RADIOLABELLING OF RC-160: PRELIMINARY RESULTS

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Abstract

Vapreotide (RC-160) was labelled with ^{125}I using Chloramine-T and Iodogen methods and with ^{99m}Tc by a direct method with sodium ditionite as reducing agent in the presence of ascorbic acid.

Several methods of purification and quality control were evaluated. Yields of the reactions and of purification steps were calculated. The results obtained for the radioiodination reactions showed higher yields when limiting Chloramine-T method was used. Labelling of RC-160 with ^{99m}Tc indicated better yields when high radioactivity concentration of the radionuclide was used.

Stability of the products obtained was assessed at different post-labelling times by selected quality control methods: Sep-Pak cartridge as purification method and chromatography by RP-HPLC and ITLC-SG using saline solution as solvent. It was demonstrated that I-125-RC-160 and Tc-99m-RC-160 were stable during five weeks (at -20 °C) and 6 hours (at room temperature) respectively.

Preliminary biodistribution of Tc-99m-RC-160 in normal rats and mice were done showing different biological behaviour compared with control animals injected with pertechnetate.

In conclusion, RC-160 was successfully labelled with both radionuclides, with radiochemical purity higher than 95%. These results encourage further research work in animal models as well as to investigate the biochemical behaviour of radiolabelled peptide.

1. INTRODUCTION

Radiolabelled receptor specific bio-molecules promise to play a major role in diagnostic as well as therapeutic applications of malignant tumours. There are several peptides which may form the basis of useful radiopharmaceuticals for imaging a wide range of major diseases, especially those related with alterations at receptor levels.

Vapreotide, a somatostatin analoge, tyrosine-3 octapeptide (RC-160), is a potential agent for diagnostic purposes [1].

In this study, techniques and methodology for labelling RC-160 with I-125 and with Tc-99m were investigated.

2. MATERIALS AND METHODOLOGY

RC-160, provided by I.A.E.A., was labelled with radioiodine using Iodogen (Pierce) and Chloramine-T (1,8 μg Cl-T, without reducing agents) (Sigma Chemical Co.). Several methods of purification and/or quality control of the radiolabelled compound were evaluated: ionic exchange resin (Dowex 1x8); Sep-Pak C-18 cartridges (Waters Associates) [2]; gel filtration (Sephadex G-25 Pharmacia, PD-10 columns); electrophoresis SDS-PAGE (gel 15%, 6x8 cm, V=100 volts, time 30, 45, 60 and 90 min); ITLC-SG (Gelman) chromatography (MeOH 85%, TCA 20% or NaCl 0.9% as solvents); RP-HPLC with UV detection at 280 nm as well as radioactive detection (0.4x30 cm, MCH-10 C18 column, Varian). HPLC analysis was performed using methanol 100% as solvent A and 0.9% NaCl as solvent B (flow rate 1ml/min) [3].Gradient was programmed to start from 40% A reaching 80% A in 20 minutes; initial composition was regenerated at 25 minutes. In line radioactivity determination was not always possible, so in those cases, samples were collected and measured in a solid scintillation counter.

RC-160 was labelled with ^{99m}Tc by direct method using sodium dithionite as reducing agent in the presence of ascorbic acid [4]. The final $Na_2S_2O_4$ concentration was 2 μg/μl reaction mixture. Freshly eluted pertechetate with 12 mCi/ml - 85mCi/ml concentration range was used. Sep-Pak purifications (using C18 cartridge) were performed. Chromatographic separation methods were the same as previously indicated for labelling with iodine. RP-HPLC control assays were performed for RC-160, ascorbic acid, sodium dithionite, ethanol, free pertechnetate and iodide. Biological distribution was assessed in normal rodents, 1 hour after i.v. administration of technetium peptide. After the sacrifice, organs and blood, muscle and skin samples were dissected, weighed and activity measured.

In order to determine the "in vitro" radiochemical stability of the RC-160 labelled with ^{125}I and with ^{99m}Tc, controls in ITLC and RP-HPLC at analitic conditions mentionned before were done.

