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Potential use of ^{68}Ga-*apo*-transferrin as a PET imaging agent for detecting *Staphylococcus aureus* infection

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Abstract

Introduction: ^{67}Ga citrate has been extensively used to detect infection and inflammation since 1971. However, its clinical utility is compromised due to several limitations. The present project explored whether ^{68}Ga-*apo*-transferrin (^{68}Ga-TF), when prepared in vitro, is a useful agent for positron emission tomography (PET) imaging of bacterial infection.
Methods: An infection was induced in male Wistar rats by injecting 5×10^5 CFU units of *Staphyococcus aureus* in the right thigh muscle. ^{68}Ga-TF was synthesized by mixing $^{68}GaCl_3$ with *apo*-transferrin (TF, 2 mg) in sodium carbonate (0.1 M, pH 7.0) and incubating at 40°C for 1 h. Animals were injected with 10–15 MBq of ^{68}Ga-TF containing approximately 0.2 mg TF and imaged at different time intervals using Siemens Biograph PET-CT.
Results: When ^{68}Ga-TF were injected in the infected rats, the infection lesion was detectable within 20 min post injection. The biodistribution showed the uptake at the lesion increased with time as shown by significantly increased standard uptake values for up to 4 h post injection. There was a considerable decrease in the background activity during the same period of study, giving higher target-to-muscle ratios. Blood pool activity at 3 h post injection was insignificant. $^{68}GaCl_3$ (when not conjugated to TF) did not localize at the infection lesion up to 120 min post injection.
Conclusion: The preliminary results suggest that ^{68}Ga-TF is capable of detecting *S. aureus* infection in the rat model, within an hour after intravenous injection.

Keywords: Infection imaging; *Staphyococcus aureus*; ^{68}Ga-*apo*-transferrin; *^{68}Ga-PET*

1. Introduction

^{67}Ga citrate has been extensively used to detect malignant tumors and infection/inflammation since 1971 [1–5]. However, the clinical utility of this agent is compromised due to several limitations such as poor image quality of ^{67}Ga-single photon emission tomography (SPET), high background activity and interference from liver and bowel activity, delayed post-injection waiting times of at least 24–48 h, the associated high radiation exposure and high cost of the radionuclide.

It is well established that transferrin (TF) is a glycoprotein with high affinity for binding iron on its domain [6], which maintains low free iron levels in serum. Restriction of iron availability in the body plays an important role in host defense [7] because iron is indispensable for the growth of most bacteria. The quantity of free iron in the body fluid of most of living species is below 10^{-12} μM, which is much lower than required for bacterial growth [7]. Therefore, it is mandatory when bacterial pathogens have to survive and proliferate in serum, they have to acquire iron from transferrin via siderophore-mediated or transferrin-binding protein (TBP)-mediated mechanisms or both [8,9]. *Staphyococcus aureus* has been shown to produce a siderophore

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and TBP to assimilate free iron from human transferrin-bound iron [10]. The available evidence clearly established a regulatory role for TF in maintaining low serum iron levels, and consequent control in the growth of bacteria in general including *S. aureus* The capability of TF to bind Ga^{3+} is similar to iron-binding mechanisms.

In the past, ^{67}Ga citrate SPET has been widely used to image bacterial infection, but had the limitation of 48-h post-injection waiting time. We hypothesize that the delayed imaging time associated with Ga^{3+}-citrate imaging might be attributed to longer transfer time required for Ga^{3+} to plasma TF in vivo. This study is focused with the aim to prepare $^{68}Ga^{3+}$-TF complex in vitro and examine if this new PET tracer is capable of detecting *S. aureus* infection. It is important to establish a faster imaging method for ^{68}Ga as its half-life is 68 min compared to 78.3 h for ^{67}Ga. Therefore, the outcome of the project will establish the potential of $^{68}Ga^{3+}$-TF to detect bacterial infection.

Although ^{68}Ga-PET was described in 1960s for tumor uptake studies, the chemistry and radiopharmacy of ^{68}Ge/^{68}Ga generator have been investigated only in the late 1970s [11,12]. Since 2000 "cationic" generators are available commercially, and a sophisticated on-line eluate post-processing was established [13]. In parallel, the ^{68}Ga radiopharmaceutical chemistry was reintroduced over the last decade. Recent studies indicated a possible role for ^{68}Ga chloride to monitor bone healing in experimental osteomyelitis [14] and in detecting pancreatic adenocarcinoma xenografts in rats [15]. These studies had several limitations and have shown low standard uptake values (SUV_{max}) even up to the 2 h post-injection time period. Preliminary report on the preparation of ^{68}Ga citrate and its use in infection imaging has been published by Rizzolo et al. [16] but their results showed low grade uptake at the lesion.

