Bioorganic & Medicinal Chemistry Letters 28 (2018) 2949–2952

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of a [^{18}F]formyl–Met–Leu–Phe derivative: A positron emission tomography imaging probe for bacterial infections

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ARTICLE INFO

Keywords:
G protein-coupled formyl peptide receptor
Positron emission tomography
Bacterial infections
Peptide probe
^{18}F

ABSTRACT

The tripeptide formyl–Met–Leu–Phe (fMLF) is a prototype of *N*-formylated chemotactic peptides for neutrophils owing to its ability to bind and activate the G protein-coupled formyl peptide receptor (FPR). Here, we developed an ^{18}F-labeled fMLF derivative targeting FPR as a positron emission tomography (PET) imaging probe for bacterial infections. The study demonstrates that the fMLF derivative fMLFXYk(FB)k (X = Nle) has a high affinity for FPR (Ki = 0.62 ± 0.13 nM). The radiochemical yield and purity of [^{18}F]fMLFXYk(FB)k were 16% and > 96%, respectively. The *in vivo* biodistribution study showed that [^{18}F]fMLFXYk(FB)k uptake was higher in the bacterial infected region than in the non-infected region. We observed considerably higher infection-to-muscle ratio of 4.6 at 60 min after [^{18}F]fMLFXYk(FB)k injection. Furthermore, small-animal PET imaging studies suggested that [^{18}F]fMLFXYk(FB)k uptake in the bacterial infected region was clearly visualized 60 min after injection.

Infectious diseases still majorly account for the cause of death, and they require social measures. Bacteriological or serological tests are undoubtedly the gold standard for diagnosing infectious diseases. However, these tests require time to obtain results. Furthermore, although these tests prove the existence of bacteria, it is impossible to distinguish which part of the living body is infected. Therefore, the local diagnosis of infectious diseases using diagnostic imaging has been clinically used for a long time. Presently, X-Ray, CT, and MRI are used for diagnostic imaging for infectious diseases, but none of them specifically cater to the spread of inflammatory lesions associated with infection; thus, if the inflammatory foci does not enlarge, accurate diagnosis presents a challenge. Therefore, nuclear medicine technology such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) that can obtain functional information have attracted attention.[1] Scintigraphy and SPECT using autologous leukocytes labeled with ^{111}In or ^{99m}Tc are considered the gold standard for diagnosing infections.[2–5] However, there are certain drawbacks as leukocytes obtained from blood sample must be labeled *ex vivo*. This process, performed in a specialized laboratory, raises the potential risk of infection of both the patient and laboratory personnel. ^{67}Ga-citrate and 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) have been in use as SPECT and PET imaging probes for the detection of inflammatory and infectious conditions.[6–8] However, none of these probes are specific for bacterial infections. Hence, novel safety and specific imaging probes for the early detection of bacterial infections are needed.[9]

Leukocytes, particularly polymorphonuclear leukocytes (PMNs), accumulate in high concentrations at the site of infectious inflammation. The tripeptide formyl–Met–Leu–Phe (fMLF) is a prototype of *N*-formylated chemotactic peptides due to its ability to bind and activate the G protein-coupled formylpeptide receptor (FPR), which is highly expressed on PMNs.[10–12] Several probes targeting FPR have been reported but clinically used probes remain to be developed.[13–19] PET offers high sensitivity and reasonable spatial resolution and allows more accurate attenuation correction. In this study, fMLF and formyl–Nle–Leu–Phe–Nle–Tyr–Lys (fXLFXYK) (X = Nle),[20] which had a high affinity for FPR, were selected as lead compounds, and several

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https://doi.org/10.1016/j.bmcl.2018.07.009
Received 14 May 2018; Received in revised form 25 June 2018; Accepted 3 July 2018
Available online 06 July 2018
0960-894X/

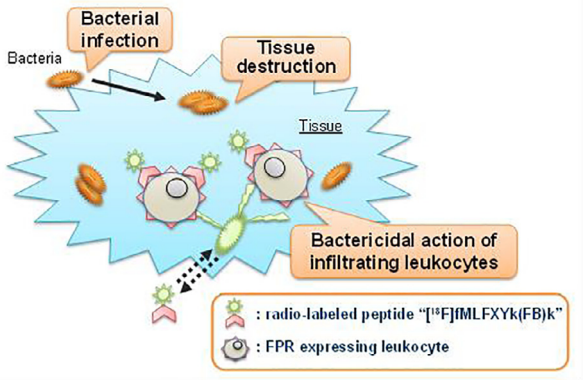


Fig. 1. Schematic of PET imaging probe for bacterial infections.

Table 1
Inhibition of [^{125}I]WKYMVm binding to FPR.

