New approaches for imaging bacteria and neutrophils for detection of occult infections

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Inflammatory and infectious diseases nowadays represent, still, a serious and significant cause of morbidity and mortality worldwide. For these diseases, rapid and accurate diagnosis plays a pivotal role for early therapeutic interventions and to discriminate infections inflammatory conditions. Indeed, the identification of bacterial cells in infections would be of great help for both therapeutic and diagnostic approaches, especially with the development of novel and tailored therapies.
In this scenario, there is a need of a non-invasive strategies to specifically localise bacterial or immune cells involved in infectious and inflammatory diseases, before planning the best treatment option for patients. This strategy would allow to save time, reduce costs and side- effects due to non-appropriate therapy. Nuclear molecular imaging offers a plethora of radiopharmaceuticals for imaging bacteria and immune cells at preclinical level, with possible translation to clinics. In this thesis, we present new and updated approaches to image infections as alternatives to currently available radiopharmaceuticals.

For bacterial infections, many compounds like antibiotics, peptides and sugars were radiolabelled with several isotopes for SPECT and PET imaging, but very few have been translated to humans. We provide an extensive overview regarding the available radiopharmaceuticals for bacterial imaging and related concerns that limit their use in humans (e.g. the lack of specific mechanisms of binding of these compounds, the lack of standardised animal models and the lack of structured guidelines).

In order to overcome these limits our first approach consists of targeting *E. coli* and *S. aureus* using an innovative infection mouse model and a new radiopharmaceuticalin comparision to three well-known and perfoming *in vitro* and *in vivo* studies. The mouse model is a Teflon cage implanted subcutaneously that allows to have a reproducible, localised and persistent infection. A ciprofloxacin derivative, ciprofloxacin dithiocarbamate (CiproCS2), was radiolabelled with ^{99m}Tc using a specific kit containing the antibiotic, succinic acid dihydrazide (SDH), ethylenediaminetetraacetic acid (EDTA), SnCl2, phosphate buffer and γ-cyclodextrin. *In vitro* studies showed a rapid but poor bacterial binding for all tested radiopharmaceuticals, whereas *in vivo* studies displayed higher infected cage/sterile cage ratio for ^{99m}Tc-UBI and ^{99m}Tc-CiproCS2 at 24 h for both *E. coli* and *S. aureus*. However, none of the tested radiopharmaceuticals seemed to be promising for discrimination of infected sites, whose accumulation still remains to be clarified.

The same strategy was applied in the subsequent study that described the radiolabelling of an antibiotic that specifically binds to lipid A of lypopolisaccharide (LPS) of Gram-negative bacteria, acting as an antimicrobial peptide. The antibiotic is polymyxin B (PMB) that is conjugated with HYNIC and radiolabelled with ^{99m}Tc, obtaining an excellent labelling efficiency and specific activity. *In vitro* studies were performed using several bacterial strains, both Gram-negative and Gram-positive as control, showing higher binding of ^{99m}Tc-HYNIC-PMB for Gram-negative bacteria than control. In the current animal model, infection was induced by the injection of three different amounts of bacteria plus Matrigel in the right thigh in comparison to only Matrigel in the contralateral thigh and imaging was performed at 1, 3 and 6 h post injection of ^{99m}Tc-HYNIC-PMB, measuring target-to-backgroud (T/B) ratio. Results showed higher T/B ratios for Gram-negative bacteria than Gram-positive controls in slightly time and bacterial amount-dependant manner, suggesting ^{99m}Tc-HYNIC-PMB as potential agent for selective diagnosis of Gram-negative infections.

In clinical practice, radiolabelled autologous white blood cells (WBCs) are the nuclear medicine gold standard for the diagnosis of infections and inflammatory disorders. They can be labelled with ^{111}In-oxine or ^{99m}Tc-HMPAO, that are able to enter into cells' cytoplasm and radiolabel cells. We tested a new chelating agent, $(S_3CPh)_2$ (S_2CPh)-complex (SSS-complex), with the aim to improve the radiochemical purity, the binding selectivity for leukocytes and its binding kinetics. The radiolabelling procedure of SSS-complex involved the use of a technetium-99m reducing kit, showing high labelling efficiency (>95%) and stable in both human serum and saline up to 24 h. Despite of high labelling efficiency to cells, ^{99m}Tc-SSS-complex showed no specific selectivity for any particular cell subset as well as a faster washout from cells than ^{99m}Tc-HMPAO. From our data, it appears that ^{99m}Tc-SSS-complex cannot be considered a valid alternative to ^{99m}Tc-HMPAO for *in vivo* imaging agent.

The procedure to label WBCs is highly time expensive and presents a high risk of cell contamination. Indeed, the Leukokit® has been developed to simplify the process. It is a commercial and disposable sterile kit, containing poly(O-2-hydroxyethyl)starch (HAES-steril 10%, HES) as a sedimentation agent to remove erythrocytes (RBCs) from WBCs. Currently, HES is not commercially available anymore and Gelofusine has been proposed as substitute sedimentation agent. Hence, we compared the labelling efficacy and the diagnostic accuracy of WBCs labelled with Leukokit® containing HES and Leukokit® containing Gelofusine. In particular, we evaluated the number and type of recovered WBCs, red blood cells (RBCs) contamination, platelets (PLTs) contamination, vitality of neutrophils, and chemotactic properties of neutrophils. Secondly, we performed a clinical comparison in terms of radiolabelling efficiency, final recovery yield and diagnostic outcome in patients affected by prosthetic joint infections, peripheral bone osteomyelitis, or vascular graft infection. Results showed a lower RBCs and PLTs contamination for HES rather than Gelofusine, but higher WBCs recovery for Gelofusine and same chemotactic properties independently from the used sedimentation agent. Then, clinical analysis reported no significantly different sensitivity, specificity and accuracy for WBCs labelled with both agents, suggesting Gelofusine as suitable substitute of HES for WBCs separation and labelling.

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