The major aim of this project is to explore if ^{68}Ga-TF complex is capable of detecting *Staphylococcus aureus* infection in a rat model. Preliminary results were presented at the annual conference of European Association of Nuclear Medicine [17].

2. Materials and methods

2.1. Materials

All chemicals, including human TF, were obtained from Sigma-Aldrich, St. Louis, MO, USA. The ^{68}Ge/^{68}Ga generator was obtained from Cyclotron, Obninsk, Russia. Cation exchanger resin AG 50W-X8 (400 mesh) was supplied by Prof. Roesch, University of Mainz, Germany.

2.2. Post-processing of ^{68}Ge/^{68}Ga generator

Pure ^{68}Ga was obtained by the on-line post-processing method described by Zhernosekov et al [13]. Briefly, the generator was eluted with 8 ml of 0.1 M HCl and the contents were bound to a cationic exchange resin. The resin was then washed with 1.0 ml solution N1, containing 0.15 M HCl in 80% acetone to remove impurities (Ti, Zn, Fe and any residual traces of ^{68}Ge), which was then discarded. Pure ^{68}Ga fraction was eluted with 0.4 ml solution N2, containing 0.05 M HCl in 98% acetone. The contents are collected into the reagent vial (10 ml Amersham vial) containing 0.25 ml of sterile water. For solvent elimination, the reagent vial was boiled for 4 min. The post-processed solution contained pure $^{68}GaCl_3$.

2.3. Synthesis of ^{68}Ga-TF

^{68}Ga-TF was prepared by mixing the post-processed pure $^{68}GaCl_3$ (180 MBq/0.5 ml) with a solution containing 2.0 mg of TF in 1.5 ml of sodium carbonate (0.1 M, pH 7.0). The reaction vial was incubated in the water bath at 40°C for 1 h. TF used in the experiments was capable of binding up to 1.0 GBq of $^{68}GaCl_3$ under the reaction conditions described. In preparing control solution, ^{68}Ga-TF was prepared under identical conditions, except saline was used instead of sodium carbonate solution. The solutions were sterilized for animal injection by final filtering with 0.22-μm filter.

2.4. Determination of radiochemical purity (RCP)

The RCP of ^{68}Ga-TF was determined by TLC/alumina chromatography, using sodium citrate (0.1 M) as the solvent (free ^{68}Ga, R_f=1 and bound ^{68}Ga, R_f=0). Stability study was performed by mixing 0.3 ml of ^{68}Ga-TF solution with 1.5 ml of human serum at 37°C. RCP was measured at 1-h interval.

2.5. Animal model

Male Wister rats (7 weeks old weighing 250–300 g) were anesthetized by intraperitoneal injection of the solution containing ketamine (100 mg/ml)/xylazine (20 mg/ml) (2:1 v/v). Infection was induced in the right thigh muscle by intramuscular injection of 5×10^5 CFU *S. aureus*, in 0.2 ml volume. Three to four days after inoculation, an abscess formed. The experimental protocol was approved by the Westmead Animal Research Ethics Committee, Westmead Hospital, Sydney West Area Health Services.

2.6. Dosage and PET imaging

Between 10 and 15 MBq of ^{68}Ga-TF (containing 0.2 mg TF) was injected intravenously in the tail vein of rats (n=5). The rats were then imaged at different time intervals (5, 30, 60, 120, 180 and 240 min post injection), and the images were acquired for 10 min each with the matrix size of 256×256 using Siemens HiRez Biograph PET-CT 16 (Siemens Medical Systems). SUV_{max} was calculated over the bacterial lesion area and over different organs by drawing regions of interest by a computer generated program, which is used routinely in our department.

3. Results

Radiochemical purity (RCP) of ^{68}Ga-TF in the carbonate medium was >99% and the yield was also >99%, as all the ^{68}Ga-activity was bound to TF. Stability study as shown by the RCP measured at 1 h interval after preparation, were >99% for 6 h without loss in the percentage of RCP. The stability was not tested beyond 6 h, as the $T_{1/2}$ of ^{68}Ga is 68 min only. Interestingly, when ^{68}Ga-TF was prepared in saline instead of carbonate medium, the resultant complex formation was very low (RCP <15%).

