“In-house” preparation of $^{99m}$Tc-EDDA/HYNIC-TOC, a specific targeting agent for somatostatin receptor scintigraphy

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Received: November 2011; Accepted: January 2012

Abstract

The use of radiolabeled peptide ligands as diagnostics and therapeutics in nuclear oncology has increased recently. One of the most frequently used radiopharmaceutical is $^{99m}$Tc-EDDA/HYNIC-TOC, a somatostatin analog with affinity for certain types of somatostatin receptors, overexpressed in tumors of neuroendocrine origin. The radiopharmaceutical is not readily available; therefore we introduced its “in house” preparation within project activities supported by the International Atomic Energy Agency (IAEA). We optimized the radiolabeling protocol, prepared a small batch of frozen kits, performed ITLC quality control and animal biodistribution during the preclinical evaluation procedures. The co-ligand exchange labeling procedure was carried out at 100°C during 10 min, resulting in radiochemical purity $>90\%$. The biodistribution scintigrams in normal Wistar rats showed rapid blood clearance after 15 min and predominant kidney accumulation after 4 h, in accordance with the data reported by other authors. Storage stability of the formulated small batch frozen kit (-20°C) was evaluated within 6 months, with radiolabeling yield ranging between 94.3% and 96.9%. We conclude that frozen kit can be a safe alternative to the freeze-dried for small batch in house production, and after the satisfactory preclinical evaluation, the “in house” prepared $^{99m}$Tc-EDDA/HYNIC-TOC can be introduced in clinical practice as specific targeting agent for somatostatin receptor scintigraphy.

Key words: radiopharmaceutical, technetium-99m, tyr$^3$-octreotide, receptor scintigraphy, in house production, quality control

Introduction

The development of Nuclear Medicine towards molecular diagnostic imaging modality in the past two decades was followed by intensive research and applicative work resulting with introduction of wide variety of new generation radiopharmaceuticals. Labeled peptides have proven their potential as targeting agents for receptor scintigraphy, especially the synthetic analogs with preferable biological half-life and in vivo stability. Octreotide, a somatostatin analog, was used to compound $^{111}$In-DTPA-octreotide (OctreoScan), the first commercial radiolabeled peptide, regulatory approved in Europe and USA for clinical application. It became the imaging agent of choice for detection of somatostatin (SST) receptors, overexpressed in neuroendocrine tumors (NET), with evidence of higher sensitivity in comparison with CT and MR scanning (Shi et al., 1998). Besides the specific somatostatin receptor imaging properties, somatostatin receptor scintigraphy can be used as tumor staging agent (Lebtahi et al., 1997), as well as a predictor of the effectiveness of therapy with somatostatin analogues (Krenning et al., 1993). Despite of the favorable features, $^{111}$In-DTPA-octreotide has limited availability, suboptimal imaging gamma energy and high cost. Therefore, efforts were made by different research groups worldwide to replace the radiometal $^{111}$In with $^{99m}$Tc, the most preferable imaging radionuclide for single photon emission computed scintigraphy (SPECT) up to date (Maina et al., 1995; Vallabhajosula et al., 1996; Decristoforo et al., 2000).

Peptide labeling with $^{99m}$Tc-pertechnetate is however a complex task and considering the small size of peptide...
molecules and limited number of donor atoms, the indirect labeling approach is shown to be the most preferable technique (Liu et al., 1997). Thus, a bifunctional chelator is used to conjugate the peptide molecule and simultaneously form a coordination bound with the reduced ⁹⁹mTc-pertechnetate. One of the earliest efficient labeling methods was reported by Macek (Macek and Béhé, 1996) and later developed by Decristoforo (Decristoforo and Mather, 1999). They used Tyr₃octreotide (TOC) conjugated with HYNIC (hydrazinonicotic acid) as a bifunctional chelator. When using HYNIC as a bifunctional chelator, the complex has to be stabilized through the addition of a co-ligand or a mixture of co-ligands. To stabilize the ⁹⁹mTc-HYNIC complex, EDDA (ethylenediamine N, N’ diacetic acid) was used as co-ligand, resulting with favorable biodistribution, low blood levels and high renal excretion of the radiopharmaceutical.

Since ⁹⁹mTc-EDDA/HYNIC-TOC was evidenced to be an efficient tool for detecting SST2 and SST5 receptor subtypes, the main targets for the somatostatin receptor scintigraphy are carcinoids, insulinomas, pituitary adenoma, pheochromocytoma, neuroblastoma, medulary thyroid carcinoma. The radiopharmaceutical is not readily available, therefore our goal was to introduce it as “in house” product within the project activities supported by the International Atomic Energy Agency (IAEA), for updating and expanding the range of nuclear oncology services provided by our institute.