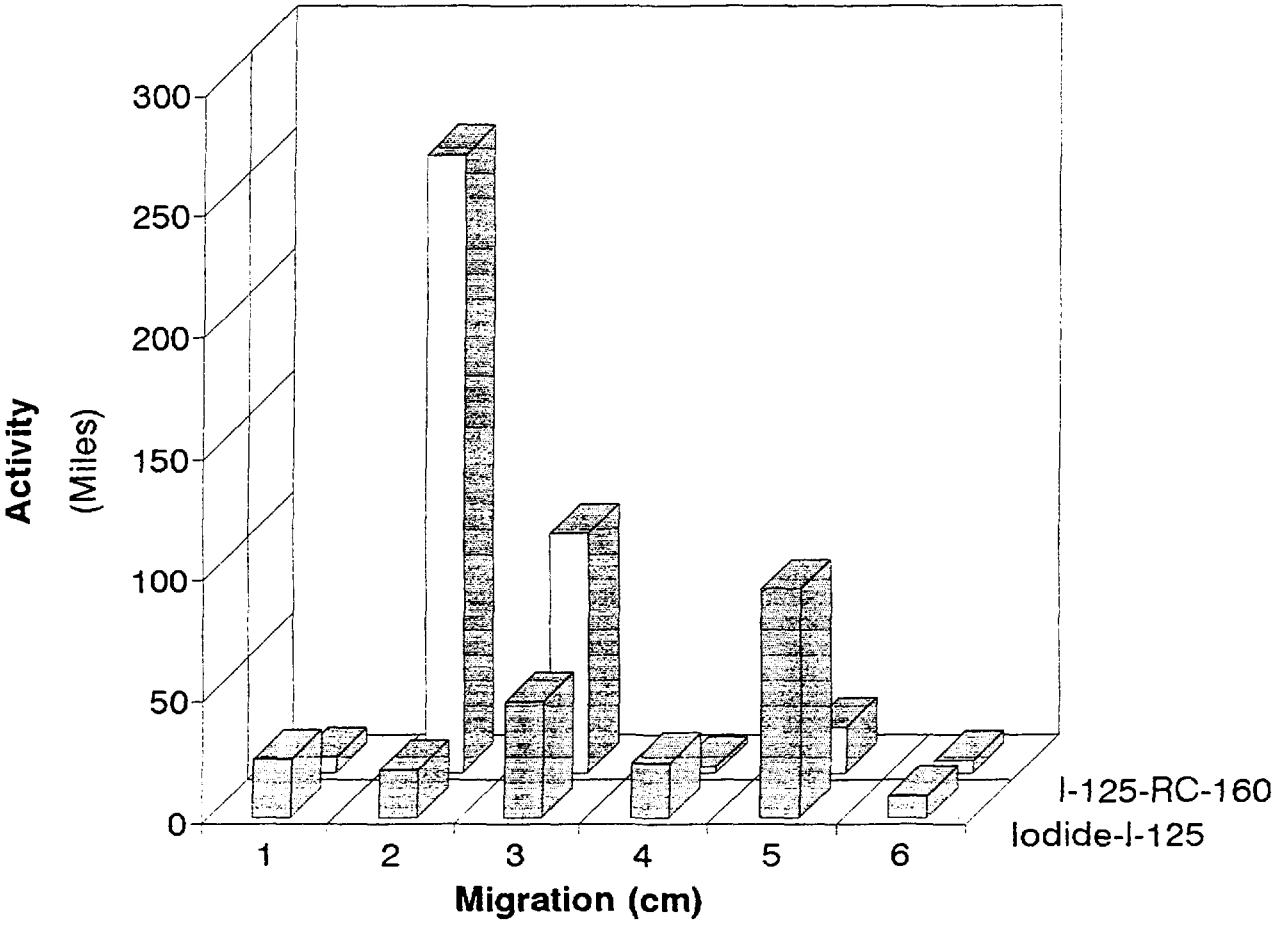

Fig. 1: SDS-PAGE Electrophoresis

Run time: 45 minutes

3. RESULTS

Sep-Pak cartridge was selected as purification method because of its capability to separate labelled molecules from free radionuclides in a simple and effective way.

Comparable results were found for the different quality control methods except for the system ITLC-SG chromatography with methanol 85%. SDS-PAGE was useful to resolve labelled radioiodinated compound from iodide with a 45 minutes run time (Fig.1). Considering the agreement among the other quality control methods, RP-HPLC and ITLC-SG/NaCl 0.9% were selected.

