Published in final edited form as:
Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2015 March ; 159(1): 60–66. doi:10.5507/bp.2014.052.

Selected ^{68}Ga-siderophores versus ^{68}Ga-colloid and ^{68}Ga-citrate: biodistribution and small animal imaging in mice

Milos Petrik[a], **Adela Vlckova**[a], **Zbynek Novy**[a], **Lubor Urbanek**[b], **Hubertus Haas**[c], and **Clemens Decristoforo**[d]

[a]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic

[b]Laboratory of Growth Regulators, Centre of the Region Hana for Biotechnological and Agricultural Research, Institute of Experimental Botany AS CR and Palacky University Olomouc

[c]Division of Molecular Biology/Biocenter, Innsbruck Medical University, Innsbruck, Austria

[d]Clinical Department of Nuclear Medicine, Innsbruck Medical University, Innsbruck, Austria

Abstract

Background—^{68}Ga-triacetylfusarinine C (TAFC) and ^{68}Ga-ferrioxamine E (FOXE) show great potential to be used as highly sensitive and selective tracers for *Aspergillus* infection imaging. Here we report on a comparison of the *ex vivo* biodistribution and small animal imaging of ^{68}Ga-TAFC and ^{68}Ga-FOXE versus ^{68}Ga-colloid and ^{68}Ga-citrate as unspecific control in mice.

Methods—The radiochemical purity of tested ^{68}Ga labelled tracers was determined by RP-HPLC or ITLC-SG. *Ex vivo* biodistribution was studied in normal DBA/2 mice 30 min and 90 min p.i. Static and dynamic imaging were performed using μPET/CT.

Results—^{68}Ga-TAFC and ^{68}Ga-FOXE showed rapid renal excretion and low blood values even 90 min p.i. ^{68}Ga-TAFC showed almost no retention in other organs while ^{68}Ga-FOXE displayed some uptake in gastrointestinal tract. ^{68}Ga-colloid and ^{68}Ga-citrate revealed significantly different *ex vivo* biodistribution. ^{68}Ga-colloid showed pronounced radioactivity retention in the liver, while ^{68}Ga-citrate displayed high blood values and significant retention of radioactivity in highly perfused organs.

Conclusions—From the results, both ^{68}Ga-TAFC and ^{68}Ga-FOXE have excellent and significantly different *in vivo* behaviour compared to ^{68}Ga-colloid and ^{68}Ga-citrate. ^{68}Ga-TAFC in particular confirmed its great potential use as a specific tracer for *Aspergillus* infection imaging.

Keywords

gallium-68; siderophores; colloid; citrate; μPET imaging; aspergillosis

Corresponding author: Milos Petrik, e-mail: milospetrik@seznam.cz.

Authorship contributions: MP, HH, CD: study design; MP, CD: manuscript writing; MP, AV: literature search; AV, MP, ZN: data collection; MP, LU: quality control; AV, MP, ZN: data analysis; MP, CD, HH, LU: data interpretation; AV, MP: figures, tables.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

Europe PMC Funders Author Manuscripts

INTRODUCTION

Triacetylfusarinine C (TAFC) and ferrioxamine E (FOXE) are common trihydroxamate-type siderophores. Siderophores are relatively low molecular weight compounds produced by bacteria, fungi and some plants for scavenging iron from the environment to make this vital mineral available to the microbial cell[1,2]. It has recently been recognised that iron plays a fundamental role in infection in general[3] and in fungal infections in particular. Schrettl et al.[4] reported that the siderophore system is essential for the virulence of *Aspergillus fumigatus*, which is the main pathogen responsible for invasive aspergillosis (IA) complications.

IA is a life-threatening infection, hazardous especially for haematopoietic stem cell and solid organ transplant recipients, as well as for patients with solid tumours and haematological malignancies[5,6]. Early and accurate diagnosis of IA is crucial for the survival of such affected patients[7]. Unfortunately, currently available methods for the diagnosis of IA lack sufficient specificity and/or sensitivity.

We have recently shown that various siderophores can be labelled with generator produced ^{68}Ga (ref.[8]), based on the similarities between iron (III) and gallium (III). Gallium-68 is a positron emitter that has recently attracted great interest for molecular imaging applications using positron emission tomography (PET) (ref.[9–11]). It can be produced from a long shelf-life and cost-effective generator system. The physical half-life of ^{68}Ga (67.7 min) permits production and application of most low molecular weight radiopharmaceuticals such as peptides, oligonucleotides, antibody fragments and potentially also siderophores.

^{68}Ga labelled TAFC and FOXE show great promise as highly selective, highly sensitive tracers for *Aspergillus* infection imaging[12,13]. This could prove indispensable in the early, accurate diagnosis of IA. For translation into clinical practice more data on the pharmacokinetics and biodistribution of these promising new tracers are warranted. Here we report on a comparison of *in vivo* biodistribution and small animal imaging of selected ^{68}Ga-siderophores (^{68}Ga-TAFC and ^{68}Ga-FOXE) versus radiochemical impurities, which can develop during ^{68}Ga labelling (^{68}Ga-colloid and ^{68}Ga-acetate) and a representative of infection imaging agents (^{68}Ga-citrate) as unspecific control in mice.

MATERIALS AND METHODS

Reagents

All chemicals obtained commercially were of the highest available purity and were used without further purification. TAFC and FOXE were gained from EMC microcollections GmbH (Tuebingen, Germany) and ^{68}Ge/^{68}Ga generator from Eckert & Ziegler Eurotope GmbH (Berlin, Germany).

