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

Auletta, Sveva

DOI:
10.33612/diss.131946200

Chapter 9

General conclusions and future perspectives

Given the high mortality and morbidity rate of infectious diseases worldwide due to increasing antibiotic resistance, their early diagnosis is of crucial importance for clinical practice as well as the discrimination between septic and sterile inflammatory processes. Currently, in clinics the scintigraphy with radiolabelled autologous white blood cells (WBC) is routinely used for imaging of infectious pathologies, but, being undirect imaging of infection, it does not allow to distinguish aseptic process from inflammation caused by the presence of bacteria. Novel attractive strategies have been and are being investigated with the goal to directly target the pathogen, exploiting several bacteria characteristics or molecules' mechanisms of action, and at the same time to have a better accurate imaging agent for diagnosis and therapy follow-up. However, very few radiopharmaceuticals that seemed to be promising in preclinical setting are available in clinical practice. This lack of bacteria-specific radiopharmaceuticals in humans is related to several issues to be considered. Among main critical factors, we might include the bacterial cells properties that can be localized, dispersed or in the form of biofilm, the bacterial mutations occurring over time, the amount and location of bacteria, if intracellular or extracellular that influence and drive the choice of molecule to radiolabel. Furthermore, other issues concern the radiopharmaceutical such as the injected dose and after how long it is injected, the imaging time points, the pharmacokinetic and pharmacodynamic, the biological activity, the a-pyrogenicity, the effect on bacterial vitality and functions.

Indeed, different approaches have been developed for bacterial imaging, if target-driven (bacterial metabolism, antimicrobial compounds, cell wall components, iron metabolism), if species-driven (Gram-negative, Gram-positive, others), if isotope-driven (γ or β emission), but, whatever approach is being considered, there are pros and cons in each one. In addition, in the literature, there are numerous published studies on bacterial imaging that used several compounds such as amino sugars, siderophores, peptides, antibiotics. Some of these also obtained promising and encouraging results, but also considerably heterogenous in bacterial strain, infection animal model, isotope, compound and time flow of the study.

In the present thesis, we proposed two different approaches to improve the management of infectious diseases. Firstly, we radiolabelled with ^{99m}Tc two compounds, ciprofloxacin dithiocarbamate and polymyxin B, to directly image bacteria in two mouse models of infection, respectively the Teflon cage and "Matrigel-mediated" infection. Secondly, we tried to get better the radiolabelling procedure of WBC that is intensively laborious and time consuming. We tested an alternative of HMPAO, called SSS-complex, to radiolabel WBC, but it cannot be considered a valid substitute. Then, it was strictly necessary to find an alternative of HES, the sedimentation agent into the Leukokit®, for commercial needs. Therefore, we tested Gelofusine as new sedimentation agent that, then, have been replaced HES, also apporting better results in terms of overall diagnostic accuracy.

This thesis highlights the difficulty of accurately localize bacteria during infective process and confirms scintigraphy with radiolabelled WBC as the main diagnostic tool in clinical routine. In the meantime, the radiolabelling of WBC with PET isotopes (e.g. ^{89}Zr, ^{64}Cu) is currently under investigation with the aim to improve the diagnostic performance of radiolabelled WBC. The PET imaging has higher spatial resolution and sensitivity than SPECT technique, surely improving the quantitative evaluation of radioactive signal from pathological tissues in infectious diseases. However, some concerns have to be taken into account after the radiolabelling procedure, such as the

following: the resulted labelling efficiency and specific activity, if the biological functions and chemotactic properties of WBC are preserved, if the used PET isotope is toxic for cells, the binding kinetics and the efflux rate from the cells.

At the moment, imaging bacteria is still a difficult and challenging task because an ideal bacteria-specific imaging agent should be highly sensitive and specific, quantitative, rapid, stable, safe and easily manufacturable as well as characterized by specific biochemical properties (e.g. lipophilicity, metabolic stability, low binding to plasma proteins).

Surely, having standardized protocols, guidelines and consensus documents would allow a better comparison between preclinical studies and, eventually, a prediction of results in humans. Being able to specifically image bacteria would allow to identify the responsive cause of an infection with the goal to improve the management, therapy decision-making and follow-up of patients with suspected infectious diseases. Indeed, the early detection of the specific pathogen responsible of that infection is still an open challenge, but it would be able to guide the choice of the most appropriate antibiotic therapy for each patient.