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RESEARCH ARTICLE

# $^{68}$Ga-Triacetylfusarinine C and $^{68}$Ga-Ferrioxamine E for *Aspergillus* Infection Imaging: Uptake Specificity in Various Microorganisms

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**Abstract**

*Purpose:* $^{68}$Ga-triacetylfusarinine C ($^{68}$Ga-TAFC) and $^{68}$Ga-ferrioxamine E ($^{68}$Ga-FOXE) showed excellent targeting properties in *Aspergillus fumigatus* rat infection model. Here, we report on the comparison of specificity towards different microorganisms and human lung cancer cells (H1299).

*Procedures:* The *in vitro* uptake of $^{68}$Ga-TAFC and $^{68}$Ga-FOXE was studied in various fungal, bacterial and yeast cultures as well as in H1299 cells. The *in vivo* imaging was studied in fungal and bacterial rat infection and inflammation models.

*Results:* $^{68}$Ga-TAFC and $^{68}$Ga-FOXE showed rapid uptake in *A. fumigatus* cultures, significantly lower in other fungal species and almost no uptake in other microorganisms and H1299 cells, except for $^{68}$Ga-FOXE in *Staphylococcus aureus*. $^{68}$Ga-TAFC and $^{68}$Ga-FOXE revealed rapid uptake in the lungs of *A. fumigatus*-infected rats, low accumulation in sterile inflammation and no uptake in bacterial abscess.

*Conclusions:* We have shown that $^{68}$Ga-FOXE and $^{68}$Ga-TAFC have high uptake in *A. fumigatus* both *in vitro* and *in vivo*. $^{68}$Ga-TAFC showed higher specificity, while $^{68}$Ga-FOXE showed higher sensitivity.

**Key words:** Siderophores, Gallium-68, Infection imaging, *Aspergillus fumigatus*, Positron emission tomography

## Introduction

Iron is an essential cofactor for a variety of important cellular processes and, therefore, can be considered as a vital nutrient for virtually all forms of life [1, 2]. Most microorganisms use special mechanisms to acquire iron including production of siderophores [3]. Mainly in the iron-poor environments, microorganisms such as *Aspergillus fumigatus* produce large amounts of siderophores to scavenge iron (III) and enable its uptake into the organism [4]. It has recently been recognised that iron plays an essential role in infection in general [5] and in fungal infections in particular. It has been shown that in particular, the siderophore system is essential for the virulence of *A. fumigatus* [6].

Siderophores are low molecular weight, iron-chelating ligands synthesised by almost all microorganisms for iron acquisition and storage; however, not all aspects of siderophore utilisation by microorganisms are fully understood. Luckey *et al.* [7] reported that some bacterial strains

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lost the ability to synthesise siderophores but retained the ability to utilise siderophores released by other microorganisms. Similarly, Haas [8], Philpott *et al*. [9] and Heymann *et al*. [10] reported that several fungal species are able to utilise siderophores produced by other fungi, indicating the lack of specificity of siderophore systems for particular microorganisms.

A great variety of different siderophores are known today, the majority being of hydroxamate, catecholate or α-hydroxycarboxylate type, each having a high selectivity for iron (III) [11]. The chemistry of iron (III) and gallium (III) is very similar and was already widely exploited in the field of nuclear medicine in the use of $^{67}$Ga-citrate. $^{68}$Ga is short-lived, generator-produced isotope that has recently become the subject of great interest for molecular imaging applications using positron emission tomography (PET) [12]. We have recently shown that various siderophores can be labelled with $^{68}$Ga [13], and $^{68}$Ga-triacetylfusarinine C (TAFC) and $^{68}$Ga-ferrioxamine E (FOXE) are able to detect *A. fumigatus* infection in a rat infection model using PET imaging [14, 15].

TAFC is a common trihydroxamate-type siderophore of many fungal species (*Aspergillus* sp., *Fusarium* sp., *etc*.). Although many different forms of fusarinines have been detected, the cyclic acetylated trimer is regarded as the product with the highest chemical stability [16]. Adjimani and Emery have even shown that TAFC is able to extract iron from other siderophores and thereby feed the producing microorganism with iron from exogenous siderophores [17].

Ferrioxamines were originally isolated and characterised as ferrioxamines A to H. All ferrioxamines are trihydroxamate-type siderophores and are either cyclic or linear. FOXE is a cyclic siderophore with high affinity to iron (III) mainly produced by actinomycetes and other bacteria [18].

Here, we report on the characterisation and comparison of *in vitro* and *in vivo* uptake of $^{68}$Ga-TAFC and $^{68}$Ga-FOXE in different microorganisms and human lung cancer cells to evaluate their specificity and sensitivity for *A. fumigatus* infection imaging.

