P409 A COMPACT MICROWAVE SYSTEM FOR RAPID, SEMI-AUTOMATED RADIOSYNTHESES

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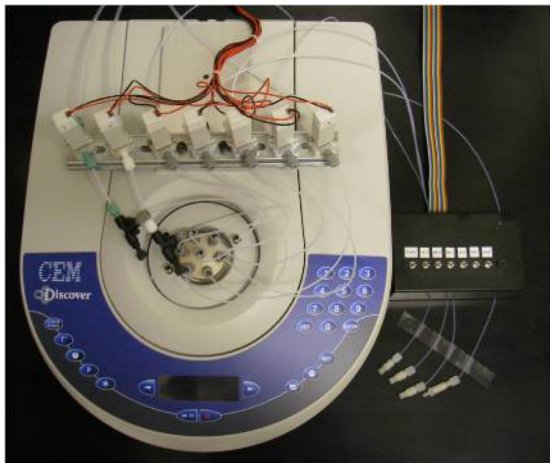
Objectives: Microwave irradiation has long been used to increase the rate of reactions, generate better, cleaner materials, and to help accelerate transformations that will not occur by any other means. Nowhere, however, does microwave irradiation have a larger potential impact than radiochemistry. Reduction of the reaction time by half or even less can serve to increase the yield of the final product significantly. Herein we describe the integration of a compact microwave system into a remote controlled radiochemical setup and the resulting impact on the overall synthetic process.

Methods: In the first part of this work, a CEM Discover microwave system was modified so that a 5 mL Wheaton V-vial, equipped with a magnetic stir bar and PEEK lid for tubing connection, could be placed into the irradiation cavity (see image). The remote controlled radiochemical setup consists of seven electrically switched solenoid valves. They were connected to the V-vial via a PEEK adapter through 1/16" Teflon tubing. Three valves were used for reagent delivery through similar tubing equipped with a Luer adapter. One port serves as a solution output, where the tubing is extended to the bottom of the vial. Another one permits venting of the vial. The final two ports are connected to pressurized nitrogen and a vacuum pump. Control of the CEM Discover system is accomplished using the Synergy software. In the second part of this work, the microwave system was miniaturized by decoupling the cavity from the microwave electronics and by shrinking the cavity to the minimum size needed for 5 mL vials.



Results: The radiosynthesis of N-succinimidyl-[^{18}F]fluorobenzoate was performed by a novel one-pot process (described in another contribution to this conference). Importantly, the aqueous [^{18}F]fluoride solution was dried in the microwave device. Furthermore, the use of microwave irradiation shortened the total time of reaction in the three steps from ca. 30 min to 4 min. The reaction vial can be cooled by a stream of compressed air. Stirring of the reaction mixture with a magnetic stir bar is possible. Final solution transfer is accomplished by pressurizing the vial with nitrogen and collecting the product from the output port. The miniaturized and decoupled microwave system took far less space than the original system. With a footprint just over a 10 cm square, it could easily fit inside a mini-cell, along with analytical equipment, fluid handling system, and/or additional reactors for multi-vessel microwave syntheses.

Conclusions: This system enables versatile one-pot chemical and especially radiochemical syntheses of any given complexity. Another step towards complete automation is the integration with standardized, standalone Automated Radiochemistry Platform (ARC-P) components (described in another contribution to this conference). The microwave reaction module could be operated in a standalone fashion via remote interface, or integrated with the Reagent Delivery and the Cartridge Purification Modules to perform syntheses by appropriate adaptation of the reaction protocols. Control of the combined system can be achieved via an integrated LabView control program.

P410 LABELING OF MAB PR81 WITH 177Lu TO PRODUCE A BIOLOGIC RADIOPHARMACEUTICAL FOR RADIOIMMUNOTHERAPY OF BREAST CANCER

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Objectives: In previous study we used PR81, labeled with ^{99m}Tc via HYNIC, in imaging of breast cancer in mouse model successfully as a scouting procedure. In this study we developed an efficient method for indirect labeling of PR81 with ^{177}Lu via DOTA as a chelator to produce a biologic radiopharmaceutical for radioimmunotherapy of human breast cancer. The quality control of new therapeutic radiopharmaceutical was also performed.

Methods: According to different mole ratio of DOTA/PR81, DOTA-NHS was added to PR81 solution and incubated for 2 h. We did plenty of experiments to determine the optimal conjugation condition of DOTA with PR81. ^{177}Lu was prepared by neutron bombarding of ^{176}Lu in the reactor. The $^{177}Lu_2O_3$ solution (10 mCi activity per 1 mg antibody) was added to DOTA-PR81 and incubated in water bath (37°C). The labeling efficiency was determined by ITLC. The amount of radiocolloids was measured by cellulose nitrate electrophoresis. In vitro stability of labeled product was determined at room temperature and in human serum by ITLC and gel filtration chromatography (FPLC) over 24 hr respectively. The integrity of labeled MAb was checked by means of SDS-PAGE. Biodistribution was studied in normal BALB/c mice at 4 and 24 hr post-injection. The immunoreactivity and the toxicity of the complex were tested on MCF7 cell line.

Results: The labeling efficiency was 86.2%±3.2, 30 minutes after reaction and radiocolloids was less than %2. In vitro stability was 82.6±3.6 and 73.4±6.7 at room temperature and in human serum over 24 h respectively. There was no significant Ab fragmentation due to labeling procedure. Biodistribution studies in normal BALB/c mice showed that there was no significant accumulation in any organ. The immunoreactivity of the complex was %73±%4.3. The complex in concentration of 1 nM and 2 nM killed 64%±3.5% and 84%±4.2% of the MCF7 cells respectively.

Conclusions: The results showed that one may consider the new complex as a potential radiopharmaceutical for treatment of human breast cancer which needs further investigations.

P411 SYNTHESIS OF NIMOTUZUMAB-DOTA RADIOCONJUGATES LABELLED WITH LUTETIUM-177 AND THEIR BIOLOGICAL BEHAVIOUR

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Objectives: Humanized anti-EGFR monoclonal antibody hR3 (Nimotuzumab, commercial name Theraloc) is beeing studied in many clinical trials for immunotherapy of various cancers. The aims of this study were to develop simple, fast and efficient methods for radiolabelling hR3-DOTA conjugates with ^{177}Lu for radioimmunotherapy, to test in vitro their relative immunoreactivity and their in vivo biodistribution and elimination in experimental animals.

