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Preclinical evaluation of new radioligand of cholecystokinin/gastrin receptors in endocrine tumors xenograft nude mice

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Abstract

The cholecystokinin(CCK)/gastrin 2 receptors (R-CCK2) are overexpressed in 90% of medullary thyroid cancers (MTC) and in 60% of small cell lung cancers but not or poorly in corresponding healthy tissues. They represent a relevant target for the diagnosis and internal targeted radiotherapy of these tumors. Although previous studies have demonstrated the feasibility of radiolabeled CCK/gastrin to target CCK-2 receptor-expressing tissues in animals and patients, some problems remained unsolved to identify an optimum candidate for in vivo targeting of R-CCK2-expressing tumors. By a rational approach and "*in silico*" drug design, we synthesized a new CCK-derivative with high affinity for the R-CCK2. The aim of this study was to achieve the radiolabeling of a new radioligand, to assess its efficacy using a published CCK radioligand (^{111}In-DTPA-CCK8) as a control for the R-CCK2 targeting.

This new CCK-derivative was radiolabeled with ^{111}In. Nude mice, bearing the human MTC TT tumors and NIH-3T3 cell line expressing a tumorigenic mutant of the R-CCK2, were injected with this radiolabeled peptide. In vivo planar scintigraphies were acquired. Thereafter, biodistribution studies (%ID/g tissue) were done.

The conditions of radiolabelling were optimized to obtain a radiochemical purity >90%. Scintigraphic images of xenograft mice showed significant tumor uptake with a target to nontarget ratio higher than two. These results were confirmed by the biodistribution studies which showed as expected a significant activity in the spleen, the liver and the kidneys.

Therefore, this new radiolabeled compound is a promised new candidate for molecular imaging and internal radiotherapy for R-CCK2 tumor targeting.

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Keywords: Cholecystokinin/gastrin receptor; CCK2 receptor; Medullary thyroid cancer; Radiolabeled peptide; Tumor targeting

1. Introduction

The successful development of radiolabeled somatostatin analogs has opened new horizons in nuclear oncology. Regulatory peptide receptors in many tumors are good candidates for malignant tissues targeting for diagnosis and internal targeted radiotherapy. However, whereas somatostatin (sst2) receptor scintigraphy has been proven to be a valuable tool for staging gastrointestinal endocrine tumors, its sensitivity and accuracy in some malignant derived-endocrine tumors like metastatic medullary thyroid cancer (MTC) or small cell lung cancer (SCLC) is limited. This is due to the fact that the sst2 receptors are not expressed in all these tumors and metastasis or that the expression of these receptors can change during the evolution of the pathology [1]. Moreover, some of the gastrointestinal endocrine tumors are sst2 negative or express, like insulinoma, other subtypes of the somatostatine receptor. The relatively recent discovery that many

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tumors overexpressed other peptide receptors, in particular the R-CCK2, has opened new area of research for the development of radiolabeled ligands targeting these receptors. Indeed, the R-CCK2 is overexpressed in up to 90% of the MTC and 60% in the SCLC while it is not or poorly expressed in the corresponding healthy tissues [2].

The cholecystokinin 2 receptor (R-CCK2, formely named CCKB/gastrin receptor) is a member of the G-protein coupled receptor (GPCR) superfamily that mediates important physiological functions by binding cholecystokinin (CCK) and gastrin peptides. Although previous studies had demonstrated the feasibility to target R-CCK2-expressing tumors in animals and patients with radiolabeled CCK/gastrin peptides (^{111}In-DTPA-CCK8 or ^{111}In-DTPA-minigastrin (DTPA: diethylene-triamine-pentaacetate)) [1,3], different adverse effects using these peptides were reported indicating that the biodistribution and stability of the radioligand must be improved for clinical application [1,4]. To this end, we have used a molecular model of the R-CCK2 occupied by CCK that we have previously built [5] to design "*in silico*" a modified CCK derivative. The synthesized CCK-derivative was tested in vivo to characterize its properties to target the R-CCK2 and to assess its efficacy compared with a published CCK radioligand (^{111}In-DTPA-CCK8) used as control.