## Materials and Methods

### Chemicals

All commercially obtained chemicals were of the highest available purity and were used without further purification. Siderophores were obtained from Genaxxon BioScience GmbH (Ulm, Germany), and $^{68}$Ge/$^{68}$Ga generator, from Eckert & Ziegler Eurotope GmbH (Berlin, Germany).

### Radiolabelling

Both siderophores were labelled with $^{68}$Ga using acetate buffer at room temperature (RT) (TAFC) or at 80 °C (FOXE) [13]. Radiochemical purity was determined using reverse-phase high-performance liquid chromatography gradient method and/or instant thin-layer chromatography on silica gel impregnated glass fibres, as described previously [13–15].

### Preparation of Microbial Cultures for In Vitro Studies

*A. fumigatus* ATCC46645, *Aspergillus terreus* DSM826, *Aspergillus flavus* ATCC9643, *Rhizopus oryzae* AS5 and *Fusarium solani* AS94 were cultured at 37 °C in liquid *Aspergillus* minimal media (AMM) [19] containing 1 % glucose and 20 mM glutamine as carbon and nitrogen source, respectively. Iron-containing media were supplemented with 30 μM $FeSO_4$, whereas for iron-limiting cultures, iron was omitted. For all other microbial strains, the iron-replete and iron-limited main cultures were at first precultured for 18 h and inoculated with a single colony at 37 °C. Such an inoculum was subsequently used for the culturing of the main cultures. The preculture medium for *Candida albicans* ATCC90028, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* ATCC9027 was yeast peptone dextrose (YPD) + 0.5 % glucose; that for *Mycobacterium smegmatis* mc$^2$155, YPD + 0.5 % Tween 80; and that for *Staphylococcus aureus*, Roswell Park Memorial Institute (RPMI) + 1 % casamino acids. With exception of *P. aeruginosa*, the iron-replete main culture medium for all these strains was the same as the preculture medium, and for iron-limiting conditions, the ferrous iron chelator dipyridyl was added to a final concentration of 200 μM. For *P. aeruginosa*, the main culture media were iron-replete and iron-limiting AMM (see above), respectively. Iron-deficient conditions were verified by detection of extracellular siderophores production, which is repressed by iron.

### Preparation of Human Lung Cancer Cells for In Vitro Studies

H1299 non-small cell human lung cancer cells (ATCC) were maintained in tissue culture flasks (Cellstar, Greiner Bio-One, Kremsmuenster, Austria) in RPMI 1640, supplemented with 10 % (*v*/*v*) heat-inactivated FBS and 1 % (*v*/*v*) PSG at 37 °C with 5 % $CO_2$ in a humidified atmosphere and grown in monolayer. On the day of the experiment, cells were removed with trypsin-EDTA and used at a density of $1\times10^6$ cells/ml for the uptake experiments.

### In Vitro Uptake of $^{68}$Ga-Siderophores in Various Microbial Media

*In vitro* uptake was studied in *A. fumigatus*, *A. flavus*, *A. terreus*, *C. albicans*, *R. oryzae*, *F. solani*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *M. smegmatis* iron-deficient and iron-sufficient cultures. For the monitoring of uptake over time, $^{68}$Ga-siderophores were incubated in the microbial media for 10, 45 and 90 min at RT with or without blocking solution (Fe-TAFC or Fe-FOXE) in 96-well plates (Millipore, Billerica, MA, USA). The uptake was interrupted by filtration of the medium and rapid rinsing with ice-cold Tris buffer. The filters were collected and counted in a γ-counter.

### *Siderophore Utilisation Growth Assay*

To exemplary confirm the ability or inability to take up TAFC, we developed a siderophore utilisation growth assay (Fig. 2). In this agar diffusion assay, the analysed species ($10^7$ conidia of *A. fumigatus* or 0.2 ml of *K. pneumoniae* preculture, respectively) was poured in 5 ml top agar (iron-limiting AMM medium + 0.7 % agar) on agar plates (iron-limiting AMM medium). Subsequently, 80 µl of 0.6 mM ligand-free TAFC solution was inoculated into a hole (5 mm diameter) punched into the middle of the plate. The plates were then incubated at 37 °C for 30 h. The ligand-free siderophore diffuses into the growth medium and chelates the present iron with the highest TAFC concentration in the vicinity of the hole. The growth of microorganisms unable to take up TAFC-iron is inhibited by high TAFC concentrations in this assay as TAFC iron is here the only iron source present.

