Separation of two labeled components of [111In]-OctreoScan by HPLC

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[111In]-DTPA-D-Phe\(^1\)-octreotide (OctreoScan\(^\circ\), Mallinkrodt) is widely used for detection of neuroendocrine tumors and has lately been proposed for radionuclide therapy. We found, using HPLC and a GF-250 column (Zorbax\(^\circ\), Hewlett Packard), that OctreoScan\(^\circ\) can be separated in two radio
cabeled components of about equal amount. The analytical conditions for a quantitative isolation indicate that the two-peptide components of OctreoScan\(^\circ\) have different lipophilicity. The isolated components are stable and do not transform into each other at room temperature during 6 hours (shelf-life of OctreoScan\(^\circ\)).

Introduction

Octreotide, an octapeptide analogue of the neuropeptide somatostatin, is widely used in nuclear medicine. The compound was labeled with radiiodine1,3 and with various radio
clements. A commercial kit (OctreoScan\(^\circ\), Mallinkrodt) with the chemical form [111In]-DTPA-D-Phe\(^1\)-octreotide is today commonly used in the clinics. The [111In]-DTPA-D-Phe\(^1\)-octreotide is used extensively in the diagnosis of hormone producing tumors using planar gamma camera and SPECT. Lately, clinical trials have been started to evaluate the possible use of [111In]-DTPA-D-Phe\(^1\)-octreotide for radionuclide therapy.

Positron emission tomography (PET) should be able to quantify information about receptor kinetics and concentrations with better temporal and spatial resolution than SPECT, as well as provide quantitative information concerning dosimetry. For this purpose, we labeled a commercial kit to obtain [111In]-DTPA-D-Phe\(^1\)-octreotide. The labeling with the short-lived positron emitting indium isotope, [111In] (T\(_{1/2}\)=69 min), changed somewhat the original labeling procedure so we decided to make an extended characterisation of the labeled product. Besides analyses recommended by the manufacturer (SEP-PAC C18 cartridge and instant thin layer chromatography (ITLC)), we applied size exclusion chromatography (SEC) using a GF-250 column (Zorbax\(^\circ\), Hewlett Packard). To our surprise, we found that this technique revealed two peaks of the labeled product of about the same size. Their sum accounted for the total radioactivity of [111In]-DTPA-D-Phe\(^1\)-octreotide measured by the SEP-PAC or ITLC method. The same double peak appeared when [111In]-labeled OctreoScan\(^\circ\) was tested in the same way, which indicated that this product contains two peptide components with dissimilar chromatographic behavior.

A thorough literature search revealed that the presence of two peaks in octreotide was known and presented in an abstract from 1992.13 The authors claimed that the separated components were diastereoisomers and that, after separation, re
equilibrated at elevated temperature or in acidic pH. Acid catalyzed dissociation and subsequent reassociation have been proposed as re-equilibration mechanism. Separation conditions were not given in the cited report.

The chromatographic behavior found indicates some structural differences in the two components and it can not be excluded that they have different targeting properties, e.g., affinity to somatostatin receptors. To be able to study this, it is then important to obtain a more or less complete separation of the components. Thus, the goal of this study was to develop a method providing a base-line separation of [111In]-DTPA-D-Phe\(^1\)-octreotide components. All investigations were performed with 111In labeled OctreoScan\(^\circ\), produced according to the manufacturer’s recommendations.

Experimental

Equipment

The chromatographic system used contained a solvent pump (model 501, Waters, Milford, Massachusetts, USA), an injector (Rheodyne\(^\circ\)) with a 20 µl sample loop for analytical work and a 500 µl loop for semi-preparative separation, a flow-through radioactivity detector (of own construction) and a fix wavelength UV detector (Pharmacia, Uppsala, Sweden). A commercial data acquisition system was used (hardware and software, Ramona\(^\circ\)). The GF-250 column (250×9.4 mm\(^2\), Zorbax), used in our study, has a zirconia stabilized silica surface, which gives a usable pH range of 3.0–8.5. A monolayer diol phase is bonded to this surface to give a reproducible, hydrophilic column designed to minimize protein adsorption.

