Bioquimia

ISSN: 0185-5751

publicacionesbioquimia@prodigy.net.mx

Sociedad Mexicana de Bioquímica A. C.

México

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Bioquimia, vol. 34, núm. 2, abril-junio, 2009, pp. 61-68

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Distrito Federal, México

Bioquímia

Artículo original
Farmacología

Radiopharmacokinetics and uptake of ^{99m}Tc-cRGD in $\alpha_v\beta_3$ integrins for imaging angiogenesis in induced malignant tumors in athymic mice

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ABSTRACT

The multistep process of angiogenesis offers several targets for therapeutic interventions. One molecular target structure is the alfa five beta three ($\alpha_v\beta_3$) integrin which is expressed on vascular endothelial cells and over-expressed in cancer tumor angiogenesis. To image neoangiogenesis in athymic mice with induced pancreatic, breast and prostate malignant tumors a new radiopharmaceutical was developed. The ^{99m}Tc-EDDA/HYNIC-cyclic-Arg-Gly-Asp-D-Phe-Lys (^{99m}Tc-cRGD) targets integrin receptors $\alpha_v\beta_3$ and was prepared with an average radiochemical purity > 95 %. ^{99m}Tc-cRGD shows high in vivo *stability, fast blood clearance and rapid renal excretion in mice. There are statistical differences between tumor/muscle ratios for the 3 tumors studied. The highest tumor/non-target ratio was found in breast cancer (7.2 after 24 h) and a representative dorsal SPECT image was obtained where the tumor showed up very clearly over the background tissue. The high resolution of the image implies that ^{99m}Tc-cRGD will be of great value in nuclear medicine as a potential radiopharmaceutical for $\alpha_v\beta_3$ integrins receptor uptake and for imaging neoangiogenesis in neoplastic tissue and to follow up cancer tumor progression.*

Key words: *Radiolabelled RGD-peptide, integrin $\alpha_v\beta_3$, molecular imaging, angiogenesis.*

RESUMEN

Los receptores de integrinas $\alpha_v\beta_3$ se encuentran en la pared endotelial de los vasos sanguíneos y están sobreexpresados, sobre todo en los nuevos vasos de los tumores malignos. Para visualizar la neoangiogénesis en tumores inducidos con células cancerosas de páncreas, mama y próstata en ratones atímicos se desarrolló un nuevo radiofármaco de tecnecio-99m (^{99m}Tc). El péptido cíclico con los aminoácidos: -Arg-Gly-Asp-D-Phe-Lys- se marcó con ^{99m}Tc por medio del ligante bifuncional ácido hidracinonicotínico (HYNIC) y del coligante etilendiaminodiacético (EDDA). El ^{99m}Tc-EDDA/HYNIC-cyclic-Arg-Gly-Asp-D-Phe-Lys (^{99m}Tc-cRGD) con pureza radioquímica > 95%, se une *in vivo* a los receptores de integrinas $\alpha_v\beta_3$ con alta especificidad. En los ratones atímicos con cáncer inducido presenta rápida depuración sanguínea y eliminación renal y hay diferencias estadísticamente significativas entre la captación del tumor comparada contra la captación en músculo, en los tres tipos de neoplasias. La relación más alta tumor/músculo fue de 7.2 a las 24 h para el cáncer de mama y se visualizó la neoangiogénesis en este tumor. La alta resolución de la imagen demuestra que en la medicina nuclear el ^{99m}Tc-cRGD será de gran valor como un radiofármaco que se une específicamente a receptores de integrinas $\alpha_v\beta_3$ y por consiguiente permite obtener imágenes moleculares de tumores malignos con alta resolución.

Palabras clave: Péptido -RGD-^{99m}Tc, integrina $\alpha_v\beta_3$, imagen molecular, angiogénesis.

