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Original article

Comparative Study of the Biodistribution of ^{99m}Tc-HYNIC-Lys3-Bombesin Obtained With the EDDA/Tricine and AN/Tricine as Coligands☆

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ABSTRACT

The aim of present investigation was to evaluate the biodistribution in healthy animals and in tumor models of the radiopharmaceuticals ^{99m}Tc-EDDA/tricine-HYNIC-Lys3-bombesin (HYNIC-Lys3-BN) and ^{99m}Tc-NA/tricine-HYNIC-Lys3-BN. Biodistribution and pharmacokinetics were carried out over 24 h. To do so, 24 healthy Wistar rats were used and were administered 37.0 ± 0.8 MBq/rat of each radiopharmaceutical. For the tumor model study, 20 CD-1 nude mice were used and prostate tumors (PC3) were implanted in all the mice. Ten days later, tumor volumes were calculated and 40.00 ± 0.04 MBq/mice of each radiopharmaceutical were injected. Both showed high radiochemical purity: 98.08 ± 0.25% for EDDA/tricine product and 95.1 ± 0.3% for the conjugate with NA/tricine. Uptake of the radiopharmaceutical with NA/tricine was significantly higher in organs of the reticulo-endothelial system of healthy Wistar rats during 24 h, specifically in the liver and spleen. Both labeled compounds showed no significant differences between their blood elimination half lives. Average of tumor growth was 0.93 ± 0.02 cm^3 and affinity for tumors showed a growing and specific binding of both radiopharmaceuticals, although it was significantly higher for the EDDA/tricine conjugate. This outcome made it possible to corroborate the direct relationship between the density of gastrin releasing peptide and its receptors (GRPr) and the variation of the accumulation of the radiopharmaceuticals in the tumor. Use of EDDA/tricine as coligand is more appropriate than NA/tricine for labeling of HYNIC-Lys3-BN with ^{99m}Tc.

Estudio comparativo de la biodistribución de ^{99m}Tc-HYNIC-Lys3-bombesina obtenido con los coligandos EDDA/tricina y AN/tricina

Palabras clave:
Bombesina
Cáncer de próstata
Biodistribución
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RESUMEN

El propósito de la presente investigación fue evaluar la biodistribución en animales sanos y en modelos de tumores de los radiofármacos ^{99m}Tc-EDDA/tricina-HYNIC-Lys3-bombesina (HYNIC-Lys3-BN) y ^{99m}Tc-AN/tricina-HYNIC-Lys3-BN. La biodistribución y la farmacocinética fueron realizados durante 24 horas para lo cual se utilizaron 24 ratas wistar sanas a las cuales se les administró 37,0 ± 0,8 MBq/rata de cada radiofármaco y para el estudio de modelo de tumor fueron utilizados 20 ratones desnudos CD-1 a los que se les injertaron tumores de próstata (PC3). Diez días después fueron calculados los volúmenes tumorales y aplicados 40,00 ± 0,04 MBq/ratón de cada radiofármaco. Ambos mostraron alta pureza radioquímica con valores de 98,08 ± 0,25% para el compuesto con EDDA/tricina y de 95,1 ± 0,3% para el conjugado con AN/tricina. La captación del radiofármaco con AN/tricina fue significativamente mayor en órganos del sistema retículo endotelial de ratas Wistar sanas durante 24 h, específicamente en hígado y bazo. No se encontraron diferencias significativas entre los tiempos medios de eliminación de la sangre para ambos compuestos. El volumen promedio de crecimiento tumoral fue 0,93 ± 0,02 cm^3 y la afinidad por tumores mostró una unión creciente y específica de ambos radiofármacos, siendo significativamente mayor para el conjugado con EDDA/tricina. Este resultado permitió corroborar la relación directa que existe entre la densidad de receptores del péptido liberador de la gastrina (PLGr) y la variación de la acumulación de los radiofármacos en el tumor. El uso de EDDA/tricina como coligando para marcar HYNIC-Lys3-BN con ^{99m}Tc, es más apropiado que AN/tricina.