Radiolabelling

$^{68}GaCl_3$ was obtained from the $^{68}Ge/^{68}Ga$ generator using 0.1N HCl as eluent. 300 μL of the fractionated generator eluate (20-80 MBq of $^{68}GaCl_3$) was added to a mixture of 10 μL of TAFC (10 μg in water) or 20 μL of FOXE (20 μg in 10% ethanol) and 30 μL of 1.1M sodium acetate solution. After 15 min at room temperature (TAFC) or 20 min at 80°C (FOXE), 100 μL of 1.1M sodium acetate solution were added to increase the pH to 6-7.

^{68}Ga-acetate/colloid was prepared using excess (150 μL) of 1.1M sodium acetate solution mixed with the fractionated $^{68}Ge/^{68}Ga$ generator eluate (300 μL) to reach a reaction pH of 6-7 for 15 min at room temperature.

^{68}Ga-colloid was prepared by mixing a ‘Monday’ fractionated $^{68}Ge/^{68}Ga$ generator eluate (300 μL) with 0.5M sodium hydroxide (55 μL). The reaction mixture was incubated for 15 min at room temperature.

Fractionated $^{68}Ge/^{68}Ga$ generator eluate (300 μL) was mixed with 0.5M sodium citrate pH ~ 5 (80 μL) for the preparation of ^{68}Ga-citrate. The reaction mixture was incubated for 15 min at room temperature.

Quality control

The radiochemical purity of the ^{68}Ga labelled tracers was determined using reversed-phase high-performance liquid chromatography (RP-HPLC) and/or instant thin layer chromatography on silica gel impregnated glass fiber sheets (ITLC-SG). Dionex UltiMate 3000 (Thermo Scientific, Waltham, MA, USA) and GABI Star (Raytest, Straubenhardt, Germany) radiometric detector were used for RP-HPLC analysis of ^{68}Ga-siderophores. A Nucleosil 120-5 C18 250 × 40 mm column (WATREX, Prague, Czech Republic) with a flow rate of 1 mL/min was used with the following gradient: acetonitrile (ACN)/0.1% trifluoracetic acid (TFA)/H_2O: 0-2 min, 0% ACN; 2-15 min, 0-36% ACN; 15-18 min, 36-60% ACN; 18-19.5 min, 60% ACN; 19.5-20 min, 60-0% ACN; 20-24 min, 0% ACN.

ITLC-SG (Varian, Lake Forest, CA, USA) was used for ^{68}Ga-citrate, ^{68}Ga-acetate/colloid and ^{68}Ga-colloid quality control. The radiochemical purity of ^{68}Ga-citrate was determined by ITLC-SG using methanol/glacial acetic acid (9:1) as mobile phase (free ^{68}Ga, $R_f = 0$ and ^{68}Gacitrate, $R_f = 1$) (ref.[14]). Two methods were used for the assessment of ^{68}Ga-acetate/colloid and ^{68}Ga-colloid using ITLC-SG as previously described[15]. The distribution of radioactivity along the ITLC-SG strips was measured on a Cyclone Plus Storage Phosphor system (PerkinElemer, Waltham, MA, USA).

Animal experiments

All animal experiments were conducted in accordance with regulations and guidelines of the Czech Animal Protection Act (No. 246/1992) with the approval of the Czech Ministry of Education Youth and Sports (MSMT-18933/2013-1), and the institutional Animal Welfare Committee of the Faculty of Medicine and Dentistry of Palacky University in Olomouc. The studies were performed using DBA/2 mice (Anlab, Prague, Czech Republic).

Biodistribution in mice

Normal DBA/2 mice (female) were retro-orbitally (r.o.) injected with a dose of 1-2 MBq per mouse of the ^{68}Ga labelled tracer. Animals were sacrificed by cervical dislocation 30 min and 90 min postinjection (p.i.). The organs (blood, spleen, pancreas, stomach, intestine, kidneys, liver, heart, lung, muscle and femur) were removed and radioactivity was measured in an automatic gamma counter (Wizard^2, PerkinElmer, Waltham, MA, USA). The results are expressed as percentage of injected dose per gram organ (%ID/g).

Animal imaging

The positron emission tomography (PET) and computed tomography (CT) images were acquired with an Albira PET/SPECT/CT small animal imaging system (Bruker Biospin Corporation, Woodbridge, CT, USA) (ref.[16]). Radiolabelled tracers were administered retroorbitally into DBA/2 mice in a dose of 5-10 MBq per mouse. Mice were subsequently anaesthetized with isoflurane (FORANE, Abbott Laboratories, Abbott Park, IL, USA) (2% flow rate) and were kept under anaesthesia during the imaging. Static PET/CT imaging was carried out 5 min, 30 min and 90 min p.i. A 5-min PET scan (axial FOV 148 mm) was performed, followed by a CT scan (axial FOV 65 mm, 45 kVp, 400 μA, at 600 projections). Dynamic imaging was carried out immediately after the injection of ^{68}Ga labelled tracer for 90 min (5-min PET scan per frame). Scans were reconstructed with the Albira software (Bruker Biospin Corporation, Woodbridge, CT, USA) using maximum likelihood expectation maximization (MLEM) and filtered backprojection (FBP) algorithms[16,17]. After reconstruction, the acquired data were viewed and analyzed with PMOD software (PMOD Technologies Ltd., Zurich, Switzerland). The 3D images were obtained using VolView software (Kitware, Clifton Park, NY, USA).

