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Labeling of biotin with [^{166}Dy]Dy/^{166}Ho as a stable in vivo generator system

G. Ferro-Flores [a,*], C. Arteaga de Murphy [b], M. Pedraza-López [b], F. Monroy-Guzmán [a], L. Meléndez-Alafort [c], J.I. Tendilla [a], R. Jiménez-Varela [a]

[a] *Departamento de Materiales Radiactivos, Instituto Nacional de Investigaciones Nucleares, Km. 36.5 Carretera México-Toluca, Ocoyoacac, Estado de México, C.P. 52045, Mexico*
[b] *Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Vasco de Quiroga 15, México, DF C.P. 14000, Mexico*
[c] *Universidad Autónoma del Estado de México, Paseo Tollocan s/n esquina con Jesus Carranza, Colonia Moderna de la Cruz, Toluca Estado de México, Mexico*

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Abstract

The aim of this work was to synthesize [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin to evaluate its potential as a new radiopharmaceutical for targeted radiotherapy. Dysprosium-166 (^{166}Dy) was obtained by neutron irradiation of enriched $^{164}Dy_2O_3$ in a Triga Mark III reactor. The labeling was carried out in aqueous media at pH 8.0 by addition of [^{166}Dy]$DyCl_3$ to diethylenetriaminepentaacetic-α,ω-bis(biocytinamide) (DTPA-Biotin). Radiochemical purity was determined by high-performance liquid chromatography (HPLC) and TLC. The biological integrity of labeled biotin was studied evaluating its avidity for avidin in an agarose column and by size-exclusion HPLC analysis of the radiolabeled DTPA-Biotin with and without the addition of avidin. Stability studies against dilution were carried out by diluting the radiocomplex solution with saline solution and with human serum at 37 °C for 24 h. The [^{166}Dy]Dy/^{166}Ho-labeled biotin was obtained with a 99.1 ± 0.6% radiochemical purity. In vitro studies demonstrated that [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin is stable after dilution in saline and in human serum and no translocation of the daughter nucleus occurs subsequent to β^- decay of ^{166}Dy that could produce release of $^{166}Ho^{3+}$. Avidity of labeled biotin for avidin was not affected by the labeling procedure. Biodistribution studies in normal mice showed that the [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin has a high renal clearance. In conclusion, the radiolabeled biotin prepared in this investigation has adequate properties to work as a stable in vivo generator system for targeted radiotherapy.

Keywords: Radiolabeled biotin; Targeted radiotherapy; Holmium-166; Dysprosium-166 [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin; In vivo generator

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1. Introduction

In nuclear medicine, a variety of therapeutic radiopharmaceuticals or targeted radiotherapy systems have been introduced for the internal therapy of malignant and inflammatory lesions. Therapeutic radiopharmaceuticals are radiolabeled molecules designed as pharmaceutical forms to deliver therapeutic doses of ionizing radiation to specific disease sites. Advances in the molecular biology, combinatorial chemistry, immunotherapy, and peptide biochemistry have provided novel molecular targeting vectors (Ferro-Flores et al., 2001; Volkert and Hoffman, 1999).

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[*] Corresponding author. Tel.: +52-55-53-29-73-06; fax: +52-55-53-29-73-06.
E-mail address: gff@nuclear.inin.mx (G. Ferro-Flores).

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The selection of the radionuclide and the chemical strategies used for radiolabeling of molecules are critical elements in the formulation of safe and effective therapeutic radiopharmaceuticals. However, developing effective targeted radiotherapy systems is a complex problem which is not simply accomplished by attaching a radionuclide to a non-radiolabeled targeting vector. Several aspects have to be taken into account during their design such as the interference of radiometal chelate with binding specificity and affinity of the biomolecule to target cells, maximizing the residence time of radioactivity at targeted sites, in vivo catabolism and metabolism of the radiopharmaceutical and, optimization of the radiomolecule clearance from non-target sites (Volkert and Hoffman, 1999).

The concept of the in vivo generator system represents a potential alternative to maximize exposure of target tissues. This strategy is based on the initial chemical separation of the daughter radionuclide from the parent radionuclide; the parent is then attached to a tissue-specific therapeutic agent or vector. Although the ingrowth of the daughter is progressing, if the time required for targeted tissue localization is significantly less than the time required to reach equilibrium conditions, the radiation dose to non-target tissues is minimal. After tissue target localization and production of the desired daughter radioisotope by decay, the target tissue would be exposed to a maximum radiation dose since the daughter should decay with emission of energetic β particles (Lambrecht et al., 1997; Smith et al., 1995; Knapp and Mirzadeh, 1994). Dysprosium-166 (^{166}Dy, $T_{1/2} = 81.5\,\text{h}$, $E^{\text{av}}_{\beta^-} = 130\,\text{keV}$) can be produced by neutron irradiation of ^{164}Dy and decays to Holmium-166 (^{166}Ho, $T_{1/2} = 26.6\,\text{h}$, $E^{\text{av}}_{\beta^-} = 665.7\,\text{keV}$) as the daughter radionuclide. Because of its nuclear properties, the ^{166}Dy/^{166}Ho radionuclide pair can be considered as an in vivo generator system.