Methods: Humanized mAb hR3 (CIM,Cuba) was conjugated to DOTA bifunctional ligand in water media (0.1 M phosphate buffer, pH=8.5) for 24 hours at 16 °C using DOTA-NHS or p-SCN-Bn-DOTA (Macrocyclics, Texas). The immunoconjugates were purified by size-exclusion chromatography on PD-10 (Amersham Biosciences) desalting column, analyzed using HPLC and labelled with ^{177}Lu produced in our laboratory. Radiochemical purity was determined by ITLC or HPLC with radio/UV detection. Human skin keratinocyte (HaCaT) and A431 cell lines were used for in vitro testing of immunoreactivity of the hR3-DOTA[^{177}Lu]Lu radioimmunoconjugates. Their biodistribution and elimination in vivo were studied in Wistar male rats.

Results: The stable hR3-[NH-DOTA]$_n$ and hR3-[p-(NH-CS-NH)-Bz-DOTA]$_n$ immunoconjugates (where n ~ 4-6) were synthesized. These conjugates were labeled with ^{177}Lu at pH=6-7 (42 °C) within 60 min with high efficiency and specific activity of ^{177}Lu of about 400 MBq/mg hR3. Radiochemical purity of the products was found to be > 95 % for a week after their preparation, when stored at 4 °C . Their affinity to EGF receptors was proved in vitro with HaCaT and A431 cell lines. Blood clearance and accumulation in a liver (Fig.1.) and excretion with urine and feces (Fig.2.) were significantly slower for the hR3-DOTA[^{177}Lu]Lu radioimmunoconjugate with a longer conjugation chain, -p-(NH-CS-NH)-Bz-.

Conclusions: The methods for synthesis of hR3-DOTA bioconjugates and their labeling with ^{177}Lu have been successfully developed. The promising results for further preclinical experiments with induced carcinomas (e.g. colorectal or pancreatic) have been received in vitro with HaCaT and A431 cell lines and in vivo with healthy experimental animals.

Research Support: The research was supported by the Grant No.1QS100480501 of the Academy of Sciences of the Czech Republic, the EUREKA Grant No.E08018 (the Czech Ministry of Education) and the IAEA Coordinated Research Project No. 13948.

Fig. 1. Time distribution of ^{177}Lu in whole blood and liver of rats after injection of DOTA-hR3 radioimmunoconjugates

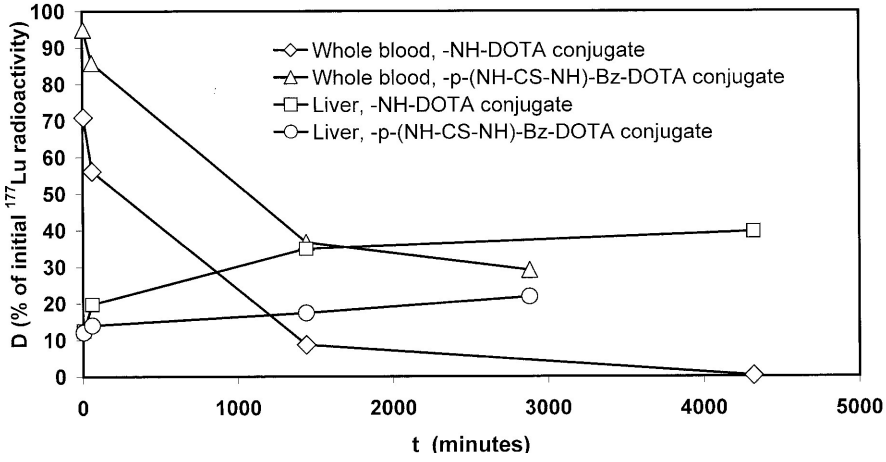
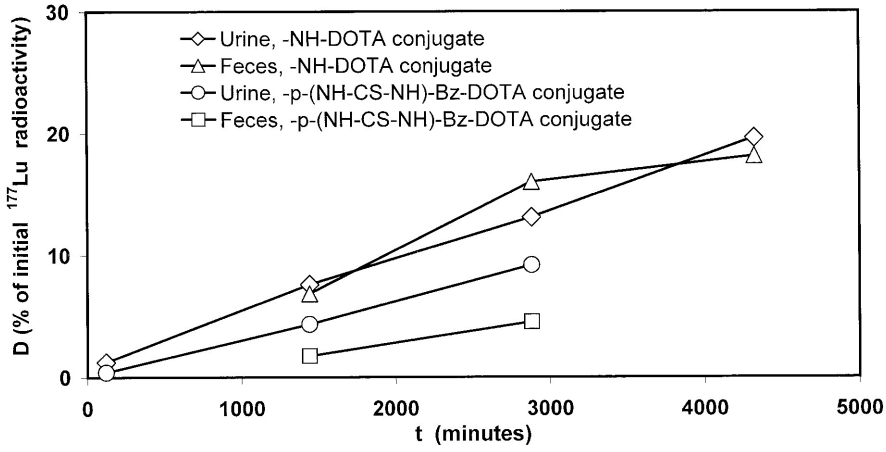


Fig. 2. Excretion of ^{177}Lu with urine and feces of rats after injection of labelled DOTA-hR3 radioimmunoconjugates



P412 SYNTHESIS AND BIOLOGICAL EVALUATION OF RHENIUM-188-LABELED RGD-PEPTIDES

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Objectives: Peptides with the sequence Arg-Gly-Asp (RGD) actively recognize and bind to $\alpha_v\beta_3$-integrins expressed on many tumor cells, leading to inhibition of the cells' invasiveness, dissemination and proliferation. Our aim was to provide a radiolabeled agent which could not only visualize $\alpha_v\beta_3$-integrin expression, but might also be useful for cancer therapy.

