

Compounding of ^{99m}Tc -labeled antimicrobial peptide for molecular imaging of bacterial infections

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Introduction

In clinical practice it is often very challenging to discriminate bacterial infection in bone, soft tissue and orthopedic devices from sterile inflammation, due to the common unspecific symptoms. The most useful diagnostic procedures for detecting infection in such conditions are histopathology and imaging studies.

Nuclear Medicine imaging techniques have been widely used to detect functional, metabolic, as well as biochemical changes at molecular level. In vitro radiolabeled autologous leukocytes have been considered “gold standard” for visualization of suspecting infections (Palestro et al., 2009), especially in orthopedic surgery. Besides ^{99m}Tc and ^{111}In – labeled leukocytes, used as single photon emitting (SPECT) radiopharmaceuticals (RF), ^{18}F -FDG and recently ^{64}Cu as positron emitting tomography (PET) labels are introduced to improve the quality of leukocyte imaging studies (Rini and Palestro, 2006). Nevertheless, they have all been proved to be non-specific in discrimination between infection and sterile inflammation (Bunyaviroch et al., 2006).

Radiolabeled antimicrobial peptides (AMPs) are new generation of radiopharmaceuticals, developed to overcome the lack of specificity issue. One of these peptides is derivate (29-41 fraction) of ubiquicidine (UBI), a natural AMP found in all

mammal tissues, which specifically binds to bacterial membrane, enters the cell and remains within the cytoplasm. Our aim was to present the compounding of ^{99m}Tc -UBI (29-41) and quality control of the radiopharmaceutical, as primary step of product introduction in Nuclear Medicine Department settings.

Materials and methods

The study was carried out at the Instituto Nacional de Investigaciones Nucleares (ININ), Mexico. The ligand UBI (29-41), used for the kit production and the compounding process, was purchased from Bachem Bioscience Inc. Radiolabeling with ^{99m}Tc was performed by direct procedure, utilizing two protocols: freeze-dried kit formulation labeling (Ferro-Flores et al., 2005) and “in-situ” unit dose labeling (Ferro-Flores et al., 2003). ^{99m}Tc as sterile, apyrogenic $\text{Na}^{99m}\text{TcO}_4$ solution was obtained from GETEC $^{99}\text{Mo}/^{99m}\text{Tc}$ generator (ININ, Mexico).

^{99m}Tc UBI freeze-dried kit compounding

UBI freeze-dried kit is two vials commercial product of ININ (Mexico), containing 25 μg UBI (29-41) and 12,5 μg SnCl_2 in the first and 40 μL 0,1 M NaOH in the second vial. In brief, 370-740 MBq of $\text{Na}^{99m}\text{TcO}_4$ was added to the second vial and the alkalized label was afterwards transferred to the first

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vial. After incubation of 15 minutes, intermediate pH was measured. The volume was then adjusted to 3 mL with 0.9% saline and final pH measured.

^{99m}Tc UBI “in-situ” unit dose compounding

For the “in-situ” compounding we assessed three different formulations with constant amount of reducing agent and variable amounts of ligand and alkalizing agent. UBI aqueous solution (1 mg/200 µL), SnCl₂ solution (1 mg/mL) and 0.1 M NaOH had been prepared prior the compounding. Formulation 1 was compounded with 50 µL UBI, 25 µL SnCl₂ and 50 µL NaOH, followed by 100 µL of 370-740 MBq Na^{99m}TcO₄. Formulation 2 consisted of 50 µL UBI, 25 µL SnCl₂, 20 µL NaOH, and 100 µL of 370-740 MBq Na^{99m}TcO₄. For formulation 3 we used 25 µL UBI, 25 µL SnCl₂, 10 µL NaOH and 100 µL of 370-740 MBq Na^{99m}TcO₄. After 5 min. incubation, volume was adjusted to 3 mL with 0.9% saline. Intermediate and final pH was measured. Before patient administration, the RF should be membrane filtrated through 0.22 µm

Radiochemical purity was determined in both kit and “in-situ” RFs by instant thin layer chromatography (ITLC) on silica gel in 0.9% saline as mobile phase. Additionally, reverse phase HPLC on a C-18 column with radioactivity detector was used, as described (Ferro-Flores et al., 2003).

Results and discussion

Successful ^{99m}Tc-UBI compounding process and the stability of the RF are dependent of alkaline pH. During the ^{99m}Tc UBI freeze-dried kit compounding, the formation of radioactive complex occurred at pH 9. After adjustment with saline, final pH was 7.5. In all three “in-situ” compounded formulations the pH value, measured after incubation, was between 9 and 10. After obtaining the final volume, the pH remained within the same range.

Average radiochemical purity (RCP) of ^{99m}Tc UBI freeze-dried kit determined by ITLC was 97.3±1.1% (N=3) (Rf ^{99m}Tc-UBI = 0.0; Rf ^{99m}TcO₄⁻ = 1.0). RCP determined by HPLC (retention time of 12.5±0.5 minutes for ^{99m}Tc UBI) was 98.8±1.3% (N=3).

Quality control results obtained for the ^{99m}Tc UBI “in-situ” formulations reveal high RCP in all 3 RFs and had not been affected by the increased quantity of the ligand and alkalizing agent. RCP by ITLC and HPLC was 97.9-98.6% and 99.5-99.8% respectively.

Conclusion

^{99m}Tc UBI compounding was successfully performed with freeze-dried kit and “in-situ” formulations, both with high PCP. In Nuclear Medicine Department settings without GMP premises for in-house kit production, compounding of the RF could be performed in Shielded Biosafety LAF cabinet using “in-situ” unit dose formulations.

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