### *In Vitro Uptake of $^{68}$Ga-Siderophores in Human Lung Cancer Cells*

*In vitro* uptake was studied in human non-small cell lung cancer cells H1299 type. H1299 cells were seeded at a density of $1\times10^6$ cells per Eppendorf tube and incubated in triplicates with $^{68}$Ga-TAFC or $^{68}$Ga-FOXE at RT for 90 min. For positive and negative control, Eppendorf tubes containing iron-deficient and iron-sufficient cultures of *A. fumigatus* were incubated in triplicates with $^{68}$Ga-labelled siderophores at RT for 90 min in parallel. The incubation was interrupted by 2-min centrifugation at 5,000×*g*. The supernatant was collected and measured in a γ-counter. Cells sediment was disturbed by 1 ml of glycine and subsequent whirling. *Aspergillus* media were washed with 1 ml of ice-cold Tris buffer. All samples were centrifuged for 2 min, and supernatants were measured in γ-counter. Thereafter, 1 ml of 1 M NaOH was added to human lung cancer cells, mixed and centrifuged for 2 min. The supernatant was again collected and measured in a γ-counter.

### *Animal Experiments*

All animal experiments were conducted in accordance with the regulations and guidelines of the Austrian and Dutch animal protection laws and with the approval of the Austrian Ministry of Science (66011/42-II/10b/2009) and Institutional Animal Welfare Committee of the Radboud University Medical Centre Nijmegen (revised Dutch Act on Animal Experimentation, 1997). Animal studies were performed using Lewis rats (Charles River Laboratories, Wilmington, MA)

### *In Vivo Imaging*

*In vivo* uptake was studied in the *A. fumigatus* rat infection model [15, 16] and in rats with sterile (turpentine oil) and bacterial (*S. aureus*) intramuscular (i.m.) inflammation. An abscess was induced in the left calf muscle with approximately $1\times10^9$ colony-forming units of *S. aureus* in 0.1 ml 50:50 % suspension of autologous blood and normal saline. A sterile inflammation was induced in the right calf muscle by injecting 0.1 ml turpentine oil intramuscularly. During the procedure, animals were anaesthetised. After 24 h, when swelling of the muscle was apparent, the tracers were injected intravenously through the tail vein.

PET imaging was obtained using an Inveon animal PET/CT scanner (Siemens Preclinical Solutions, Knoxville, TN, USA) [15]. Static PET scans of 30 min were recorded at 30 min after i.v. injection of $^{68}$Ga-siderophore or 2-deoxy-2-[$^{18}$F]fluoro-D-glucose ($^{18}$F-FDG), the time established in the previous studies [15].

## Results

### *Radiolabelling and In Vitro Stability*

Both $^{68}$Ga-TAFC and $^{68}$Ga-FOXE showed high radiochemical purity and *in vitro* stability as described in the previous studies [13–15]. Figure 1 displays chemical structures of studied $^{68}$Ga-siderophores.

### *In Vitro Uptake of $^{68}$Ga-Siderophores in Various Microbial Media*

*In vitro* uptake of $^{68}$Ga-TAFC and $^{68}$Ga-FOXE was highly dependent on the mycelia iron load (Tables 1 and 2). Both compounds showed rapid uptake by iron-starved *A.*

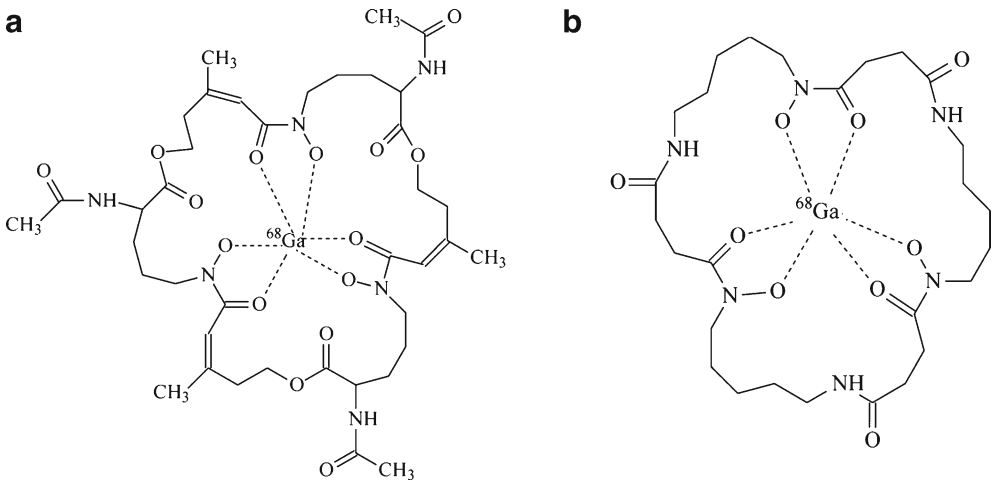


**Fig. 1.** Chemical structures of **a** $^{68}$Ga-TAFC and **b** $^{68}$Ga-FOXE.

**Table 1.** Comparison of $^{68}$Ga-FOXE and $^{68}$Ga-TAFC uptake in various microorganisms 10, 45 and 90 min after incubation in iron-deficient cultures and in human lung cancer cells (H1299)