Biotin, ^{1}H-thieno[3,4-*d*]imidazole-4-pentanoic acid, hexahydro-2-oxo-, (3a*S*,4*S*,6a*R*)-(9Cl), is a 244 Da vitamin found in low concentration in blood and tissue (Vitamin H). Avidin is a tetrameric protein, and each subunit binds one molecule of biotin. In radioimmunodiagnosis and radioimmunotherapy practice, the pretargeting avidin–biotin strategy has shown that target to non-target radioactivity ratios can be significantly improved (Grana et al., 2002; Breitz et al., 2000; Knox et al., 2000; Cremonesi et al., 1999). In addition, the biotin content of cancerous tumors is higher than that of normal tissue and it has been found in the cell nucleus due to a specific transfer of biotin to histones by human serum biotinidase (Hymes and Wolf, 1999).

Our group is developing reagents that are optimized for use in the pretargeting approach to cancer therapy. As part of radiopharmaceutical development, we have focused on an effort for determining which design factors make biotin conjugates optimal for in vivo use. Recently, we synthesized ^{153}Sm-DTPA-Biotin to evaluate its potential in antibody pretargeting strategies for radioimmunotherapy (Correa-González et al., 2003; Ferro-Flores et al., 1999). The samarium complex was highly stable in human serum due to the fact that the nitrogen of the amide bond susceptible to hydrolysis by enzyme biotinidase, was coordinated to Sm(III) producing a protective effect.

On the basis that Sm, Ho, and Dy are lanthanides, and therefore with similar chemical properties, the aim of this work was to examine the feasibility of labeling diethylenetriaminepentaacetic-α,ω-bis(biocytinamide) (DTPA-Biotin) with ^{166}Dy/^{166}Ho and to evaluate whether or not the in vitro and in vivo stability of ^{166}Dy-DTPA-Biotin and ^{166}Ho-DTPA-Biotin complexes is maintained when the daughter ^{166}Ho is formed.

2. Materials and methods

2.1. Production of Dysprosium-166/Holmium-166 chloride solution

^{166}Dy was produced by double neutron capture of the highly enriched ^{164}Dy$_2$O$_3$ (^{164}Dy, 99%, from Oak Ridge, NL). Irradiations were performed at the central thimble of a Triga Mark III reactor (Instituto Nacional de Investigaciones Nucleares, ININ, México) at a neutron flux of 3×10^{13} N/s/cm^2. Typically, 50 mg of ^{164}Dy$_2$O$_3$ was irradiated for 20 h. Following irradiation, the target was allowed to decay for 2 days, then 100 μl of 12N HCl was added and the mixture stirred for 3 min. To this solution 500 μl of injectable water was added and heated for 2 min at 90 °C. During the irradiation and decay time, an activity of approximately 111 MBq of Holmiun-166 (^{166}Ho) was produced by β^- decay of the parent isotope ^{166}Dy. The average

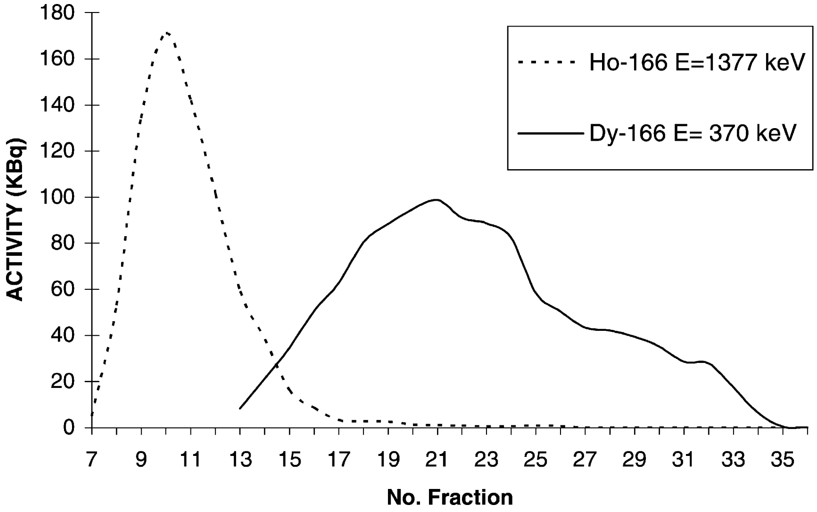

Fig. 1. Typical chromatographic profile of the [^{166}Dy]Dy/^{166}Ho separation using a 200–400 mesh AG50W-X8 ion-exchange resin and 0.2 M α-HIBA as mobile phase.

radioactive concentration of the [^{166}Dy]Dy/^{166}Ho chloride solution was ∼444 MBq/ml.

