with or without PTA. Rapid cerebral accumulation was observed with PTA loading. Plateaus of the time-activity curves were shifted within 15 min for PTA loaded monkey. The plateau levels of radioactivity in the brain were significantly increased up to 1.5 times compared with the control condition, while the relative changes of regional cerebral accumulation were not observed. It was suggested that the PTA treatment increased the free fraction of ^{123}I-IMP, and then brought rapid and high cerebral accumulation in *in-vivo*.

The displacement method could be easily applied to human study in the present condition. Thus, the displacement of radiopharmaceuticals binding to serum protein would provide better images of contrast as well as shortened imaging time and reduction of radiation dose for patients.

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Keywords: Serum Protein Binding, Cerebral Accumulation, Amino-Acid Fluids

BIODISTRIBUTION OF [^{227}Th]EDTMP: COMPARISON WITH [^{227}Th]CITRATE, [^{153}Sm]EDTMP, AND [^{223}Ra]CHLORIDE

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Objectives: Thorium-227 ($T_{1/2}$ = 18.7 d) is a promising α-emitting nuclide for radionuclide therapy. It has a suitable half life and emits α-particles with an average energy of 5.9 MeV decaying to ^{223}Ra ($T_{1/2}$ = 11.4 d). Successively the daughter nuclide ^{223}Ra emits 4 α-particles with energy of 26.6 MeV decaying to stable ^{207}Pb. The ^{227}Th-^{223}Ra series are a potential *in vivo* generator system for the treatment of skeletal metastases. Radium-223 has already been applied for phase 2 clinical trials for the treatment of bone metastases. In general, thorium has been known as a bone seeking element and are also accumulates in other soft tissues. In this work, in order to increase ^{227}Th uptakes in bone and decrease those in soft tissues, ^{227}Th was labelled with bone seeking chelating agent, ethylenediamine-tetra-methylenephosphonic acid (EDTMP). We examined the biodistribution of ^{227}Th-EDTMP over a period of 14 days in mice and compared it with the biodistribution of ^{227}Th-citrate, ^{153}Sm-EDTMP, and ^{223}Ra-chloride. Thoriumn-227-citrate is used as a typical cationic thorium, and ^{153}Sm-EDTMP is used understanding the biobehavior of an EDTMP compound. The biodistribution of ^{223}Ra-chloride was also studied to evaluate the dosimetoric efficacy of ^{227}Th-EDTMP.

Methods: Male 7-week-old ICR mice were administered 100 μL of ^{227}Th-EDTMP solution or ^{227}Th-citrate solution or ^{153}Sm-EDTMP solution or ^{223}Ra-chloride solution via tail vein. After several time points, mice were sacrificed and the femur, blood, liver, spleen, and kidney were excised and weighed. The uptake rates of ^{227}Th, ^{153}Sm, and ^{223}Ra were determined by γ-ray spectrometry.

Results and Discussion: ^{227}Th-EDTMP was found to show high uptake and long-term retention in bone. Bone uptake rates rapidly reached the maximum level and retained at a constant level throughout the 14-day experimental period. The clearance of ^{227}Th-EDTMP from blood and soft tissues were rapid. These biodistribution patterns were comparable to those of ^{153}Sm-EDTMP. On the other hand, ^{227}Th-EDTMP showed the large difference with ^{227}Th-citrate in the biodistribution in soft tissues. From the basis of the results, ^{227}Th-EDTMP would initially bind to bone by bridging of ^{227}Th to hydroxyapatite by EDTMP. Successive ^{223}Ra stays in the place and shows long-term retention in the bone. Their clearance was rapid from soft tissues. Dosimetric evaluation showed that the absorbed dose of ^{227}Th-EDTMP and ^{223}Ra-chloride in bone were comparable.

Conclusions: ^{227}Th-EDTMP showed selective accumulation and long-term retention in bone, with rapid clearance from soft tissues.