### Material and methods

- Chemicals with high purity grade were purchased by Merck, Germany (SnCl₂·2H₂O, or granted by IAEA (EDDA from Fluka Chemie Gmbh, Tricine from Sigma-Aldrich Chemie GmbH).
- The peptide conjugate (HYNIC-TOC) was provided by Radioisotope Centre POLATOM, Poland
- ⁹⁹mTc-pertechnetate was obtained as fresh eluate from commercial ⁹⁹Mo/⁹⁹mTc generator (ELUMATIC-III) supplied by Cis biointernational, France
- For the product purification SepPak C-18 mini cartridges (Waters, Milford, USA) were used, and for the final sterilization of the radiopharmaceutical 0.20 μ sterile Millipore filters
- For the quality control TLC-SG (Merck, 5553 Silica gel 60) aluminum sheets were used
- Biodistribution studies were performed on normal female Wistar rats in accordance with EC Directive 86/609/EEC for animal experiments

### Preparation of the radiopharmaceutical

In order to optimize the radiopharmaceutical production protocol concerning own facilities two approaches were implemented:

1. Production of a single dose wet labeled ⁹⁹mTc-EDDA/HYNIC-TOC
2. Production of a small-batch frozen kits for labeling with ⁹⁹mTc-pertechnetate

The optimized labeling conditions (pH, temperature, reaction time, specific activity) applied during the wet labeling procedure were identical during the labeling of frozen kits.

**Wet labeling protocol** was conducted according the publication of Decristoforo (Decristoforo and Mather, 1999), via co-ligand exchange as follows: in a sterile glass vial 20μg of HYNIC-TOC were mixed with 1 mL of solution mixture (1:1) consisting of 20 mg EDDA and 10 mg tricine, 15 μg of SnCl₂·H₂O dissolved in 0,1 M NCl and incubated with addition of 1000 MBq ⁹⁹mTc-pertechnetate (up to final volume of 2 mL) in water bath at 70 °C for 30 minutes (protocol 1). The solutions were freshly prepared and purged with sterile nitrogen. To optimize the reaction time we followed the von Guggenberg (von Guggenberg et al., 2003) modification of the previous labeling protocol and heated the reaction mixture at 100 °C for 10 minutes (protocol 2). The pH of the labeled product was 7 (measured semi-quantitative by Merck pH-indicator strips). Finally, the radiopharmaceutical was SepPak purified and sterile filtered.

**Kit formulation** was prepared for a mini batch of 12 vials. We modified the protocol published for freeze-dried kit formulation by von Guggenberg (von Guggenberg et al.,2004). Initially, 150 mg of EDDA and 300 mg of tricine were dissolved in 14 mL sterile H₂O (in a vial) by heating in water bath. 12 mL of the co-ligand mixture were transferred into 50 mL sterile evacuated closed vial through 0,20 μ Millipore filter. 250 μL (1 mg/mL) of HYNIC-TOC solution was added with syringe in the vial and mixed. Finally, 150 μL of instant dilution of SnCl₂·2H₂O in 0,1 M HCl consisting 240 μg was injected and mixed properly. The pH of this solution was 4. Finally, the solution was dispensed in 12 sterile evacuated vials through Millipore GV (low protein binding) filter in 1 mL volume. The vials were filled with sterile nitrogen and stored frozen at -20 °C.

The labeling procedure of the frozen kit was performed after thawing at room temperature, adding 0,5 mL of 0,2N Phosphate buffer and 0,5 mL of ⁹⁹mTc-pertechnetate. The mixture was incubated for 10 minutes in boiling water bath. The final pH was 7. The radiopharmaceutical was SepPak purified and sterile filtered.

**SepPak purification procedure**

A SepPak C-18 mini cartridge was initially activated with 5 mL of 100% ethanol, washed with 5 mL of 0.9% saline and dried with 5 mL of air. The radiolabeled solution was passed through the cartridge which was washed afterwards with 5 mL of saline. The labeled peptide was eluted from the cartridge with 0.6 mL of 80% ethanol through a low-protein binding sterile filter, and finally diluted with 5 mL of saline. The content of the ethanol in the final solution was not likely to induce side effects.

Maced. pharm. bull., 57 (1, 2) 65 - 70 (2011)
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Quality Control

Radiochemical purity

Instant thin-layer chromatography (ITLC) was performed on Silica gel strips with different mobile phases: Methylethylketone (MEK) to determine the percentage of the free $^{99m}$TcO$_4$ fraction (Rf=1), acetonitrile (ACN) 50% for the $^{99m}$Tc-colloid fraction (Rf=0). For the non-peptide bound $^{99m}$Tc-co-ligand 0.1M Sodium citrate buffer (pH=5) (Rf=1) and optionally 0.9% NaCl (Rf=1) was used. Radiochemical purity was calculated by subtraction the sum of the impurities from 100%. Radiochromatograms were obtained by Veenstra Radiochromatogram scanner VCS 101, with a software package.

