[^{99m}Tc(CO)$_3$] - BiotinNTA Derivative for Tumor Pretargeting

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1. Introduction

Tumor pretargeting using monoclonal antibodies in combination with biotin, labeled with radionuclides, could be useful for cancer diagnosis and treatment. [1]
Biotin nitrilrotriacetic acid (BiotinNTA) having functional groups of three –COOH was used as biotin derivative for antibody pretargeting strategies.
In this investigation, ^{99m}Tc(CO)$_3$-BiotinNTA was prepared to determine its potential use in antibody pretargeting strategies for tumor diagnosis.
^{99m}Tc is considered to be ideal radionuclides for the development of radioimaging of tumor. [2,3]
Radiolabeling of the BiotinNTA was performed in PBS buffer (pH=7.4) at 75°C, 30 min. The radiolabeling efficiency and radiochemical purity were analyzed by a reverse-phase high performance liquid chromatography (RP-HPLC).
The radiolabeled complex was tested *in vitro* for stability in plasma and biological affinity against avidin using magnetic particle seperator in PBS buffer (pH7.4).

2. Methods and Results

2-1. Preparation of[^{99m}Tc(CO)$_3$] Precursor

^{99m}Tc(CO)$_3$ was prepared directly from saline (0.9% NaCl/H_2O) in a closed vial.
Small amounts sodium boranocarbonate, sodium tetraborate·10H_2O, sodium titrate and sodium carbonate were used for preparation of kit vial. A HPLC trace is shown in Figure.1
Upon HPLC analysis, no remaining unreactive $^{99m}TcO_4^-$ was observed. This means that, $^{99m}TcO_4^-$ changed to ^{99m}Tc(CO)$_3$ completely.

2-2. Preparation of Radiolabeled ^{99m}Tc(CO)$_3$-BNTA

450 μLsolution of the BiotinNTA in PBS buffer (PH 7.4) were placed in a 10mL glass vial.
The concentrations ranged form from 10^{-3} to 10^{-7} M. The vial was sealed and flushed with nitrogen. For the radiolabling with ^{99m}Tc(CO$_3$) moiety, 50μL of [^{99m}Tc(CO$_3$)(H$_2$O)$_3$]$^+$ was injected into the prepared ligand vial followed by incubation at 75°C for 30min and cooled in ice bath. The radiolabeling efficiency and radiochemical purity were analyzed by a reverse-phase high performance liquid chromatography (RP-HPLC).

The radio-HPLC analysis of ^{99m}Tc(CO)$_3$-BNTA solution showed a single peak with retention time of 19 min. (Figure. 1)
The ligand concentration on the non-carrier-added level necessary to achieve a radiochemical purity >90 % (30 min reaction at 75 □) were reached at a concentration of 1.0×10^{-4} M.

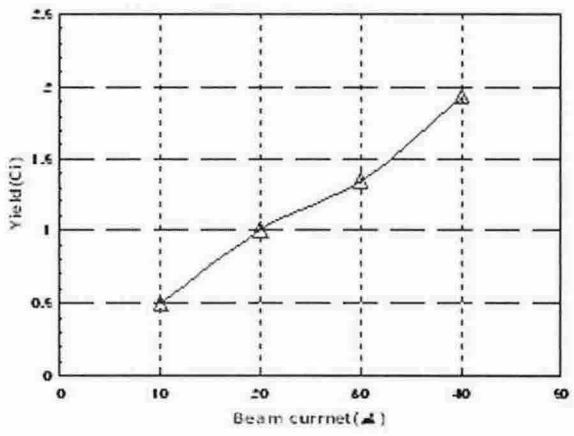

Figure. 1 HPLC trace of ^{99m}Tc(CO)$_3$ and ^{99m}Tc(CO)$_3$-BiotinNTA

2-3. Affinity of ^{99m}Tc(CO)$_3$-BNTA for Avidin and in Vitro Plasma Stability

An aliquot of the labeled biotin conjugate was added to avidin in a tip vial and incubated for 10 min. The mixture was separated avidin beads and solution by using magnetic particle separator.
The percentage of ^{99m}Tc-BiotinNTA bound to the avidin was calculated by dividing avidin bead radioactivity over the total activity of both solution fraction plus the avidin bead.
For measurement of the plasma stability of the ^{99m}Tc-BiotinNTA, the solutions of the complex was adjusted with physiological saline to a concentration of 37 MBq/mL. Aliquots of 25 μL of this solutions was added to 475 μL human plasma and incubated for 30 min.
The plasma stability of ^{99m}Tc(CO)$_3$-BNTA bound to the avidin is expressed as the percentage of the activity that was capable of specific binding to avidin after incubation for 2hr. The results of the in vitro stability of ^{99m}Tc-BNTA in human plasma showed that the complex was stable. Compound revealed a very good plasma stability (> 90 %, after 24 hr) and showed high affinity (> 90 %) when measured after 30 min incubation at 30℃

3. Conclusion

This $^{99m}Tc(CO)_3$-BiotinNTA can be applied for pretargeting strategies for tumor diagnosis. The promising results will establish the scaffold for the in vivo testing of this compound.

Reference

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