Keywords: alpha-Particle Emitter, Thorium-227, Bone Metastases

SURFACE MODIFICATION OF THE MAGNETITE NANOPARTICLE AND ITS APPLICATION IN RADIOIMMUNOTHERAPY

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The development of high-effectivity and high-specificity radiopharmaceuticals are of considerable interest in many areas of research, from molecular and cellular biology to molecular imaging and medical diagnostics/therapy. Magnetic nanoparticles offers great potential applications in a variety of biomedical field, such as retinal detachment therapy, cell separation, tumor hyperthermia, improved MRI diagnostic contrast agents and as magnetic field-guided carriers for localizing drugs or radioactive therapies. Magnetic nanoparticles have been covalently linked to biorecognition molecules such as peptides, antibodies, nucleic acids or small-molecule ligands for in vivo cancer targeting and imaging.The bioconjugated magnetic nanoparticle could be dually targeted both magnetically and biologically.

Rhenium-188 (β^-_{max}=2.12 MeV (79%), 1.96 Mev (20%); γ=155 keV (15%); therapeutic range=2.1 mm; $T_{1/2}$ =16.9 h) is an attractive therapeutic radioisotope which is produced from decay of the reactor-produced tungsten-188 parent ($T_{1/2}$=69 d) and thus conveniently obtained on demand by elution from the $^{188}W/^{188}Re$ generator system.

This paper reports a noval multifunctional bioconjugated magnetic nanoparticle labelled with Re-188 for tumor targeted radioimmunotherapy.

• Synthesis of magnetite nanoparticles and its surface coating with silica shell.

The magnetite nanoparticles was prepared by a partial reduction method, with ferric chloride as the iron source, ammonia as the alkaline source and sodium sulfite as the reducing agent.Its surface was then coated with silica shell by tetraethyl orthosilicate (TEOS) After 12 hours of stirring at 40° water bath. A small portion of the obtained precipitate was dried under vacuum and used for characterization.The size of the silica coated magnetite was about 25nm.

• conjugation of the magnetite nanoparticle with Herceptin

The silane-coupling agent, SG-Si900, was added to the methanol suspension of silica-coated magnetite nanoparticles until about a concentration of 20 wt% was achieved. After about 5 minutes ultrasonic bath, the mixture was refluxed with stirring for above 12 hours at 60°C.The reacted SG-Si900 was removed by several times washing with methanol. Herceptin, a humanized anti-p185-HER-2/neu monoclonal antibody directed against the extracellular domain of the HER-2/neu receptor, was coated to functional magnetic nanoparticles by the cross-linker with glutaraldehyde to prepare immuno-magnetic nanoparticles (IMN).The mean diameter of IMN was about 60nm.

• Labeling of the immuno-magnetite nanoparticle with ^{188}Re

The optimum labeling conditions include: $SnCl_2 \cdot 2H_2O$ 8 mg/ml, citric acid 20 mg/ml, vitamin C 8 mg/ml,reaction volume 500 ml and reaction time 3 h, with a

labeling efficiency of about 90%. The labeled particles are stable up to 72 h in BSA and the immunoactivity remaind up to 90% after the labeling of ^{188}Re.

• Conclusion

A novel bioconjugated magnetite nanoparticle was prepared and labeled with ^{188}Re with more than 90% labeling efficiency and over 90% immunoactivity remaind.The further study is needed to evaluate its feasibility as radioimmunotherapy drug targeted both magnetically and immunologically for malignant tumor.

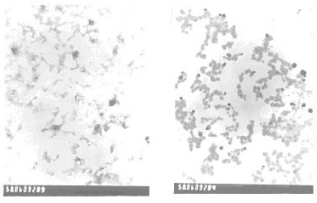

Fig.1 the TEM(50K amplified) picture of the immuno-magnetite nanoparticle (left) and histidine immobilized magnetite nanoparticle (right)

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Keywords: Rhenium-188, Magnetite Nanoparticle, Radioimmunotherapy

LABELING OF ANTI-CD20 WITH Re-188: THEORETICAL DOSIMETRY CONSIDERATIONS

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Therapies using ^{90}Y-anti-CD20 or ^{131}I-anti-CD20 have demonstrated their efficacy in patients with B-cell non-Hodgkin's lymphoma (NHL). Rhenium-188 is a radionuclide useful for radioimmunotherapy. The aim of this study was to develop a procedure for efficient labelling of anti-CD20 with ^{188}Re from lyophilised formulations to achieve high radiochemical yield, high specific activity and preservation of the molecular recognition after a simple kit reconstitution without further purification. The MIRD cellular theoretical dose for Re-188 was calculated and compared with that produced by Y-90 and I-131.

