Vancomycin-based tracers for bacteria-targeted imaging of infection

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November 20, 2024
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In this thesis, novel vancomycin-based PET tracers were developed and characterized to improve bacteria-targeted imaging of (Gram-positive) infections. The research was conducted using various *in vitro, in vitro,* and *post mortem* models of infection.

Bacterial infections remain a serious challenge in most fields of medicine and they are responsible for major patient morbidity and mortality (1), and healthcare costs. Fracture-related infections (FRIs), specifically, occur in between 1% and 30% of all trauma patients (2). These infections were found to cause a fivefold to tenfold prolonged hospital stay compared to patients who do not suffer from an infection. There are a number of factors contributing to the occurrence of bacterial infections in the hospital environment, such as the increasingly aging population, an increase in elective surgeries and use of biomaterials, including orthopaedic implants for fracture care, such as osteosynthesis plates, nails, and joint prosthesis. With advances in healthcare, there is also an increasing population of immunocompromised patients, who are especially vulnerable in the context of bacterial infections. The rise of antimicrobial resistance and the limited clinical introduction of new antimicrobials are further complicating patient care. Importantly, in order to provide optimal care for patients who are suspected of having a bacterial infection, faster and more accurate diagnostic tools are vitally important. The consequences of a missed bacterial infection can be dire, resulting at best in prolonged hospitalisation, but potentially also loss of limbs, sepsis or even death.

Currently, culturing is performed on samples collected from patients in order to reach a diagnosis. However, the performance of these culture-based approaches suffers from a number of limitations, such as false negative culture results due to prior antibiotics usage, insufficient blood or tissue collection or sample processing delays (3). Even after a positive culture, it can take a few more days before information regarding identity and susceptibility of the microorganism are available. Contamination with other bacteria or microorganisms, during sample collection or during culturing, can misdirect clinical diagnosis, and give rise to inadequate, or unnecessary, treatment regimens (4).

[^{18}F]FDG has found its place in infection imaging, where it can be used to differentiate between normal tissue and infected or inflamed tissue. However, it is precisely this inability to differentiate between infection and inflammation, underscoring the need for bacteria-targeted tracers.

The main aim of the research described in this PhD thesis was to develop and characterise novel ^{18}F-vancomycin-based PET tracers for bacteria-specific detection of infections. Three tracers ([^{18}F]FB-vancomycin, [^{18}F]BODIPY-FL-vancomycin, and [^{18}F]PQ-VE1-vancomycin, figure 1) were developed. The syntheses were initially explored manually, and after having established the labelling protocols, adapted for automated labelling on an Eckert & Ziegler Modular-Lab PharmTracer synthesis module. In characterising the tracers, it was found that [^{18}F]PQ-VE1 was conjugated to the primary amine of vancomycin, whilst [^{18}F]FB and [^{18}F]BODIPY-FL were both found to be conjugated to the secondary amine of vancomycin. Synthesis times and molar activities were determined for all three tracers. Importantly, the conjugation site did not appear to influence the stability of the tracers. Both [^{18}F]PQ-VE1-vancomycin and [^{18}F]BODIPY-FL-vancomycin were stable in both phosphate-buffered saline (PBS) and human serum, whilst [^{18}F]FB-vancomycin was highly unstable in human serum and used *S. aureus* growth medium. Since [^{18}F]FB-vancomycin was unstable, this tracer was not used in the *in vitro* binding experiments. Both [^{18}F]PQ-VE1-vancomycin and