| Medium (iron deficient) | $^{68}$Ga-TAFC | | | $^{68}$Ga-FOXE | | | $^{68}$Ga-TAFC | | | $^{68}$Ga-FOXE | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 min | 45 min | 90 min | 10 min | 45 min | 90 min | 10 min | 45 min | 90 min | 10 min | 45 min | 90 min |
| | Mean % uptake/mg protein ($n$=4) ± SD | | | Mean % uptake/mg protein ($n$=4) ± SD | | | Mean % uptake ($n$=4) ± SD | | | Mean % uptake ($n$=4) ± SD | | |
| *Aspergillus fumigatus* | 50.2±17.8 | 161.9±60.5 | 406.4±228.2 | 242.6±81.6 | 698.5±127.5 | 1,117.0±144.5 | 4.0±1.6 | 11.1±4.0 | 23.5±15.1 | 14.1±2.7 | 55.5±7.7 | 66.3±11.8 |
| *Aspergillus flavus* | 4.9±0.8 | 11.8±3.1 | 13.1±2.7 | 44.7±15.6 | 128.9±26.7 | 214.0±23.3 | 0.6±0.1 | 1.6±0.3 | 2.1±0.8 | 6.7±1.3 | 21.2±9.5 | 39.1±5.3 |
| *Aspergillus terreus* | 14.6±6.8 | 13.1±6.0 | 8.8±1.5 | 50.7±15.0 | 187.4±41.6 | 223.8±23.9 | 1.1±0.4 | 1.2±0.4 | 0.8±0.3 | 5.6±1.8 | 16.9±3.0 | 22.4±6.6 |
| *Candida albicans* | 0.7±0.7 | 3.0±4.5 | 1.2±1.6 | 0.7±0.4 | 1.3±0.8 | 1.3±1.1 | 0.1±0.1 | 0.1±0.2 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 |
| *Rhizopus oryzae* | 19.6±1.5 | 63.8±31.2 | 193.7±54.4 | 42.2±18.1 | 177.4±56.4 | 365.0±196.0 | 1.7±0.5 | 5.5±1.6 | 13.7±2.1 | 4.6±0.9 | 15.2±2.1 | 32.0±9.5 |
| *Fusarium solani* | 42.5±15.0 | 48.0±15.7 | 64.3±25.8 | 116.8±21.7 | 413.7±154.4 | 603.2±189.2 | 2.7±0.2 | 3.1±0.3 | 4.0±0.7 | 12.4±1.2 | 35.3±2.7 | 49.0±7.2 |
| *Pseudomonas aeruginosa* | 1.9±0.7 | 2.7±0.7 | 0.9±0.4 | 0.2±0.1 | 0.3±0.3 | 0.4±0.1 | 0.2±0.1 | 0.2±0.1 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 |
| *Klebsiella pneumoniae* | 0.9±0.1 | 1.2±0.2 | 1.9±0.2 | 0.6±0.2 | 0.9±0.1 | 1.7±0.1 | 0.3±0.1 | 0.4±0.1 | 0.6±0.1 | 0.2±0.1 | 0.3±0.1 | 0.5±0.1 |
| *Staphylococcus aureus* | 2.9±0.8 | 6.2±2.8 | 6.8±1.2 | 365.5±138.2 | 495.0±87.0 | 484.0±58.2 | 0.4±0.1 | 1.1±0.6 | 1.0±0.1 | 36.4±1.0 | 45.3±8.8 | 48.7±3.3 |
| *Mycobacterium smegmatis* | 2.0±1.4 | 1.5±0.6 | 1.8±0.5 | 0.6±0.5 | 0.6±0.5 | 3.0±3.7 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.2±0.2 | 0.1±0.1 | 0.1±0.1 |
| Human lung cancer cells (H1299) | n.d. | n.d. | 0.3±0.2 | n.d. | n.d. | 0.2±0.2 | n.d. | n.d. | 0.1±0.1 | n.d. | n.d. | 0.1±0.1 |