2.2. Separation of [^{166}Dy]Dy from ^{166}Ho

The [^{166}Dy]Dy/^{166}Ho separation was performed using a 0.8 cm × 20 cm glass column packed with 200–400 mesh AG50W-X8 ion exchange resin. The column was pre-equilibrated and eluted with 0.2 M α-hydroxyisobutyric acid (α-HIBA, Aldrich Chemical Co.) at pH 4.2. Fractions of 5 ml were collected and analyzed by γ-spectrometry using a HPGe detector (Canberra). The 1377 keV γ-rays of ^{166}Ho and 370 keV γ-rays of ^{166}Dy were used for detection. Fig. 1 illustrates a typical chromatographic profile of the Dy–Ho separation. Solutions of [^{166}Dy]Dy were freed from α-HIBA after separation by addition of 1 M HCl to pH 2 and loaded again on the column eluting with 1 M HCl. In this paper, we refer to the radiolabeled biotin complex as [^{166}Dy]Dy/^{166}Ho since immediately after Dy–Ho separation, the ingrowth of the ^{166}Ho is progressing to the equilibrium level.

2.3. Preparation of radiolabeled—DTPA-Biotin complex

The biotin used in this investigation is DTPA-Biotin (Sigma Chemical Co.). Sterile and apyrogenic V-vial was prepared to contain 12.0 mg (11.4 μmol) of the DTPA-Biotin compound in 3.0 ml of 0.1 M bicarbonate buffer (pH 8.0), then 50 μl (∼2 μmol, Dy) of [^{166}Dy]DyCl$_3$ solution were added to the preparation. The mixture was reacted for 30 min at room temperature. Finally, for animal administration, the labeled biotin was sterilized by passage through a 0.22 μm membrane filter (Gelman Sciences Co.).

2.4. Radiochemical and chemical quality control

2.4.1. HPLC chromatography

Quality control of the labeled [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin was performed by reverse phase high-performance liquid chromatography (HPLC) analysis employing a C-18 column (ODS, YMC, Inc.) with a gradient from 100 to 30%, water and 0 to 70% acetonitrile in 20 min (flow rate 1.0 ml/min) and with in line radioactivity and UV detection.

2.4.2. TLC chromatography

For thin layer chromatography aluminum cellulose sheets (Merck) were utilized as the stationary phase and a ternary mixture of methanol:water:ammonium hydroxide (20:40:2) as the mobile phase. The procedure involved spotting a 5–10 μl sample of the radiopharmaceutical onto a chromatography strip 8 cm in length. [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin traveled with the solvent front $R_f = 0.9–1.0$ and the Dy^{3+}/Ho^{3+} species remained at the origin ($R_f = 0$). Strips were cut into eight pieces and the radioactivity of each 1 cm fraction was measured with a HPGe detector or NaI(Tl) gamma counter detector.

2.5. In vitro stability of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin complex

2.5.1. Stability to dilution in 0.9% NaCl

Stability studies against dilution were carried out by diluting the [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin complex solution from 2- to 100-fold with saline solution at room temperature. After 1 and 24 h the radiochemical purity of the [^{166}Dy]Dy/^{166}Ho complex solution was determined by TLC or reverse phase HPLC as mentioned earlier.

2.5.2. Stability in human serum

Size-exclusion HPLC analysis was used to estimate the stability of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin preparation toward incubation at 37 °C for 24 h in fresh human serum. The radiolabeled biotin was analyzed by size-exclusion HPLC using a ProteinPak 125 gel filtration column (Waters) at a flow rate 1.0 ml/min with 0.1 M phosphate pH 7.4 as eluant and with on line radioactivity and UV detection. In this system [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin shows 12.4 ± 0.2 min as retention time. The labeled biotin was added to serum at 37 °C with the concentration at approximately 100 μg/ml. After 1 h the sample was also analyzed by reverse phase HPLC in order to determine if [^{166}Dy]Dy/^{166}Ho-DTPA was formed by the effect of human serum biotinidase.

2.5.3. Avidity of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin for avidin

2.5.3.1. HPLC. Size-exclusion HPLC radiochromatograms (ProteinPak 125, Waters, phosphate buffer 0.1 M, 1.0 ml/min) were obtained from the radiolabeled DTPA-Biotin with and without the addition of avidin (Sigma Co.) in a 5 M excess.

