Glycomics and Lipidopics

Extended Abstract

Synthesis of Labeled Rifabutin Dithiocarbamate: A Potential *Mycobacterium Tuberculosis* Imaging Agent

Syed Qaiser Shah

Abstract:

In this investigation, Rifabutin dithiocarbamate (RFND) was labeled with Technetium-99m (99mTc) using tricarbonyl technique. The labeled RFND was further characterized in terms of radiochemical purity, stability in saline & serum, in vitro bacterial binding, biodistribution in animal model rats and for scintigraphic accuracy in animal model rabbit. Finally different radiobiological characteristics of the 99mTc(CO)3-RFND was compared with the recently reported 99mTc-RFN. It was observed that the dithiocarbamate form of RFN showed better radiochemical purity, stability in saline, bacterial binding, biodistribution and targeted imaging than the recently reported 99mTc-RFN. These better radiobiological parameters posed 99mTc(CO)3-RFND as a more reliable agent for tuberculosis imaging. In the last century, one of the major achievements of the scientists is the development of vaccines and antibiotics that up to a higher extent eliminated or managed majority of the infectious diseases. Besides such tremendous achievements in the diagnosis and treatment of infectious diseases, infection still remains the focus of investigators and even these days infection is believed to be the major cause of morbidity and mortality. Due to the advancement in clinical pathology, the infectious diseases can be detected through simple laboratory tests and successfully treated with appropriate drugs. However, it is observed that a major fraction of those infections resulting in death could be owing to conditions complicated to detect in its early stages. Early and in time detection in such situations could help in appropriate treatment and hence decrease the chances of death. Nuclear Medicine Scintigraphic Technology (NMST) provides a different alternative for localization of suspected bacterial infection due its higher sensitivity. In case of deep tissue infection, bone infection, acute life threatening infections which need early appropriate management e.g appendicitis, severe chronic infections occurring due to drug-resistance; and opportunistic infections in immune-compromised persons, one could take advantage of NMST. Such quarries like infection being there or not and its site, severity and potential cause could be answered by using NMST. However, to reply these quarries accurately, the prerequisite is a reliable radio-drug that can accumulate at the site of infection. The radio-drug intended for scintigraphy must answer the above mentioned quarries, 56but it shall not be toxic, show higher uptake in the target areas, low dose, and low cost easy availability.

The reported agents and its derivatives have shown promising specific target (infectious area) to non-target (non infectious area) ratios in its very early stages, besides normal circulatory and excretory behavior. However, the appearance of multidrug resistant bacteria's like *Mycobacterium tuberculosis* (MBT), is a serious threat for the clinicians to detect and manage such infections in its early time. In this scheme, labeling of Rifabutin dithiocarbamate (RFND) with ^{99m}Tc was examined using tricarbonyl technique. The feasibility of the tricarbonyl labeling procedure is based on the poorly attached H_2O of the $^{99m}Tc(OH_2)_3(CO)_3]^+$ precursor which can be easily substituted. The labeled RFND was further characterized in terms of radiochemical purity, stability in saline & serum, *in vitro* bacterial binding, biodistribution in animal model rats and for scintigraphic accuracy in animal model rabbit. Finally different radiobiological characteristics of the $^{99m}Tc(CO)_3$-RFND was compared with the recently reported ^{99m}Tc-RFN. Rifabutin (RFN) was obtained from Chengdu Yuyang High-Tech Developing Co., Ltd. China, and all chemicals & solvents from Sigma. In this work HPLC of Shimadzu, well counter of Ludlum, Dose calibrator of Capintech and Gamma camera of Nuclearmedizine, were used. Rifabutin (RFN) was derivatized to Rifabutin dithiocarbamate (RFND) using the method reported earlier[28]. Briefly, 0.002 mol of RFND and 2.4 mg of NaOH were mixed in a clean sterilized vial. Thereafter, 22 ml of tetrahydrofuran (THF) was added to the reaction vial followed by shaking for 30 min in an ice bath. Then, 2 ml carbon disulfide (CS_2) was added and left the reaction vial for 8 h in an ice bath for continuous shaking. After that the mixture was processed for continuous stirring up to 12 h at room temperature followed by recovery through re-crystallization. The RFND was characterized by advance spectroscopic techniques. Synthesis of 99mTc(CO)3-RFND & Radiochemical purity Sodium pertechnetate 1 mCi (0.2 ml) was mixed with 2 mg (dissolved in 0.4 ml normal saline) of RFND followed by pH adjustment (pH 10) using 0.1 mol / L HCL in a clean nitrogen gas filled sterilized vial. Thereafter, the mixture was transferred to an Isolink kit followed by incubation for optimum labeling at 25 ˚C for 15 min. High-performance liquid chromatography (HPLC) was used to