*n.d.* no data

**Table 2.** Comparison of $^{68}$Ga-FOXE and $^{68}$Ga-TAFC uptake in various microorganisms 10, 45 and 90 min after incubation in iron-sufficient cultures and in human lung cancer cells (H1299)

| Medium (iron sufficient) | $^{68}$Ga-TAFC | | | $^{68}$Ga-FOXE | | | $^{68}$Ga-TAFC | | | $^{68}$Ga-FOXE | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 min | 45 min | 90 min | 10 min | 45 min | 90 min | 10 min | 45 min | 90 min | 10 min | 45 min | 90 min |
| | Mean % uptake/mg protein ($n$=4)±SD | | | Mean % uptake/mg protein ($n$=4)±SD | | | Mean % uptake ($n$=4)±SD | | | Mean % uptake ($n$=4)±SD | | |
| *Aspergillus fumigatus* | 7.4±2.0 | 6.7±1.8 | 8.1±2.4 | 15.5±4.0 | 99.0±10.4 | 207.0±30.1 | 1.1±0.6 | 1.0±0.6 | 0.6±0.1 | 2.9±0.7 | 17.4±1.8 | 31.4±1.5 |
| *Aspergillus flavus* | 4.8±2.9 | 1.6±0.6 | 0.8±0.2 | 4.6±2.2 | 8.1±1.5 | 11.0±2.4 | 0.9±0.6 | 0.4±0.2 | 0.2±0.1 | 1.1±0.6 | 2.2±0.1 | 3.1±1.0 |
| *Aspergillus terreus* | 1.1±0.4 | 2.4±0.6 | 2.2±1.1 | 12.6±1.8 | 21.2±5.4 | 68.5±19.4 | 0.2±0.1 | 0.2±0.1 | 0.3±0.1 | 1.8±0.1 | 4.5±1.2 | 8.1±1.3 |
| *Candida albicans* | 0.2±0.2 | 1.2±1.5 | 0.6±0.5 | 0.9±0.4 | 0.5±0.3 | 1.2±1.0 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.2±0.2 | 0.1±0.1 | 0.1±0.1 |
| *Rhizopus oryzae* | 0.5±0.4 | 1.1±0.9 | 0.5±0.1 | 2.4±0.9 | 12.4±4.2 | 27.1±5.7 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.4±0.1 | 1.7±0.1 | 4.0±0.8 |
| *Fusarium solani* | 10.1±2.3 | 16.5±4.6 | 17.8±4.5 | 27.6±4.7 | 110.8±14.7 | 150.0±55.6 | 1.2±0.2 | 1.3±0.1 | 1.6±0.2 | 3.7±0.4 | 14.0±2.2 | 15.8±2.6 |
| *Pseudomonas aeruginosa* | 0.9±0.3 | 1.3±0.2 | 1.5±0.2 | 0.5±0.2 | 0.9±0.1 | 0.8±0.2 | 0.1±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 | 0.1±0.1 |
| *Klebsiella pneumoniae* | 1.2±0.2 | 1.6±0.3 | 1.9±0.1 | 0.6±0.1 | 1.3±0.3 | 1.7±0.3 | 0.3±0.1 | 0.4±0.1 | 0.6±0.1 | 0.2±0.1 | 0.3±0.1 | 0.5±0.1 |
| *Staphylococcus aureus* | 3.5±0.3 | 6.7±1.3 | 7.2±1.1 | 4.1±0.5 | 6.8±1.7 | 11.0±1.6 | 0.7±0.1 | 1.0±0.1 | 1.3±0.1 | 0.9±0.1 | 1.5±0.3 | 2.0±0.2 |
| *Mycobacterium smegmatis* | 1.5±0.9 | 1.1±0.4 | 1.1±0.3 | 0.5±0.3 | 0.2±0.2 | 0.6±0.7 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 |
| Human lung cancer cells (H1299) | n.d. | n.d. | 0.3±0.2 | n.d. | n.d. | 0.2±0.2 | n.d. | n.d. | 0.1±0.1 | n.d. | n.d. | 0.1±0.1 |

*n.d.* no data

*fumigatus* cultures (Table 1), which could be partly blocked with excess of Fe-siderophore and significantly lower uptake by *A. fumigatus* grown under iron-sufficiency (Table 2). Tables 1 and 2 summarise the uptake values of both $^{68}$Ga-TAFC and $^{68}$Ga-FOXE in various microorganisms and H1299 cells. In bacterial (*P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *M. smegmatis*) or yeast (*C. albicans*) cultures, $^{68}$Ga-TAFC revealed virtually no uptake in both iron-deficient and iron-sufficient media. $^{68}$Ga-FOXE showed similar results to $^{68}$Ga-TAFC except for *S. aureus*. In *S. aureus* iron-deficient media, clear uptake of $^{68}$Ga-FOXE was observed. In fungal cultures (*A. flavus*, *A. terreus*, *R. oryzae*, *F. solani*), both $^{68}$Ga-siderophores, especially $^{68}$Ga-FOXE, showed certain uptake in iron-deficient media, which was substantially lower in comparison with the uptake in *A. fumigatus* cultures.