2.5.3.2. Avidin–agarose column. An aliquot of the labeled biotin conjugate (100 μl) was loaded onto an immobilized avidin–agarose column (Pierce Co.), incubated for 10 min and washed with 60 ml of phosphate buffer saline (PBS) at pH 7.4. The radioactivity of the eluted sample and of the column was assayed by using a dose calibrator (Capintec Radioisotope Calibrator, Model CRC-7). The percentage of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin bound to the avidin–agarose column was calculated by dividing column radioactivity over the total activity of both sample liquid fraction plus the column. In order to evaluate the non-specific link of ^{166}Dy/^{166}Ho to the avidin column, the same procedure was performed but [^{166}Dy]Dy/^{166}HoCl$_3$ was used instead of the [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin complex.

2.6. In vivo distribution of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin complex

Female Balb-c mice (27–30 g) were used in these studies which were performed in accordance with the Mexican regulations regarding animal care and handling.

An i.v. injection of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin in a volume of 100 μl was given into the tail vein of the mice. Biodistributions were obtained as follows: the mice were killed 0.25, 0.5, 1, and 3 h postinjection, the blood samples and relevant organs (blood, kidney, stomach, intestine, liver, spleen, lung, heart, bone, and muscle) were removed and weighed and then counted in a pre-calibrated NaI(Tl) detector system. The uptake in each organ was calculated and expressed as % ID/g. All sample counts were corrected for background and physical decay by using standards of comparable volume and the adequate geometry for blood and organs.

Imaging was performed 30 min after the radiopharmaceutical injection on an E-CAM, siemens scintillation camera with a pinhole. The anesthetized live mouse was placed in a prone position with limbs spread out and secured with surgical tape. The image was taken for 35 min and stored in a 256 × 256 matrix.

3. Results

The radio-HPLC analysis of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin solution showed a main peak with retention time of 5.8 ± 0.3 min. This radiochromatographic profile correlated with the UV-chromatogram of DTPA-Biotin (Fig. 2). In this system, free $^{166}Dy^{3+}$

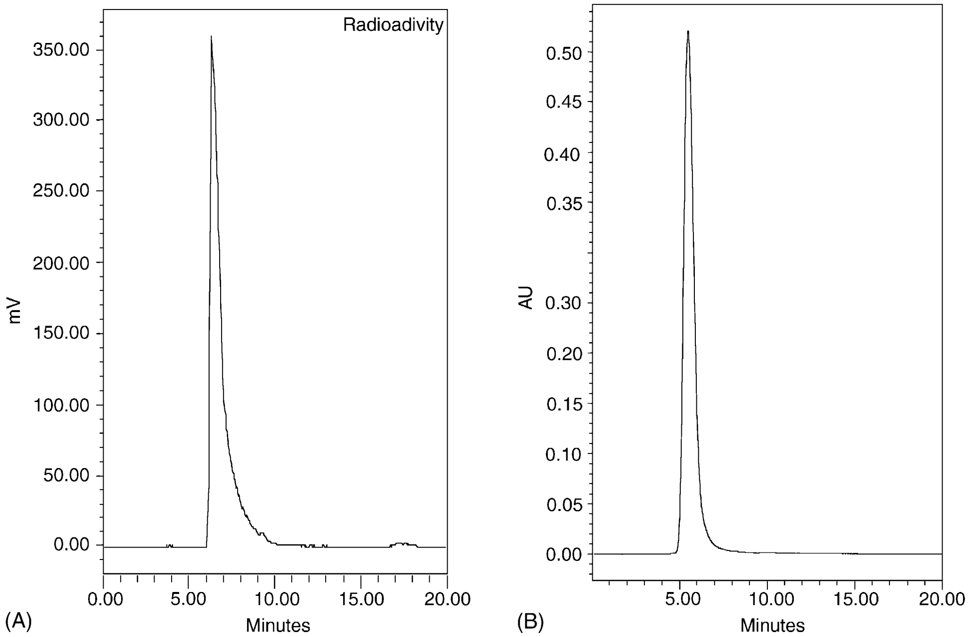

Fig. 2. (A) Reverse phase radio-HPLC chromatogram for [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin and (B) reverse phase UV-HPLC chromatogram for DTPA-Biotin (ODS column, gradient from 100 to 30%, water and 0 to 70% acetonitrile in 20 min, flow rate 1.0 ml/min).

and ^{166}Ho^{3+} as chloride forms, present a retention time of 2.4 ± 0.2 min. [^{166}Dy]Dy/^{166}Ho-DTPA have a retention time of 3.8 ± 0.2 min, which was not observed during any preparation of the labeled biotin complex. The radioactivity recovery was 96 ± 2.3% determined by reverse phase HPLC and the radiochemical purity 99.1 ± 0.6% determined by TLC. In all labelings ($n = 10$) the amount of free ^{166}Dy^{3+} or ^{166}Ho^{3+} was less than 1.5% (TLC analyses).

Size-exclusion HPLC radiochromatograms obtained for the radiolabeled DTPA-Biotin ($T_r = 12.4 \pm 0.2$ min) with and without the addition of avidin, showed a quantitative shift of the radioactivity profile to shorter retention time (avidin retention time $T_r = 9.2 \pm 0.3$ min) in the presence of the avidin demonstrated the avidity of the labeled biotin complex for avidin (Fig. 3).