Sterility

The small-batch of frozen kits was tested on sterility, in compliance with the internal GMP Production Protokol.

Biodistribution

For biodistribution studies 7.4 MBq of the radiopharmaceutical (consisting 1-2 μg of peptide) in a volume of 300 μL was injected into the tail vein of the rats. Animal scintigrams were obtained after 15, 60 min, 2h and 4h p.i. in a supine position, with a single-head Simens (e.cam) gamma camera, using a pinhole collimator. Biodistribution within the organs was performed 4h p.i.; blood sample was collected with heparinized syringe by heart puncture, the animals were sacrificed afterwards and other organs of interest (liver, kidneys, spleen, pancreas, adrenals, gut and muscle) extracted. The organs were weighed and radioactivity measured respectively in well-type gamma scintillation counter. The results were expressed as percentage of the injected dose per gram of the organ (% ID/g).

Results and Discussion

For the optimization of the labeling protocol, the radiochemical impurities obtained by ITLC-SG for the different wet labeling protocols were displayed (Table 1) and compared.

The proportion of free pertechnetate was low (0.53% for protocol 1 and 0.36% for protocol 2) as well as the $^{99m}$Tc-colloid impurities (3.4% and 2.46% respectively). Although the results for the radiochemical impurities were similar in both protocols, the 10 min. boiling water (protocol 2) was chosen as more convenient, as suggested by von von.

Table 1. Labeling conditions and radiochemical impurity of the wet labeled $^{99m}$Tc-EDDA/HYNIC-TOC

<table>
<thead>
<tr>
<th>Labeling conditions</th>
<th>$^{99m}$TcO$_4$ (MEK)</th>
<th>$^{99m}$Tc-RH (ACN 50%)</th>
<th>$^{99m}$Tc-non peptide bound (0.1N Citrate buff.)</th>
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<tbody>
<tr>
<td>70°C, 30 min.</td>
<td>0.53 ± 0.15</td>
<td>3.4 ± 0.72</td>
<td>5.4 ± 0.82</td>
</tr>
<tr>
<td>100°C, 10 min.</td>
<td>0.36 ± 0.11</td>
<td>2.46 ± 0.76</td>
<td>5.63 ± 1.5</td>
</tr>
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Fig. 1. Gamma camera images of $^{99m}$Tc-EDDA/HYNIC-TOC a) 15 min and b) 4h post injection into normal Wistar rats
Guggenberg et al (2003). The percentage of the overall radiolabeled impurities was less than 10%, accepted as upper limit and reported by other authors (Plachcinska et al., 2003; von Guggenberg et al., 2004; Melero et al., 2009). For the assessment of non-peptide bound impurities 0.9% NaCl was used elsewhere (Bangard et al. 2000; Plachcinska et al.2004). We obtained lower percentage for the above mentioned impurities with 0.9% NaCl (2.8% ±1.06) compared to 0.1 M Citrate buffer mobile phase. For more precise detection of peptide related side products, HPLC analysis with radiation detector would be preferable method, but it was not available in our laboratory during this study.

Animal biodistribution studies, integrated in our quality control protocol, were carried out with wet labeled kits. The results from the imaging study are presented in Fig. 1.

The radiopharmaceutical was cleared rapidly from the blood, as shown on the 15 min static image, and the most intense radioactivity was observed in the kidneys and bladder. The late, 4 h image confirmed the predominant renal uptake, as reported by Decristoforo et al. (1999). The proportion of the injected dose/g in the kidneys was 2.95% (see Fig. 2), and with lesser extent in the gut (1.16%) and the liver (1.15%). No activity was observed in the thyroid, which was in accordance with the low percentage of free 99mTc-pertechnetate impurity found by ITLC-SG quality control.

Although the wet labeling protocol was confirmed as efficient and reliable procedure for 99mTc labeling of HYNIC-TOC, it was more time and labor consuming in comparison with frozen kit labeling. Therefore, prior introducing the radiopharmaceutical in the clinical routine, we decided to produce a small kit batch. After the preparation of frozen kits, which were found sterile, their storage stability on -20 °C was tested within the period of 6 months. Protocol 2 was used for the radiolabeling, after adding 0,2N phosphate buffer to the mixture. The labeling yield during period observed (expressed as mean of duplicate assessment) is displayed on Fig. 3. The range of the radiolabeling yield was between 94.3% and 96.9%. After 6 months, no decrease of radiolabeling yield was observed.