RESULTS

Radiolabelling and analytics

Both ^{68}Ga labelled siderophores displayed high radiochemical purity (≥ 98%) and *in vitro* stability as previously described[8]. Fig. 1 shows the proposed chemical structures and radiochromatograms of ^{68}Ga-TAFC and ^{68}Ga-FOXE, with a single main peak for both ^{68}Ga-siderophores (^{68}Ga-TAFC retention time = 15.8 min and ^{68}Ga-FOXE retention time = 13.6 min). Free ^{68}Ga analyzed using RP-HPLC showed a single peak at the retention time (Rt) of 3 min as presented in Fig. 1. ^{68}Ga-citrate, ^{68}Ga-acetate/colloid and ^{68}Ga-colloid were determined using ITLC-SG methods. ^{68}Ga-citrate and ^{68}Ga-colloid were prepared with a radiochemical purity of ≥ 95% and ^{68}Ga-acetate/colloid mixture contained ≥ 75% of ^{68}Ga-acetate.

Ex vivo biodistribution

In normal DBA/2 mice (Table 1), both ^{68}Ga-TAFC and ^{68}Ga-FOXE showed rapid renal excretion with highest activity retained in kidneys (1.88±0.53 %ID/g for ^{68}Ga-TAFC and 0.75±0.24 %ID/g for ^{68}Ga-FOXE) and low blood values (0.24±0.10 %ID/g for ^{68}Ga-TAFC and 0.04±0.01 %ID/g for ^{68}Ga-FOXE) at 90 min after application. ^{68}Ga-TAFC showed almost no retention in other organs, while ^{68}Ga-FOXE displayed some uptake (0.92

± 0.09 %ID/g (30 min p.i.) and 0.84±0.05 %ID/g (90 min p.i.)) in the gastrointestinal tract, which was confirmed by μPET imaging. ^{68}Ga-acetate/colloid, ^{68}Ga-colloid and ^{68}Ga-citrate revealed significantly different *ex vivo* biodistribution compared to ^{68}Ga-siderophores (see Table 1). High blood values (17.0±1.0 %ID/g at 90 min p.i.) and pronounced retention of radioactivity in highly perfused organs (4.1±1.1 %ID/g for spleen, 4.3±0.5 %ID/g for liver, 5.2±1.2 %ID/g for heart, 7.2±0.7 %ID/g for lung at 90 min p.i.) were found in mice injected with ^{68}Ga-acetate/colloid. ^{68}Ga-citrate showed comparable *in vivo* behaviour as ^{68}Ga-acetate/colloid. The highest level of radioactivity was found in the blood (19.9±1.5 %ID/g at 90 min p.i.) and highly perfused organs revealed significant levels of retained activity (5.9±2.7 %ID/g for spleen, 3.8±0.3 %ID/g for liver, 9.0±0.7 %ID/g for heart, 10.9±1.4 %ID/g for lung at 90 min p.i.). In contrast, ^{68}Ga-colloid displayed predominant liver uptake (36.2±2.7 %ID/g at 90 min p.i.).

Animal imaging

μPET imaging of normal DBA/2 mice confirmed rapid renal excretion of ^{68}Ga-TAFC showing almost all injected activity in bladder at 45 min p.i. (Fig. 2 A and 3 A). ^{68}Ga-FOXE revealed similar biodistribution compared to ^{68}Ga-TAFC. The only relevant exception was an evident uptake of ^{68}Ga-FOXE in gastrointestinal tract, conspicuous from 50 min frame of dynamic imaging (Fig. 2 B and 3 B). In contrast, both ^{68}Ga-acetate/colloid and ^{68}Gacitrate showed very slow *in vivo* kinetics with high retention of radioactivity in the blood pool (Fig. 2 C and E). ^{68}Ga-colloid displayed rapid liver uptake slowly increasing over time (Fig. 2 D). Static imaging (Fig. 4) confirmed and supported the results gained from *ex vivo* biodistribution and dynamic imaging.

DISCUSSION

The development of ^{68}Ga-radiopharmaceuticals has increased enormously in the last five years. The renewed popularity of ^{68}Ga was initiated mainly by the development of new, simple to use ^{68}Ge/^{68}Ga generator systems, by the fact that positron emission tomography (PET) has become a routine clinical and preclinical imaging modality and owing to the favourable chemical properties of ^{68}Ga and developed ^{68}Ga labelling strategies including automation[11,18].

The commercially available ^{68}Ge/^{68}Ga generator from Eckert & Ziegler Eurotope GmbH (Berlin, Germany) used for our study provides ^{68}Ga in its ionic form as $^{68}Ga^{3+}$. Elution is performed using hydrochloric acid solution, since acidic conditions are required to inhibit hydrolysis of the $^{68}Ga^{3+}$ ion (pH < 3), which is a requirement for chemical processing and successful radiolabelling[10].

The in-house accessibility, favourable radiochemical properties of $^{68}Ga^{3+}$ and chemical similarities with Fe^{3+}, which is chelated by siderophores, led us to attempt ^{68}Ga labelling of selected siderophores. These attempts were successful and we have shown that various siderophores can be labelled with ^{68}Ga (ref.[8]). Moreover, we have demonstrated that ^{68}Ga-TAFC and ^{68}Ga-FOXE are highly selectively accumulated in the infected tissue in *Aspergillus fumigatus* rat infection model[12,13] and ^{68}Ga-TAFC revealed high *in vitro*

specificity towards *Aspergillus fumigatus*[19]. Our studies showed, that ^{68}Ga-TAFC in particular appears to be promising candidate for the noninvasive detection of *Aspergillus* infections by PET.

In this work, we studied *in vivo* kinetics and biodistribution of different ^{68}Ga labelled tracers: ^{68}Ga-siderophores (TAFC and FOXE), ^{68}Ga-citrate, ^{68}Ga-colloid and ^{68}Ga-acetate/colloid. ^{68}Ga-citrate was chosen as a representative of infection imaging tracer due to successful application of ^{67}Ga-citrate for SPECT imaging in the past[20] and recent reports on the clinical use of ^{68}Ga-Citrate[21]. ^{68}Ga-colloid is a radiochemical impurity, which can occur during ^{68}Ga labelling at higher pH and could influence the biodistribution of improperly prepared ^{68}Ga-siderophores. ^{68}Ga-acetate/colloid mixture was studied to compare the *in vivo* behaviour of ^{68}Ga-TAFC and ^{68}Ga-FOXE versus reaction mixture used for ^{68}Ga labelling of these siderophores.