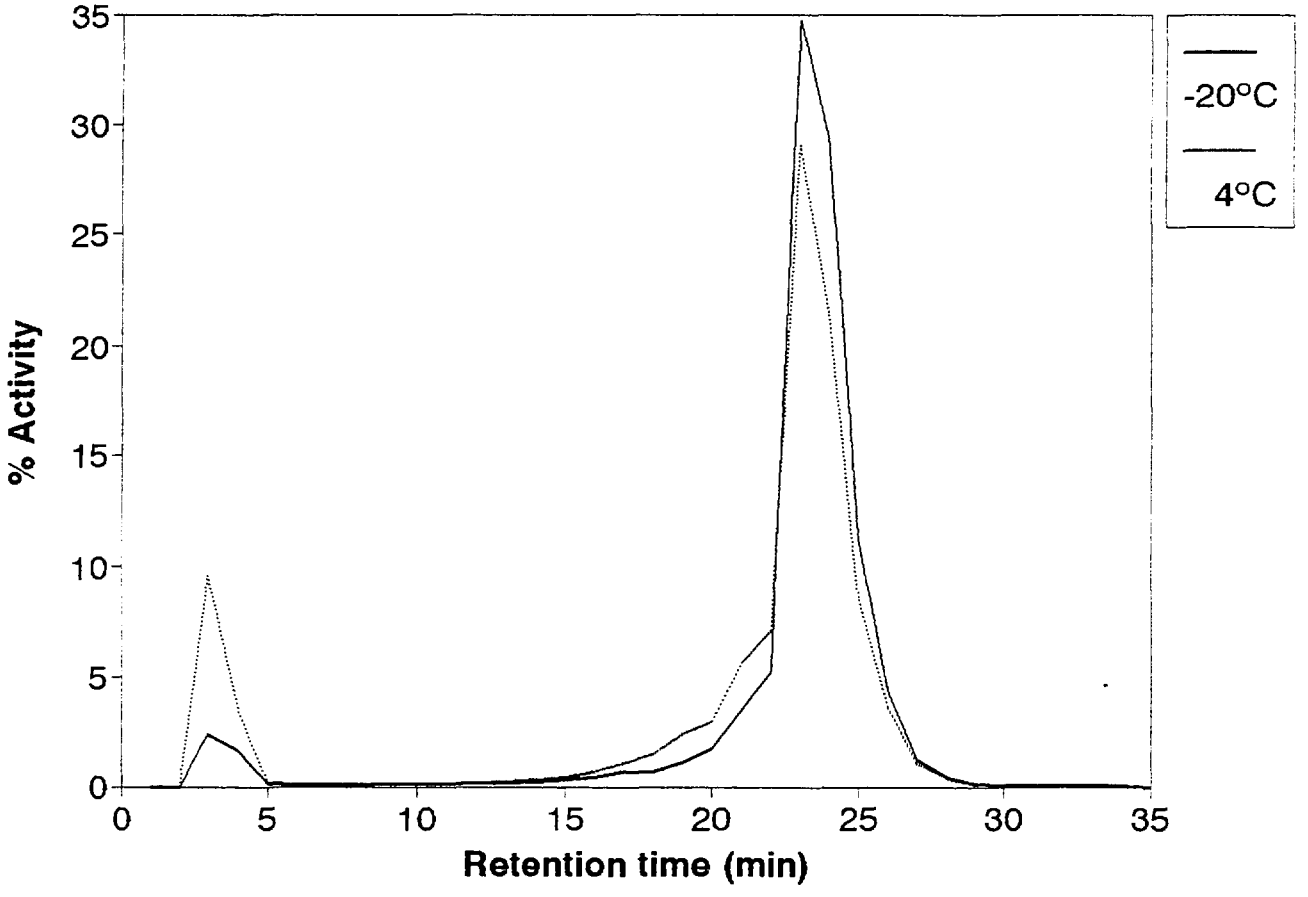

Fig. 2: ^{125}I-RC-160

HPLC analysis 37 days after labelling

The radioiodination reactions showed higher yields when Chloramine-T was used: 60% or more versus less than 50% for iodogen method. Specific activities were 240μCi/μg-274μCi/μg and the radiochemical purity was 98% (calculated by HPLC). Loss in the radiolabelling steps, including adsorption was up to 20%.

Total yield for radiolabelling of RC-160 with ^{99m}Tc was better when high concentration radioactivity solutions were used. More than 90% of ethanolic fraction activity from Sep-Pak elution were recovered in the second and third half mililiters and less than 2% remain in the cartridge. The hydrophilic radiochemical impurities by HPLC were less than 4% and was consistent with the results of ITLC-SG chromatographic system.