^{188}Re-anti-CD20 was prepared by a direct labelling method using sodium tartrate as weak competing ligand. Different lyophilised formulations were prepared to optimise tartrate and stannous chloride concentration, pH and reaction time. To evaluate the biological recognition a comparative study of the *in vitro* binding of ^{188}Re-anti-CD20, ^{125}I-anti-CD20 (positive control) and ^{188}Re-anti-CEA (negative control) to normal B lymphocytes was performed. Biodistribution studies in normal mice were accomplished to assess the *in vivo* ^{188}Re-anti-CD20 complex stability. To estimate the theoretical dose delivered to the nucleus from the cell-bound radiolabeled antibody, the cumulative disintegrations during a period of 7 d were calculated for Re-188, Y-90 and I-131. S values were obtained from the MIRD cellular data considering different cell sizes.

Rhenium-188 labelled anti-CD20 was obtained with high radiochemical purities (> 97 %) and high specific activity (0.5-0.7 GBq/mg) 1-1.5 h after addition of sodium perrhenate solution to a kit containing 4.4 μM anti-CD20, 4 mM anhydrous stannous chloride, and 140 mM dihydrate sodium tartrate at pH 4. The binding of ^{188}Re-anti-CD20 to cells was in the same range as ^{125}I-anti-CD20 (> 80 %) and was significantly different to cell binding of ^{188}Re-anti-CEA (< 10 %). No evidence of free rhenium-188 release was found at 2, 4 and 24 h after ^{188}Re-anti-CD20 administration in mice. Lyophilised kits showed high stability during the storage at 4°C for 6 months. Re-188 dose values were between that produced by Y-90 and I-131 in almost all sizes considered for lymphoma cells (Table 1)

Despite the advantages of rhenium-188 as a therapeutic radionuclide, it has been perceived by many to have a too short half-life for RIT because of the slow anti-CD20 tumour uptake. However RIT depends on several factors such as the type of antibody, the radionuclide and the target tumour and host. Lymphomas are very radiosensitive tumours and there is evidence that radiolabelled antibodies act as sensitizers in a combined modality therapy. The intention is not to kill the cell directly but to irradiate the environment of the tagged cell to produce the additive effect of beta-radiation-induced cytotoxicity and initiating signalling of apoptotic pathways within the cells.

Optimal reaction conditions were defined enabling high radiochemical purities of ^{188}Re-anti-CD20 to be obtained routinely and therefore potentially useful in the treatment of non-Hodgkin's lymphoma.

Table 1. Theoretical Absorbed dose per 1 Bq of labelled anti-CD20 bound to the cell surface during 7 d

Cell Dimensions (μm)		**Nucleus dose (Gy)**		
Whole cell	Nucleus	I-131	Y-90	Re-188
10	8	131	35.80	16.84
12	10	91.70	25.31	63.77
14	10	62.34	17.34	42.33
14	6	56.47	15.87	33.43
16	14	52.40	14.63	37.57
16	12	47.88	13.47	30.87
18	16	41.69	11.67	30.43
18	12	36.14	10.23	24.70
20	16	31.17	8.82	21.7
20	10	27.51	7.84	20.73