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Recibido: 29-07-2009
Aceptado: 20-05-2009

INTRODUCTION

Angiogenesis is a complex natural physiologic process that results in the formation of new vessels in a tissue and during angiogenesis a group of cell membrane receptors (transmembrane heterodimeric glucoproteins), called integrins modulate cell migration, cell-cell interactions and cell binding to the extracellular matrix.[1-3]

Angiogenesis is increased in several pathologic entities including cancer. The integrins enhance vascular and lymphatic permeability and are directly involved in cancer tumor-induced angiogenesis.[2,4] Several studies have shown that there is a correlation between $\alpha_v\beta_3$ receptor expression, angiogenesis and the metastatic potential of the corresponding cancer tumor.[5]

The multistep process of angiogenesis offers several targets for therapeutic interventions. One target structure is the $\alpha_v\beta_3$ integrin, which is overexpressed in vascular endothelial cells, and recognizes certain specific amino acid sequences.[4,6] It is of interest to study the development of targeting radiopharmaceuticals for $\alpha_v\beta_3$ integrin receptors that could serve as antiangiogenic therapeutics.[7] Radiolabeled peptides containing the specific amino acid sequence —arginine-glycine-aspartic acid— (RGD) have been used for targeting $\alpha_v\beta_3$ integrin receptors and thus imaging angiogenesis.

RGD peptides have been labeled with ^{99m}Tc, ^{111}In, ^{90}Y, ^{123}I, ^{64}Cu, ^{68}Ga. and ^{18}F.[8-12] There is especial interest in positron emitters for PET (positron emission tomography) integrin $\alpha_v\beta_3$ expression imaging in cancer tumors. It was shown that constraining the mobility of the linear RDG, by forming a cyclic peptide, increased their integrin receptor affinity *in vitro*.[13] Tetramer and octamer RDG peptides have been labeled with copper-64 for PET imaging of induced glioblastomas in a mice model.[14] Gallium-68 was conjugated to cyclic RGDs through a macrocyclic chelator.[11,12] For ^{18}F labeling chemistry many linkers and conjugated molecules in cyclic, monomeric and polymeric structures have been used for PET. Some radiopharmaceuticals are: ^{18}F-galacto-RGD, the dimeric cyclic ^{18}F-E[c(RGDyK)]2 and the ^{18}F-bombesin heterodimer. The tetrameric fluorinated aldehydes that were conjugated to an amino-oxy-bearing RGD peptide and linked to a polyethyleneglycol prosthetic group gave the best biodistribution in mice.[15-19] Nevertheless ^{99m}Tc labeling is still the best option for *in situ* radiopharmaceutical preparations useful for the hybrid SPECT/CT (single-photon emission computed tomography/computed tomography).[20]

A considerable number of synthetic peptides modified from the five amino acid parent or lead structure arginine-glycine-aspartic acid-phenilalanine-valine amino acids (RGDfV) have been assayed. Kok et al. reported that substituting the valine of the parental cRGDfV peptide for a lysine amino acid shows high affinity for $\alpha_v\beta_3$ integrins.[7] The cyclized-Arg-Gly-Asp-D-Phe-Lys (cRGDfK), as a monomer or dimer, can be technetium-99m labeled for targeting $\alpha_v\beta_3$ receptors. The lysine side chain provides primary amine functionality for coupling bifunctional chelating agents such as hydrazinonicotinic acid (HYNIC).[3,21-23]

Decristoforo et al. described the radiolabeling of a HYNIC-derivatized cyclic monomeric RGD peptide containing tyrosine and lysine amino acids [c(RGDyK (HYNIC)] using tricine and ethylendiaminodiacetic acid (EDDA) as coligands.[24]

The aim of this research was to easily prepare a ^{99m}Tc-EDDA/HYNIC-cyclic-Arg-Gly-Asp-D-Phe-Lys (^{99m}Tc-cRGD) radiopharmaceutical, to determine its biopharmacokinetics, estimate $\alpha_v\beta_3$ integrin receptor uptake and to image angiogenesis in athymic mice with induced malignant pancreatic, breast and prostate tumors.

METHODS

Preparation of ^{99m}Tc-EDDA/HYNIC-cyclic-Arg-Gly-Asp-D-Phe-Lys (^{99m}Tc-cRGD)

The cyclic-Arg-Gly-Asp-D-Phe-Lys pentapeptide (cRGD) (Bachem, USA) was conjugated to hydrazinonicotinic acid (HYNIC) via *o*-(7-azabenzotriazolyl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU). The HYNIC-cRGD conjugate was > 98%, chemically pure as analyzed by reversed phase high-performance liquid chromatography (HPLC, Waters instrument running Millenium software with both radioactivity and UV photodiode array in-line detectors).

The lyophilized kit was prepared with the HYNIC-cRGD conjugate plus ethylendiaminodiacetic acid (EDDA) and tricine as coligands and $SnCl_2$ as a reducing agent.