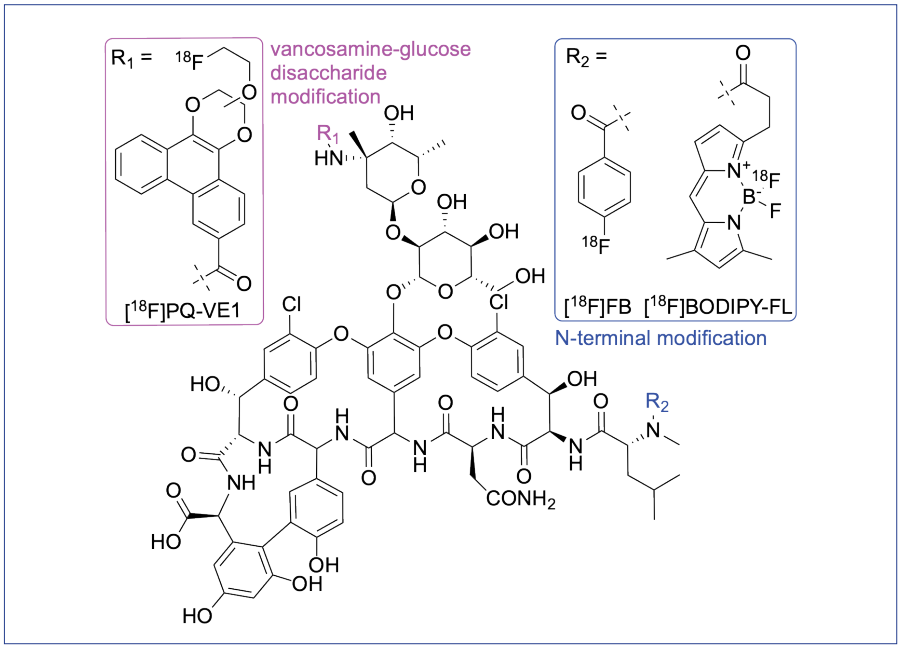

Figure 1. Chemical structures of vancomycin, [18F]PQ-VE1, [18F]FB, and [18F]BODIPY-FL. Figure from (5).

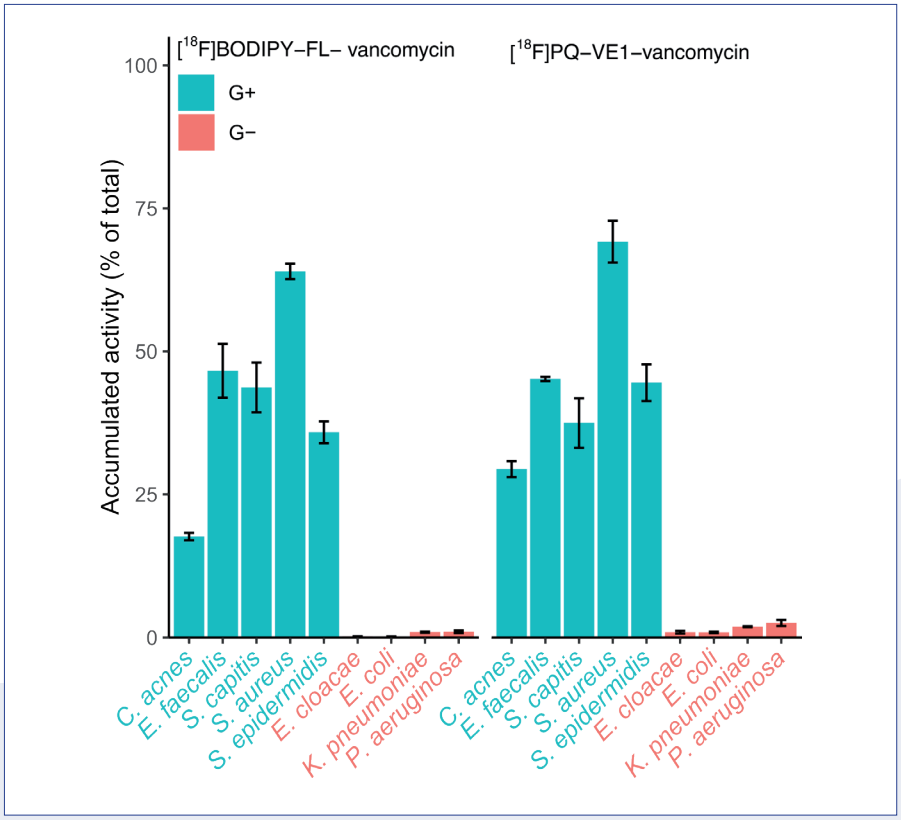

Figure 2. Accumulation of [18F]PQ-VE1-vancomycin and [18F]BODIPY-FL-vancomycin at Gram-positive strains (green) but not at Gram-negative strains (red). Figure adapted from (5).