3.1. ^{68}Ga-TF PET for imaging S. aureus infection

When ^{68}Ga-TF (10–15 MBq/0.2 ml) was injected into the infected rats, the lesion was detectable within 20 min post injection, although intense, focal uptake was seen only after 50 min post injection (Fig. 1A). The accumulation of activity at the lesion increased with time as shown by significantly increased SUV_{max} at the target from 20 min for up to 4 h post injection (Fig. 1B). There was a considerable decrease in the cardiac activity during the initial 90 min post-injection period, but a small percentage of activity was measurable for up to 4 h post injection of the study. The activity associated with liver and bowel decreased rapidly up to 90 min of the post-injection period. The low level of activity then persisted for up to 4 h post-injection time. There was marginal increase in the activity associated with the spine over the entire period of the study, whereas muscular uptake increased marginally during the initial 60 min post-injection time but then decreased steadily over the period of the study. Since the background activity was washed out in a time dependent manner, the ratio of tumor to muscle increased progressively over the entire period of the study from 2.2 to 7.5 from 10 to 4 h post injection (Fig. 1B).

3.2. ^{68}Ga-TF PET for imaging S. aureus and Proteus mirabilis infection

The results in one particular rat showed an avid uptake of the agent, as expected at the site corresponding to *S. aureus* -induced infection in the right thigh. It also showed an additional lesion, which was clearly defined intense focal uptake in the lower abdominal area, which was persistent during the entire period of study (Fig. 2). Physical examination of the additional lesion site indicated to be an

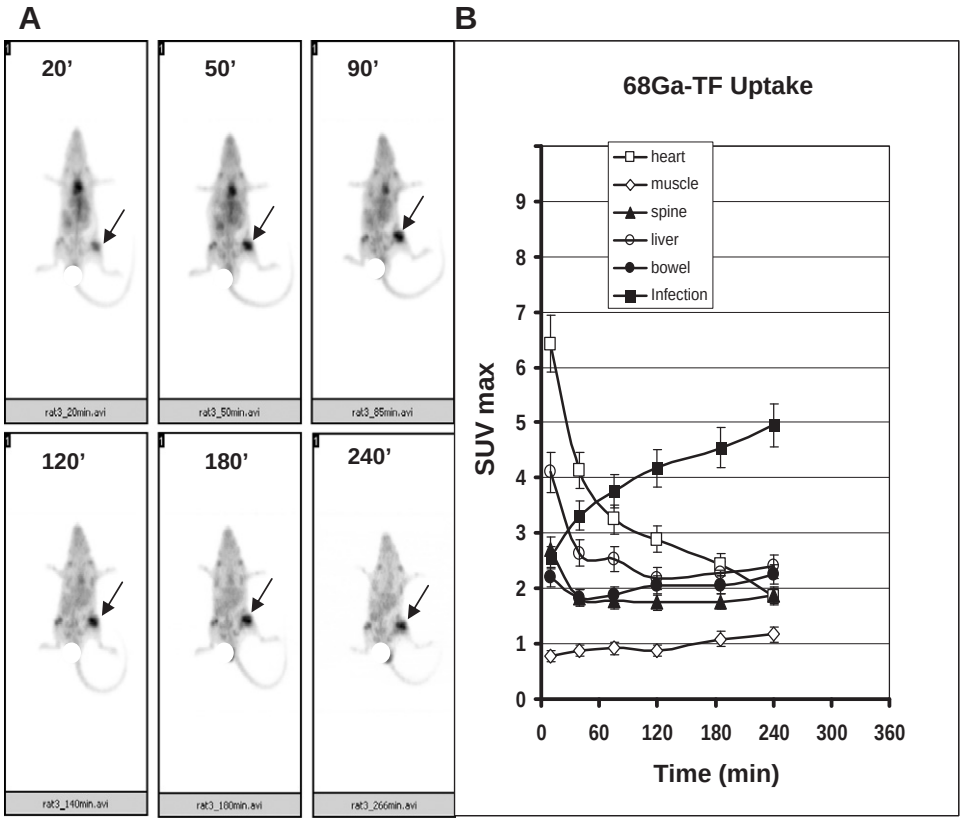

Fig. 1. ^{68}Ga-TF uptake by *S. aureus* infection in a rat model: 6^{8}Ga-TF (10–15 MBq/0.2 ml) was injected into an anaesthetized rat and images were acquired at different time intervals (up to 4 h) (A). Infection was visualized within 20 min post-injection, but intense uptake was seen after 50 min up to 4 h post-injection images (arrows). SUV_{max} was calculated for each time point over different organs as described in the methods section and plotted in the graph as shown (B). Progressive increase of SUV_{max} at the target and the concomitant decrease in cardiac blood pool activity could be visualized. The mean ± S.D. were calculated and were plotted in the graph.