The radiopharmaceutical ^{99m}Tc-EDDA/HYNIC-cyclic-Arg-Gly-Asp-D-Phe-Lys (^{99m}Tc-cRGD) was prepared by adding ^{99m}Tc-pertechnetate obtained from a GETEC ^{99}Mo/^{99m}Tc generator (ININ-Mexico) to the lyophilized sterile kit and incubated for 15 min in boiling water.[25]

Evaluation of radiochemical purity

Radiochemical purity analyses were performed by solid phase extraction (Sep-Pak C-18 cartridges), reverse phase high-performance liquid chromatography (HPLC) and by instant thin-layer chromatography on silica gel (ITLC-SG, Gelman Sciences) with three different mobile phases: 2-butanone to determine the amount of free $^{99m}TcO_4^-$ (Rf = 1); 0.1 M sodium citrate pH 5 to determine ^{99m}Tc-EDDA coligand and $^{99m}TcO_4^-$ (Rf = 1) and, methanol: 1 M ammonium acetate (1:1 v/v) for ^{99m}Tc-colloid (Rf = 0).[25]

Cancer cell lines

Three cancer producing cell lines were used:

a) The AR42J murine pancreatic cancer cell line (ATTC, Rockville, MD, USA) over-expresses somatostatin receptors (SS). The cells were routinely cultivated at 37 °C in 5% CO_2 and 85% air humidified atmosphere in Dulbecco's minimum essential culture medium (DMEM) (Life Technologies, Inc, Gaithersburg, MD) which was supplemented with 5% fetal-bovine-serum and 100 U/mL of penicillin and 100 μg/mL streptomycin.
b) The MCF-7 cell line is derived from human breast cancer and overexpresses cholecystokinin receptors (CCK). The cells were cultivated in DMEM and 10 nM estradiol and incubated in 5% CO_2 at 37 °C, 85% atmosphere and humidity.
c) The PC-3 human prostate cancer cell line PC-3 was originally obtained from ATCC (USA) and overexpresses gastrin releasing peptide receptors (GRP). PC-3 cells were routinely grown in "RPMI" culture medium supplemented with 10% newborn calf serum and streptomycin (100 μg/mL) at 37 °C, with 5% CO_2 atmosphere and 85% humidity.[26]

Induced cancer tumors in athymic mice

Animal experiments were carried out in compliance with the Official Mexican Norm (NOM 062-Zoo-1999): rules and regulations for safe and adequate laboratory animal handling.

Athymic adult male mice (20-22 g) were kept in sterile cages with beds of sterile wood shavings, constant temperature, humidity, noise and 12:12 light periods. Water and feed (standard PMI 5001 feed) were given *ad libitum*. The cancer cells (1 x 10^6 in 0.1 mL) were implanted in 36 athymic mice by subcutaneous injection into the animal's back (12 mice injected with the AR42J cells, 12 mice injected with the MCF-7 cells and 12 mice injected with the PC-3 cells). Tumor growth was monitored frequently and the length and width were measured in cm^2 with calipers. The final volume (cm^3), after sacrifice, was expressed in grams.

In vivo biokinetics and cancer tumor uptake

When the tumors became visible (< 1 cm^2) a mean of 18.5 MBq of the radiopeptide, was injected in a tail vein (n = 3) for each time interval. After 0.5, 1, 3 and 24 h the mice were sacrificed in a CO_2 chamber. The tumor and the organs or tissues (blood, heart, lung, liver, spleen, pancreas, kidneys, thigh muscle and femur) were excised, blotted dry and put into plastic test tubes, previously weighed. The weight of the organ and the anatomic characteristics of the tumors were recorded. Activity of the tumor and organs was measured in a crystal scintillation [NaI (Tl)] well-type detector. The mean of 3 diluted aliquots of the injected activity (I.A.) were considered as the 100% uptake. Percentage uptake or activity A (number of disintegrations per unit time) of each organ or tissue was calculated and the results were expressed as the percentage of injected activity per gram of tissue (%IA/g). Tumor/blood and tumor/muscle ratios were calculated. Each value represents the mean and SD of three animals.

To evaluate the kinetics of the system a compartmental model was developed using the OLINDA/EXM code. The program allows the user to enter kinetic data and fit it to one or more exponential terms. The total number of disintegrations (N), in source regions, was calculated, integrated over time and expressed per unit of initial activity in the source region: MBq-h/MBq (previously considered as residence time in the MIRD code) equation 1.[27]

$$N_{SOURCE} = \int_{t=0}^{t=\infty} A_{SOURCE}\,dt \qquad (1)$$

Image acquisition

Imaging of mice injected with ^{99m}Tc-cRGD was performed with a Siemens E-cam SPECT single head gamma camera with a pinhole collimator.