^{68}Ga-TAFC and ^{68}Ga-FOXE showed high radiochemical purity and similar retention times using previously described RP-HPLC method[12], while ^{68}Ga-citrate, ^{68}Ga-colloid and ^{68}Ga-acetate/colloid displayed completely different analytical behaviour using ITLC-SG. Subsequent *in vivo* small animal imaging showed rapid elimination of both ^{68}Ga-siderophores mainly via kidneys. This supports the findings of high *in vivo* stability of these complexes with only intact ^{68}Ga-TAFC and ^{68}Ga-FOXE found as urinary excretion products[12]. The only significant difference in the biodistribution of ^{68}Ga-TAFC and ^{68}Ga-FOXE was found in certain uptake of ^{68}Ga-FOXE in gastrointestinal tract in later time points, which could be caused by the binding of ^{68}Ga-FOXE to intestinal microflora. This hypothesis is supported by our previous study, testing of uptake specificity of ^{68}Ga-TAFC and ^{68}Ga-FOXE in various microorganisms[19], which showed clear uptake of ^{68}Ga-FOXE not only in *Aspergillus fumigatus*, but also in another tested fungal and bacterial species, indicating higher specificity of ^{68}Ga-TAFC for imaging *Aspergillus fumigatus* infections. μPET/CT imaging of ^{68}Ga-citrate in mice revealed slow excretion of the radioactivity with high blood pool values, which is in accordance with the investigations of Kumar et al.[22], who have studied ^{68}Ga-citrate for diagnostic imaging of infection in rats. ^{68}Ga-acetate/colloid displayed comparable *in vivo* behaviour to ^{68}Ga-citrate. Both citrate and acetate are weak chelators *in vivo*. ^{68}Ga is rapidly released from the weak complex and bound to transferrin, ferritin and other iron-binding proteins[23], which explain the high blood pool values for both tracers. The *in vivo* imaging of ^{68}Ga-colloid showed clear rapid liver uptake slowly increasing in time. The *ex vivo* biodistribution data showed perfect correlation with *in vivo* dynamic and static imaging.

CONCLUSIONS

Both studied ^{68}Ga labelled siderophores displayed excellent and significantly different *in vivo* behaviour compared to ^{68}Ga-citrate, ^{68}Ga-colloid and ^{68}Ga-acetate/colloid, and especially ^{68}Ga-TAFC confirmed its great potential to be used as specific tracer for *Aspergillus* infection imaging. These data on normal biodistribution and pharmacokinetics of ^{68}Ga-TAFC and ^{68}Ga-FOXE are essential for further translation of these promising compounds into the clinic.

ACKNOWLEDGMENTS

We would like to thank the staff of the Animal Facilities of Institute of Molecular and Translational Medicine of Faculty of Medicine and Dentistry of Palacky University in Olomouc. We gratefully acknowledge the financial support of Biomedreg project CZ.1.05/2.1.00/01.0030, National Programme of Sustainability LO1304 and the Austrian Science Foundation (FWF; grant L676-B18 to C.D. and I1346-B2 to H.H.).

References

1. Neilands JB. Siderophores: structure and function of microbial iron transport compounds. J Biol Chem. 1995; 270:26723–6. [PubMed: 7592901]
2. Hider RC, Kong X. Chemistry and biology of siderophores. Nat Prod Rep. 2010; 27:637–57. [PubMed: 20376388]
3. Weiss G. Iron and immunity: a double-edged sword. Eur J Clin Invest. 2002; 32:70–8. [PubMed: 11886435]
4. Schrettl M, Beckmann N, Varga J, Heinekamp T, Jacobsen ID, Jöchl C, Moussa TA, Wang S, Gsaller F, Blatzer M, Werner ER, et al. HapX-mediated adaption to iron starvation is crucial for virulence of Aspergillus fumigatus. PLoSPathog. 2010; 6(9):e1001124.doi: 10.1371/journal.ppat.1001124
5. Baddley JW, Andes DR, Marr KA, Kontoyiannis DP, Alexander BD, Kauffman CA, et al. Factors associated with mortality in transplant patients with invasive aspergillosis. Clin Infect Dis. 2010; 50:1559–67. [PubMed: 20450350]
6. Pagano L, Akova M, Dimopoulos G, Herbrecht R, Drgona L, Blijlevens N. Risk assessment and prognostic factors for mouldrelated diseases in immunocompromised patients. J Antimicrob Chemother. 2011; 66:i5–14. [PubMed: 21177404]
7. Reichenberger F, Habicht JM, Gratwohl A, Tamm M. Diagnosis and treatment of invasive pulmonary aspergillosis in neutropenic patients. Eur Respir J. 2002; 19:743–55. [PubMed: 11999005]
8. Petrik M, Haas H, Schrettl M, Helbok A, Blatzer M, Decristoforo C. In vitro and in vivo evaluation of selected ^{68}Ga-siderophores for infection imaging. Nucl Med Biol. 2012; 39:361–9. [PubMed: 22172389]
9. Fani M, André JP, Maecke HR. 68Ga-PET: a powerful generator based alternative to cyclotron-based PET radiopharmaceuticals. Contrast Media Mol Imaging. 2008; 3:53–63. [PubMed: 18383455]
10. Decristoforo C. Gallium-68 – a new opportunity for PET available from a long shelf-life generator – automation and applications. Curr Radiopharm. 2012; 5:212–20. [PubMed: 22642389]
11. Velikyan I. Prospective of ^{68}Ga-radiopharmaceutical development. Theranostics. 2014; 4:47–80. [PubMed: 24396515]
12. Petrik M, Haas H, Dobrozemsky G, Lass-Flörl C, Helbok A, Blatzer M, Dietrich H, Decristoforo C. ^{68}Ga-Siderophores for PET imaging of invasive pulmonary aspergillosis: proof of principle. J Nucl Med. 2010; 51:639–45. [PubMed: 20351354]
13. Petrik M, Franssen GM, Haas H, Laverman P, Hörtnagl C, Schrettl M, Helbok A, Lass-Flörl C, Decristoforo C. Preclinical evaluation of two ^{68}Ga-siderophores as potential radiopharmaceuticals for *Aspergillus fumigatus* infection imaging. Eur J Nucl Med Mol Imaging. 2012; 39:1175–83. [PubMed: 22526953]
14. Rizzello A, Di Pierro D, Lodi F, Trespidi S, Cicoria G, Pancaldi D, Nanni C, Marengo M, Marzola MC, Al-Nahhas A, Rubello D, et al. Synthesis and quality control of 68Ga citrate for routine clinical PET. Nucl Med Comm. 2009; 30:542–5.
15. Ocak M, Antretter M, Knopp R, Kunkel F, Petrik M, Bergisadi N, Decristoforo C. Full automation of ^{68}Ga labelling of DOTA-peptides including cation exchange prepurification. Appl Radiat Isot. 2010; 68:297–302. [PubMed: 19906533]