Methods: 900 ml of freshly prepared ^{188}Re tricarbonyl complex was added to 100 ml of 4 mg/ml of the linear peptide HGRGD(D) F (P6) or the cyclic peptide C(HCRGDFC) (P7) solution in a vial and incubated for 30 min at 70-80 °C under shaking at 1200 rpm. The final concentration of each peptide was 10^{-5} M. Both radiolabeled peptides were incubated with new-born calf serum or PBS at 37 °C and room temperature. Toxicity of the radiolabeled peptide to red blood cells was determined by a hemolysis test. The biodistributions of the two radiolabeled peptides were studied in groups of 5 male KM mice with osteosarcoma S180 tumor cells.

Results: The radiolabeling efficiencies of ^{188}Re-RGD were typically 93 to 95%. After 4 hours incubation in serum, there were still 91% and 89% present in peptide-bound form for^{188}Re-P6 and ^{188}Re-P7, respectively. No hemolysis of red blood cells was observed during in vitro tests. Biodistribution experiments indicated that the two labeled peptides cleared blood fast and were rapidly eliminated via the hepatobiliary and urinary system. ^{188}Re-P6 was cleared more slowly from most tissues and organs than ^{188}Re-P7. Autoradiography of S180-tumor-bearing mice taken after 2 h visualized the tumors very clearly against the normal tissue background. Tumor uptake of the radiolabeled RGD peptides seems to be specific, since it can be blocked by the injection of non-radioactive P7. This effect was shown in two additional S180-tumor-bearing mice after intravenously injecting 50 μg of P7 30 min before the administration of 20 MBq of ^{188}Re-P7 and imaging at 2 hours later. There was a clear absence of radioactivity accumulation in the tumor.

Conclusions: The two RGD-containing peptides were radiolabeled via fac-$[^{188}Re(CO)_3(H_2O)_3]^+$ with high radiolabeling efficiency(>90%). Initial biodistribution experiments showed that both peptides were selectively accumulated in the tumor, cleared rapidly from the blood compartment, and were rapidly eliminated via the hepatobiliary and urinary system. The radioactivity concentration in tumors was higher for ^{188}Re-P6 than for ^{188}Re-P7.

P413 PREPARATION AND BIODISTRIBUTION OF NEW 32P-CP-PLLA MICROPARTICLE

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Objectives: L-Polylactide (PLLA) is a good biocompatibility and biodegradable polymer. ^{32}P-chromic phosphate-L-polylactide (^{32}P-CP-PLLA) microparticle is a new control release preparation of the therapy radionuclide ^{32}P. The objective of the study is on preparation and biodistribution of ^{32}P-CP-PLLA.

Methods: ^{32}P-CP-PLLA microparticles were prepared by an SED process. Nude mice with 1.42±0.43 cm^3 pancreatic cancer xenograft were used to study the biodistribution of ^{32}P-CP-PLLA. The brain, heart, liver, spleen, kidney, lung, thyroid, muscle, bone and tumor were counted for radioactivity in the Liquid Scintillator at 1d, 3d, 7d ,14d and 28d post implantation. Six mice bearing tumor was also imaged by SKYLIGHT SPECT at 10min, 2h, 8h, 1d, 3d, 7d, 14d and 28d after implantation.

Results: The microparticle exhibits as a cylinder, the diameter length, height and mass were 0.85~0.9 mm, 2.2~2.5mm and 0.9~1.1mg, respectively. The radioactivity in the ^{32}P-CP-PLLA microparticles were restrained fully in the tumor(>98%). The microparticle disintegrated seldom at 28d.

Conclusions: The new microparticle is a successful dosage form. It could restrain diffusion of colloidal ^{32}P from tumor tissue to the other outer-tumor organs and deposit colloidal ^{32}P in the tumors for longer time.

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P414 177Lu-DOTA-CYCLOGASTRIN: STABILITY ASSESSMENT AND EVALUATION IN VITRO

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Objectives: Minigastrin analogs showing affinity to cholecystokinin subtype 2 receptors (CCK-2) are of increasing interest for the diagnosis and therapy of medullary thyroid carcinoma and other CCK-2 expressing tumors. Gastrin receptor scintigraphy has proven a higher tumor detection rate in comparison with somatostation receptor scintigraphy and FDG-PET (1). However, the radioligands delevoped so far either show a high kindney retention or a limited stability in vivo and are therefore suboptimal for therapeutic applications. We have therefore developed a cyclic DOTA-derivatized minigastrin analog and evaluated the stability and cell uptake after radiolabelling with Lu-177.

Methods: Reaction conditions for the labelling with Lu-177 where optimized varying peptide amount, buffer for pH adjustment, temperature and reaction time. For stability assessment ^{177}Lu-DOTA-cyclogastrin was incubated in human serum and blood, rat kidney and liver homogenates, and commercially available proteases from aspergillus oryzae and from bovine pancreas. Degradation was assessed by radio-HPLC. For metabolite characterization the non radiolabeled reference compound was incubated under the same conditions and the metabolites separated by UV detection. MALDI-TOF mass spectrometry allowed characterisation of the formed metabolites. Initial binding studies were performed on CCK-2 expressing AR4-2J rat pancreatic tumor cells.

Results: Radiolabelling under optimized conditions resulted in one single peak on radio-HPLC, with limited formation of oxidative side products. Degradation with halflives of 198 h in human serum and 39.2 h in blood resulted to be very low, but more pronounced in blood. In contrast, degradation in kidney and liver homogenates was very fast. After 30 min incubation in kidney homogenates the percentage of intact radioligand was <30%, whereas in liver homogenate still >80% intact radioligand was observed at the same time point. Degradation in protease solutions resulted in the formation of additional metabolites, which were not observed in the other incubation media. Results of MALDI TOF mass spectrometry showed formation of oxidative side products and cleavage at the C-terminal receptor specific tetrapeptide sequence during degradation. Initial uptake studies in AR4-2J cells showed receptor specific internalization.

Conclusions: ^{177}Lu-DOTA-cyclogastrin showed the highest stability in vitro comparing different developed radioligands. Complementary stability studies in blood and tissue homogenates enabled identification of metabolites. Characterisation of cleavage at the receptor specific peptide sequence during degradation suggests the need to explore alternative stabilisation strategies, taking into account the necessity to preserve receptor specificity.

Research Support: FFG-Project No. 815843/V0200-Z07-A, Cost BM0607 Reference: (1) Gotthardt, M et al. Eur. J. Nucl. Med. Mol. Imaging. 2006, 33, 1273-1279.