Statistical strategy

The mean % uptake for each lot of 3 mice per time interval was calculated. The statistical method em-

ployed was the t-Student's test for the activity of the tumor *vs.* muscle for each time interval after administration. The significance level was $p = 0.05$.

RESULTS

The average radiochemical purity of ^{99m}Tc-cRGD was > 95% in all cases. Free $^{99m}TcO_4$ was < 2 %; 2-3% of ^{99m}Tc-EDDA and < 1.0% of ^{99m}Tc-colloid.

The biodistribution of ^{99m}Tc-cRGD in the three models of induced cancer tumors are summarized in *table I*. The radiopeptide shows favorable pharmacokinetic properties such as rapid blood clearance and renal elimination. Activity was accumulated mainly in the liver, spleen and kidneys.

There was a high *in vivo* stability in the induced tumors (pancreas, breast and prostate). Low muscle uptake (background activity) was observed in all the mice. Twenty four hours after radiopharmaceutical administration the mean percentage of the injected activity per gram of tissue (%IA/g) found in the murine pancreatic cancer tumor was 0.73 ± 0.36% (AR42J cells); 1.31 ± 0.24% in breast cancer (MCF-7 cells) and 0.67 ± 0.02% in the prostatic cancer (PC-3 cells), *(Table I)*. The anatomical characteristics for the xenograft pancreatic tumor were of a large, friable hemorrhagic mass; the breast tumor was white, compact and hard; the prostatic tumor was white with a discreet beige coloring, compact and hard to the touch. The tumor/blood ratios of % IA/g tissue in 24 h after administration were 14.6 for the AR42J cell line; 10.9 for the MCF-7 cell line; 9.5 for the PC-3 cell line. The tumor to muscle ratio after 24 h in the pancreatic tumor induced with the AR42J cell line was 4.0; in the hard, compact breast cancer (MCF-7 cell line) was 7.2 and for the PC-3 cell line (prostate) the ratio was 3.5 *(Table II)*. The reason for these differences might be that in the hemorrhagic pancreatic tumor the activity leaks out, in the compact dense breast tumor it remains inside the tumor and in the poorly vascular prostatic tumor the activity is minor.

Table I. Biodistribution data for ^{99m}Tc-cRGD in an athymic mice model bearing induced tumors expressed as % injected activity / gram of tissue (%IA/ g tissue), at different time intervals (0.5 to 24 h).

Organ	Time p.i. (h)	% IA /g tissue		
		AR42J (Pancreas)	MCF-7 (Breast)	PC-3 (Prostate)
Blood	0.5	3.15 ± 0.48	1.66 ± 0.21	0.88 ± 0.52
	1	2.86 ± 0.38	0.68 ± 0.10	0.70 ± 0.10
	3	0.31 ± 0.05	0.31 ± 0.02	0.34 ± 0.07
	24	0.05 ± 0.03	0.12 ± 0.04	0.07 ± 0.01
Liver	0.5	2.14 ± 0.57	2.15 ± 0.24	2.14 ± 0.04
	1	2.79 ± 0.47	1.60 ± 0.18	2.45 ± 0.43
	3	2.31 ± 0.60	1.51 ± 0.13	1.57 ± 0.40
	24	0.83 ± 0.31	0.91 ± 0.15	1.16 ± 0.47
Spleen	0.5	2.53 ± 0.03	1.72 ± 0.19	2.02 ± 0.37
	1	1.09 ± 0.07	1.56 ± 0.65	2.11 ± 0.20
	3	1.11 ± 0.19	1.42 ± 0.40	1.48 ± 0.28
	24	0.53 ± 0.18	1.16 ± 0.21	1.89 ± 0.57
Kidneys	0.5	3.75 ± 0.74	4.04 ± 0.12	6.62 ± 0.79
	1	9.75 ± 1.84	3.07 ± 0.01	3.02 ± 0.14
	3	2.65 ± 0.41	3.02 ± 0.30	2.28 ± 0.36
	24	1.37 ± 0.44	1.95 ± 0.04	1.53 ± 0.33
Muscle	0.5	0.90 ± 0.06	0.73 ± 0.14	0.65 ± 0.04
	1	1.09 ± 0.14	0.46 ± 0.09	0.58 ± 0.06
	3	0.33 ± 0.05	0.32 ± 0.02	0.34 ± 0.06
	24	0.18 ± 0.03	0.18 ± 0.03	0.19 ± 0.05
Tumor	0.5	2.26 ± 0.24	2.00 ± 0.60	1.59 ± 0.27
	1	2.84 ± 0.14	1.71 ± 0.52	1.48 ± 0.19
	3	2.15 ± 0.15	1.61 ± 0.37	1.23 ± 0.29
	24	0.73 ± 0.36	1.31 ± 0.24	0.67 ± 0.02