Introduction

Interest in small neuropeptides for use in the areas of visualization and cancer therapy has been growing in recent years.[1–5] Several types of malignant cells express greater concentration of receptors for neuropeptides on their surface than the normal cells of the same tissue. The said overexpression can be used as a potential target to obtain images and for treatment of malignant lesions, using the mentioned radiolabeled biomolecules.[6,7] It has been demonstrated that the small peptides are more effective than the antibodies and their fragments since they penetrate the tumors better, show faster clearance of the normal tissue and have a better accumulation ratio in the tumor compared to the healthy tissues.

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Bombesin (BN) is a tetradecapepetide isolated from the skin of batrachians that has the sequence (pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2) that has great affinity for the gastrin releasing peptide receptors (GRPRs).[4,7] These receptors are overexpressed in small cell lung cancer or adenoids, in malignant prostate tumors, in thyroid gland medullary carcinoma, in melanomas, malignant cancers of the breast, in gastrointestinal cancer, colon, duodenal, pancreas cancer, in some uterine neoplasms and in the hypothalamus and pituitary gland.[4,8,10,11] It has been recently demonstrated that the ^{99m}Tc-N_2S_2-Tat-(49–57) BN, a fragment of the native molecule, is capable of internalizing itself in the nucleus of malignant cells of the breast and prostate. This makes it a promising agent for the therapy[12] of the said tumors. Currently, the alternative of using radiolabeled nanopeptides of BN for the diagnosis and therapy of different tumors using the pretargeting system, a variant that foretells promising results, is being investigated.[13]

The native BN has been labeled with ^{125}I and ^{131}I by nucleophilic addition on the tyrosine residue and alternatively with ^{99m}Tc, ^{111}In, ^{64}Cu, ^{188}Re, ^{177}Lu, ^{142}Pr and ^{153}Sm by radio-complex formation. Some products are used to obtain images and others for therapeutic purposes, according to the type of radioactive emission.[8,9] For the BN labeling, the C-terminal portion is conserved totally. This is the portion responsible for its biological activity and homologues areas are formed by modifications in the N extreme, such as increase, decrease or replacement of amino acids and/or addition of complex molecules of the radionuclide. Several labeling strategies have been used to achieve high purity. One of those used most is based on the use of bifunctional chelating agents (BFCAs).[14] For its labeling with ^{99m}Tc, the BN chain may be modified without protection in lysine (Lys^3) since the conjugation of any bifunctional chelating agent as diaminedithol (DADT) does not affect its functional activity. However, the disadvantage of the complex formed is its high liposolubility.[15] In order to obtain a less lipophilic complex and therefore one having lower biodistribution in the liver and spleen, the diethylenetriaminepentaacetic acid analogue (DTPA)-[Lys^3, Tyr^4] BN was prepared. This had better biodistribution properties.[16] Conjugation of the mercaptoacetyl triglycine (MAG3) to the BN has been reported during the synthesis of the peptide in solid phase in order to introduce the reduced ^{99m}Tc and provide greater stability to the radiocomplex. However, the results showed excessive hepatobiliary excretion so that the said procedures has been used less.[17,18]