## *Siderophore Utilisation Growth Assay*

In this assay (Fig. 2), *K. pneumoniae* displayed in contrast to *A. fumigatus* a clear growth inhibition zone, which is in perfect agreement with the *in vitro* $^{68}$Ga-TAFC uptake assay that indicated that *K. pneumoniae* lacks TAFC uptake (see Table 1). As a control, the plates were inoculated with ferric TAFC instead of ligand-free TAFC. In this case, iron traces that are not chelated by TAFC are available for growth. In this set-up, the growth of *K. pneumoniae* was not inhibited, which demonstrates that the growth inhibition is indeed due to iron chelation by TAFC and not by a potential iron-independent antibacterial activity of TAFC.

## *In Vitro Uptake of $^{68}$Ga-Siderophores in Human Lung Cancer Cells*

No uptake was observed in human lung cancer cells (H1299) for both $^{68}$Ga-siderophores (see Tables 1 and 2). Almost all the radioactivity was found in the supernatant containing cell media, and negligible radioactivity was observed in the glycine and NaOH supernatants for both compounds.

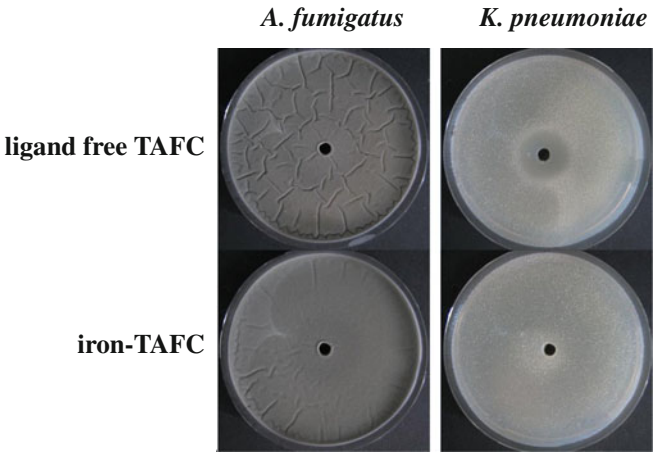


**Fig. 2.** Siderophore utilisation assay demonstrating that growth of *K. pneumoniae* is in contrast to that of *A. fumigatus* inhibited by ligand-free TAFC.

## *In Vivo Imaging*

*In vivo* PET imaging in the *A. fumigatus* rat infection model showed rapid focal accumulation of $^{68}$Ga-siderophores in the lungs. Whereas no *in vivo* uptake in the lung region was detected in non-infected animals, and the only visible organs were the kidneys and bladder (see Fig. 3).