Keywords: Rhenium-188, Radioimmunotherapy NHL, Anti-CD20

MORPHOLINO OLIGOMERS CAN BE RADIOLABELED WITH ^{188}Re AT HIGH SPECIFIC ACTIVITY USING MAG_3

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A logical progression of tumor pretargeting with antibody-morpholinos (MORF, a DNA analogue oligomer) and radiolabeled complementary MORF is from diagnosis with ^{99m}Tc as label to radiotherapy with ^{188}Re as label. The replacement of ^{99m}Tc with ^{188}Re for therapeutic investigation is logical based on the chemical similarities and the availability of ^{188}W/^{188}Re generators. **OBJECTIVE:** To develop a simple method using MAG_3 as chelator and capable of providing a stable and quantitative ^{188}Re labeled MORF and, especially, with the high specific radioactivity required for therapeutic investigations. **METHODS**: A procedure developed in this laboratory for ^{99m}Tc labeling of MAG_3-coupled MORF was modified for ^{188}Re labeling by adjusting the pH, concentration of tin (II), concentration of MAG_3-MORF and the heating time in a boiling water bath. The stability of the radiolabled MORF against reoxidation was determined by storage after removal of the excess tin(II) on a size-exclusion P4 column while the stability in serum was tested by incubating in serum at 37 °C. The biodistribution of ^{188}Re labeled MORF was also determined in normal CD-1 mice and the results compared to that previous obtained in identical fashion with ^{99m}Tc labeled MORF. **RESULTS**: An optimized labeling procedure was as follows: 50 μL ^{188}Re perrhenate eluate was added to a mixture of 30 μL (> 0.5 μg) MAG_3-MORF solution in 0.25 M pH 5.2 NH_4AcO buffer, 10 μL tartrate solution (50 mg Na_2tartrate·$2H_2O$ /mL), and 20 mL of $SnCl_2.2H_2O$ (10 mg/mL in 20 mM HCl containing 1 mg/mL of sodium ascorbate) followed by heating at 100°C for 1 h. This procedure provides a labeling efficiency of over 90%. Furthermore, quantitative labeling was achieved at a concentration of MAG_3-MORF as low as $< 10^{-6}$ M such that the limit on specific radioactivity depended mainly on the specific radioactivity of the perrhenate generator eluate. The ^{188}Re labeled MORF was stable against both reoxidation and in 37 °C serum for at least 24 h. The biodistribution of ^{188}Re-MORF was almost identical to that of ^{99m}Tc-MORF. Most importantly, a specific radioactivity of ^{188}Re-MORF of at least 200 μCi/μg was achieved with an upper limit yet to be determined. **CONCLUSION**: Using MAG_3, MORF was radiolabeled rapidly with ^{188}Re and the label shown to be stable against reoxidation and stable in serum. The method is capable of providing specific radioactivity suitable for radiotherapy investigations.

Keywords: Radiolabeling, Radiotherapy, Immunotherapy, Rhenium-188

RADIOLABELING OF CATIONIC DEXTRAN WITH YTTRIUM-90

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Recently, ^{99m}Tc radiolabeled cationic agents such as cationic dextran and avidin have shown selective accumulation in superficial bladder tumors by intravesical instillation compared to normal tissue leading to the conclusions that these tumors apparently display cation-exchanging properties [1,2]. The effects were pronounced and charge dependent with high ratios between normal and tumour tissue. As a consequence of these findings, we are trying to develop ^{90}Y radiolabelled cationic dextran (Dx) derivative suitable for intravesical instillation therapy on this patient category. Herein, we report radiosyntheses of ^{90}Y-DTPA-Dx and in vitro investigation of this agent.

Dextran-40 was oxidized with sodium periodate at room temperature to yield reactive aldehyde groups. The activated dextran was subsequently reacted with lysine and 2-(p-NH2-Bz)-6-methyl-DTPA (1B4M-DTPA) at room temperature for 10 h. The conjugate was then stabilized by reducing Schiff bases with sodium cyanoborohydirde for another 2.0 h and purified with a Sephadex G25 column. The concentration of DTPA in final conjugate was determined by spectrophotometric method [3]. The molar ratio of DTPA/dextran in final DTPA-Dx-Lys conjugate was 2.5.