P415 PREPARATION AND BIODISTRIBUTION OF 153Sm-BLEOMYCIN COMPLEX FOR TUMOR THERAPY

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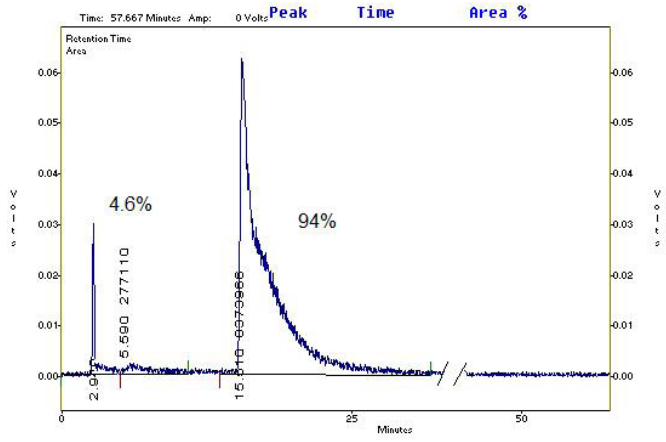
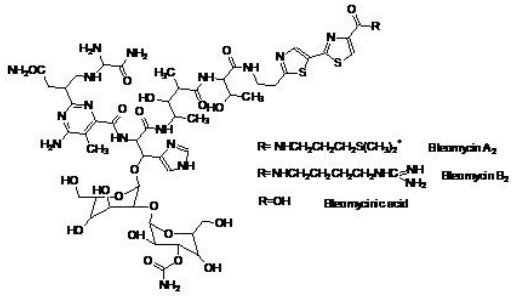
Objectives: Samarium-153 is one of the most important therapeutic nuclides due to low-energy beta emission, interesting for targeted therapy modalities especially in solid tumors. In this work, the DNA targetting antineoblastic agent, bleomycin (BLM) was labeled using $^{153}SmCl_3$ followed by quality control experiments.

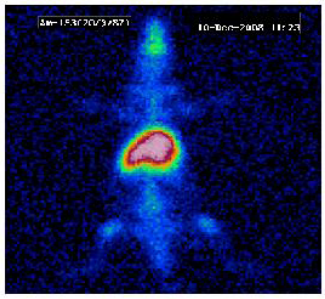
Methods: ^{153}Sm ($T_{1/2}$ = 46.7 h) was prepared by neutron irradiation of 98.7% enriched $^{152}Sm_2O_3$ (100 μg) at a thermal neutron flux 5×10^{13} n.cm^{-2}.s^{-1} for 5 days (specific activity ≈ 780 mCi/mg). The final evaporated activity was reacted with BLM, in normal saline at room temperature followed by radiochemical purity determination (HPLC), stability and biodistribution studies using scarification and SPECT imaging up to 48 hour.

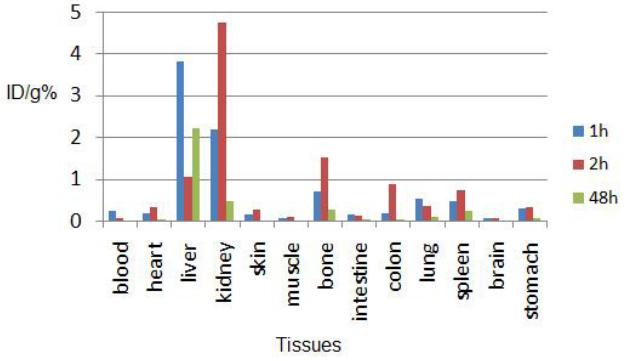
Results: ^{153}Sm-Bleomycin (^{153}Sm-BLM) was prepared with radiochemical purity over 94% shown by HPLC. ^{153}Sm-BLM excreted via urinary tract as well as entrohepatic pathway shown by biodistribution and co-incidence imaging studies.

Conclusions: ^{153}Sm-BLM is a potential therapeutic tumor targetting agent prepared in this work and further tumor accumulation studies are under invetigation on fibrosarcoma-bearing models to evaluate its efficacy.









P416 PREPARATION AND PRELIMINARY BIOEVALUATION OF 166Ho-OXINE-LIPIODOL: A POTENTIAL AGENT FOR THERAPY OF LIVER CANCER

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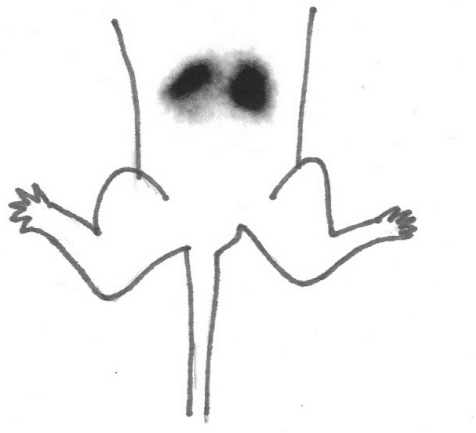
Objectives: Intra-arterial administration of β^- emitting radionuclides in the form of suitable radiopharmaceuticals is one of the promising modalities for the treatment of liver cancer. ^{166}Ho [$T_{½}$ = 26.9 h, $E_{\beta(max)}$ = 1.85 MeV, E_{γ} = 81 keV (6.4%)] could be considered as an attractive radionuclide for use in the therapy of liver cancer owing to its high energy β^- particle emission, short half-life and feasibility of its production with adequately high specific activity and radionuclidic purity using moderate flux reactors. On the other hand, lipiodol, an esterified lipid of poppy seed oil, could be envisaged as the vehicle to deliver localized dose of ionizing radiation to liver cancer cells following intra-arterial hepatic infusion since it is selectively retained in the vascular periphery of the proliferating cells. Therefore, an attempt has been made to prepare ^{166}Ho dispersed in lipiodol medium and study its biological behavior in animal model.