There is a statistical difference ($p < 0.05$) in % IA/g tumor *vs* muscle for the 3 tumors and for each mean time interval *(Table III)*.

Figure 1 shows 0.5-24 h uptake tumor data for each cell line inductor. The optimal time for activity concentration was observed between 1 h to 3 h for the three cell lines. The total number of disintegrations N in the source organs is shown in *Table IV*.

The back bearing induced human breast cancer tumor showed the highest uptake (6.15%). A dorsal SPECT image of one representative athymic mouse 3 h after injection is shown in *figure 2*.

DISCUSSION

For their growth cancer tumors require new blood vessel formation via angiogenic factors and one of these factors is the $\alpha_v \beta_3$ integrin. Besides alfa5 beta3 receptors the 3 tumors studied overexpress specific receptors: somatostatin in pancreatic cancer, cholecystokinin (CCK) in breast cancer and gastrin releasing peptide (GRP) in prostate cancer and the 3 tumors showed different degrees of radiopharmaceutical uptake which might be due to the differences in vasculature and to the amount of integrins present in the blood vessels regardless of other specific receptors.

As mentioned above, angiogenesis is a complex natural physiologic process that refers to the remodeling of the vascular tissue and it is characterized by the branching out of new blood vessels from preexisting vessels. Pathologic angiogenesis is found in cancer tumors and in several other pathologic entities. Angiogenesis can be imaged because of integrin targeting and uptake of radiopharmaceuticals. Early attempts using direct ^{99m}Tc labeling approaches were reported without showing specific *in vivo* uptake.[28,29] Other groups of researchers have reported indirect ^{99m}Tc labeling using HYNIC conjugated to peptide analogues.

Janssen et al. have compared the tumor targeting characteristics of a monomeric radiolabelled RGD-peptide with those of a dimeric analogue.[3] Both peptides were radiolabelled with ^{99m}Tc via HYNIC to form ^{99m}Tc-HYNIC-c(RGDfK) and ^{99m}Tc-HYNIC-E[c(RGDfK)]2. *In vitro*, the IC_{50} showed a 10-fold higher affinity of the dimer as compared to the monomer (0.1 *vs.* 1.0 nM). In athymic female BALB/c mice with subcutaneously growing OVCAR-3 ovarian car-

Table II. Tumor-to-organ ratios in an athymic mice model bearing induced tumors at different time intervals (0.5 to 24 h).

	Time p.i. (h)	AR42J (Pancreas)	MCF-7 (Breast)	PC-3 (Prostate)
Tumor/blood	0.5	0.71	1.20	1.80
	1	0.99	2.51	2.11
	3	6.93	5.19	3.61
	24	14.6	10.91	9.57
Tumor /muscle	0.5	2.51	2.73	2.44
	1	2.60	3.71	2.55
	3	6.51	5.03	3.61
	24	4.05	7.27	3.52

Table III. Statistical analysis of the tumor activity *vs* muscle activity for each induced tumor per time intervals (t-Student's test) n = 3 for each time.

AR42J	%IA/g tissue							
	0.5 h		1 h		3h		24 h	
	Muscle	Tumor	Muscle	Tumor	Muscle	Tumor	Muscle	Tumor
Mean	0.93	2.43	1.08	2.84	0.32	2.15	0.17	0.73
Variance	0.003	0.092	0.045	0.039	0.006	0.045	0.001	0.171
	t = 8.40		t = 8.58		t = 11.42		t = 2.66	
	p = 0.001		p = 0.013		p = 0.008		p = 0.037	
MCF7								
Mean	0.73	2.00	0.46	1.71	0.32	1.61	0.18	1.31
Variance	0.031	0.543	0.011	0.400	6.11E-4	0.201	4.0E-4	0.090
	t = 2.90		t = 3.36		t = 4.97		t = 6.47	
	p = 0.044		p = 0.028		p = 0.007		p = 0.003	
PC3								
Mean	0.65	1.59	0.58	1.48	0.34	1.23	0.19	0.67
Variance	0.002	0.151	0.006	0.054	0.005	0.127	0.004	0.001
	t = 4.57		t = 6.32		t = 4.25		t = 9.83	
	p = 0.019		p = 0.003		p = 0.013		p = 0.002	