[^{166}Dy]Dy/^{166}Ho-labeled biotin was 99 + 0.5% bound to the avidin–agarose column, therefore, its biological properties were not affected by the radiolabeling procedure. The free [^{166}Dy]Dy/^{166}HoCl$_3$ bound to the avidin–agarose column was less than 1% and it represents the non-specific bonding of ^{166}Dy^{3+} or ^{166}Ho^{3+} to avidin column.

The results of the in vitro stability of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin in saline dilution, showed that the complex was very stable since no significant change or decomposition (<4%) was detected by the TLC and HPLC analyses (Fig. 4).

When the [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin complexes were analyzed by size-exclusion HPLC after incubation in human serum at 37 °C for 24 h, approximately 2.5% of radioactivity was found to be associated with the proteins (Fig. 5). The analysis by reverse phase radio-HPLC showed that a minimum amount of [^{166}Dy]Dy/^{166}Ho-DTPA (<5.0%, $T_r = 3.8 \pm 0.2$ min) was formed as result of a possible biotin complex hydrolysis by enzyme biotinidase present in the human serum.

Biodistribution data of significant organs are showed in Table 1. The [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin complexes have high renal clearance and no accumulation is observed in metabolic organs (Fig. 6). If any amount of ^{166}Dy^{3+} or ^{166}Ho^{3+} were released as consequence of the β$^-$ decay of the ^{166}Dy, they would be captured almost in their entirety by the liver as all free lanthanide ions (Correa-González et al., 2003; Ferro-Flores et al., 1999).

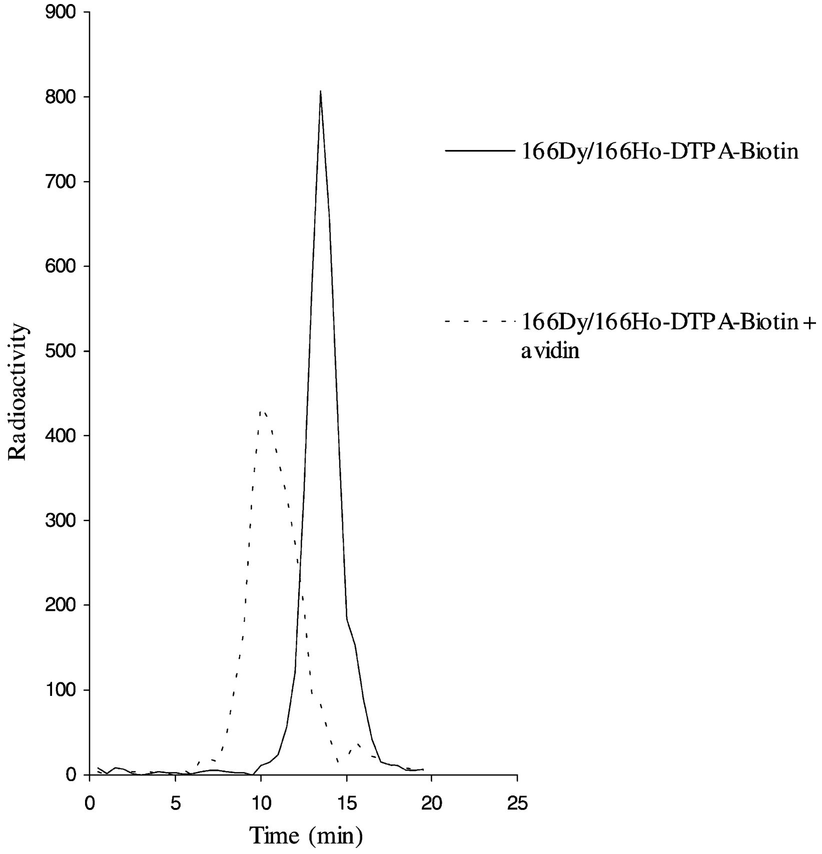

Fig. 3. HPLC radioactivity profile to determine avidity of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin for avidin. A pronounced shift of the radiolabeled biotin to a high molecular weight (avidin retention time) after addition of avidin is observed.

