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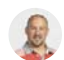
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ORIGINAL ARTICLES

Aspects on radiolabeling of 177Lu-DOTA-TATE: After C18 purification re-addition of ascorbic acid is required to maintain radiochemical purity

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Abstract

Purpose: Radiolabeled peptides like 177Lu-DOTA-TATE are vulnerable to radiolysis, which results in decreased radiochemical purity (RCP) of these radiopeptides. Gentisic acid (GA) and ascorbic acid (AA) are well known ingredients to reduce the effects of radiolysis. Currently, there is a trend to change the procedure from a manual to a cassette-based automated labeling and to introduce a C18 solid phase extraction (SPE) post-radiolabeling in order to remove non-incorporated 177Lu from the injection solution. However, with the introduction of SPE purification, GA and AA might effectively be removed from injection solution with a concordant dramatically drop of the RCP. Therefore we investigated the impact of tC18 SPE purification on the RCP of 177Lu-DOTA-TATE.

Methods: We compared the manual radiolabeling procedure with the cassette-based automated radiolabeling procedure with/out tC18 SPE purification cartridge. The effect of tC18 purification on RCP of 177Lu-DOTA-TATE was investigated by HPLC as function of the post-radiolabeling time and the concentration of activity.

Results: After tC18 SPE purification, GA and AA were effectively removed and resulted in volume-dependent decrease in RCP, e.g. <95% after 5h in 20 mL. Re-addition of AA directly after tC18 SPE purification resulted in a RCP ≥95% at 72h. In addition, with the cassette-based automated radiolabeling procedure we also found 28% of the original activity remaining in the activity-containing vial and tubing vs. < 1% with the manual procedure.

Conclusion: Re-addition of AA post tC18 SPE purification is required to maintain RCP of 177Lu-DOTA-TATE.

Keywords

Lutetium-177, 177Lu-DOTA-TATE, Cassette-based, Automated radiolabeling, Radiochemical purity, Ascorbic acid, Quencher, Gentisic acid, Radiolysis

1 Introduction

Radiolabeled somatostatin analogues, such as [DOTA0,Tyr3]octreotate, further referred as DOTA-TATE have been subject of intensive research during the last 2 decades and play an important role in somatostatin receptor imaging and peptide receptor-targeted radionuclide therapy (PRRT) e.g. 177Lu-DOTA-TATE[1-7].

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RCP of 177Lu-DOTA-TATE is an essential factor for successful PRRT. Because of the high doses of 177Lu-DOTA-TATE (7.4 GBq 177Lu per PRRT administration), the peptide is subject to radiolysis. The degree of radiolysis is influenced by several factors like the amount of DOTA-TATE, temperature, time, the total activity, the volumic activity, quencher(s) et cetera [8-12].

In the current publication we present a comparative study to investigate the effect of gentisic acid (GA) and ascorbic acid (AA) as quenchers during and after (manual and cassette-based automated) the radiolabeling 177Lu-DOTA-TATE procedures.

There is currently a trend to move from manual radiolabeling to a cassette-based automated radiolabeling procedure; therefore we investigated these two different procedures in parallel. Sep-Pak Light tC18 SPE purification (further referred as tC18) is included as default in the cassette-based automated procedure. However, the tC18 purification of the reaction mixture after radiolabeling potentially removes GA and/or AA effectively. The aim of this study was to compare radiolabeling procedures (manual vs. cassette-based automated) with and without tC18 purification and to investigate the impact on the RCP of 177Lu-DOTA-TATE as function of time until the moment of administration to patient.

Materials and chemicals

Reagents and solvents were used in the highest quality grade without further purification.

GA and water for trace analyses (Trace-SELECT®) were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Hydrochloric acid 30% (HCl) Ultrapur was obtained from Merck KGaA (Darmstadt, Germany). AA was purchased from WÖRWAG Pharma GmbH & Co. KG (Böblingen, Germany). DOTA-TATE as kit formulation [13] was provided by Erasmus MC Rotterdam (Rotterdam, The Netherlands). Ethanol (99.5%), Aqua ad iniectabilia and isotonic 0.9% NaCl (further referred as saline) were purchased from B.Braun Melsungen AG (Melsungen, Germany). tC18 cartridges were obtained from Waters GmbH (Eschborn, Germany). 177LuCl3 with specific activities in the range 740-1000 GBq/mg was bought from IDB-Holland (Baarle Nassau, the Netherlands).