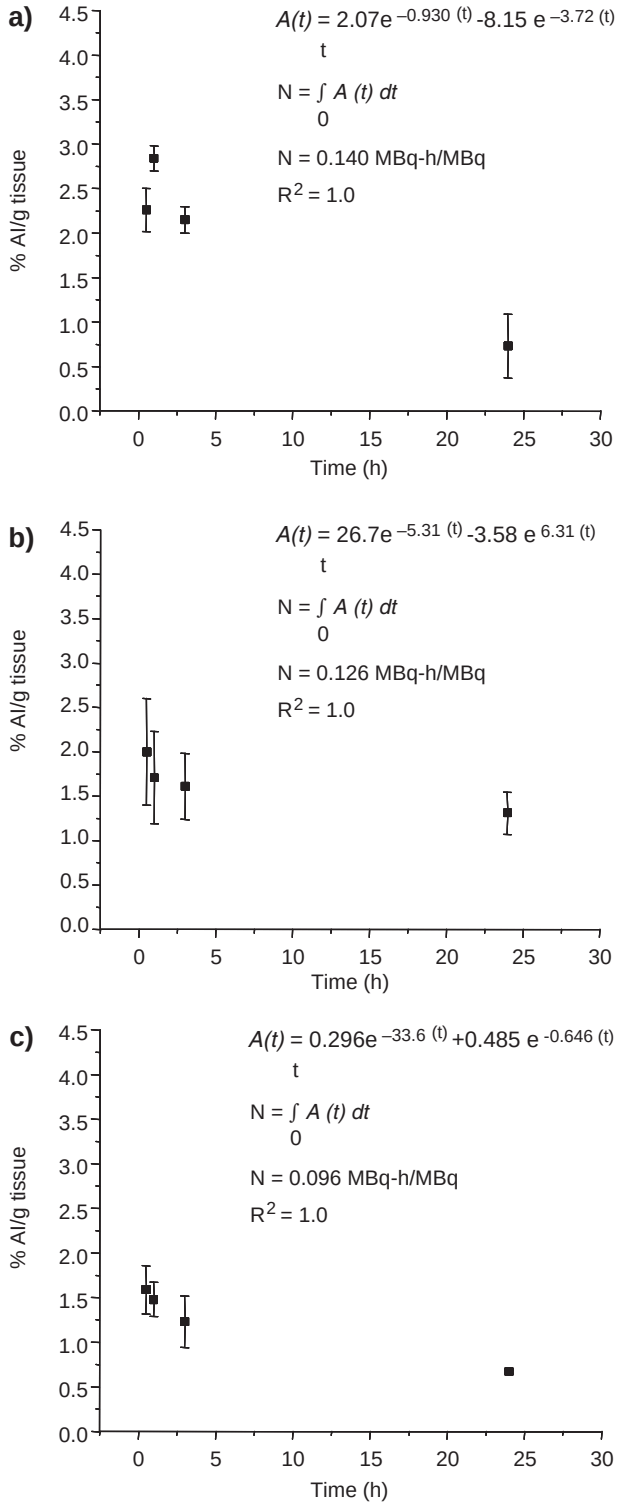

Figure 1. Biokinetics of ^{99m}Tc-cRGD in cancer tumors induced with cell lines a) AR42J (murine pancreas cancer); b) MCF-7 (human breast cancer) and c) PC-3 (human prostate) in 0.5 to 24 h.

Table IV. Total number of disintegrations (N) in the source organs of athymic mice with induced cancer tumors expressed per unit activity administered, (OLINDA/EXM).