Methods: ^{166}Ho was produced by thermal neutron bombardment on natural Ho_2O_3 target (100% ^{165}Ho) at a flux of ~$6x10^{13}$ n/cm^2.s for 7 days. Since direct incorporation of ^{166}Ho in lipiodol is not feasible, dispersion of a lipophilic complex of ^{166}Ho was attempted. Toward this, 8-hydroxyquinoline (oxine) was chosen as the ligand, since it is known to form stable lipophilic complexes with lanthanides. ^{166}Ho-oxine complex was prepared by incubating an ethanolic solution of oxine with $^{166}HoCl_3$ in presence of ammonium acetate buffer at room temperature for 30 min. The complex thus obtained was subsequently dispersed in lipiodol by stirring at 50°C for 30 min. The in-vitro stability of ^{166}Ho-oxine-lipiodol was studied by incubating the preparation in normal saline as well as in human serum at 37°C. The biological behavior of ^{166}Ho-oxine-lipiodol was studied by biodistribution and scintigraphic imaging in normal Wistar rats following administration of the agent via hepatic artery.

Results: ^{166}Ho was produced with a specific activity of 9.25-11.10 TBq/g (250-300 Ci/g) and radionuclidic purity of ~100%. ^{166}Ho labeled oxine complex was prepared in high yield (~97%) under optimized reaction conditions. More than 95% of the ^{166}Ho activity could be dispersed in lipiodol within 30 min. The resulting radiolabeled preparation was found to exhibit good stability in normal saline and human serum upto 3 d at 37°C (95.5±2.3% and 84.7±3.9%, respectively). The biodistribution and imaging studies revealed satisfactory hepatic retention (88.43±2.85% of injected activity after 2 d) with insignificant uptake in any other major organ/tissue except skeleton (6.44±1.07% at 2 d post-injection). The whole-body image of a normal Wistar rat recorded after 48 h following the administration of ~74 MBq (2 mCi) of ^{166}Ho-oxine-lipiodol is shown below.

Conclusions: ^{166}Ho-oxine-lipiodol preparation exhibited promising features in preliminary studies and warrants further investigation.



P417 IN VITRO STUDIES TO ASSESS MODE OF CELL DEATH BY IODINE-131 THERAPEUTIC RADIOPHARMACEUTICAL USED IN NUCLEAR MEDICINE

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Objectives: ^{131}I is an important therapeutic isotope that has been incorporated into several cancer treatment strategies, including labeling of targeting antibodies for lymphomas and breast carcinomas. These radiopharmaceuticals are administered intravenously or orally in a dosage that may vary from 1.85-9.25 GBq for every patient. Apoptosis, or programmed cell death is highly desired for an effective therapy of tumors. Thus it is imperative to study the possible impact of exposure of radioiodine to assess the nature of cellular damage during therapy. The aim of this study was to assess the toxicity to cells in terms of apoptosis induced when exposed to ^{131}I using in vitro studies.

Methods: U937 histiocytic lymphoma and MCF-7 ER-positive breast carcinoma cell lines were used for this study. Toxicity to the cells were measured by parameters such as MTT assay and lactate dehydrogenase (LDH) released while apoptosis was observed by increase in caspase-3 activity and expression of anti-apoptotic gene such as bcl-2. The cells were cultured at 37°C with 5% CO_2 in RPMI and DMEM supplemented with 10% fetal bovine serum. 10^5 cells were incubated for 4h at 37°C with varying concentrations of NaI^{131} activity (3.7-185MBq). The supernatant after incubation with radioactivity was analyzed using a LDH assay kit. The colored product formed was proportional to the amount of LDH released and was measured in a spectrophotometer. Caspase-3 enzyme activity is a suitable marker, which increases when there is apoptosis. The cells which were exposed to I-131 were harvested after centrifugation, lysed in cell lysis buffer and caspase-3 activity was measured by colorimetric assay kit with Ac-DEVD-pNA (Acetyl-Asp-Glu-Val-Asp p-nitroaniline) as the substrate. In order to analyse the expression of anti-apoptotic gene Bcl-2 and Bcl_{xl} RNA was isolated from radiation treated cells as well as untreated cells using a column purification kit and reverse transcription PCR was carried out.

Results: Dose dependent cell toxicity ($p<0.001$, t-test) which is normally expected was observed from LDH released. It was observed that the caspase-3 activity increased by >30% in all cases, with a down-regulation of Bcl-2 gene in cells when cells were exposed to low dose (3.7MBq), whereas the cells exposed to higher dose (185MBq) did not exhibit down regulation of Bcl-2 gene to the same extent.

Conclusions: The above studies show that radioiodine induces apoptosis in cells exposed to low dose as observed by the down-regulation of bcl-2 and increased caspase-3 activity.

P418 PREPARATION AND PRELIMINARY BIOEVALUATION STUDIES OF 90Y-OXINE IN LIPIODOL FOR LIVER CANCER THERAPY

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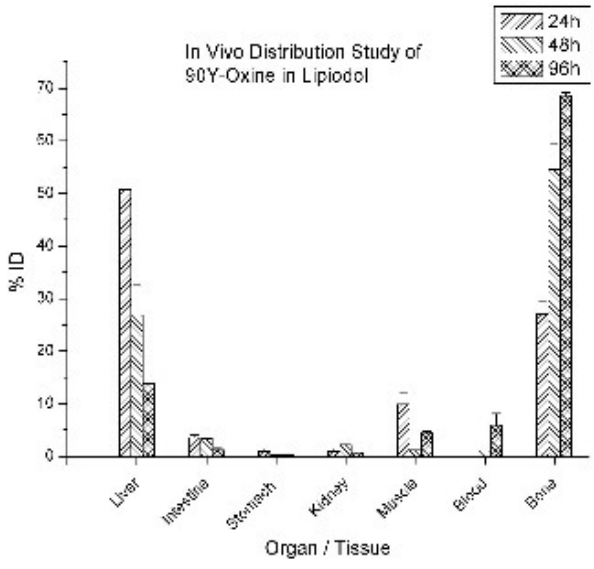
Objectives: Liver cancer is a common malignancy with a high associated mortality rate (≥5 year survival in less than 5% of cases). β^- emitters incorporated in lipophilic complexes or particulate preparations are being evaluated for their therapeutic efficacy in liver cancer, the objective being to have a radiopharmaceutical which can be retained in liver to deliver therapeutic dose with minimum collateral damage. ^{90}Y ($T_{1/2}$ = 64h, E_{max} = 2.28 MeV) is a pure beta emitter which, when combined with an appropriate lipophilic delivery agent, has good potential as a radiopharmaceutical for liver cancer therapy. In this study, we assess the potential of ^{90}Y–labeled oxine in lipiodol for use as a radiopharmaceutical in liver cancer therapy.