The DTPA-Dx-Lys conjugate was then radiolabeled with ^{90}Y in 0.4 M acetate buffer, pH 5.5 for 20 min at room temperature. The labeling yield was determined by ITLC-SG with EDTA 4 mM, pH 4.5 as solvent. The radiolabeled conjugate was purified on a Sephadex G25 column. The purified conjugate was analyzed by HPLC with a Superdex 75 column with an on-line radioactivity detection.

The conjugate ^{90}Y-DTPA-Dx-Lys was prepared in high yield (>98%). The radiochemical purity determination demonstrated the stability of the conjugate over a wide range of pH values over a time course of 24 h in saline and serum.

In summary, the cationic dextran was successfully labeled with ^{90}Y in high yield and stability. The studies to evaluate its biodistribution in animal tumor xenograft models are in progress.

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Keywords: Dextran, Radiolabelling, Yttrium-90

PREPARATION OF ^{188}Re-HEDP AND ITS PRE-CLINICAL STUDY

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Introduction

The initial goal was to develop a therapeutic agent that would localize in metastases and emit both a beta particle suitable for therapy and a gamma ray suitable for imaging and calculating dosimetry. 1-hydroxyethylidene diphosphonate (HEDP) is known to concentrate markedly in bone. Rhenium-188 with short physical half-life(16.9hrs) is an attractive radionuclide for therapy. Its maximum beta-energy is 2.1MeV with a 15% abundance of gamma ray emission (155KeV). In particular, ^{188}Re is available from an in-house generator system, which makes it very convenient for clinical use.

Experiments and results

Preparation of ^{188}Re-HEDP kit The influence of some factors on the labeling efficiency of ^{188}Re-HEDP had been systematically investigated. According to the optimal labeling conditions, the kit formulation was developed. The kit consists of ligand (HEDP), reductant($SnCl_2$), antioxidant(Vc), excipient and so on. After the HEDP kit had been reconstituted with the ^{188}Re-solution, the radiochemical purity of the ^{188}Re-HEDP injection was over 95 %.

Stabilities of the HEDP kit and ^{188}Re-HEDP injection. The influence of light, temperature and humidity on stability was studied. The results indicated that the kits were stable when it had been stored at room temperature for 3 months. When the kits were exposed to strong light, high humidity or at 40°, they were stable for ten days but not stable at 60°. It can be stable for 6 months at 2~8°. ^{188}Re-HEDP injection was stable at room temperature, strong light or high humidity. However it was unstable at 40° and 60°.

Study of the biodistribution of ^{188}Re-HEDP injection in animal. Kunming mice weighing 20±2 g each were used to determine the tissue biodistribution of ^{188}Re-HEDP. The mice were sacrificed at 1 h, 3h, 24h, 48h and 72 h (five mice at each time) after injection of approx. 214 MBq/kg ^{188}Re-HEDP in a volume of 0.2 mL via the tail vein.The results indicted the high uptake in the skeletal tissue and the quick clearance in the blood.

Study of the pharmacokinetics of ^{188}Re-HEDP injection. The mice were injected separately with 395,217,44MBq/kg 188Re-HEDP. The radioactivities of the blood samples collected at 0.5min, 5min, 10min, 0.25h, 0.5h, 1h, 2h, 3h, etc were measured. The results showed that the blood clearance curve corresponded to a two-phase distribution (Fig. 1).

Long-term toxicity examination of ^{188}Re-HEDP injection. Three dose of 74, 222 and 444 MBq/kg were injected through i.p. to SD rats and other three doses of 37, 74, 148 MBq/kg through i.v. to Beagle dogs once two weeks for a treatment course and it lasted for 4 courses. After the administration, these animals were observed for 27 days convalescence. At 7th and 27th day after the last injection, respectively, half of the animals in each group were killed and the hematological, biochemical and histological examinations were performed. The results show that the safe dose was lower than 333MBq/kg once for rats.The safe dose of one application is 74 MBq/kg for beagle dogs.

Conclusion

The above results suggest that ^{188}Re-HEDP is a very good candidate for the treatment of bone pain due to the high uptake in the skeletal tissue and the quick clearance from the blood.