	Total number of disintegrations (N) MBq-h/MBq Induced cancer tumor		
Organ	Pancreas	Breast	Prostate
Blood	0.061	0.040	0.030
Heart	0.030	0.037	0.031
Lung	0.074	0.061	0.057
Liver	0.181	0.193	0.015
Spleen	0.143	0.154	0.154
Pancreas	0.038	0.043	0.030
Kidneys	0.279	0.342	0.251
Intestine	0.211	0.255	0.244
Muscle	0.024	0.029	0.027
Bone	0.016	0.058	0.034
Tumor	0.140	0.126	0.096

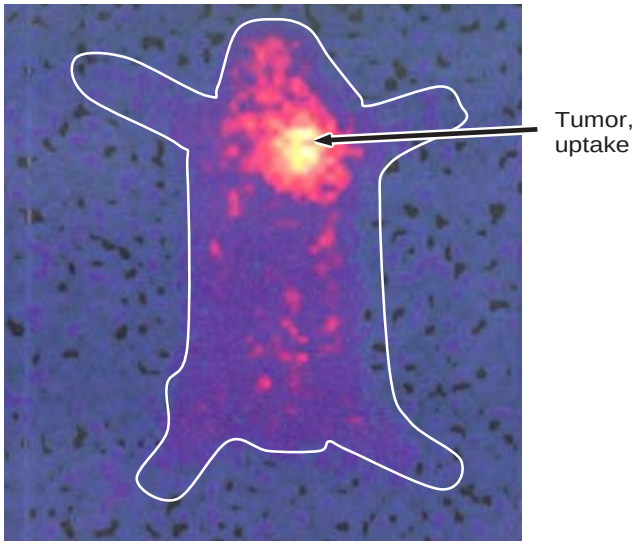

Figure 2. A selected image of an athymic mouse with induced tumor of human breast cancer 3 h after injection of ^{99m}Tc-cRGD was taken after dissection of internal viscera to highlight the tumor uptake (tumor/muscle ratio 6.15),

cinoma xenografts, the dimeric RGD peptide showed better retention in the tumor than the monomeric analogue, most likely due to the bivalent interaction with the target cell.

The cyclic HYNIC-derivatized monomeric RGD peptide [c(RGDyK(HYNIC)] was successfully ^{99m}Tc labeled using EDDA and tricine as coligands.[24]

Evaluation of radiolabeling of linear and cyclic RGD peptides was determined using a carbonil precursor $[^{99m}Tc(H_2O)_3(CO)_3]^+$ and the uptake of the

cRGDfK-His was higher than that with the linear peptide.[30]

^{99m}Tc-RGD has been used in many studies as a dimer or as a trimeric, tetrameric, octameric and multimeric molecules for imaging of tumor integrin $\alpha_v \beta_3$ expression, with good tumor uptake and favorable pharmacokinetics However all these peptides need to be synthetized.[3,11,14,31]

We used the commercial cyclic pentapeptide containing a phenylalanine amino acid (cyclic-Arg-Gly-Asp-D-Phe-Lys) which was conjugated with HYNIC and EDDA and tricine as coligands to form ^{99m}Tc-EDDA/HYNIC-cyclic-Arg-Gly-Asp-D-Phe-Lys (^{99m}Tc-cRGD).

Our research has led us to conclude that a highly stable, radiochemically pure, freeze dried kit can be ^{99m}Tc radio labeled in any hospital radiopharmacy. The ^{99m}Tc-cRGD targets $\alpha_v \beta_3$ integrin receptors in cancer tumor angiogenesis and allows noninvasive imaging of malignant tumors in a murine model. This could be a promising radiopharmaceutical for imaging cancer tumor angiogenesis in human beings.

The advantage of this lyophilized formulation of the cRGD peptide conjugated to HYNIC and with the added coligands EDDA and tricine over other reported formulations is that cRGD kit is easily labeled in one step with technetium-99m in any hospital radiopharmacy. The radiopharmaceutical ^{99m}Tc-cRGD targeted $\alpha_v \beta_3$ integrin receptors in athymic mice with 3 xenografted pancreas, breast and prostate cancer tumors and the tumor to muscle ratio are high allowing a better resolution of the tumor uptake over the muscle background in the SPECT image.

This non invasive nuclear medicine procedure demonstrated that ^{99m}Tc-cRGD will be of great value as a radiopharmaceutical for $\alpha_v \beta_3$ integrin receptor uptake and for imaging angiogenesis in neoplastic tissue.

ACKNOWLEDGMENTS

This study was supported by the CONACyT-Mexico 52633-M project.

Our gratitude to Jesús Sepúlveda-Méndez MD; Ma. Minerva Méndez-Fernández NMT; Ma. Guadalupe Soto-Gutiérrez NMT; Manuel Maury-Ruiz NMT; Armando Jiménez-Yedra NMT for obtaing the mice images.

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