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Scintigraphic imaging of *Staphylococcus aureus* infection using ^{99m}Tc radiolabeled aptamers

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HIGHLIGHTS

- ^{99m}Tc radiolabeled *S. aureus* aptamers were evaluated for infection imaging.
- Identification of *S. aureus* infected areas was possible by scintigraphic images.
- High target to non-target ratios were obtained using the ^{99m}Tc radiolabeled aptamers.

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ABSTRACT

Staphylococcus aureus is a specie of great medical importance associated with many infections as bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device related infections. Early identification of infectious foci is crucial for successful treatment. Scintigraphy could contribute to this purpose since specific radiotracers were available. Aptamers due to their high specificity have great potential for radiopharmaceuticals development. In the present study scintigraphic images of *S. aureus* infectious foci were obtained using specific *S. aureus* aptamers radiolabeled with ^{99m}Tc.

1. Introduction

Nuclear medicine could contribute to an accurate diagnosis of bacterial infections, since specific radiopharmaceuticals were developed. Infection specific radiopharmaceuticals can be used for diagnosis, decision-making in therapy and follow-up treatments. A variety of radiopharmaceuticals is used to detect infection, but long-term clinical use has shown that these probes cannot distinguish between infection and inflammation mainly because they are not specific to the target bacteria (Ferro-Flores et al., 2012).

The plethora of biological targeting molecules of radiopharmaceuticals ranges from antibodies, antibodies fragments, proteins, peptides and nucleic acid (Aerts et al., 2014). Nucleic acid aptamers are RNA or DNA oligonucleotides able of binding to a target molecule with high affinity and selectivity. The term “aptamers" derives from the Latin word "aptus," meaning “to fit” and the Greek word “meros”, meaning “part or region”. Nucleic acid aptamers are selected by an in vitro selection process termed SELEX (Systematic Evolution of Ligands by EXponential enrichment) through repeated rounds of partitioning and amplification from large random synthetic oligonucleotide library (Tuerk and Gold, 1990). They have been selected to a variety of target molecules such as proteins, peptides, amino acids, nucleotides, drugs, vitamins, organic and inorganic compounds, and whole cells (You et al., 2003).

Aptamers has advantages to other biomolecules as radiopharmaceuticals. Aptamers can be easily produced by in vitro conditions with high reproducibility and free of contaminants. They seem to be non-toxic and non-immunogenic, have small size (10–20 kDa) and fast clearance. The chemical synthesis allows alterations to become them more nuclease-resistant with modification at the 3' and 5' ends or by using modified nucleotides (Missailidis and Perkins, 2007). Lyophilized aptamers can be stored for years and, once reconstituted; they

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can be boiled or subjected to many freeze-thaw cycles (Evtugyn et al., 2014). Besides, they were able to discriminate between closely related molecules, such as the presence or absence of a methyl group or hydroxyl group, or the ability to distinguish between D and L amino acids (Geiger et al., 1996). Due to the high specificity, large variety of potential targets, and the possibility of labeling with different radionuclides, aptamers have great potential for radiopharmaceuticals development. Since their discovery, several aptamers have been used as targeting molecule of radiopharmaceuticals in preclinical studies (Gijs et al., 2016).

Staphylococcus aureus is both a commensal bacterium and a human pathogen that may cause bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device related infections. This bacterium is the most common agent in skin and soft tissue infections, often being isolated from infected surgical wounds that can be foci for systemic infections. The early identification of infectious foci is critical to successful treatment of *S. aureus* infections (Robert and Chambers, 2005). In this study aptamers specific to *S. aureus* were radiolabeled with ^{99m}Tc and evaluated by scintigraphic imaging of bacterial infection-bearing mice.

2. Material and methods

2.1. Chemicals

The aptamers SA20, SA23 and SA34 (Cao et al., 2009) were synthesized by Integrated DNA Technologies (IDT) with the flowing modifications: an amino group linked to a 6 carbons spacer at the 3'end and an inverted thymidine at the 5' end. The ^{99m}Tc was obtained from a molybdenum generator (IPEN- São Paulo/Brazil). All other chemicals and reagents used were of analytical grade.

2.2. Microorganisms

Staphylococcus aureus (ATCC 25923) cells were cultured on BHI solid (Himedia Laboratories Pvt Ltd.) in petri dishes at 37 °C and subcultured every seven days.

2.3. Animals

Swiss mice were kept in cages with wood shavings, water and common food in ordinary shelves. All protocols were approved by the local Ethics Committee for Animal Experimentation of the Federal University of Minas Gerais (CEUA/UFMG), Protocol no. 108/2014.