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![Fig. 2. Biodistribution of 99mTc-EDDA/HYNIC-TOC in normal Wistar rats 4h.p.i (n=2)](image)

![Fig. 3. Storage stability of the frozen kit formulation up to 6 months post production (p.p.= immediately post production)](image)

![Fig. 4. Radiochromatograms (ITLC) of 99mTc-EDDA/HYNIC-TOC kit after 6 months storage at -20 °C: a) MEK (Rf=1) free 99mTc-pertechnetate; b) 50% ACN (Rf=0) 99mTc-colloid; c) 0.9% NaCl (Rf=1) 99mTc-non peptide bound coligand + free 99mTc-pertechnetate](image)
The radiochromatograms obtained from the ITLC strips for the three main radiochemical impurities (Fig. 4) illustrate the good quality of our radiopharmaceutical and indicate that frozen kit can be a safe alternative to the freeze-dried for small batch in house production.

We could not compare our stability findings with those published in the literature available, because they are mainly related to the in house wet labelling procedures (Meler et al., 2009; Pursuwan et al., 2010) or in house freeze dried kits (von Guggenberg et al., 2004; Gandomkar et al., 2006; Tasdelen, 2011). In house production of $^{99m}$Tc-EDDA/HYNIC-TOC is usually carried out within Coordinated Research Projects or Technical Cooperation Projects of IAEA in labs worldwide due to its restricted availability. The only commercially released kit so far is Tektrotyd from Radioisotope Centre POLATOM, Poland.

**Conclusion**

In house kit production, performed in compliance with GMP and GRP regulations within hospital premises is sometimes the only way to introduce novel radiopharmaceuticals, especially in developing countries. Following the proscribed and optimized procedures, we produced $^{99m}$Tc-EDDA/HYNIC-TOC, a specific SST receptor targeting agent which fulfilled the quality control criteria for radiochemical purity, sterility, normal biodistribution and stability. Our future activities will be focused on the clinical application and evaluation on selected group of patients.

**Acknowledgements**

This work was supported by IAEA Technical Co-operation Project MAK3/004 "Developing of 99mTc labeled peptides and monoclonal antibodies".

We wish to thank C. Decristophoro and E. von Guggenberg from the University Clinic for Nuclear Medicine, Innsbruck, Austria for sharing their experience in EDDA/HYNIC-TOC labeling, and preclinical and clinical evaluation of the radiopharmaceutical.

**References**


Резиме

“In house” производство на ⁹⁹ᵐTc-EDDA/HYNIC-TOC, специфичен насочувачки агенс за соматостатин - рецепторна сцинтиграфија

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Ключни зборови: радиофармацевтик, технециум-99m, тир3-октреотид, рецепторна сцинтиграфија, домашно производство, контрола на квалитет.

Употребата на радиообележени пептидни лиганди како дијагностички и терапевтични во нуклеарната онкологија е зголемена во последно време. Еден од најчесто користени радиофармацевтици е ⁹⁹ᵐTc-EDDA/HYNIC-TOC, соматостатински аналог кој специфично се врзува за одредени типови соматостатински рецептори со висок дензитет кај тумори од невроендокрино потекло. Раdioфармацевтикот не е широко комерцијално достапен и поради тоа го воведовме како “in house” производ, како дел од проект поддржан од Меѓународната агенција за атомска енергија (МААЕ). Во рамките на процедурите за предклиничка проценка, оптимиран е протоколот за радиообележување, произведена е мала серија китови, извршена е контрола на квалитет со инстант тенкослојна хроматографија и се направени биодистрибуциони студии. Процесот на радиообележување на пептидот, изведен со измена на ко-лиганди на температура од 100 °C за време од 10 мин., резултираше со радиохемиска чистота > 90%. Биодистрибуционите сцинтиграми кај нормални Wistar стаорци покажаа брз клиренс од циркулацијата по 15 мин. и доминантна акумулација во бубрезите по 4 часа, што е во согласност со податоците објавени од други автори. Беше проценета стабилноста на препаратот, проizведен во мала серија, на -20 °C при складирање за време од 6 месеци, при што ефикасноста на радиообележување се движеше од 94,3% до 96,9%. Нашите резултати покажаа дека смрзнатите китови можат да бидат сигурна алтернатива на лиофилизираните, во услови на домашно производство на мали серии, а по задоволителната предклиничка проценка ⁹⁹ᵐTc-EDDA/HYNIC-TOC може да биде воведен во клиничката практика како специфичен насочувачки агенс за соматостатин - рецепторна сцинтиграфија.