Table 1
Biodistribution (% ID/g tissue) in normal Balb-c mice at different times following the i.v. injection of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin ($n = 3$)

Organ	Time (h)			
	0.25	0.5	1	3
Blood	2.71 ± 0.58	0.97 ± 0.11	0.13 ± 0.02	0.08 ± 0.02
Kidney	11.34 ± 1.84	3.24 ± 0.30	2.51 ± 0.30	1.49 ± 0.11
Stomach	0.04 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.02 ± 0.02
Intestine	0.08 ± 0.04	0.11 ± 0.05	0.09 ± 0.02	0.06 ± 0.02
Liver	0.16 ± 0.03	0.07 ± 0.01	0.09 ± 0.02	0.03 ± 0.01
Spleen	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Lung	0.21 ± 0.07	0.12 ± 0.06	0.04 ± 0.01	0.03 ± 0.01
Bone	0.18 ± 0.04	0.06 ± 0.01	0.06 ± 0.02	0.01 ± 0.01
Muscle	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Heart	0.19 ± 0.05	0.11 ± 0.04	0.05 ± 0.03	0.03 ± 0.01

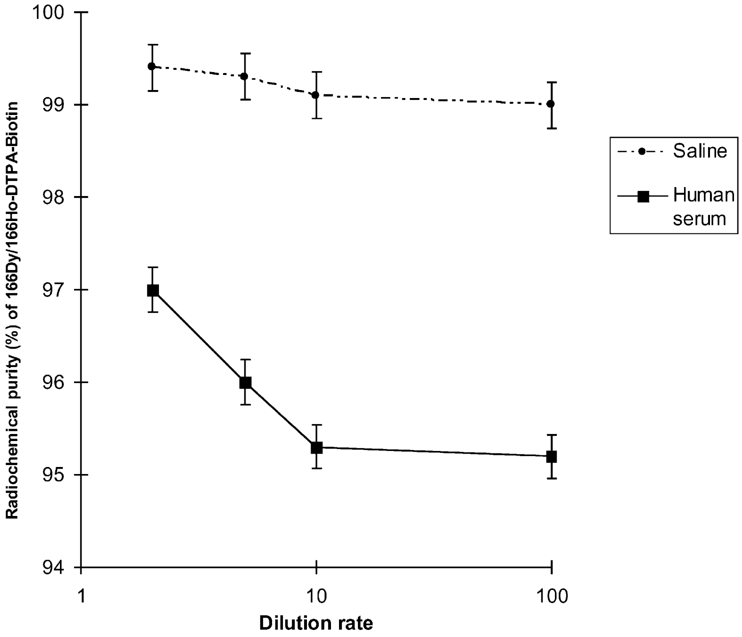

Fig. 4. In vitro stability of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin to dilution from 2- to 100-fold in 0.9% NaCl at 1 and 24 h.

4. Discussion

A crucial aspect of the in vivo generator concept relies on the similar chemistry of the parent/daughter isotopes so that the daughter radionuclide would not escape from the ligand sphere carrying the parent radionuclide to the biological target (Smith et al., 1995). Dy and Ho, like other lanthanides, have a preference for “hard” donor groups such as nitrogen and oxygen atoms and the similar ionic radii of 3+ oxidation state (1.027 and 1.015 Å) suggest comparable complexation geometry which could explain the stability found for this labeled biotin.

The stability in fresh human serum of this radiopharmaceutical could also be explained by the fact that the nitrogen of the amide bond, susceptible to hydrolysis by enzyme biotinidase, is coordinated to Dy(III)/Ho(III) producing a protective effect (Ferro-Flores et al., 1999).

The absence of hepatic uptake after the administration of the labeled biotin, demonstrates that Dy and Ho remain bound to the molecule in vivo and that no translocation of the daughter nucleus occurs subsequent to β^- decay of ^{166}Dy that could produce release of ^{166}Ho^{3+}. These results were expected because as explained before, the lanthanide elements exhibit uniform chemistry, therefore the change in the atomic number as a result of β^- decay of the central lanthanide ion has minimal effects on the chemical binding (Lambrecht et al., 1997). Under these considerations we could propose that as in the case of samarium, the Dy^{3+} or Ho^{3+} ion is probably neutralized by three carboxylate groups of the DTPA-Biotin ligand and coordinated to it, where the coordination sphere of Dy(III)/Ho(III) is totally satisfied with nitrogen and oxygen donors with a coordination number of 9. The metal center could be shielded from the medium producing in vivo stability. It is quite probable that the two biotins are in the adequate spatial orientation to permit the molecule to be recognized by the corresponding avidin (Ferro-Flores et al., 1999).

For radionuclide therapy, a fast renal excretion of the radiopharmaceutical is desirable in order to minimize radiation toxicity to non-target tissues. Because of its pharmacokinetic behavior, [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin could be used in radioimmunotherapy by the pretargeting approach. In this strategy the biotinylated monoclonal antibody,