2 General methods

The manual radiolabeling procedure was performed in a temperature-controlled heating block from CardiRad (Lohja, Finland) and cassette-based automated radiolabeling procedure was performed in a Modular-Lab Pharm Tracer module (EZAG, Berlin, Germany) using the C4-Y90-00-standard synthesis and C1-PR-00 pressure test cassette (EZAG, Berlin, Germany).

2.1 Cassette-based automated radiolabeling procedure

The cassette was prepared according to the Y-Lu-INCASSETTE-TEST protocol (EZAG, Berlin, Germany). For standard patient radiolabeling, 240 µg DOTA-TATE in 0.6 mL (400 µg/mL) of the DOTA-TATE kit formulation [13] was transferred automatically and quantitatively in to a glass reaction vial (RV) and eventually 7.5 GBq 177LuCl3 (0.3 mL) was added in to RV. Subsequent program steps are consecutively executed and the radiolabeling was running automatically according to the LU-177-DOTA-PEPTIDES-PT-V.X.X protocol (according EZAG). RV was heated for 30 min at 80°C. After 5 min cooling to ambient temperature the reaction mixture was transferred from the RV to the preconditioned tC18 cartridge. Pre-conditioning of the tC18 cartridge was performed with an ethanol/water mixture (5 mL, 50:50% v/v). This tC18 procedure was introduced in order to remove non-incorporated 177Lu from the final product. The content of RV was transferred to the tC18 cartridge and rinsed with 3 mL saline. 177Lu-DOTA-TATE was desorbed from the tC18 cartridge with 2.5 mL of ethanol/water (50:50% v/v). An aliquot of the final mixture was taken and subjected to quality control by ITLC and HPLC. The eluate plus 17.5 mL saline solution (final volume 20 mL) were

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filtered with means of a sterile Millex-GV 0.22 µm filter into a 25 mL glass vial (product vial or PV). Finally, a filter integrity test was performed. The cassette-based automated radiolabeling procedure process takes about 60 min.

2.2 Manual radiolabeling procedure

The manual radiolabeling procedure was performed directly in the activity-containing vial (AV), containing 7.5 GBq 177LuCl3 in 0.3 mL. For standart patient radiolabeling 0.6 mL (240 µg DOTA-Tate) of the DOTA-TATE kit formulation [13] was added to the AV, and incubated for 30 min at 80 °C [13]. After cooling down to ambient temperature non-incorporated 177Lu was complexed by the addition of 0.25 mL DTPA-solution (4 mg/mL) to the reaction mixture [14-15]. An aliquot of the reaction mixture was taken and quality control was performed using ITLC and HPLC. The residual was diluted with 5 mL saline solution, filtered with means of a sterile Millex-GV 0.22 µm filter into PV and finally adjusted to a final volume of 20 mL. The manual radiolabeling procedure takes about 40 min.

3 Studies on RCP of 177Lu-DOTA-TATE

RCP is defined as the % of the activity of the radionuclide present in the desired radiopharmaceutical form of the total radioactivity. RCP of 177Lu-DOTA-TATE was investigated with/out tC18 purification and re-addition of quenchers as shown in experiments 1-4, see Table 1. In order to investigate the dilution of the quenchers two additional radiolabeling were performed (experiment 5-6, see Table 1). One radiolabeling included tC18 purification (experiment 5, see Table 1), while another radiolabeling (experiment 6) was performed without the tC18 purification. Both samples were diluted with saline up to a final volume of 20 mL, at constant concentration of ~ 0.5 GBq/mL. In addition, experiment 7 was performed without tC18 purification post radiolabeling and was diluted to a patient dose (7.4 GBq in 100 mL). RCP was determined by HPLC, as described below.

Table 1. Different post radiolabeling procedures of 177Lu-DOTATATE

Experiment	1	2	3	4	5	6	7
tC_{18} purification	-	+	+	+	-	+	-
Ethanol/H_2O 50/50% (2.5 mL)	-	+	+	+	-	+	-
Re-addition AA or GA (100 mmol/L)	-	-	AA	GA	-	-	-
Final volume (mL)	5	5	5	5	20	20	100
Total activity (GBq)	2.5	2.5	2.5	2.5	10	10	7.4
0.5 GBq/mL	+	+	+	+	+	+	-