Methods: 2 mg oxine (8-hydroxyquinoline) was dissolved in minimum volume of ethanol. After removal of ethanol, 74-111 MBq of ^{90}Y-chloride was added to the vial containing oxine, and the pH adjusted to ~6.5-7.0. The reaction was allowed to proceed for 60min at 80-85°C. The labeled product was extracted into dichloromethane and its stability was assessed by TLC in $CHCl_3$:CH_3OH (90:10) as the mobile phase. The dichloromethane was purged off and the product ^{90}Y-oxine was extracted into lipiodol for use in animal studies. Retention of the labeled complex in lipiodol was tested by partition with saline. Preliminary in vivo evaluation studies were performed in rat model to assess retention of the labeled preparation in the liver. Liver cancer model was raised in wistar rats which were given diethylnitrosamine mixed in drinking water for ~90 days. For the experiment, the ^{90}Y-labeled preparation was administered into the liver of the animal via hepatic vein injection under anesthesia. In vivo retention of the preparation was monitored from biodistribution studies.

Results: Greater than 90% yield of radio-labeling was obtained under optimized conditions. The labeled product was stable at ambient temperature (22-25°C) for a week. More than 90% retention of the product in vitro in lipiodol was observed for this period of stability. The results of the biodistribution study are outlined in the figure. It was observed that that at 24h there is a decrease in the radioactivity retained in the liver with corresponding bone accumulation. At 96h, accumulation in the bone rises to ~68% of injected dose. Significant accumulation of ^{90}Y in bone with time could probably be due to the metabolic breakdown of the lipophilic product in the liver tissue.

Conclusions: While ^{90}Y is a promising isotope for liver cancer therapy, the retention and pharmacokinetic behavior of ^{90}Y-oxine in lipiodol when injected into the liver indicates that further work needs to be done to develop a suitable agent that would be retained sufficiently long in the liver to administer therapeutic dose and on biodegradation would be excreted without accumulation in skeleton.



P419 PRODUCTION AND DEPLOYMENT OF THERAPEUTIC RADIONUCLIDES: FEASIBILITY IN INDIAN SCENARIO

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Objectives: The interest in the in-vivo radiopharmaceuticals for therapy using a variety of particulate emitting radionuclides has been growing. The aim of this paper is to enumerate our experience in India in this area, with particular emphasis on the emerging nuclides.

Methods: A large number of beta emitting nuclides such as ^{32}P, $^{125/131}$I, ^{142}Pr, ^{153}Sm, ^{166}Ho, ^{170}Tm, ^{177}Lu, $^{186/188}$Re, etc. were produced in nuclear reactors and ^{90}Y was obtained from ^{90}Sr separated from the waste arising from processed spent nuclear fuel. Two novel generator techniques, namely one based on supported liquid membrane(SLM) and the other on electrochemical separation, were developed for separation of radiopharmaceutical grade ^{90}Y from ^{90}Sr. ^{90}Y and ^{177}Lu, owing to their attractive physical features, as well as production logistics were used to label molecules and tested as potential radiopharmaceuticals. ^{32}P was used to prepare mould brachytherapy sources for treatment of superficial cancers in a novel way and used for limited clinical trials in patients.

Results: ^{32}P, ^{131}I, ^{153}Sm were produced in large quantities regularly, due to the amenable production logistics. $^{142/143}$Pr, ^{170}Tm and ^{141}Ce with potential for therapy could also be produced in our reactors. Among the newer nuclides, ^{177}Lu could be easily produced by (n,γ) route owing to the large thermal cross-section(σ=2100b). For example, irradiation of >64% enriched ^{176}Lu irradiation for 21 days at $\Phi \sim 9*10^{13}$ n/s/cm^2 could give >850 MBq/mg ^{177}Lu which was suitable for receptor specific radiopharmaceuticals such as ^{177}Lu-DOTATATE. Reaction such as (n,p), (n,γ, followed by fission/ β^-/EC decay) could give NCA grade high specific activity products. e.g. ^{32}S(n,p)^{32}P; ^{130}Te(n,γ;β^-)^{131}I.; ^{124}Xe(n,γ;EC)^{125}I; ^{235}U(n,f) ^{90}Sr; The possible utility of the long lived nuclide ^{170}Tm in bone pain palliation could be demonstrated by animal studies, using ^{170}Tm-EDTMP. Both the novel generators for obtaining ^{90}Y, yielded radiopharmaceutical grade ^{90}Y, in high yields, high concentrations and in a form amenable for radiolabeling molecules. These user friendly systems were demonstarted at 3.7 GBq levels and have great potential for scaling up and automation. Further, a novel sensitive QC method based on extraction paper chromatography could be developed for real-time quick analysis of the ^{90}Y obtained from ^{90}Sr/^{90}Y generators and validated. This technique is capable of detecting ppm levels of ^{90}Sr in ^{90}Y. Due to the key role played by the IAEA in bringing the medical and scientific fraternities together, we could demonstrate of the use of ^{177}Lu-EDTMP as a bone pain palliating agent and take up ^{177}Lu-DOTATATE for pre-clinical studies in humans. ^{32}P mould therapy could be shown to be an excellent mode of therapy for basal cell cancers.

Conclusions: We could demonstrate that several important therapeutic radionuclides such as ^{32}P, $^{125/131}$I, ^{142}Pr, ^{153}Sm, ^{166}Ho, ^{170}Tm, ^{177}Lu, $^{186/188}$Re could be produced in medium flux reactors. Two types of ^{90}Sr/^{90}Y generators could be developed and a simple technique for QC of the ^{90}Y. Among these nuclides, ^{32}P, ^{170}Tm, ^{177}Lu and ^{90}Y are specially suitable for therapy in countries such as India owing to amenable logistics.

Research Support: This paper reviews the work carried out at the Radiopharmaceuticals Division, BARC in the area of therapeutic radionuclides and their use. The author expresses deep gratitude to all the colleagues of Radiopharmaceutiacls Division, BARC and the colleagues from Reactor Group and Fuel Reprocessing Division, BARC, without whose work, this paper could not have been possible. The author also is grateful to Director, Radiochemistry & Isotope Group for his support to the program and to the IAEA for enabling several aspects of this work through CRPs.

