ChemComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2013, **49**, 564

Received 12th October 2012,
Accepted 26th November 2012

DOI: 10.1039/c2cc37460a

www.rsc.org/chemcomm

Gd^{3+} cFLFLFK conjugate for MRI: a targeted contrast agent for FPR1 in inflammation†

Graeme J. Stasiuk,[ab] Helen Smith,[b] Marzena Wylezinska-Arridge,[c] Jordi L. Tremoleda,[c] William Trigg,[d] Sajinder Kaur Luthra,[d] Veronique Morisson Iveson,[d] Felicity N. E. Gavins*[b] and Nicholas J. Long*[a]

Formyl Peptide Receptors (FPRs) are vital in the host inflammatory response, playing an important regulatory role in multiple diseases. A Gd(III) DOTA conjugate of cFLFLFK has been synthesised which targets and visualises FPR1 upon leukocytes in the inflammatory response *via* magnetic resonance imaging for the first time.

Magnetic resonance imaging (MRI) is a powerful non-invasive diagnostic tool used in clinical medicine. It provides highly detailed three-dimensional images of organs, tissues and blood-flow within the human body, through visualizing water protons within the subject. As a result of the high abundance of water molecules in biological systems, it is able to detect a wide variety of pathological conditions, such as heart disease,[1] cancer[2] and musculoskeletal disorders.[3] Paramagnetic metals such as gadolinium within a stable chelate have been used very successfully as contrast agents in MRI, enhancing signal intensity to aid in the diagnosis of numerous disease states over the last 25 years.[4] Targeted Gd-based contrast agents, is an area of research that has been currently expanding, in particular for proteins involved in disease states of the circulatory system.[5]

The host inflammatory response is a complex process characterised by multiple molecular and cellular phenomena, including the rolling, adherence and emigration of leukocytes from vasculature into parenchymal tissue at sites of inflammation.[6] These phenomena contribute to widely recognised clinical manifestations of inflammation *i.e.* pain, fever, redness, swelling and in the case of chronic inflammation (such as rheumatoid arthritis) loss of function.[7] Although inflammation is critical for pathogen-removal, effective resolution and tissue recovery is dependent on a fine balance between pro- and anti-inflammatory mediators, with stringent temporal and quantitative restrictions on each.[7] Neutrophils are the most abundant leukocyte in humans and typically the first type of white cell recruited to sites of inflammation.[7,8] The ability to detect and quantify neutrophilic infiltration into the tissue with a non-invasive technique, such as MRI, will enable the identification of inflammatory lesions and provide a useful screening tool for multiple disease states.

The FPRs are a family of seven transmembrane domain G-protein-coupled receptors (GPCRs), found on leukocytes.[9] FPR1 is a highly abundant cell surface receptor on neutrophils, expressed under inflammatory conditions, which mediates emigration to the site of injury. This has been widely demonstrated through immunohistology and a variety of other *in vitro* techniques.[10] Several agonists and antagonists bind to members of the FPRs with various potencies and specificities; in particular, the peptide antagonist cFLFLFK, which specifically binds FPR1.[11]

cFLFLFK is a hexapeptide with a cinnamoyl group at the c-terminus, which has previously been modified with a PEG-DOTA motif by Locke *et al.* for use as a positron emission tomography (PET) imaging agent with ^{64}Cu, a PEG-TKPPR-^{99m}Tc for Single Photon Emission Computed Tomography (SPECT) and with a cyanine-7 for optical imaging.[12]

In this paper we show the design, synthesis and validation of the first MRI contrast agent that specifically targets FPR1 receptors in inflamed tissues.

The desired Gd(III) contrast agent was synthesised from 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and cFLFLFK as shown in Scheme 1, with an overall yield of 65%. Conjugation of the DOTA motif was achieved using EDC and NHS at stoichiometric ratio to allow for only one carboxyl

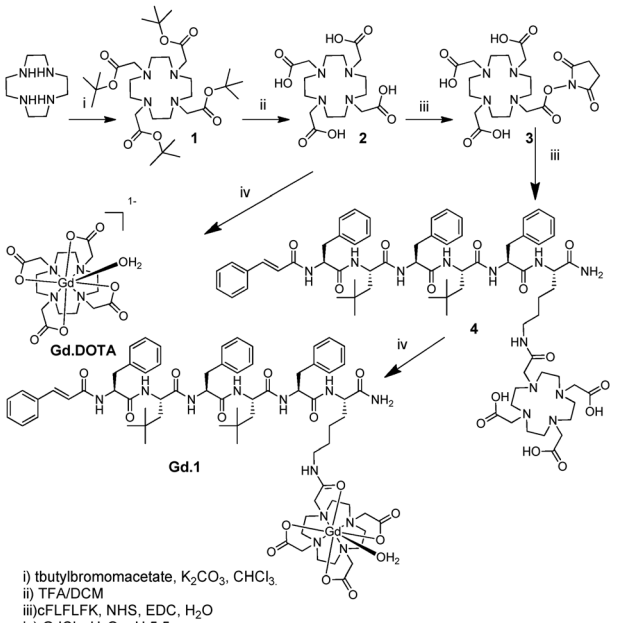
[a] *Department of Chemistry, Imperial College London, South Kensington Campus, London SW7 2AZ, UK. E-mail: n.long@imperial.ac.uk; Fax: +44 (0)20 7594 5804; Tel: +44 (0)20 7594 5781*