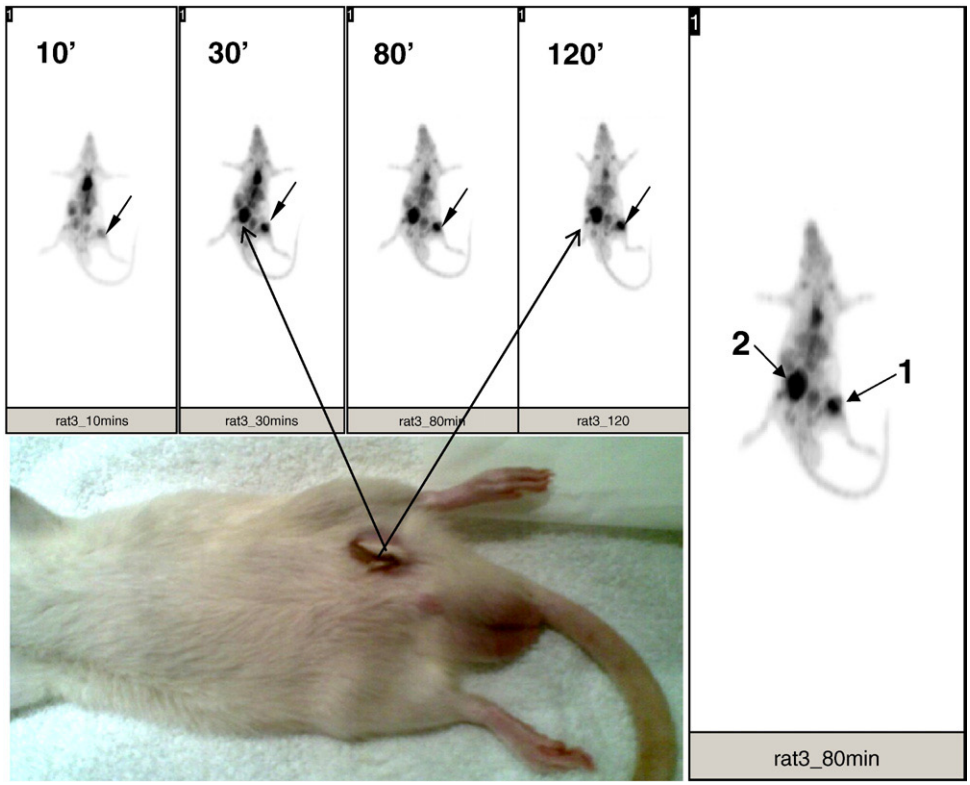

Fig. 2. ^{68}Ga-TF uptake by *S. aureus* and an additional infection lesion. ^{68}Ga-TF (10-15 MBq/0.2 ml) was injected into an anaesthetized rat and images were acquired at different time intervals as shown in the figure. Intense uptake was seen at or after 30 min post-injection images at the site corresponding to *S. aureus* injection (Arrow 1). An additional intense, focal uptake was seen in the lower right abdominal area, which was identified to be infection due to skin abscess carrying *P. mirabilis* organisms (Arrow 2).

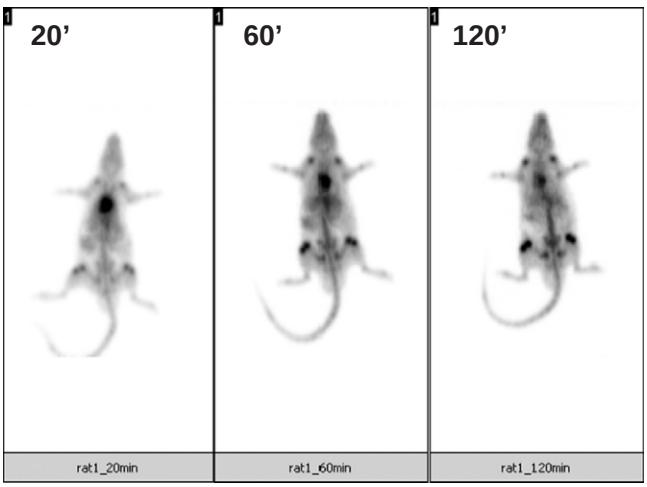

Fig. 3. $^{68}GaCl_3$ uptake by *S. aureus* infection in a rat model: $^{68}GaCl_3$ (10–15 MBq/0.2 ml) was injected into an anaesthetized rat and images were acquired at different time intervals (up to 120 min). Infection was not visualized up to 120 min post-injection, but an increased uptake was seen bilaterally at the growth plate of the hind leg joints. Cardiac blood-pool, liver and muscle uptake were persistently high with very little renal excretion, which was consistent with minimal bladder activity.