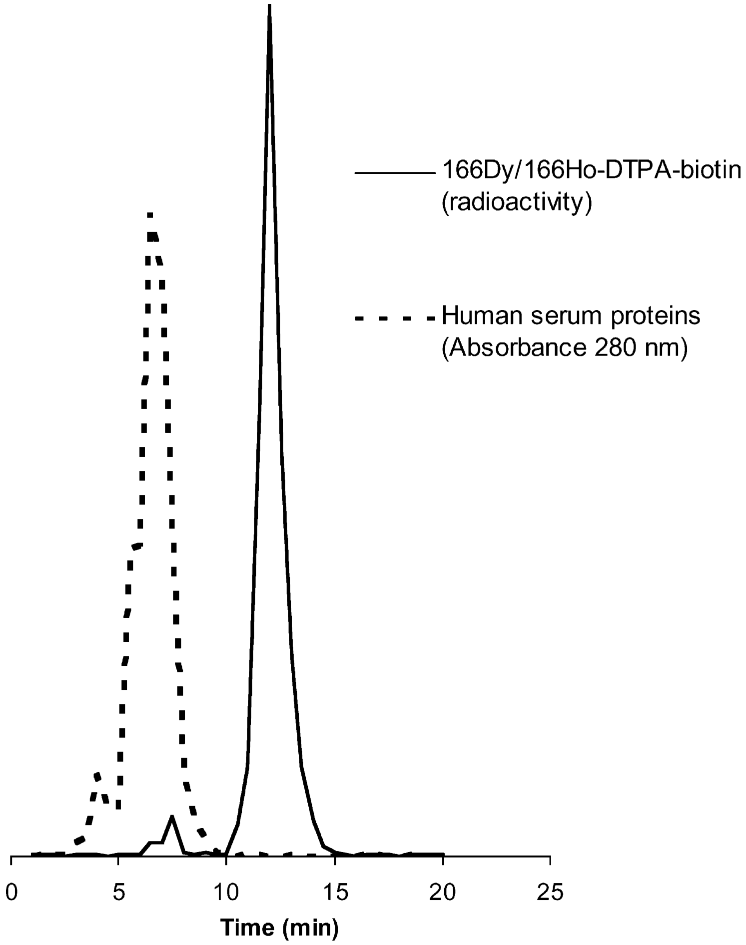

Fig. 5. Size-exclusion HPLC radiochromatogram of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin after 24 h incubation at 37 °C in human serum, showing a minimum shift of the radiolabeled biotin to high molecular weight corresponding to protein human serum.

highly specific but with very slow clearance, is injected followed by an excess of cold avidin and, as a third step and after the ratio tumor-bound to non-tumor-bound antibody has reached its maximum value, the [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin could be administered. It is important to mention that this labeled biotin might be used with different kinds of monoclonal antibodies such as those for treatment of lymphomas or colon cancer employed successfully in radioimmunotherapy.

Despite the fact that the in vivo generator strategy is primarily based on the initial chemical separation of the daughter radionuclide from the parent radionuclide, we could propose that because of the fast clearance of [^{166}Dy]Dy/^{166}Ho-labeled biotin from metabolic organs, this radiopharmaceutical could be administered while in equilibrium since the time required for target tissue localization would also be fast. The radiation dose to the tumor would not be affected significantly producing minimal radiation toxicity to non-target tissues. This procedure would facilitate the preparation of the radiopharmaceutical since the Dy/Ho radiochemical separation step could be avoided and the radiochemical yield increased.

In therapy trials (Grana et al., 2002; Breitz et al., 2000; Knox et al., 2000; Cremonesi et al., 1999) patients have been treated with yttrium-90 (^{90}Y) labeled DOTA-Biotin by the pretargeting strategy. However,

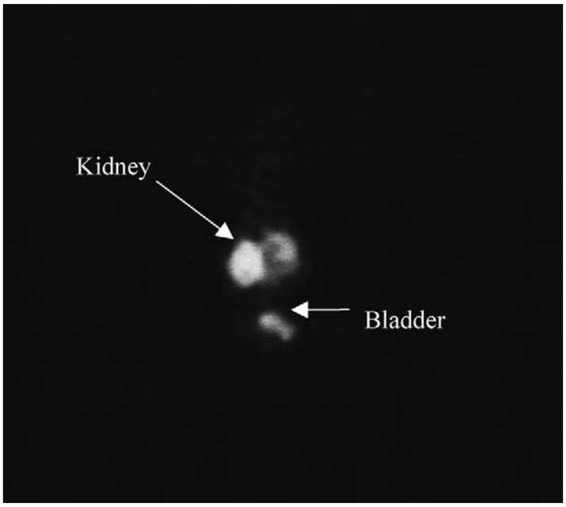

Fig. 6. Gamma camera scintigraphic image of a mouse 30 min after administration of [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin. Radiopharmaceutical showed only renal clearance.

^{90}Y is not a γ emitter radionuclide, and therefore is unsuitable for scintigraphy imaging, restricting the absorbed dose calculation. ^{166}Dy and ^{166}Ho produce 82 and 81 keV γ emissions, respectively, useful for scintigraphic image of their biological distribution that allows the in vivo absorbed dose to be calculated. In addition, a higher tumor uptake could be expected for [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin than ^{90}Y-DOTA-Biotin because of the dimeric structure of the labeled biotin prepared in this investigation. The total radiation dose received by a tumor per unit of captured activity of ^{166}Dy/^{166}Ho in equilibrium is higher than that produced by ^{90}Y (data not presented).