[b] *Division of Brain Sciences, Department of Medicine, Imperial College London, Du Cane Road, London, W12 ONN, UK. E-mail: f.gavins@imperial.ac.uk; Fax: +44 (0)20 7594 6548; Tel: +44 (0)20 7594 6586*

[c] *Biological Imaging Centre, Imperial College London, Du Cane Road, London, W12 ONN, UK*

[d] *GE Healthcare, The Grove Centre, White Lion Road, Amersham, Buckinghamshire, HP7 9LL, UK*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2cc37460a



Scheme 1 Synthesis of **Gd·1**.

group on the DOTA to react with the amine on the n-terminal lysine residue, forming an amide bond and providing the proligand (**4**) which was purified *via* HPLC. $GdCl_3.6H_2O$ was reacted with the proligand (**4**) in H_2O at pH 5.5 to give **Gd·1** in 88% yield. Relaxivity is a measure of how effective the contrast agent would be in an MRI experiment. The r_1 = 5.4 mM^{-1} s^{-1} (400 MHz, 9.4 T) value is within the range for a Gd chelate with a hydration state of 1, which is expected for the eight coordinate macrocycle and confirmed by the lifetime measurements for the terbium analogue **Tb·1** (Table S1, ESI†). **Gd·DOTA**, the gold standard commercial contrast agent, has an r_1 = 4.2 mM^{-1} s^{-1}.[13] The slight increase in relaxivity of **Gd·1** can be attributed to its larger mass and even at fields such as 9.4 T it presents a high relaxivity for a single chelate agent.[14]

In order to validate the specificity of **Gd·1** for FPR1, receptor-binding assays were undertaken. The receptor binding constant was obtained using Human Embryonic Kidney (HEK) cells stably expressing FPR1. The assay was a competition study with ^{125}I-labelled fMLP-analogue and **Gd·1**. The K_d = 4.5 nM, which is a slight change from the native peptide with a K_d = 2 nM,[15] thus illustrating that the **Gd·1** still binds FPR1 in a similar manner as the native peptide. Therefore the choice of conjugation upon the lysine residue does not disrupt the binding to FPR1. This confirmation of high relaxivity for a single molecule contrast agent and the specific binding to FPR1 provides a very useful targeted contrast agent for disease states indicated by inflammation.

In order to investigate the imaging capabilities of **Gd·1** with regards to FPR1, *in vivo* experiments were set up. C57BL/6 mice were treated for 24 hours with lipopolysaccharide (LPS. 10 μg per mouse intraperitoneally (i.p.)). LPS is a bacterial fragment that disrupts the vascular endothelium, causing sites of inflammation and the recruitment of activated neutrophils, following which, 1 mmol kg^{-1} of **Gd·1**/**Gd·DOTA** was injected intravenously.

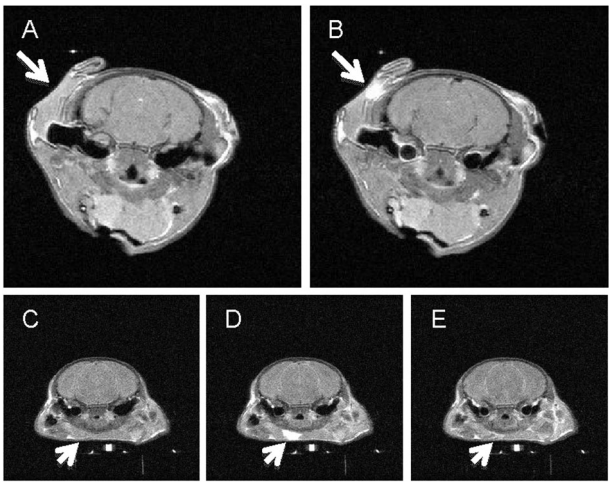


Fig. 1 MRI images of a mouse brain. (A) Before injection and (B) 80 minutes after injection with **Gd·1**. (C) Pre-injection (D) 1 minute and (E) 80 minutes after injection with **Gd·DOTA**. 1 mmol Kg^{-1} (Gd·agent) 37 °C, Arrows indicate site of increased signal intensity due to Gd agent.