16. Sánchez F, Orero A, Soriano A, Correcher C, Conde P, González A, Hernández L, Moliner L, Rodríguez-Alvarez MJ, Vidal LF, Benlloch JM, et al. ALBIRA: a small animal PET/SPECT/CT imaging system. Med Phys. 2013; 40:051906. [PubMed: 23635276]
17. Sanchez F, Moliner L, Correcher C, Gonzalez A, Orero A, Carles M, Soriano A, Rodriguez-Alvarez MJ, Medina LA, Mora F, Benlloch JM. Small animal PET scanner based on monolithic LYSO crystals: performance evaluation. Med Phys. 2012; 39:643–53. [PubMed: 22320773]
18. Rösch F. Past, present and future of ^{68}Ge/^{68}Ga generators. Appl Radiat Isot. 2013; 76:24–30. [PubMed: 23245638]
19. Petrik M, Haas H, Laverman P, Schrettl M, Franssen GM, Blatzer M, Decristoforo C. ^{68}Ga-Triacetylfusarinine C and ^{68}Ga-Ferrioxamine E for *Aspergillus* infection imaging: uptake specificity in various microorganisms. Mol Imaging Biol. 2014; 16:102–8. [PubMed: 23818006]
20. Hughes DK. Nuclear medicine and infection detection: the relative effectiveness of imaging with ^{111}In-oxine-, ^{99m}Tc-HMPAO-, and ^{99m}Tc-stannous fluoride colloid-labeled leukocytes and with ^{67}Ga-citrate. J Nucl Med Technol. 2003; 31:196–201. [PubMed: 14657285]
21. Nanni C, Errani C, Boriani L, Fantini L, Ambrosini V, Boschi S, Rubello D, Pettinato C, Mercuri M, Gasbarrini A, Fanti S. ^{68}Ga-citrate PET/CT for evaluating patients with infections of the bone: preliminary results. J Nucl Med. 2010; 51:1932–6. [PubMed: 21078801]
22. Kumar V, Boddeti DK, Evans SG, Angelides S. ^{68}Ga-Citrate-PET for diagnostic imaging of infection in rats and for intra-abdominal infection in a patient. Curr Radiopharm. 2012; 5:71–5. [PubMed: 22074481]
23. Silvola JM, Laitinen I, Sipilä HJ, Laine VJ, Leppänen P, Ylä-Herttuala S, Knuuti J, Roivainen A. Uptake of 68gallium in atherosclerotic plaques in LDLR-/-ApoB100/100 mice. EJNMMI Res. 2011; 1(1):14.doi: 10.1186/2191-219X-1-14 [PubMed: 22214258]