2.4. Aptamers radiolabeling with ^{99m}Tc and radiochemical purity determination

Labeling with ^{99m}Tc was performed by the direct method (Correa et al., 2014). For the labeling reaction 111.6 μmol of tricine and 28.3 μmol of EDDA were added to 300 μL of 0.9% saline. Then, 10 μL of a mixture of aptamers SA20, SA23 and SA34 (200 pmol/μL each) followed by 100 μL of $SnCl_2{\cdot}2H_2O$ (8.9 mM in HCl 0.25 N) were added. The pH was adjusted to 7.0 with NaOH 1N. The bottle was sealed and vacuum was performed. The activity of 481 MBq of a ^{99m}Tc-pertechnetate solution ($Na^{99m}TcO_4$) was added. The solution was boiled in water bath for 15 min and then cooled. An oligonucleotide DNA library (random sequences) was labeled in the same way and used as control.

The radiochemical purity (RP) of ^{99m}Tc-aptamer complex was assessed by ascending instant thin-layer chromatography (TLC) using silica gel-coated fiber glass sheets and two solvent systems: (1) 100% acetone to determine the percentage of TcO_4^- and (2) NaCl 0.9% (w/v) solution with 5% NH_4OH to determine the percentage of TcO_2. The labeled product (^{99m}Tc-aptamer) remained at the point of application when 100% acetone was used as the mobile phase (Rf = 0) and the labeled product moved with the solvent front when NaCl 0.9% (w/v) solution with 5% NH_4OH was used as the mobile phase (Rf = 1). The RP was determined according to the following equation:

$$\textit{Radiolabeling yield}\ (\%) = 100 - (\%\ TcO_4^- + \%\ TcO_2)$$

2.5. Determination of radiolabeled aptamers binding to plasma proteins

A blood sample (6 mL) was collected from Swiss mice and the plasma fraction was separated by centrifugation (700*g*). EDTA (0.1 M) was used as anticoagulant. Aliquots (triplicate) of 50 μL of plasma were incubated with 45 μL of ^{99m}Tc-aptamers, at 37 °C, for 1 h and 3 h. After that, 1.5 mL of acetonitrile was added in each tube. The soluble and insoluble plasma fractions were separated by centrifugation (700*g*) and the radioactivity in the fractions was measured in a gamma counter (Wallac Wizard 1470-020 Gamma Counter, PerkinElmer Inc., Waltham, EUA). The percentage of radioactivity in the insoluble plasma fraction was determined by dividing the count in the insoluble plasma fraction by the total count (soluble fraction + insoluble fraction) and multiplying this value by 100.

2.6. Blood clearance

The ^{99m}Tc-aptamers (3.0 MBq) were administrated in Swiss mice (n = 7) through the tail vein. A small incision was made in the distal tail to enable rapid and reliable blood collection. Blood samples (20 μL) were collected at 5, 10, 15, 30, 60, 90, 120, and 240 min after administration. The samples were weighted and the radioactivity was measured in gamma counter. The percentage of injected activity per gram (%ID/g) and the mean ± SD in each sample were determined. The data were plotted as a function of time and blood clearance analysis was performed using GraphPad PRISM version 5.01 software. The distribution half-life and elimination half-life were calculated using non-linear regression.

2.7. Biodistribution studies

Three groups of Swiss mice (20–25 g) (n = 7) were used. The group 1 included animals infected with *S. aureus* that were inoculated with the ^{99m}Tc-aptamers. The group 2 comprised animals infected with *S. aureus* that were inoculated with a ^{99m}Tc-radiolabeled library (control). The group 3 consisted of healthy non-infected mice (which received 100 μL of saline in the right thigh) inoculated with the ^{99m}Tc-aptamers. The animals were anesthetized with a mixture of xylazine (15 mg/kg) and ketamine (80 mg/kg). The group 1 and group 2 were infected intramuscularly in the right thigh with 1 x 10^6 cells of *S. aureus* suspended in 100 μL of saline. A visible swelling was observed on infected thigh of all animals 24 h after the intervention.

Twenty-four hour after infection foci induction, 100 μL (35.9 MBq) of ^{99m}Tc-aptamers solution were injected by the tail vein of each animal of groups 1 and 3. The group 2 mice were submitted to the same procedure using the ^{99m}Tc-library. The groups 2 and 3 were used as controls.

The mice were euthanized at 3 h after the injection and tissue samples (blood, liver, spleen, stomach, heart, lung, kidneys, right thigh infected muscle and left thigh muscle) were dissected, weighed and their activities measured in a gamma counter. The results were expressed as the percentage of injected dose per gram of tissue (%ID/g). Target/non-target ratios were obtained from the analysis of radiation measured in the infected muscle of the right thigh in relation to radiation measured in the muscle of the left thigh.

2.8. Scintigraphic imaging

The animals were anesthetized by intraperitonial injection, as above mentioned, and placed in the prone position on a γ-camera equipped with a low-energy collimator (Nuclide TH 22, Mediso, Hungary). Five