P420 IODINE-131 SALIVA SECRETION IN ABLATION TREATMENT FOR THYROID CANCER PATIENTS

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Objectives: The high magnitude of ^{131}I activities administered for well-differentiated thyroid cancer treatment can lead to side effects. Among nauseas, thyroiditis, hipothyroidism, sialadenitis is a very common side effect observed after ^{131}I administration for treatment. In the absence of thyroid tissue, secondary tissues – iodide specific uptake, such as salivary glands cells, rise at the element body retention process. In addition, among nuclear medicine professionals, there is no consensus about suitable restrictions as function of time, that must be observed by the released patient to reduce^{131}I contamination by saliva. This situation constitutes a special radiation protection concern in cases involving children as patients or as close relatives of the treated patients. The aim of this study is to evaluate the curve of ^{131}I secreted by salivary glands as a function of time after the administration of the radionuclide to thyroid cancer patients for tissue ablation purposes.

Methods: Well-differentiated thyroid cancer patients from University Hospital Clementino Fraga Filho (HUCFF) of Federal University of Rio de Janeiro (UFRJ) followed-up in the present study are female, adult, non smokers, non alcoholic, without additional detected health diseases. Along the first 48-72 h following ^{131}I administration for ablation purposes, saliva samples were collected systematically, sealed and counting rate was assessed using a NaI(Tl) scintillator detector.

Results: Analysis of the graphs shows two distinct behavior patterns between oral administration of $Na^{131}I$ in solution and in capsules. In the pattern observed in samples collected from patients that received $Na^{131}I$ solution, the maximum counting rates values were obtained from 14 to 16 hours after the radiopharmaceutical administration; while for patients that received $Na^{131}I$ in capsules, maximum counting rates values were observed previously, from 4 to 6 hours after ablation dose administration. After reaching maximum counting rates values, saliva samples concentration of iodine levels fell rapidly, but did not reach basal levels within the period of follow-up.

Conclusions: As the study is at an early stage, the preliminary results suggest the possibility of conducting an evaluation of ^{131}I secretion in saliva using the proposed protocol. Factors like the use of lemon juice could influence the behavior of ^{131}I secretion in saliva. The use of $Na^{131}I$ in solution or in capsules is another potential factor. In this study, it was observed two standards that can be defined according to these variables of radioiodine administration.It is necessary to continue this study following more patients and associating the results with possible factors of disturbance of ^{131}I secretion in saliva.

P421 EVALUATION OF GUM ARABIC STABILIZED GOLD-198 NANOPARTICLES IN PROSTATE CANCER BEARING MICE

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Objectives: To evaluate an alternative to treating prostate cancer that utilizes radioactive Au-198 nanoparticles (^{198}AuNP) which can deliver a higher payload of therapeutic dose per unit. These nanoparticles are rapidly produced using tris-hydroxymethyl phosphine alanine (THPAL), a non-toxic reducing agent derived from an amino acid. The nuclear properties of ^{198}Au are suitable for radiotherapeutic applications ($t_{1/2}$ = 2.7 days, E_{bmax} = 1.37 MeV). SPECT can be used to track the 411 keV (95%) gamma emitted from the ^{198}AuNP. Au-197 has high nuclear cross sections achieving reasonable specific activity.

Methods: ^{198}Au was produced at MURR and provided as $H^{198}AuCl_4$ in a final concentration of 0.05 M HCl. Gum Arabic was dissolved in Milli-Q water, heated to 90°C, and $H^{198}AuCl_4$ and carrier $HAuCl_4$ (0.1 M Au) was added immediately followed by THPAL. The solution was allowed to stir at room temperature for 30 min to generate the ^{198}Au nanoparticles. UV-Visible spectrophotometry showed the expected Plasmon peak at 546 nm. We used SCID mice bearing a flank model of human prostate cancer derived from a subcutaneous implant of 10 million PC-3 cells for pharmacokinetic studies. For the therapy study, unilateral solid tumors were allowed to grow 3 weeks, and animals were randomized (denoted Day 0) into control and treatment groups (n=7). On Day 8, 30 μL of gum Arabic ^{198}AuNP(408 μCi) was injected directly into the tumor to deliver an estimated 70 Gy. Control animals received 30 μL Dulbecco's PBS. Tumors were measured twice each week.

Results: Gum Arabic ^{198}AuNP were evaluated by intravenous (IV), intraperitoneal (IP), and intratumoral (IT) injections. High liver uptake was observed from IV and IP injections with 65.2 + 2.30% ID/g and 8.9 + 3.3% ID/g 24 h p.i. with less than 5% ID/g in the tumors. IT data showed promising results as little to no leakage of ^{198}AuNP was observed. After 24 h, 87.0 + 16.9% ID/g remained in the tumors. These results allowed for further evaluation of ^{198}AuNP for radiotherapy in PC-3 (human prostate) bearing mice. By Day 17, tumors in the control group were 2-fold larger than in the treatment group ($p = 0.005$). By day 28 , tumors in the control group were 5-fold larger than those of the treatment group ($p = 0.005$). End of study biodistribution data on Day 31 showed that 52.3 ±10.5% of the ID remained in the residual whole tumor samples.

Conclusions: Using gum Arabic ^{198}AuNP to treat prostate cancer can become an alternative to current treatment options. Since very little leakage is detected, using ^{198}AuNPs is beneficial in that a majority of the dose is retained in the tumors. Translation of this methodology to ^{199}Au nanoparticles will take advantage of the lower gamma emission (159 keV) and its higher specific activity.