To establish the therapeutic possibilities for [^{166}Dy]Dy/^{166}Ho-labeled biotin it will be necessary to obtain biodistribution tumor-bearing mice and to determine the absorbed radiation dose.

5. Conclusions

[^{166}Dy]Dy/^{166}Ho-DTPA-Biotin was obtained with a 99.1 ± 0.6% radiochemical purity. In vitro studies demonstrated that the radiolabeled biotin is stable after dilution in saline and in human serum and no translocation of the daughter nucleus occurs subsequent to β^- decay of ^{166}Dy. Avidity of labeled biotin for avidin was not affected by the labeling procedure. Biodistribution studies showed that the [^{166}Dy]Dy/^{166}Ho-DTPA-Biotin complexes have high renal clearance and a negligible accumulation in metabolic organs was observed. Therefore, the [^{166}Dy]Dy/^{166}Ho-labeled biotin prepared in this investigation has adequate properties to work as a stable in vivo generator system for targeted radiotherapy.

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References

Breitz, H.B., Welden, P.L., Beaumier, P.L., Axworthy, D.B., Seiler, C., Su, F.M., Graves, S., Bryan, K., Reno, J.M., 2000. Clinical optimization of pretargeted radioimmunotherapy with antibody-streptavidin conjugate and ^{90}Y-DOTA-Biotin. J. Nucl. Med. 41, 131–140.

Correa-González, L., Murphy, C.A., Pedraza-López, M., Ferro-Flores, G., Murphy-Stack, E., Mino-León, D., 2003. Uptake of ^{153}Sm-DTPA-bis-Biotin and ^{99m}Tc-DTPA-bis-Biotin in rat AS-30D-hepatoma cells. Nucl. Med. Biol. 30, 135–140.

Cremonesi, M., Ferrari, M., Chinol, M., Stabin, M.G., Grana, C., Prisco, G., Robertson, C., Tosi, G., Paganelli, G., 1999. Three-step radioimmunotherapy with yttrium-90 biotin: dosimetry and pharmacokinetics in cancer patients. Eur. J. Nucl. Med. 26, 110–120.

Ferro-Flores, G., Ramírez, F.M., Tendilla, J.I., Pimentel, G.G., Murphy, C.A., Meléndez, L., Ascencio, J.A., Croft, B.Y., 1999. Preparation and pharmacokinetics of samarium(III)-153-labeled DTPA-Biotin. Characterization and theoretical studies of the samarium (III)-152 conjugate. Bioconjug. Chem. 10, 726–734.

Ferro-Flores, G., Arteaga de Murphy, C., Villarreal, B.J.E., Pedraza, L.M., García, S.L., Tendilla, J.I., 2001. Development of targeted radiotherapy systems. In: Fifth Mexican Symposium on Medical Physics. AIP Conference Proceedings, vol. 593. pp. 63–70.

Grana, C., Chinol, M., Robertson, C., Maceta, C., Bartolomei, M., De Cicco, C., Fiorenza, M., Gatti, M., Cliceti, P., Paganelli, G., 2002. Pretargeted adjuvant radioimmunotherapy with yttrium-90-biotin in malignant glioma patients: a pilot study. Br. J. Cancer 86, 207–212.

Hymes, J., Wolf, B., 1999. Human biotinidase isn't just for recycling biotin. J. Nutr. 129, 485S–489S.

Knapp, F.F., Mirzadeh, S., 1994. The continuing important role of radionuclide generator systems for nuclear medicine. Eur. J. Nucl. Med. 21, 1151–1165.

Knox, S.J., Goris, M.L., Tempero, M., Weiden, P.L., Gentner, L., Breitz, H., Adams, G.P., Axworthy, D., Gaffigan, S., Bryan, K., Fisher, D.R., Colcher, D., Horak, I.D., Weiner, L.M., 2000. Phase II trial of yttrium-90-DOTA-Biotin pretargeted by NR-LU-10 antibody/streptavidin in patients with metastatic colon cancer. Clin. Cancer Res. 6, 406–414.

Lambrecht, R.M., Tomiyoshi, K., Sekine, T., 1997. Radionuclide generators. Radiochim. Acta 77, 103–123.

Smith, S.V., Di Bartolo, N., Mirzadeh, S., Lambrecht, R.M., Knapp, F.F., Hetherington, E.L., 1995. [^{166}Dy]Dysprosium/[^{166}Ho] Holmium in vivo generator. Appl. Radiat. Isot. 46, 759–764.

Volkert, W.A., Hoffman, T.J., 1999. Therapeutic radiopharmaceuticals. Chem. Rev. 99, 2269–2292.