Reducing Renal Uptake of ^{177}Lu Labeled CCK Derivative using Basic Amino Acids

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1. Introduction

In peptide-receptor radionuclide therapy (PRRT), radiolabelled somatostatin analogues such as octreotide have great potential against neuroendocrine tumors [1]. Other radiolabeled peptides and antibodies are under investigation. Radiolabeled peptides have been designed to target the relative receptors overespressed in tumor cells, such as integrin αvβ3, gastrin-releasing peptide receptor (GRPR), melanocortin-1 receptor (MC1-R), glucagon-like peptide-a receptor (GLP-1R), and cholecystokinin (CCK) receptor [2].

Most of these peptides are eliminated from the body via the kidney and are partly reabsorbed in the proximal tubular cells [3].

However, the high renal uptake of the radiolabeled peptides may lead to renal toxicity. The radiation dose to the kidney is a dose-limiting factor in PRRT [4]. The current PRRT protocol uses an infusion of positively charged amino acids such as lysine and arginine [5]. Lysine is usually preferred, and its d- and l-forms are equally effective [5].

In this study we investigated various amino acid solutions to reduce the renal uptake of ^{177}Lu-DOTA-CCK derivative.

2. Methods and Results

2.1 Radiolabeling of ^{177}Lu-DOTA-CCK derivative

The cholecystokinin receptor has been demonstrated as a possible target for PRRT. It was overexpressed in several tumor cells, including medullary thyroid cancer, neuroendocrine tumors, small cell lung cancer, and others [6]. Full-length CCK is a 34-residue peptide and the shorter form, CCK-8, has the structure DYMGWMDF-NH_2 [7]. We synthesized a modified analog of CCK-8, DOTA-Nle-cyclo(Glu-Trp-Nle-Asp-Phe-Lys-NH_2) (Fig. 1). Tetraazacyclododecane tetraacetic acid (DOTA) is used as a chelator for labeling with ^{177}Lu.

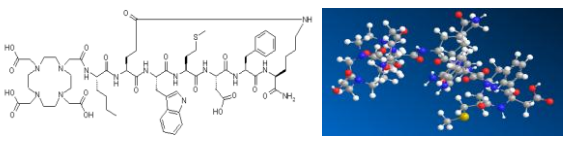

Fig.1 Structure of DOTA-CCK derivative

10^{-6} mole/ml of DOTA-CCK derivative in a 50mM sodium acetate buffer (pH 5.5) was mixed with 3.7 MBq of $LuCl_3$ and heated for 15 min at 90 ℃.

The radiolabeling yield was analyzed using a Waters Chromatograph equipped with an X-Terra C-18 column. The column was eluted with a binary gradient system with a flow rate of 1.0 ml/min using an elution solvent of 0.1% TFA in water and 0.1% TFA in ACN.

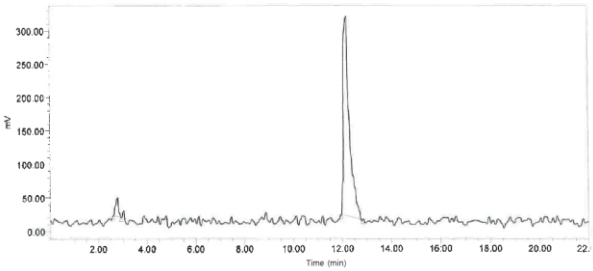

Fig. 2. HPLC result of ^{177}Lu-DOTA-CCK derivative.

To determine the partition coefficient (log *P*), 37 KBq of a ^{177}Lu-DOTA-CCK derivative was added in an equal volume mixture of 1-octanol and a PBS buffer and incubated for 15min at room temperature. 100 μl of fractions from both 1-octanol and PBS layers were transferred and the radioactivity was measured using a Wallac 1470 Wizard automated gamma counter (PerkinElmer Life Science). The log *P* value of the ^{177}Lu-DOTA-CCK derivative was -2.4. This means a relatively high hydrophilicity of this radiolabeled peptide.

Elimination of radiolabeled hydrophilic peptides from the body occurs preferably via the kidneys. However, renal reabsorption of radiolabeled peptide delivers high radiation doses to the kidneys, causing dose-limiting nephrotoxicity [4]. Coadiministration of competitive inhibitors of reabsorptions such as basic amino acids is currently used for kidney protection in clinical PRRT [5]. Rolleman et al. reported the combination of 25 g of lysine and 25 g of arginine was more effective than 50 g of lysine and caused relatively few side effects such as vomiting [5].

We investigated the potency of amino acid mixtures to inhibit the renal reabsorption of the ^{177}Lu-DOTA-CCK derivative. Groups of 4 female Balb/C mice were used in all experiments. Solutions of 60mg/ml of L-lysine or L-arginine or a combination of lysine and arginine were prepared. 0.2 ml (12 mg/mouse) amino acids mixtures were coinjected intravenously with 74kBq (2 μCi) of ^{177}Lu-DOTA-CCK derivative via the

tail vein. The mice (n=3) were sacrificed 2 h after injection, and the radioactivity levels in the kidney were determined using a gamma scintillation counter. These were expressed as a percentage of the injected dose per gram of organ (% ID/g).

The effects of amino acid mixtures on kidney uptake of ^{177}Lu-DOTA-CCK are shown in Figure. 3. A dose of 12 mg/mouse of arginine led to about a 20% reduction in kidney uptake (0.27 %ID/g vs. 0.22%ID/g). The effect of 12 mg of lysine (0.19 %ID/g) is similar to the combination of 6 mg of lysine and 6 mg of arginine (0.19 %ID/g) with a 30% reduction.

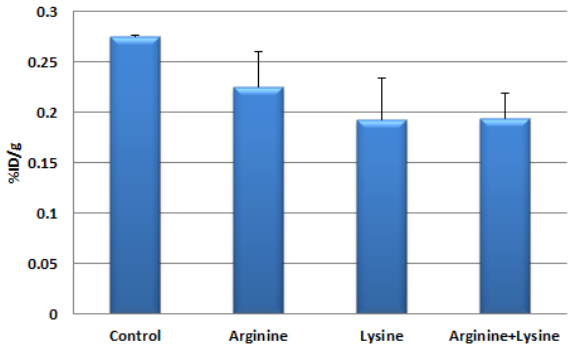

Fig. 3. Kidney radioactivity concentrations 2 h after intravenous injection of ^{177}Lu-DOTA-CCK derivative in mouse. Results are presented as mean %Injected Dose (%ID)/gram.

3. Conclusions

Renal uptake of ^{177}Lu-DOTA-CCK derivative is effectively reduced by the administration of positively charged amino acids. The administration of 12 mg of L-lysine was as effective in reducing the renal uptake as 6 mg of lysine and 6 mg of arginine combinations. Further studies will be performed to identify the most potent inhibitor of renal reuptake of radiolabeled peptides and minimize the chance of unwanted side effects.

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