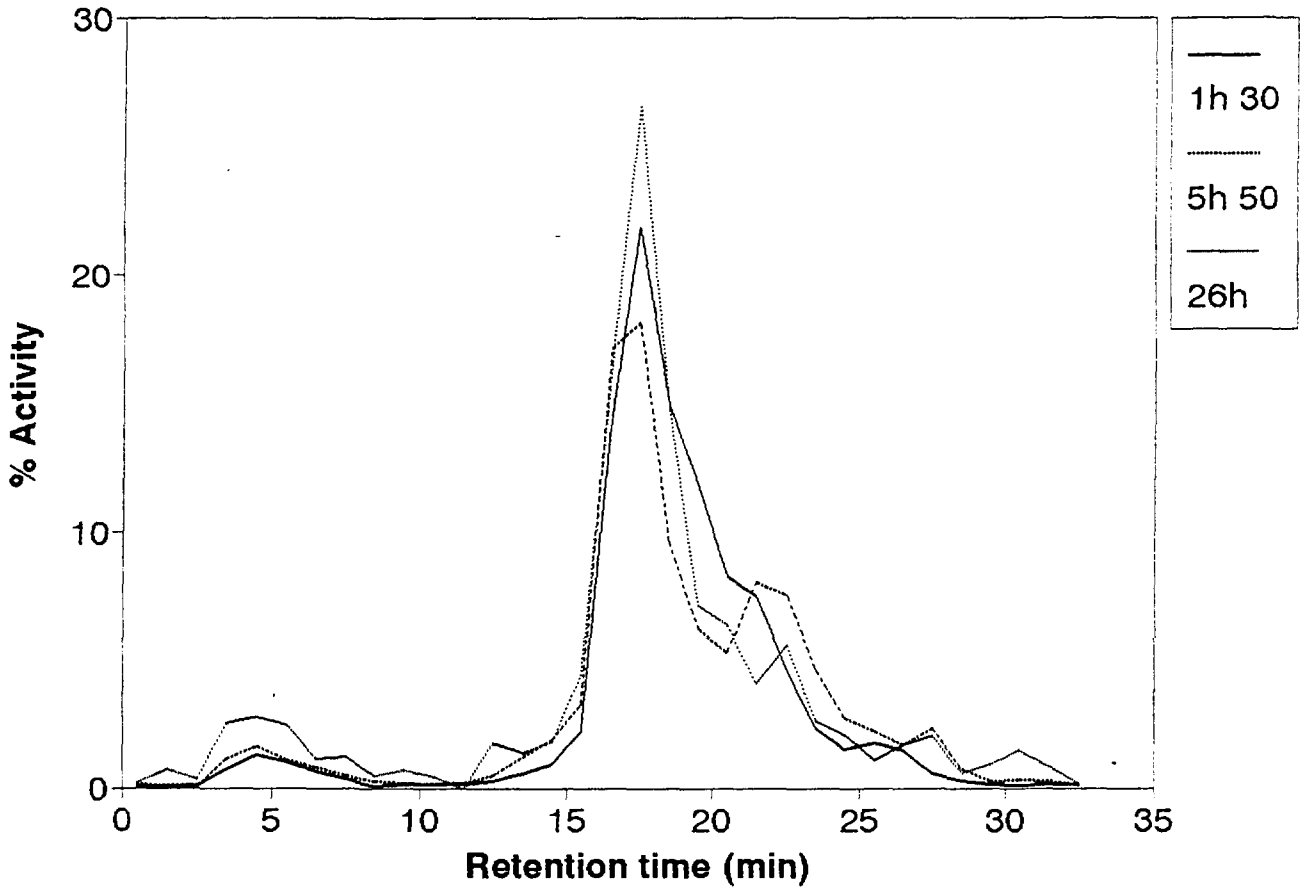

Fig.3: ^{99m}Tc-RC-160

HPLC at different times post labelling.

Biodistribution results indicated high blood cleareance (less than 0.10% ID/g at 1 hour) and high uptake in intestines (more than 80% of injected activity) which is in agreement with the lipophilic characteristics of the compound. Very small or negligible activity was find at heart, lung, thyroid, muscle, brain and stomach. Uptake in adrenals and pancreas was not significatively different from the results obtained for pertechnetate so longer biodistribution times are going to be tried.

Figures 2 and 3 showed the typical HPLC profiles for the labelled molecules at different times post-labelling and storage conditions. It was verified that at -20°C, the radioiodinated molecules do not have a significative increase of hydrophilic impurities (4% ± 2%) during 5 weeks, but at 4°C, an increase of up to 13% for the same period of time was observed. For the ^{99m}Tc labelled molecule, no significant difference was observed in radiochemical purity during 6 hours. At longer times, the hydrophilic species increase from less than 3% to more than 10% (at t=24 hours stored a room temperature).

4. CONCLUSIONS

RC-160 was successfully labelled with ^{125}I and with ^{99m}Tc, leading to high radiochemical purity compounds with standardized physicochemical quality control methods and well recognized "in vitro" stability. Therefore, these results and the local experience achieved trough these activities will encourage further basic research activities mainly in the field of biological and biochemical behaviour of radiolabelled peptides for peripheric membrane receptors.

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