[18F]BODIPY-FL-vancomycin showed strong affinity towards Gram-positive bacteria *in vitro* (figure 2), with the highest observed affinity for *Staphylococcus aureus (S. aureus)* (a species frequently associated with prosthetic joint infections (PJIs) and FRIs). In an effort to negate the binding of the 18F-labelled PET tracers, samples co-incubated with unlabelled vancomycin showed a decreasing accumulation at higher concentrations of unlabelled vancomycin. As these tracers have an antibiotic backbone, the minimum inhibitory concentration (MIC) was determined. This showed that the conjugation of either BODIPY-FL-vancomycin or PQ-VE1-vancomycin results in an increase of mass required for inhibition, compared to vancomycin. Lastly, the biodistribution of all three tracers was determined. [18F]FB-vancomycin, again, showed rapid degradation, with excretion of the degradation products through the kidneys and bladder. [18F]BODIPY-FL-vancomycin showed predominantly renal clearance, whilst [18F]PQ-VE1-vancomycin showed a distinct hepatobiliary component, and an overall slower clearance compared to [18F]BODIPY-FL-vancomycin.

The potential applications of [18F]PQ-VE1-vancomycin and the fluorescent tracer vancomycin-IRDye800CW (NIR fluorescent vancomycin tracer) were explored in a human *post mortem* infection model. Osteosynthesis plates, partly covered with a *Staphylococcus epidermidis* biofilm were incubated with either [18F]PQ-VE1-vancomycin or vancomycin-IRDye800CW, both tracers, or PBS, and placed on the femora and tibiae. Subsequently, imaging was performed, with either a NIR fluorescence camera, or a clinical PET/CT camera. Surgical debridement was performed, following the clinical routine. After the debridement, imaging was repeated. This showed that [18F]PQ-VE1-vancomycin was able

to differentiate between the biofilm-covered side and the non-biofilm-covered side of the osteosynthesis plates. Furthermore, vancomycin-IRDye800CW allowed visualisation of the extent of the debridement, in real-time.

[^{18}F]BODIPY-FL-vancomycin and [^{18}F]PQ-VE1-vancomycin were used in two different murine model of infection. Myositis, an infection of the hind leg quadriceps muscle, was induced by injection of the Gram-positive bacterium (*S. aureus* Xen36), or the Gram-negative bacterium *(Escherichia coli*). Alternatively, sterile Cytodex beads were injected to induce sterile inflammation. Sterile PBS was used as control. Forty-eight hours after injection of the inoculum, the animals were injected with either PET tracer, or [^{18}F]FDG as control, and imaged in a microPET camera. The PET data showed that both [^{18}F]BODIPY-FL-vancomycin and [^{18}F]PQ-VE1-vancomycin were able to effectively differentiate between infection and inflammation. Microscopy of hind leg muscle tissue confirmed the presence of (the respective) bacteria.
The second model used was a murine model of bone- and joint infections. Unlike the myositis infection model, the Kirschner-wire infection model more closely resembles the often more chronic nature of low-grade infection seen in patients suffering from FRI and PJI. In this model, a stainless-steel Kirschner-wire was placed in the distal femur of mice. The surgical site was subsequently inoculated with bacteria to induce infection, or with PBS to induce sterile inflammation. The infection was monitored for up to fourteen days, and subsequently, the animals were injected with [^{18}F]BODIPY-FL-vancomycin, [^{18}F]PQ-VE1-vancomycin, or [^{18}F]FDG as control in the PET-arm of the study. In parallel, animals were injected with the fluorescent tracer vancomycin-IRDye800CW. As expected, [^{18}F]FDG showed uptake under all conditions, including the unaffected contralateral hind leg. [^{18}F]BODIPY-FL-vancomycin and [^{18}F]PQ-VE1-vancomycin showed the highest accumulation at the site of the Gram-positive bacterial infection (*S. aureus*). Using vancomycin-IRDye800CW, distinct accumulation at the site of Gram-positive infection was observed. Comparatively, vancomycin-IRDye800CW also showed the highest target/non-target ratios.

In this thesis it is shown that vancomycin as bacteria-targeting agent holds great promise for infection imaging, enabling the identification of Gram-positive bacterial infections by either fluorescence or PET imaging. Utilising the specificity of vancomycin towards Gram-positive bacteria, we successfully synthesised and characterised three potential tracers. The promising results obtained with [^{18}F]BODIPY-FL-vancomycin and [^{18}F]PQ-VE1-vancomycin in *in vitro*, *in vivo* and *post mortem* models of infection underscore the potential of vancomycin-based PET in diagnosing infections that are otherwise difficult to diagnose with current modalities. It will be really exciting to see how vancomycin-based PET imaging will develop in the coming years towards clinical translation including its role in supporting trauma surgeons treating fracture-related infections. ♦

References

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