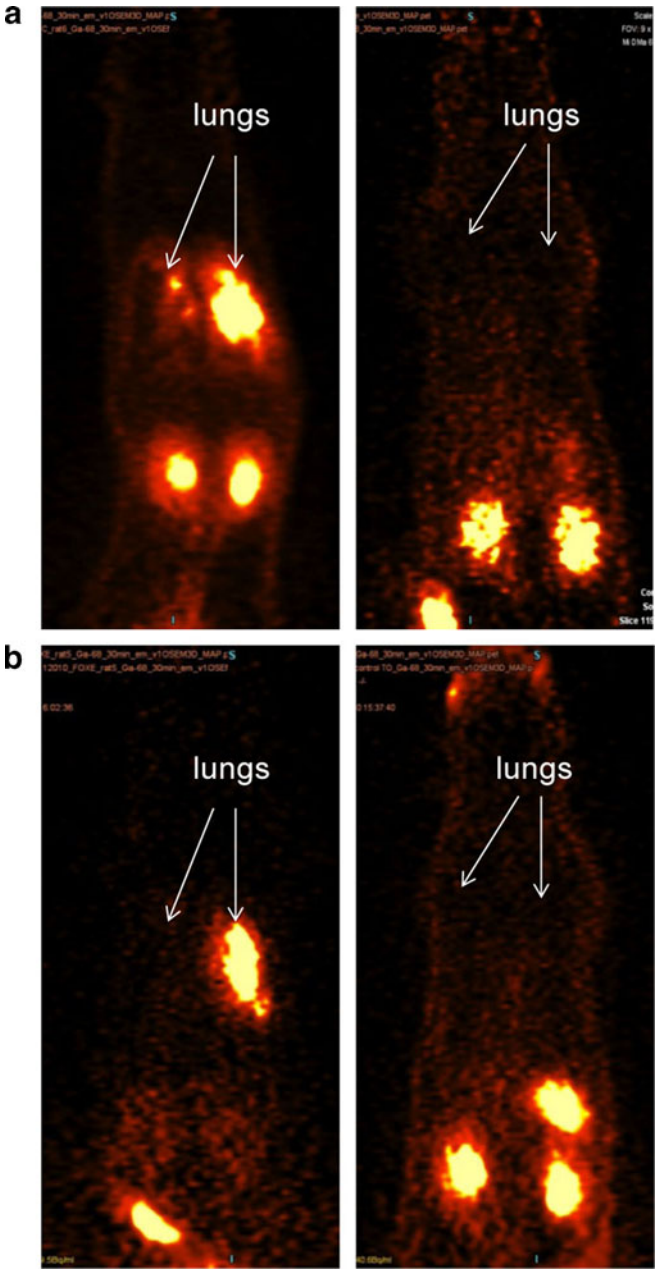


**Fig. 3.** *In vivo* imaging of the *A. fumigatus* rat infection model (*left-hand side images*) and non-infected (*right-hand side images*) animals using **a** $^{68}$Ga-TAFC and **b** $^{68}$Ga-FOXE, 1 h. i.v. postinjection.

Both $^{68}$Ga-TAFC and $^{68}$Ga-FOXE revealed moderate clearly detectable uptake in the muscle with turpentine oil and no uptake in the *S. aureus* abscess, whereas $^{18}$F-FDG showed evident uptake in both sites of induced inflammation (see Fig. 4).

## Discussion

Invasive aspergillosis (IA), which is mainly caused by *A. fumigatus*, is becoming one of the leading infective causes of morbidity and mortality in immunocompromised patients [20–22]. A key factor for the patient survival is an early and accurate diagnosis of IA. Currently a number of tests and techniques are used in clinical practice, unfortunately lacking enough sensitivity and/or specificity that the diagnostics of IA continues to be highly challenging [23].

The question of specificity of the diagnostic method is extremely important for subsequent therapy management. There are many different types of chemotherapeutic drugs that are used against specific pathogens or tumours. Many radiotracers used in nuclear medicine clinical practice for inflammation or infection imaging (*e.g.* $^{18}$F-FDG, $^{67}$Ga-citrate) are not specific, even though some experimental developments claim to be able to distinguish between inflammation and infection [24]. We have shown that the $^{68}$Ga-labelled siderophores TAFC and FOXE are able to image *A. fumigatus* infection *in vivo* at an early onset of infection [15]. The mechanism of $^{68}$Ga-siderophores action is based on the active transport via highly specific transporters, which are upregulated during *A. fumigatus* infection, resulting in rapid accumulation of the radiotracer at the site of infection, allowing early detection of infection having appropriate clinical impact. A specific uptake mechanism utilising the nutrition pathway of the microorganisms also potentially should allow uptake in infected tissues that are normally not accessible by hydrophilic radiotracers such as the brain. This specific mechanism also holds the promise that $^{68}$Ga-siderophores could be highly specific agents for *A. fumigatus* infection imaging, and we tried to address this in the presented paper.

TAFC is a common siderophore of many pathogenic fungal species [16]. It is interesting to note that transport systems for fusarinines in bacteria are unknown so far [3]. Although other fungal siderophores, such as ferrichromes and coprogen, are utilised by *Escherichia coli*, a fusarinine outer membrane receptor is still lacking. Whereas TAFC is produced and utilised by many fungal species, FOXE is a siderophore produced by bacteria (*Streptomyces* spp.) and used by many bacteria including *S. aureus* [25, 26], but also by several fungal species, as we shown here. We have tested the specificity of $^{68}$Ga-TAFC and $^{68}$Ga-FOXE *in vitro* on the set of representative microorganisms as well as in the human lung cancer cells. Both compounds showed no uptake in the human lung cancer cells. The distinction between infection and tumour is very important, especially considering that one of the major groups of immunocompromised patients suffer various malignancies including lung cancer. $^{68}$Ga-TAFC displayed no uptake in any tested bacterial cultures (*P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *M. smegmatis*), whereas $^{68}$Ga-FOXE was clearly taken up by *S. aureus*. This finding was in contrast with subsequent *in vivo* tests using the i.m. rat bacterial (*S. aureus*) infection model. No *in vitro* uptake was observed for $^{68}$Ga-FOXE in the remaining tested bacterial cultures (*P. aeruginosa*, *K.*