These proof of principle *in vivo* experiments demonstrate the binding of **Gd·1** to FPR1. This is seen in Fig. 1A and B: pre-injection on the left (1A), shows the base line and instantly after injection there is a significant increase in signal intensity around the right ear at eighty minutes (1B). The signal intensity increases over a twenty minute period as the agent passes around the circulation system, allowing for binding to the FPR1 receptors on blood-borne neutrophils. Histology of FPR1 shows that a high expression (along with FPR2) is present on neutrophils.[11] The signal intensity then levels off and is retained for up to eighty minutes. This demonstrates that **Gd·1** binds to FPR1 on neutrophils/leukocytes, which have localised around sites of inflammation, in this case behind the right ear.

This is compared to **Gd·DOTA** (Fig. 1C–E) which is a commercially available blood pool agent, showing signal increase at the site of injection, but which after 80 minutes shows no detectable increase in intensity. This illustrates the efficiency of **Gd·1** in binding FPR1 on the surface of neutrophils. From the study it is clear that **Gd·1** can be used to observe the movement of leukocytes to the sites of injury.

In conclusion, we have shown that cFLFLFK can be conjugated with DOTA and coordinated to lanthanide metals, forming a mono-aqua coordinated complex. Gd(III) has been used to create the first targeted MRI contrast agent that binds to neutrophils expressing FPR1. It has subsequently been shown to image neutrophils during an inflammatory response for up to four hours, with excellent contrast at high fields (9.4 T). This has far reaching implications for the identification of disease states involved in the inflammatory process and could also ultimately be used as a screening tool in drug discovery.

We would like to thank Drs Ji-Liang Gao, Philip Murphy and Professor Mauro Perretti for the HEK-FPR1 cells, and Professor Sir Steve Bloom, Professor Mohammad Ghatei, Andrew Hogben and Dr James Minnion for help with the receptor binding assays. The BBSRC and GE Healthcare are thanked for funding and an Industry Interchange award.

Notes and references

1 K. M. Donahue, D. Burstein, W. J. Manning and M. L. Gray, *Magn. Reson. Med.*, 1994, **32**, 66–76.
2 P. A. Sukerkar, K. W. MacRenaris, T. R. Townsend, R. A. Ahmed, J. E. Burdette and T. J. Meade, *Bioconjugate Chem.*, 2011, **22**, 2304–2316.
3 S. Blemker, D. Asakawa, G. Gold and S. Delp, *J. Magn. Reson. Imaging*, 2007, **25**, 441–451.
4 P. Caravan, *Chem. Soc. Rev.*, 2006, 512–523; R. E. Mewis and S. J. Archibald, *Coord. Chem. Rev.*, 2010, **254**, 1686–1712.
5 R. Uppal, K. L. Ciesienski, D. B. Chonde, G. S. Loving and P. Caravan, *J. Am. Chem. Soc.*, 2012, **134**, 10799–10802; A. Mishra, S. Gottschalk, J. Engelmann and D. Parker, *Chem. Sci.*, 2012, **3**, 131.
6 K. Ley, C. Laudanna, M. Cybulsky and S. Nourshargh, *Nat. Rev. Immunol.*, 2007, **7**, 678–689.
7 C. Nathan, *Nature*, 2002, **420**, 846–852; Y. Le, Y. Yang, Y. Cui, H. Yazawa, W. Gong, C. Qui and J. M. Wang, *Int. Immunopharmacol.*, 2002, **2**, 1–13.
8 A. D. Kennedy and F. R. Deleon, *Immunol. Res.*, 2009, **43**, 25–61.
9 R. D. Ye, F. Boulay, J. M. Wang, C. Dahlgren, C. Gerard, M. Parmentier, C. N. Serhan and P. M. Murphy, *Pharmacol. Rev.*, 2009, **61**, 119–161.
10 V. M. Loitto, B. Rasmusson and K. E. Magnusson, *J. Leukocyte Biol.*, 2001, **69**, 762–771; P. Mandal, M. Novotny and T. A. Hamilton, *J. Immunol.*, 2005, **175**, 6085–6091.
11 F. N. E. Gavins, *Trends Pharmacol. Sci.*, 2010, **31**, 266–276.
12 L. W. Locke, M. D. Chordia, Y. Zhang, B. Kundu, D. Kennedy, J. Landseadel, L. Xiao, K. D. Fairchild, S. S. Berr, J. Linden and D. Pan, *J. Nucl. Med.*, 2009, 790–797; Y. Zhang, L. Xiao, M. D. Chordia, L. W. Locke, M. B. Williams, S. S. Berr and D. Pan, *Bioconjugate Chem.*, 2010, **27**, 1788–1793; L. Xiao, Y. Zhang, Z. Liu, M. Yang, L. Pu and D. Pan, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 3515–3517.
13 P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293–2352.
14 A. E. Merbach and E. Toth, *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, Wiley, 2001.
15 C. J. Palestro, *J. Nucl. Med.*, 2009, **50**, 671–673.