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P422 PRECLINICAL COMPARATIVE STUDY OF 177LU-LABELED BOMBESIN ANALOGUES FOR HUMAN PROSTATE TUMOR DIAGNOSIS AND TREATMENT

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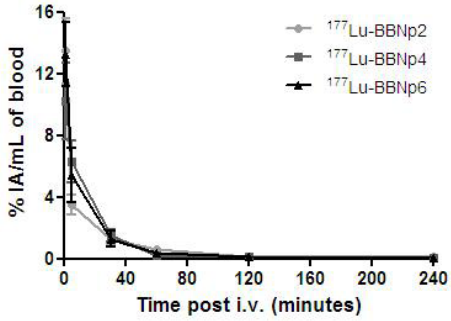
Objectives: Prostate cancer (PC) is one of the most frequently diagnosed cancer in men in the world. Treatment options have varied, but once the tumor has metastasized, treatment become less effective and the cancer can progresses to a hormone refractory state characterized by high morbidity and mortality. Bombesin (BBN) receptors – in particular, the GRP receptor – have been shown to be overexpressed in PC and could be an alternative as target for its diagnosis and treatment by radionuclide therapy. A large number of BBN analogues had already been synthesized for this purpose but most of the studied analogues exhibit high abdominal accumulation, especially in pancreas. This abdominal accumulation may represent a problem in clinical use of radiolabeled bombesin analogues due to serious side effects to patients. Herein we describe the results of radiolabeling with lutetium-177 (^{177}Lu), stability and in vivo studies in normal and prostate tumor bearing mice of new bombesin analogues, DOTA-X-BBN(6-14), where X is a spacer of two (BBNp2), four (BBNp4) or six amino acids (BBNp6). The spacers were inserted to improve bombesin in vivo properties and to reduce its uptake in non-tumor sites.

Methods: Preliminary studies were done to determine the best labeling conditions of bombesin analogues and both ITLC and HPLC were applied to evaluate the radiochemical purity of the preparations. The stability of the radiolabeled peptides was assayed either after storing at 4º C or incubation in human serum at 37º C. Partition coefficient of labeled BBN analogs were determined. Finally, biodistribution, pharmacokinetics and single photon emission tomography studies were performed at different times post intravenous administration in normal Balb-c and Nude mice bearing PC-3 human prostate tumor cells xenografts.

Results: The bombesin analogues were successfully labeled with high yield (> 98%) after reacting with 92.5 MBq of $^{177}LuCl_3$ at 90° C for 30 minutes and the mixtures kept stable for more than 96 hours at 4ºC and 1 hour in human serum. The peptides exhibit low lipophilicity, in according to their partition coefficient. In vivo studies showed that the fast blood clearance in the first 60 min p.i., indicating rapid excretion, which is performed mainly by renal pathway.



In addition, biodistribution studies showed low abdominal accumulation and significative tumor uptake of ^{177}Lu-labeled derivatives, especially of ^{177}Lu-BBNp6.

PC-3 tumor uptake of radiolabeled bombesin derivatives different times post intravenous administration.

Bombesin analogue	1 hour	4 hours	24 hours
^{177}Lu-BBNp2	0.32±0.03	0.2±0.01	0.05±0.01
^{177}Lu-BBNp4	0.37±0.06	0.17±0.04	0.04±0.01
^{177}Lu-BBNp6	0.88±0.11	0.09±0.02	0.03±0.01

Moreover, scintigraphic images showed that the studied analogues can also be a tool in PC diagnosis.

Conclusions: Our results showed that these new ^{177}Lu-labeled bombesin derivatives are potential candidates for prostate tumor imaging and treatment.

Research Support: CNPq and IPEN/CNEN.

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P423 TARGETING TUMOR ANGIOGENESIS USING 90Y-DOTA-MALEIMIDE-CYCLIC (RGDfC)

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Objectives: Targeting of tumor angiogenesis-related receptors is a promising approach to advancing a cancer diagnosis and treatment. $\alpha_v\beta_3$ integrin is a receptor that mediatesa cellular interaction with an extracellular matrix and plays a crucial role in a tumor growth, metastasis and angiogenesis. Radiolabeled cyclic-RGD peptides can be applicable for a non-invasive tumor imaging of a αvβ3 integrin expression and a targeted therapy. In this study, we prepared a new DOTA conjugated cyclic-RGD peptide, DOTA-Maleimido-cyclic(RGDfC), and radiolabeled with Y-90 for therapy through the targeting of a tumor $\alpha_v\beta_3$ integrin expression. Furthemre, biodistribution study was performed by using tumor bearing mouse.

Methods: For the synthesis of DOTA-Maleimido-cyclic(RGDfC), cyclic(RGDfC) was synthesized by applying standard Fmoc strategy. After cleavage of cyclic((RGDfC) from the resin, DOTA-maleimide was conjugated and purified using RP-HPLC to form a stable thioether compound, DOTA-Maleimido-cyclic(RGDfC). For the radiolabeling, the solution vial was prepared containing 10mg of DOTA-Maleimido-cyclic(RGDfC) in 0.5M Sodium Acetate buffer (pH 5.5). After injecting radioisotope Y-90(185 MBq) to the vial, the vial was heated for 20min at 90°C and cooled in ice bath. HPLC analysis was performed using the gradient system using 0.1% TFA in water,0.1% TFA in ACN. Biodistribution studies were performed using female balb/C nude mice implanted with Calu6 human lung cancer cells, subcutaneously.The mice were used for in vivo biodistribution studies 2 weeks post inoculation of tumor cells, when tumors reached a weight of approximately 0.2g. ^{90}Y-DOTA and 90Y-DOTA-maleimido-cyclic(RGDfC)(0.111MBq) were injected intravenously into the tumor bearing mice, respectively. The mice were sacrificed at 2, 24h (n=5) after injection and extracted tissues. The radiactivities were determined using liquid scintillation counter (Perkin Elmer).

Results: We synthesized a DOTA-conjugated RGD derivative and radiolabeled with Y-90 with a high labeling yield(>98%). ^{90}Y-DOTA Maleimido-cyclic(RGDfC) revealed a fast renal clearance in normal mice and specific uptake in athymic mice. Tumor uptake was 1.48±0.24 %ID/g(2 h post injection) and 0.80±0.08 %ID/g(24 h postinjection) and the blood-to-tumor ratio was 14.47 and 13.56, respectively.

Conclusions: We prepared a new Y-90 labeled cyclic-RGD peptide which revealed specific uptake in Calu6 human lung cancer cells xenografted in athymic mice. The biodistribution of ^{90}Y-DOTA-Maleimido-cyclic(RGDfC) shows the potential for the targeted therapy of tumor. Furthermore, the prepared RGD peptide is agood candidate for a noninvasive evaluation of $\alpha_v\beta_3$ integrin expression when apply the diagnostic radioisotopes including In-111, Ga-68 and Cu-64.

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