Table 1 The biodistribution of ^{188}Re-HEDP in mice

ID%/g X±SD n=5

1h	3h		24h	48h	72h
Blood	0.91±0.51	0.25±0.04	0.17±0.10	0.06±0.04	0.09±0.08
Heart	0.23±0.09	0.09±0.02	0.05±0.01	0.05±0.04	0.07±0.05
Lung	0.56±0.18	0.29±0.07	0.16±0.05	0.08±0.04	0.22±0.11
Liver	0.34±0.11	0.23±0.06	0.17±0.02	0.19±0.09	0.10±0.09
Spleen	0.18±0.06	0.09±0.02	0.11±0.07	0.10±0.09	0.09±0.07
Kidney	2.41±0.90	1.64±0.49	0.98±0.11	0.81±0.16	0.47±0.12
Intestine	0.32±0.15	0.17±0.04	0.09±0.04	0.08±0.07	0.17±0.15
Bile	2.05±1.08	2.79±1.15	1.28±0.52	0	0
Muscle	0.22±0.18	0.23±0.19	0.03±0.01	0.03±0.01	0
Femur	29.39±5.72	31.22±7.53	32.40±6.36	28.56±4.96	26.05±4.02
Pancreas	0.20±0.08	0.09±0.02	0.04±0.02	0.05±0.04	0.03±0.03
Marrow	1.06±0.52	3.48±0.71	0.38±0.06	0	0
Spermary or ovary	0.29±0.21	0.13±0.04	0.06±0.02	0.08±0.06	0.07±0.05
Bladder	0.90±0.67	1.59±1.47	0.20±0.02	0.16±0.10	0.34±0.33

Acknowledgement: This study is supported by the Key project of the knowledge innovation program of Chinese Academy of Sciences(No.KJCXI-SW-08)

Keywords: Rhenium-188, HEDP, Bone Pain Palliation

IMPROVED METHOD FOR LABELING OF ANTI-CEA HUMANIZED CHIMERIC ANTIBODY RCH24 WITH ^{131}I

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Objective: To provide a effective radioactive agent for radioimmunoguided surgery, a improved method for ^{131}I labeling antibody was developed, which is of speediness, high efficiency, high specific activity and less loss of immunoreactivity.

Methods: N-BromoSuccinimine(NBS)was used for ^{131}I (^{125}I) labeling. The labeling yield from different conditions and the immunoreactivity from different specific activity were tested. In addition, the stability of ^{131}I-RCH24 was monitored under the conditions with and without protective agent HAS for 48 h period. The biodistribution and g imaging in nude mice with human colonic cancer LS174 T cells xenografts were performed, using ^{125}I-RCH24.

Results: The labeling yield was more than 90% (n=15) under the condition, 10-16mg NBS/mg RCH24, reacting 50-60 sec at room temperature, and the radiochemistry purity was more than 99% after PD-10 column purification. The immunoreactivity was more than 75% (n=3) when the specific activity reached 740 MBq ^{131}I/mg RCH24. The release of free ^{131}I from ^{131}I-RCH24 was less than 3% with presence of HSA during 24 h. SDS-PAGE showed single intact belt of ^{131}I-RCH24 during 0-6 h storage. HPLC revealed consistency of UV-peak and Radio-peak with 7.1-7.3 min retention time. The tumor uptake (ID/%) was 16.02±1.97, 21.05±4.38, 19.78±2.15 and 19.65±5.63, and the radioactive ratio of tumor/blood was 2.15±0.42, 3.55±0.79, 6.98±1.88, and 8.44±1.56 at 24 h, 48 h, 96 h and 168 h postinjection, respectively. The clear images of xenografted tumors were obtained during 24 h-168 h postinjection.

Conclusions: ^{131}I-RCH24 prepared in the improved method showed good stability and excellent tumor targeting, and seems to be promising for radioimmunoguided therapy of patients with colonic cancer.

Keywords: Monoclonal Antibody, Radiolabeled, Iodine-131