Syed Qaiser Shah

Biochemistry Section, Institute of Chemical Sciences, University of Peshawar, Peshawar, KPK, Pakistan

characterize $^{99m}Tc(CO)_3$-RFND in terms of radiochemical purity by the method reported earlier. Briefly, 10 µL of $^{99m}Tc(CO)_3$-RFND was administered to the HPLC system fitted with UV detector operating at 254, and a flow scintillation counter, C-18 column and binary pump. Thereafter, for 15 min, a flow rate of 1 ml / min the column was eluted with water and methanol (W:M). The effluent was collected in separate vials followed by counting for activity. Mycobacterium Tuberculosis (MBT) Uptake MBT uptake of $^{99m}Tc(CO)_3$-RFND was assessed adopting the method reported earlier. Briefly, 0.8 mL acaetic acid (0.01 M) containing approximately 1 x 10^8 colony forming units (CFU) of MBT and 0.2 mL sodium phosphate buffer was incubated at 4 °C for 60 min. The mixture was centrifuged at 1500 rpm for 15 min and after removing the supernatant was re-centrifuged after suspending in 1.5 mL sodium phosphate buffer. Subsequently, the supernatant was removed again and the bacterial pellets were counted for activity. The percent *in vivo* uptake of the $^{99m}Tc(CO)_3$-RFND was assessed in healthy and artificially infected animal model rats. The animal was divided into two groups i.e. A and B. To group A animals, approximately 1 x 10^8Colony Forming Units (CFU) in 0.2 mL saline MBT was intramuscularly injected into the left leg of the anaesthetized rat Sprague-Dawley rat (weight range, 200–250g) for creating infection. After eight hours, equimolar amount of sterile oil was injected to the right leg of the same rat for creating inflammation followed by intravenous admission of 0.5 mCi $^{99m}Tc(CO)_3$-RFND. To group B animals, the above process was repeated without administration of MBT. Thereafter, the rats were sacrificed at different intervals after intravenous injection of radio-drug as per procedure of the Pakistan Nuclear Regulatory Authority (PNRA), Ethics Committee, Pakistan Atomic Energy Commission (PAEC). Thereafter, % *in vivo* uptake of the $^{99m}Tc(CO)_3$-RFND in one gram of blood, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle was measured using gamma counter. Imaging with 99mTc(CO)3-RFND Healthy rabbits (weight: 3.0 to 4.0 kg) were used in the assessment of imaging profile of the instant radio-drug. 0.5 mL MBT containing 1 x 10^8CFU was injected to the left leg of the healthy rabbit and after 08 h, to the right leg of the same rabbit 0.5 mL sterile oil was injected. Finally, the rabbit was placed face up on the bed of the gamma camera followed by intravenous injection of 2 mCi $^{99m}Tc(CO)_3$-RFND. Whole body images were acquired using Low Energy General Purpose (LEGP) collimator at different intervals.

Syed Qaiser Shah

Biochemistry Section, Institute of Chemical Sciences, University of Peshawar, Peshawar, KPK, Pakistan