Europe PMC Funders Author Manuscripts

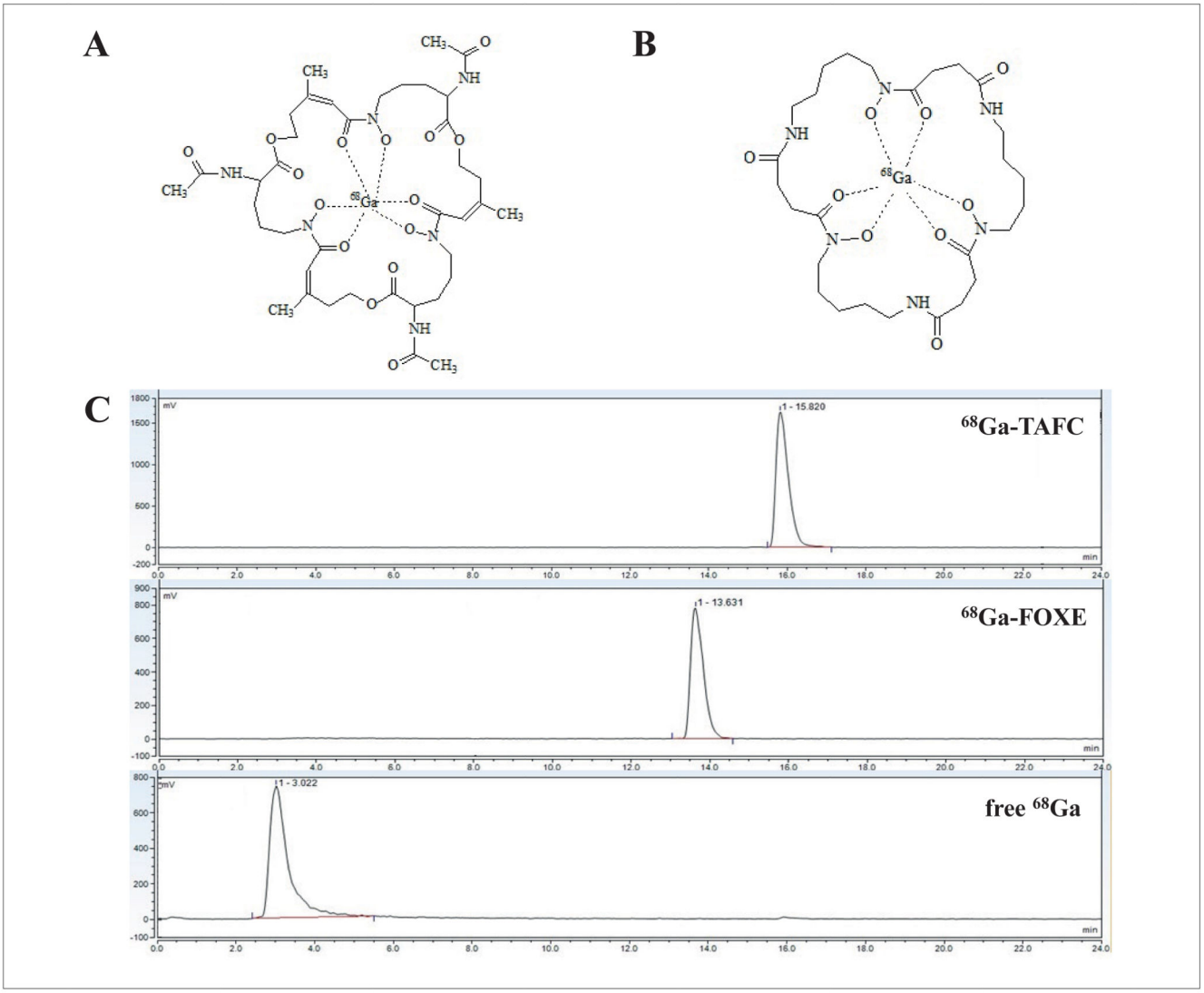

Fig. 1.

Proposed chemical structures of ^{68}Ga-TAFC (A) and ^{68}Ga-FOXE (B) and HPLC-radiochromatograms of ^{68}Ga-TAFC, ^{68}Ga-FOXE and free ^{68}Ga (C).