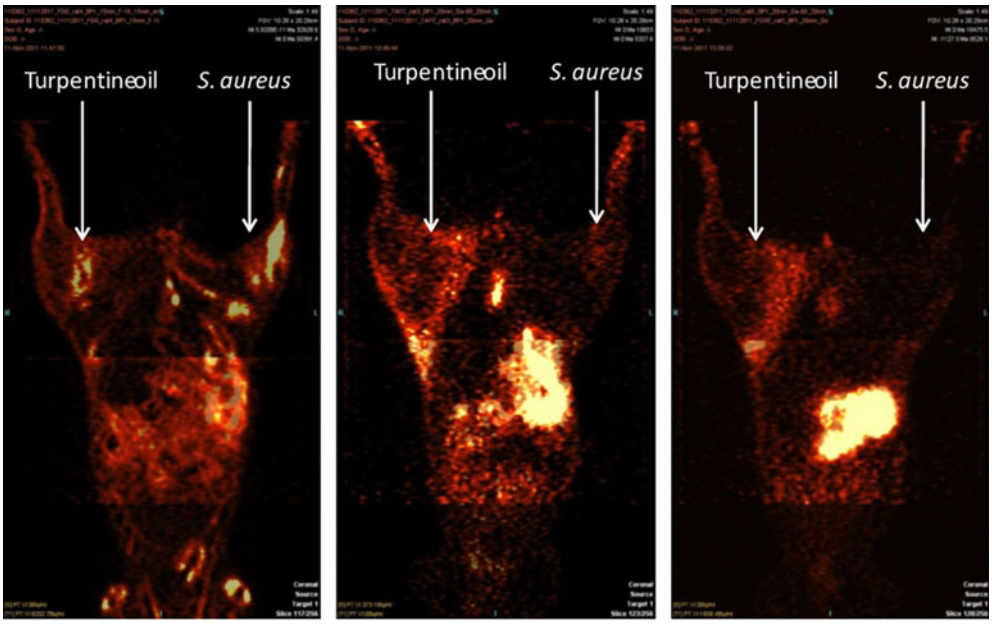


**Fig. 4.** Comparison of **a** $^{18}$F-FDG, **b** $^{68}$Ga-TAFC and **c** $^{68}$Ga-FOXE uptake in the muscle of rats i.m. injected with turpentine oil and *S. aureus* abscess.

*pneumoniae* and *M. smegmatis*). *C. albicans* was chosen as widely occurring representative of yeasts which is potentially pathogenic in immunocompromised patients. Both compounds were not taken up by *C. albicans* at all. The last group microorganisms studied *in vitro* covered selected fungal species. $^{68}$Ga-TAFC showed certain uptake in *R. oryzae* and *F. solani* cultures, but surprisingly very low uptake in *Aspergillus* species (*A. flavus*, *A. terreus*) in relation to *A. fumigatus*. $^{68}$Ga-FOXE displayed evidently higher uptake in all tested fungal media. In summary, the *in vitro* uptake of both $^{68}$Ga-TAFC and $^{68}$Ga-FOXE in *A. fumigatus* cultures was significantly higher compared to all tested microorganisms and human lung cancer cells, and the *in vitro* studies indicated higher specificity of $^{68}$Ga-TAFC.

*In vivo* PET imaging was based on our previous studies [14, 15] and was extended by testing of $^{68}$Ga-siderophores and $^{18}$F-FDG in the rat model of sterile (turpentine oil) and bacterial (*S. aureus*) i.m. inflammation. Both $^{68}$Ga-siderophores displayed rapid and highly selective focal accumulation in the lungs of *A. fumigatus* infected rats, whereas in non-infected animals, no radioactive signal was detected in the lungs area. *In vivo* imaging in the sterile and bacterial inflammation model showed no *in vivo* uptake in the *S. aureus* abscess for both $^{68}$Ga-siderophores. This finding is in contrast to the *in vitro* data of $^{68}$Ga-FOXE in *S. aureus* cultures as mentioned before. $^{68}$Ga-TAFC and $^{68}$Ga-FOXE revealed detectable uptake in the area of induced sterile inflammation. This uptake might be due to the severity of the inflammation, causing non-specific extravasation of the tracers. As expected, $^{18}$F-FDG showed much higher uptake in both i.m. sites of inflammation. *In vivo* studies confirmed that $^{68}$Ga-siderophores can be used for *Aspergillus* infection imaging.

## Conclusions

We have shown that both $^{68}$Ga-TAFC and $^{68}$Ga-FOXE can be used for imaging of IA. Moreover, $^{68}$Ga-TAFC showed high *in vitro* specificity towards *A. fumigatus* compared to other tested microorganisms and human lung cancer cells. Both $^{68}$Ga-siderophores showed highly selective accumulation in the infected lungs in the *A. fumigatus* rat infection model using PET imaging with $^{68}$Ga-FOXE being slightly superior in terms of sensitivity. The final answer of the usefulness and applicability of these compounds for early diagnosis of IA in patients lies now in the clinical studies.

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*Conflict of interest.* The authors declare that they have no conflict of interest.



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