2. Materials and methods

The molecular model of the R-CCK2 occupied by CCK built by the group of Dr. Silvente-Poirot was used to design 'in silico' a new CCK-derivative [5]. After synthesis, the CCK-derivative was coupled to DTPA and radiolabeled with ^{111}In. Six nanomoles of peptide were labelled with 74 MBq of $^{111}InCl_3$ (Mallinckrodt Tyco Healthcare). The radiolabeling conditions used for the published radioligand ^{111}In-DTPA-CCK8 were the same. The peptide-bound fractions of ^{111}In were evaluated before injection by instant thin-layer chromatography. Animal models were used; the animal studies were performed according to the French legislation. Xenograft tumors were established from the human MTC TT cell line (American Type Culture collection) and from an established NIH-3T3 fibroblast cell line expressing a tumorigenic mutant of the R-CCK2 (NIH-3T3-E151A-R-CCK2 mutant) [6] after sub-cutaneous injection of 3×10^6 cells. The human MTC TT cell line and the NIH-3T3-E151A-R-CCK2 mutant were cultured at 37 °C in ham's F12K medium supplemented with 10% fetal-calf serum (Sigma Chemie) and DMEM supplemented with 10% fetal-calf serum respectively.

The volume of the tumors was approximatively the same for all mice used in this study when imaging was performed. Approximatively, ten Megabecquerel of radiolabeled peptide were intravenously injected in the tail vein of mice. In vivo, images were performed at 1 and 24 h post injection. An acquisition of planar images was performed during 40 min using a gamma camera (Millennium VG®-GEHC) equipped with low energy high resolution collimators. Image analysis was done on a Xeleris workstation (GEHC-Waukesha). For each image, two elliptical regions of interest (ROI) were drawn: the first surrounding on the tumor (specific uptake) and the second on the muscles (nonspecific uptake) and corresponding counting statistics extracted (maximum and mean of counts and standard deviation). The number of pixels was the same for each image. ROI in mice scintigraphy without tumor were used as a control. Thereafter ex vivo biodistribution studies were done using a gamma counter (Wallac Wizard®). The injected dose percentage per gram (%ID/g) for each sample was calculated.

3. Results

Molecular modeling and 'in silico' drug-design experiments have been used in this study to control the key points of interaction between the R-CCK2 and the modified ligand are maintained as predictive of high affinity and agonist activity. Based on this approach, a new ^{111}In-CCK-derivative has been developed. The affinity of the CCK-derivative was in the nanomolar range. The conditions of radiolabelling were achieved to obtain a radiochemical purity from 20% to more than 85% for ^{111}In-DTPA-CCK8 and 90% for the new ^{111}In-CCK-derivative. Images at 1 h post injection showed an important background activity. Scintigraphic studies at 24 h of xenograft mice with the new CCK-derivative showed significant tumor uptake with a target to nontarget ratio in the two xenograft model: NIH-3T3-E151A-R-CCK2 mutant cell line [6] and MTC TT cell line (Fig. 1). The tumor/muscles ratio obtained from the ROIs was greater than two. Images of a mouse control without tumor showed physiological uptake of this radiolabeling CCK-derivative, as expected, in the stomach, the spleen, the liver and the kidneys and any uptake in the brain, the bone and the muscles (Fig. 1). These results were further confirmed by ex vivo biodistribution studies showing an expected significant activity in these organs (Fig. 2). Compared to the scintigraphy with the published ^{111}In-DTPA-CCK8 [1], the scintigraphy with this new ^{111}In-CCK-derivative demonstrated that the radiolabeling was as good as the published data (Fig. 3) for targeting the CCK2 receptor.

4. Discussion

The MTC represents 3–12% of all thyroid cancers. This metastatic cancer is unfrequent but with a 10-years survival of only 30%, cleary worse than the respective survival rates of patients with others forms of differentiated thyroid tumors. This pathology is a malignant dedifferenciation of the calcitonin-producing parafollicular cells. The problem for the diagnosis, the staging and the detection of residual disease is that although the thyrocalcitonin is positive, indicating the presence of the tumor, the conventional imaging is frequently negative leaving us with no target [1]. On the other hand, in the case of progressive and/or