Europe PMC Funders Author Manuscripts

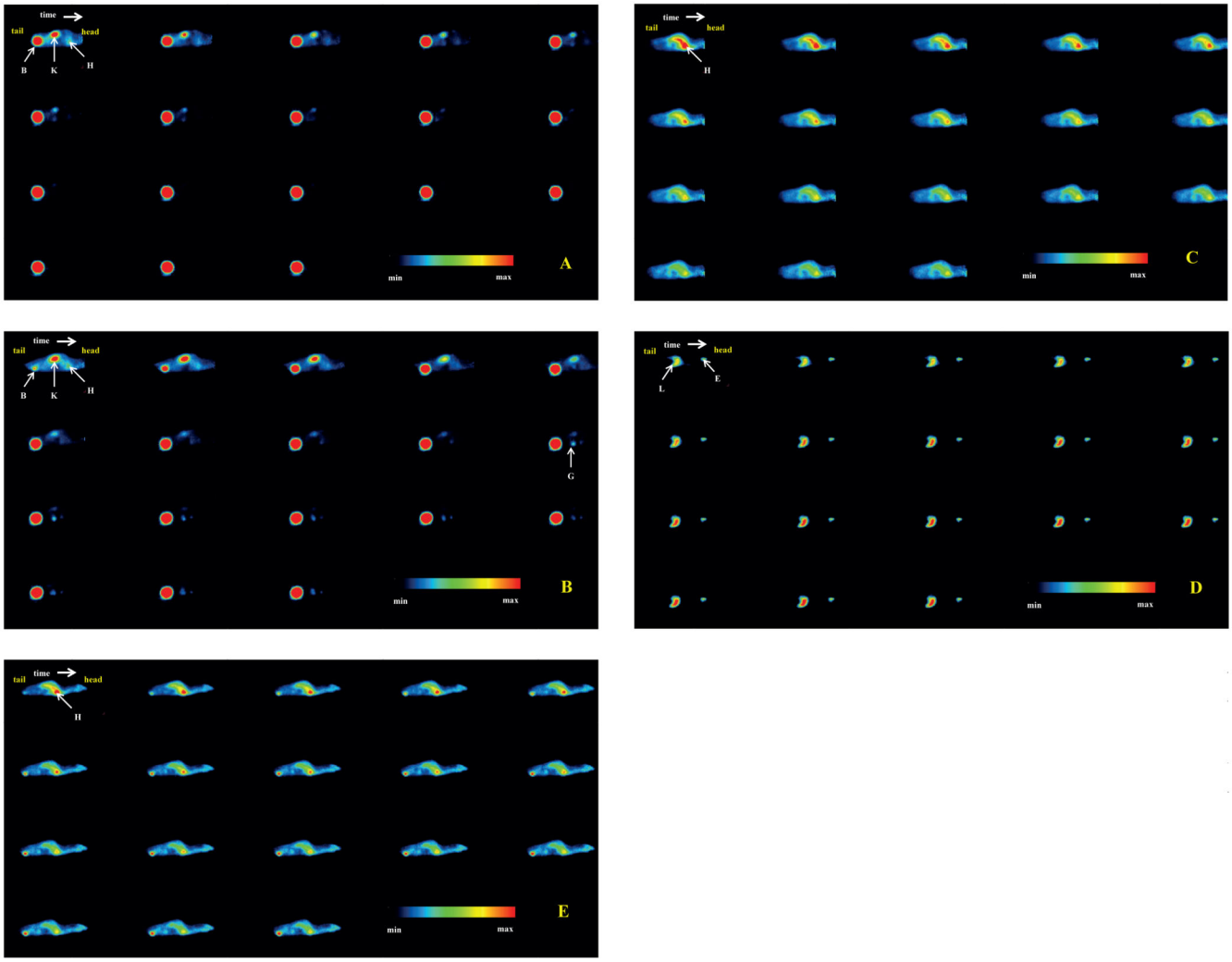

Fig. 2.

Dynamic μPET imaging of ^{68}Ga-TAFC (A), ^{68}Ga-FOXE (B), ^{68}Ga-acetate/colloid (C), ^{68}Ga-colloid (D) and ^{68}Ga-citrate (E) up to 90 min p.i. (sagittal slices; injected dose: 5-10MBq; anaesthesia: 2% isoflurane; scan duration: 5-minute PET scan per frame (18 frames); B – bladder, E – eye, G – gastrointectinal tract, H – heart, K – kidney, L - liver).

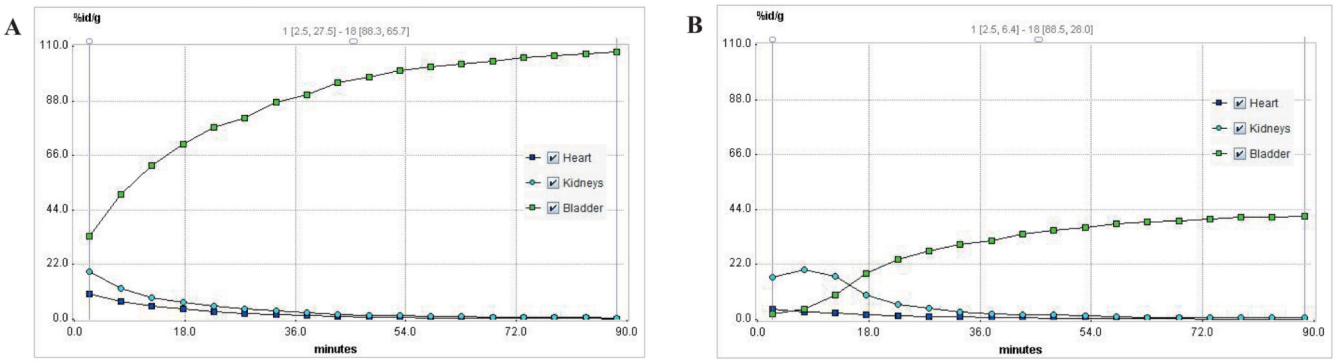

Fig. 3.

Time activity curves of selected regions of interest (heart, kidneys, bladder) of ^{68}Ga-TAFC (A) and ^{68}Ga-FOXE (B).