It has been possible to characterize the binding of ^{99m}Tc-BN to the GRP receptors in animal models, saturating the tumors with unlabeled peptide. It was demonstrated that accumulation in them was eight times less at 2 h and 10 times less at 24 h compared to non-saturated tumors. This makes it possible to assume that the binding is mediated by the recognition of the mentioned receptors on the surface of the PC-3 cells of the prostate.[18] The new Lys3-BN analogue has been conjugated with 6-hydrazinonicotinamide (HYNIC)/ethylenediamine diacetic acid (EDDA)/tricine and with HYNIC/nicotinic acid (NA)/tricine lyophilized to label with ^{99m}Tc with a single step.[8] The use of EDDA/tricine and NA/tricine may indicate variations regarding the radiochemical purity and stability of the labeling with ^{99m}Tc, since both co-ligands contribute different amounts and electron donor amino groups that intervene in the formation of the coordination complex, which benefits EDDA/tricine.[19] Accumulation in organs of the reticuloendothelial system as liver, spleen and bone marrow are closely related to the net load of the compound, to its liposolubility and its stability.[20–22] The complex formed with EDDA/tricine bestows lower liposolubility to the radiopharmaceutical than that formed with NA/tricine, which therefore affects the visualization of tumors that express the GRP receptor.[21] Considering the variations in the chemical structure and in the electrical load bestowed by the different chelators to the radioconjugates of BN, the differences that exist between ^{99m}Tc-EDDA/tricine-HYNIC-Lys3-BN and ^{99m}Tc-NA/tricine-HYNIC-Lys3-BN in regards to the biokinetics, biodistribution and accumulation on tumors of the PC3 cell line, which express abundant GRP receptors, will be evaluated.

Materials and methods

Materials

The HYNIC-Lys3-bombesin used in the present work was synthesized in the Pichem, Austria laboratories. The ^{99m}technetium was obtained from a ^{99}Mo/^{99m}Tc GETEC generator produced by ININ, Mexico. The prostate PC3 cells came from American Type Culture Collection (ATCC, Manassas). Cell culture medium used was the Dulbecco's modified Eagle's medium (DMEM) (Cell Concepts, Umkirch, Germany) with 10% fetal calf serum, penicillin, steptomycin, tin chloride II ($SnCl_2{\cdot}2H_2O$). EDDA, nicotinic acid and acetonitrile and methylethylketone for control of radiochemical purity were acquired in Sigma Aldrich (St Louis, MO USA). The ITLC-SG chromatographic strips used for quality control came from Gelman Sciences, Ann Arbor, Michigan, USA. Animals used in the biodistribution experiments and in the tumor models were treated according to the international ethics guidelines for handling experimental animals. All the pH measurements were performed in PHM84 equipment, Radometer Copenhagen, Denmark. Radioactivity was measured in a NAL (TI) solid scintillation detector Nuclear Enterprise SR8, USA; calibrated to measure ^{99m}Tc.

Methods

Labeling of HYNIC-Lys3-bombesin with ^{99m}Tc with EDDA/tricine and NA/tricine

In sterile vials, 25 mg of HYNIC-Lys3-BN, 40 mg of tricine, 3 mg of EDDA or 2 g of NA was added in 0.5 l of 0.1 M phosphate buffer, pH 6.0. All the components were dissolved in 1.5 ml of phosphate buffer at a pH of 4.2. This solution was gassed with nitrogen for several minutes and 10 μl of a $SnCl_2{\cdot}2H_2O$ solution at 1 mg/ml and 0.5 ml of a solution of TcO_4^- (2.0 ± 0.7 GBq) were added. The solution was heated for 5 min in 100° water bath and then left to rest until room temperature before proceeding to the quality analysis. Ten consecutive readings were made to obtain the final pH of each solution and the standard deviation of them was calculated.

Control of radiochemical purity

Chromatography in ITLC-SG

The radiochemical purity of the conjugates obtained was verified by instant thin layer chromatography with a two-solvent system: methylethylketone (MEK) and an acetonitrile solution (ACN) at 50% in water. In MEK, $^{99m}TcO_4^-$ presents a Rf = 1, the radiocolloids should show a Rf = 0, as well as the radioconjugate. In the ACN solution, the $^{99m}TcO_4^-$ has a Rf = 0, the radiocolloids $^{99m}TcO_2$ should present a Rf = 0 and the radioconjugate a Rf = 1.

Human PC-3 cell culture

The cultures of PC-3 prostate cancer cells were performed in 75 cm^3 flasks (Falcon) at 37 °C in a humidified atmosphere at 5% of CO_2. The PC-3 prostate cancer cells were cultured in DMEM supplemented with 10% inactivated fetal calf serum and 1% of penicillin/streptomycin.[23]