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An ultra-pure germanium detector (ORTEC, Oak Ridge, TN, USA), calibrated for energy and efficiency, was connected to an 8192 channel PC-based multichannel analyser (The Nucleus inc., Oak Ridge, TN, USA). Alternatively, radioactivity measurement were made using an isotope calibrator (Capintec, Ramsey, NJ, USA).

Material

Buffers and eluents (mono- and di-sodium phosphate, sodium citrate, ammonium sulphate and urea) were all made of chemicals of reagent grade.

A commercial kit (OctreoScan®, Mallinckrodt Medical B.V., Petten, The Netherlands) was used for preparation of [111In]-DTPA-D-Phe1-octreotide for clinical use. The labeling was carried out according to the manufacturer's instructions. Briefly, [111In]indium chloride (122 MBq in 1.1 ml of 0.02M HCl) was added to a vial containing 10 μg of lyophilized DTPA-D-Phe1-octreotide. Water of HPLC-grade was used in the preparation of buffer solutions. The mixture was incubated during 30 minutes before analysis. The following routine analyses were performed before patient administration:

The SEP-PAC C18 method: A small sample of the [111In]-DTPA-D-Phe1-octreotide was applied on to an activated 1.0×1.5 cm2 Sep-Pac C18 column (Waters, Milford, MA, USA), which then was eluted with 5 ml of water (Fraction A), followed by 5 ml of methanol (Fraction B). The fractions and the column itself (Column) were measured for radioactivity and the labeling yield was calculated as a ratio Fraction B/(Fraction A + Fraction B + Column).

The ITLC method: A small aliquot of [111In]-DTPA-D-Phe1-octreotide was applied on to a pre-heated glass fibre sheet impregnated with silica gel (ITLC™ SC; Gelman Sciences Inc., Ann Arbor, MI, USA). A sodium citrate buffer (0.1M, pH 5) was the mobile phase. After development, the sheet was cut into two pieces, A (including the start point and two-thirds of the developed length) and B (the rest of the plate including the front). The radioactivity of A and B were measured and the labeling yield was determined as a ratio A/(A+B).

HPLC chromatography conditions

Especially for this study, samples of [111In]-labeled OctreoScan® were further HPLC analysis. They were stored in a refrigerator (if not stated otherwise) until analysed within 6 hours after preparation. All buffers were sparged with helium before use. Chromatograms were obtained under isocratic conditions at a nominal flow rate of 1 ml/min. When changing the mobile phase, at least 150 ml of the new eluent was used to equilibrate the column. A calibration kit (Gel Filtration Calibration Kit, Pharmacia, Uppsala, Sweden) was used to confirm the ability of the chromatographic system to separate compounds of different molecular weights.

Results and discussion

Initially, we applied size exclusion chromatography (SEC) mainly to characterize our preparation of [111In]-D-Phe1-DTPA-octreotide, and to exclude eventual high-molecular weight indium-binding protein impurities in that. The GF-250 column is commonly used in biochemical research and has good resolution, high stability and makes it possible to work in a wide range of sample masses and volumes.

The results of our first analysis were surprising (Fig. 1). The chromatogram clearly showed the existence of two peaks in a ratio of about 1:1. The elution volumes (15.1 and 15.7 ml, respectively) for the labeled peptide peaks were significantly larger than that of the low molecular peaks (e.g., 11.6 ml for sodium azide). The SEP-PAC and ITLC analyses showed that both compounds behaved as OctreoScan®. Further experiments with 111In-OctreoScan®, prepared from a commercial kit, confirmed the existence of these two peaks.

Since we were using 111In-OctreoScan® for therapy, it was of interest to see if there were any differences in the receptor binding of these two peaks. In the initial experiments the resolution of the two peaks was poor and did not allow preparative separation for further biological investigation. Therefore, we continued to optimize the separation conditions systematically. At least 10 different batches were tested. The results of both SEP-PAC and ITLC analyses demonstrated that the labeling efficiency was more than 96% for each sample. The results of the patient investigations demonstrated typical distributions of 111In-OctreoScan®.

![Figure 1. Chromatogram of 111In-OctreoScan® showing the existence of two labeled peaks: radiodetector (curve 1), UV-detector (curve 2). No UV-peak is associated to the polypeptide due to high specific radioactivity (GF-250 column and phosphate buffer as the mobile phase, pH 7.0, elution rate 1 ml/min).](image-url)