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

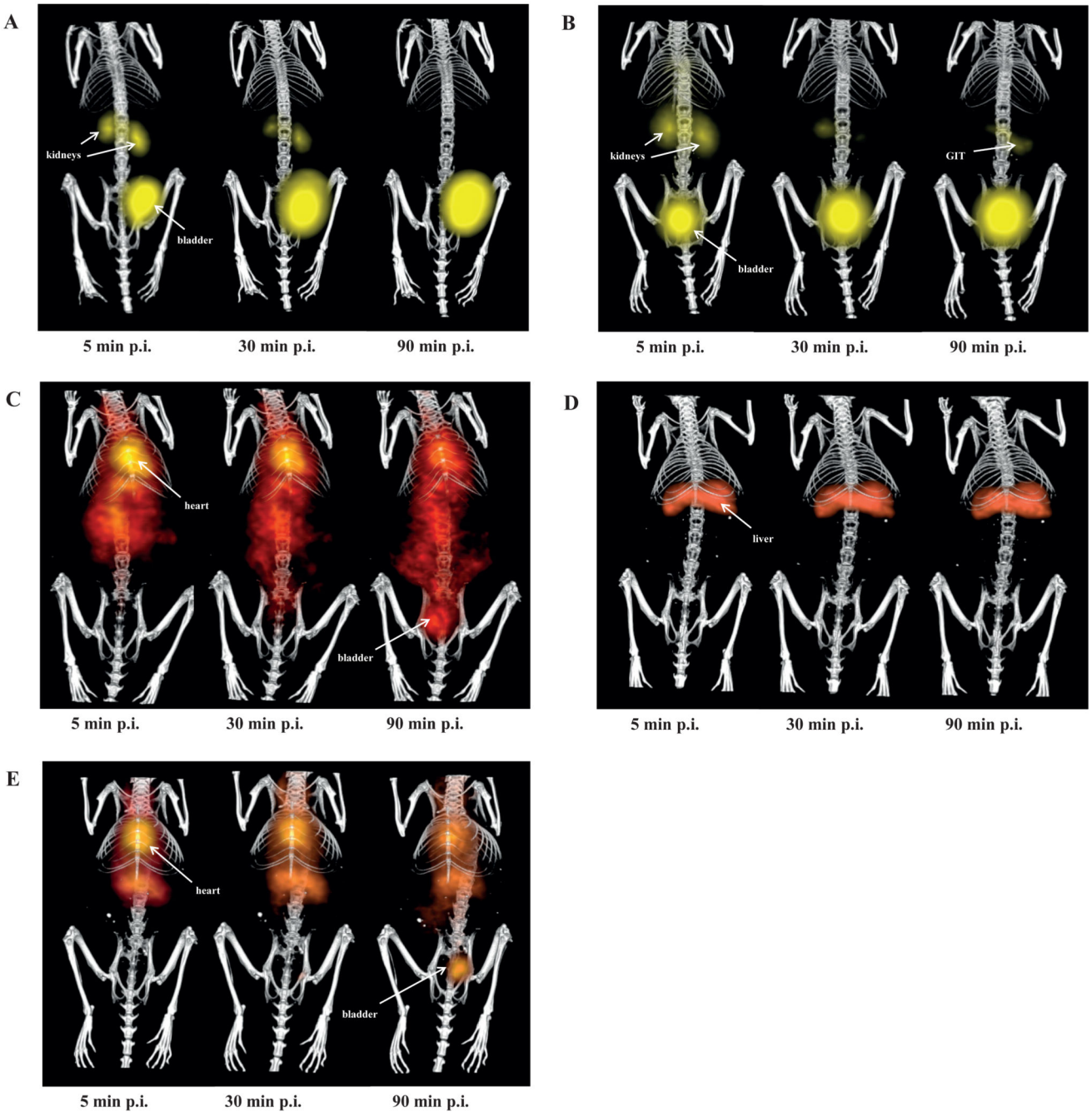

Fig. 4.

Static μPET/CT imaging of ^{68}Ga-TAFC (A), ^{68}Ga-FOXE (B), ^{68}Ga-acetate/colloid (C), ^{68}Ga-colloid (D) and ^{68}Ga-citrate (E) 5 min, 30 min and 90 min p.i. (supine position; injected dose 5-10MBq; anaesthesia: 2% isoflurane; scan duration: 5-minute PET scan followed by 15-minute CT scan).

Europe PMC Funders Author Manuscripts

Table 1.

Biodistribution of ^{68}Ga-TAFC, ^{68}Ga-FOXE, ^{68}Ga-acetate/colloid, ^{68}Ga-colloid and ^{68}Ga-citrate in normal DBA/2 mice 30 min and 90 min p.i.

Organ	^{68}Ga-TAFC		^{68}Ga-FOXE		^{68}Ga-acetate/colloid		^{68}Ga-colloid		^{68}Ga-citrate	
	30 min	90 min	30 min	90 min	30 min	90 min	30 min	90 min	30 min	90 min
Blood	2.92 ± 1.48	0.24 ± 0.09	1.04 ± 0.10	0.04 ± 0.01	21.7 ± 2.64	17.0 ± 1.00	10.8 ± 0.32	8.40 ± 1.04	20.8 ± 2.60	19.9 ± 1.53
Spleen	0.68 ± 0.12	0.09 ± 0.04	0.35 ± 0.13	0.05 ± 0.01	5.01 ± 0.06	4.05 ± 1.08	9.72 ± 2.68	5.91 ± 2.67	3.49 ± 0.70	4.16 ± 0.64
Pancreas	1.04 ± 0.39	0.13 ± 0.01	0.52 ± 0.23	0.06 ± 0.02	3.54 ± 0.45	2.57 ± 0.47	1.85 ± 0.42	1.51 ± 0.30	4.16 ± 0.16	3.70 ± 0.28
Stomach	1.64 ± 0.48	0.35 ± 0.41	0.63 ± 0.16	0.06 ± 0.01	2.35 ± 0.21	2.84 ± 0.45	1.38 ± 0.13	1.32 ± 0.01	3.21 ± 0.54	3.62 ± 0.29
Intestine	1.37 ± 0.35	0.49 ± 0.25	0.92 ± 0.09	0.84 ± 0.05	2.78 ± 0.42	3.85 ± 0.30	1.29 ± 0.15	1.55 ± 0.07	3.30 ± 0.38	4.29 ± 0.23
Kidneys	11.8 ± 3.60	1.88 ± 0.53	3.12 ± 0.29	0.75 ± 0.24	5.59 ± 0.27	4.44 ± 0.31	2.46 ± 0.63	2.73 ± 0.53	5.46 ± 1.40	5.79 ± 0.32
Liver	0.79 ± 0.30	0.11 ± 0.02	0.86 ± 0.02	0.13 ± 0.01	4.84 ± 0.85	4.34 ± 0.49	36.7 ± 5.92	36.2 ± 2.67	3.38 ± 0.34	3.86 ± 0.35
Heart	1.10 ± 0.36	0.08 ± 0.02	0.46 ± 0.12	0.05 ± 0.01	7.06 ± 0.71	5.25 ± 1.16	3.35 ± 0.31	2.65 ± 0.50	8.12 ± 1.28	9.05 ± 0.77
Lung	2.51 ± 0.97	0.27 ± 0.12	0.95 ± 0.15	0.10 ± 0.01	8.45 ± 0.33	7.24 ± 0.66	4.76 ± 0.89	5.57 ± 1.25	10.6 ± 3.76	10.9 ± 1.46
Muscle	0.65 ± 0.31	0.35 ± 0.15	0.22 ± 0.06	0.06 ± 0.03	1.22 ± 0.23	1.51 ± 0.51	0.68 ± 0.10	0.63 ± 0.06	1.79 ± 0.23	1.93 ± 0.19
Femur	0.55 ± 0.06	0.76 ± 0.49	0.15 ± 0.05	0.12 ± 0.09	1.64 ± 0.32	1.84 ± 0.24	1.20 ± 0.05	1.17 ± 0.07	2.51 ± 0.37	2.81 ± 0.50

Data are presented as % injected dose per gram organ (%ID/g ± Sd) (n = 3).