Labelings were performed using 7.5 GBq of 177LuCl3 (0.3 mL) and 0.6 mL of kit Erasmus MC matrix. Thereafter post radiolabeling procedures were performed either with/out tC18 purification, re-addition of AA or GA, and diluted in different final volumes (5, 20 and 100 mL) with saline. RCP of 177Lu-DOTA-TATE was monitored as function of time

4 Analytical methods

4.1 Incorporation by ITLC

ITLC-SG glass fibre sheets were purchased from PALL Life Sciences (Port Washington, NY, USA). Small portions (1-3 µL) were added on the ITLC-SG strips and sodium citrate buffer (0.1 M, pH 5) was used as mobile phase as described [14, 16]. Activity was recorded by Gina Star TLC and analyzed using Raytest miniGita software (Straubenhardt, Germany). Calculation of incorporation was performed as described [16].

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4.2 HPLC

HPLC methods were performed using a Dionex-3000 HPLC system with a variable wavelength Dionex GmbH detector (Idstein, Germany) containing a Waters 4.6 mm × 250 mm, 5µm Symmetry C18 column (Eschborn, Germany) . The gradient elution system utilized mobile phase A (methanol) and mobile phase B (0.06 M sodium acetate buffer, pH 5.5). Gradient was performed with a flow rate of 1 mL/min starting with 100 % B for 6.5 min; and was changed to 50% A and 50% B within 0.5 min and increased to 60% A over 20 min.

Thereafter, the mobile phase A was increased within 0.2 min to 100 % and was kept constant for 4.8 min. Finally, the gradient parameters returned to the initial starting conditions. The data were analyzed using Chromeleon Client Software Version 6.8.9. from Dionex GmbH (Idstein, Germany).

5 Results

5.1 Radiochemical yield

With the cassette-based automated radiolabeling procedure we obtained radiochemical yields of 71 ± 18 % (n=12). The manual labeling achieved radiochemical yields ≥ 99% (n=3), independent of activity (range 2.5-10 GBq) or final volume (range 5-20 mL).

5.2 Radiolabeling without tC18 Purification

RCP of 177Lu-DOTA-TATE was measured by HPLC up to 168 h post-radiolabeling. Fig. 1A shows a typical HPLC radiochromatogram of 177Lu-DOTA-TATE which was prepared as described [13]. Fig. 1B shows the HPLC radiochromatogram of 177Lu-DOTA-TATE in the absence of quenchers. Fig. 2A clearly demonstrates that 177Lu-DOTA-TATE without the tC18 purification post-radiolabeling remained stable (RCP ≥95% at 72h post radiolabeling, experiment 1 and 2, see Table 1).

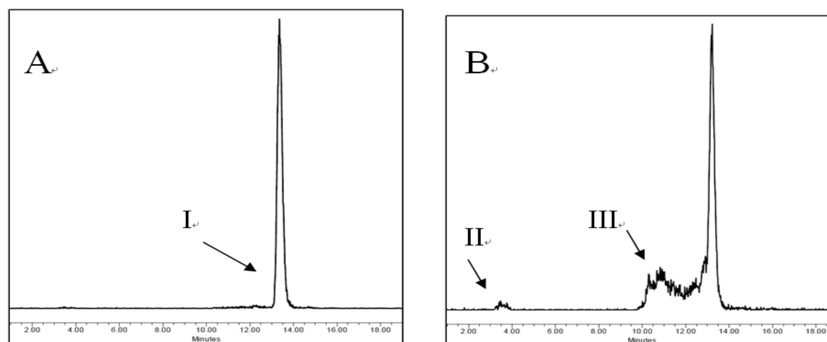

Figure 1. Typical RP-HPLC radiochromatogram of 177Lu-DOTA-TATE
(A) with a RCP of >95% and ^{177}Lu-DOTA-TATE (B) with a RCP <95%. Peaks (I): ^{177}Lu-DOTA-TATE, (II):^{177}Lu-DTPA and ^{177}Lu and (III): radiolysed fragments of ^{177}Lu-DOTA-TATE. These fragments were not further characterised. X-axes are expressed in time (min) and Y-axes in mV.

After increasing the volume up to 20 mL at constant volume activity (experiments 5, Table 1), 177Lu-DOTA-TATE remained stable (RCP ≥95% ~ 24h post-radiolabeling) as shown in Fig. 2B, while the RCP of 177Lu-DOTA-TATE in a patient dose (7.4 GBq/100 mL) rapidly decreased below 95% within 12 h (Fig 2C, experiment 7, see Table 1).

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