infected wound and an abscess on the skin. When biopsied, microbiology identified the organism as *P. mirabilis*. Therefore, the study indicated that ^{68}Ga-TF was capable of detecting infection caused by both *S. aureus* and *P. mirabilis* and may be extrapolated to all anaerobic bacteria.

3.3. $^{68}GaCl_3$ PET for imaging S. aureus infection

When unconjugated $^{68}GaCl_3$ (10-15 MBq/0.2 ml) was injected (instead of ^{68}Ga-TF complex) into an anaesthetized rat there was no focal uptake of the agent at the site corresponding to infection, for up to 120 min post-injection (Fig. 3). Instead, an increased uptake was seen bilaterally at the growth plate of the hind leg joints. Cardiac blood-pool, liver and muscle uptake were persistently high with very little renal excretion, which was consistent with minimal bladder activity.

4. Discussion

The cellular uptake of ^{67}Ga is a complex mechanism which is not fully understood. Several theories support that ^{67}Ga-uptake is dependent on multiple factors: adequate blood supply, increased capillary permeability [18], uptake by leukocytes [19] and direct uptake by bacteria [20]. The

most generally accepted theory is that ^{67}Ga is delivered to infected or inflammatory lesions primarily by binding to plasma transferrin and to some extent by lactoferrin and bacterial siderophores [19,21]. Transferrin is a glycoprotein with high affinity for binding iron on its domain. This domain belongs to the "transferrin super family" comprised of other iron-binding molecules such as lactoferrin, melanotransferrin and ovotransferrin [22,23].

The clinical utility of ^{67}Ga-SPET is somehow compromised due to several limitations. The major limitation is the delayed post-injection waiting time of at least 24-48 h. In this context, one of our concerns related to ^{68}Ga imaging was the short half-life of ^{68}Ga (68 min), which may question the fundamental concept of using this radionuclide for longer periods of study. We examined whether the ^{68}Ga-TF complex, is capable of detecting bacterial infection. The approach might provide circumstantial experimental evidence for in vivo binding of Ga^{3+} to plasma transferrin. Interestingly, the results of our study clearly indicated that bacterial infection in the rat model was convincingly defined, as an intense focal uptake, within 60 min of injecting ^{68}Ga-TF. Therefore, it is reasonable to conclude ^{68}Ga-TF complex is responsible to accumulate at bacterial and inflammatory lesions [6,13–15]. Available literature suggests the total amount of TF in humans is approximately 240 mg/kg [24]. Therefore, it is reasonable to assume in our study, the mass of injected TF (0.2mg/rat) in ^{68}Ga-apo-transferrin is too small to induce any unexpected pharmacological effect to influence the biodistribution of the agent.

The results of our one particular study revealed that ^{68}Ga-TF was capable of detecting infection caused by both the gram-positive *S. aureus* and gram-negative *Proteus mirobilis*. This was an incidental finding but gave an insight to the potential of this agent to detect more than one bacterial infection and probably other anaerobic bacterial infections. We do not have experimental evidence to support such claims, which is beyond the scope of this study.

When unconjugated $^{68}GaCl_3$ was used, instead of ^{68}Ga-TF as an imaging agent, it failed to define the infection lesion for up to 120 min post-injection. The preliminary results may suggest that $^{68}GaCl_3$ in the free form is not capable of localizing at the infection lesion. The results of this experiment also strongly support, the specificity of the tracer to the site of lesion is dictated by transferrin binding to $^{68}GaCl_3$ and not by diffusion due to the increased perfusion at the site of infection.

5. Conclusion

$^{68}GaCl_3$ can bind TF to give high specific activity preparation in small volume. The preliminary results suggest that ^{68}Ga-TF is capable of detecting *S. aureus* induced infection in the rat model, within an hour after intravenous injection, indicating its high potential for clinical utility.

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