Compound	Peptide sequence	*Ki* (nM)[a]	Water Solubility
1	fMLF	4.85 ± 2.24	high
2	fXLFXYK(FB)	0.65 ± 0.26	low
3	fXLFXYK(FB)kkk	21.3 ± 2.21	high
4	fXLFXYkkkk(FB)	86.7 ± 33.8	high
5	fXLFXYkkk(FB)	15.8 ± 4.40	high
6	fXLFXYkk(FB)	1.05 ± 0.30	low
7	fMLFMYK(FB)	0.27 ± 0.08	low
8	fXLFMYK(FB)	1.03 ± 0.18	low
9	fMLFXYK(FB)	0.20 ± 0.04	low
10	fMLFXYk(FB)k	0.62 ± 0.13	high

[a] Values are mean ± standard error of the mean in 3 independent experiments.

[^{18}F]fluorobenzoyl ([^{18}F]FB) peptides were synthesized by linking [^{18}F]FB group with C-terminal-Lys, which does not affect the binding affinity for FPR, and their utility as PET probes for bacterial infections was evaluated (Fig. 1).

Table 1 reports the Ki values of fMLF analogs. The binding affinity of fMLF and its analogs for FPR was measured by competition binding assay with [^{125}I]WKYMVm using recombinant membrane protein highly expressing FPR. fMLF (**1**) [Ki = 4.85 nM] was utilized as a reference compound due to its ability to appreciably antagonize [^{125}I]WKYMVm binding to the FPR. fXLFXYK(FB) (**2**) that linked FB group to C-terminal-Lys of lead compound fXLFXYK has a high affinity for FPR (Ki = 0.65 nM), but it is difficult to apply to an *in vivo* imaging probe due to its low solubility in water.[13] The introduction of several basic amino acid residues such as Lys into the C-terminal of **2** increases solubility: fXLFXYK(FB)kkk (**3**) acquires high solubility in water. To acquire both high solubility in water and affinity for FPR, the affinities of some analogs such as fXLFXYkkkk(FB) (**4**), fXLFXYkkk(FB) (**5**), and fXLFXYkk(FB) (**6**) were compared. A small amount of Lys residue improves the affinity for FPR: **6** has high affinity (Ki = 1.05 nM) compared with **4** (Ki = 86.7 nM). The introduction of an FB group into Lys[6] increases its affinity for FPR than does its introduction into C-terminal-Lys: **3** has a high affinity (Ki = 21.3 nM) compared with **4**. On the other hand, fXLFXYK(FB) analogs replacing Nle[1] or Nle[4] with Met were evaluated (compounds **7**–**10**). Compared with **2**, fMLFMYK(FB) (**7**) replacing Nle[1] and Nle[4] with Met has a high affinity (Ki = 0.65 vs 0.27 nM). On the substitution of the Met[4] residue in the peptide **7**, enhanced affinity for FPR is observed with Nle [fMLFXYK(FB), **9**, Ki = 0.20 nM], whereas there was a reduction in Met[1] residue with Nle (fXLFMYK(FB), **8**, Ki = 1.03 nM). fMLFXYk(FB)k (**10**) that has Met[1] and Nle[4] and introduces a Lys residue into the C-terminal and the FB group into Lys[6] acquired a high affinity for FPR (Ki = 0.62 nM) and moderate solubility in water.

Therefore, we decided to analyze the high affinity of fMLFXYk(FB)k for FPR by docking simulation. Structure models of FPR were created using the crystal structure of the bovine rhodopsin receptor (Protein Data Bank code 1U19), and pairwise sequence alignments for the modeling target and structure templates were the same as previously described.[21] A homology model was built using the MOE 2009.10. After manual sequence alignment was performed, the homology model was built without optimization in that permittivity was dependent on the distance using the AMBER99 force field. MOE 2009.10 ASE Dock was used for ligand docking simulation. Molecular docking simulation results for fMLFXYk(FB)k with FPR using MOE 2009.10 ASE Dock is shown in Fig. 2a. Visual inspection of the binding mode for fMLFXYk(FB)k at the binding site is shown in Fig. 2b. The carbonyl oxygen of the formyl group forms a H-bond with Arg^{201}, whereas the amide NH binds with Asp^{106}. The side chain of Met^{1} interacts with the hydrophobic portion of Leu^{109}, Trp^{254}, and Tyr^{257}, and the side chain of Phe^{3} interacts with the hydrophobic portion of Leu^{78}, Trp^{257}, and Phe^{291}. Additionally, the fluorophenyl group interacts favorably with the hydrophobic transmembrane domain. The docking simulation results showed that fMLFXYk(FB)k has a high affinity for FPR. The structure of the complex of fMLFXYk(FB)k and FPR can be used to design fMLF derivatives.

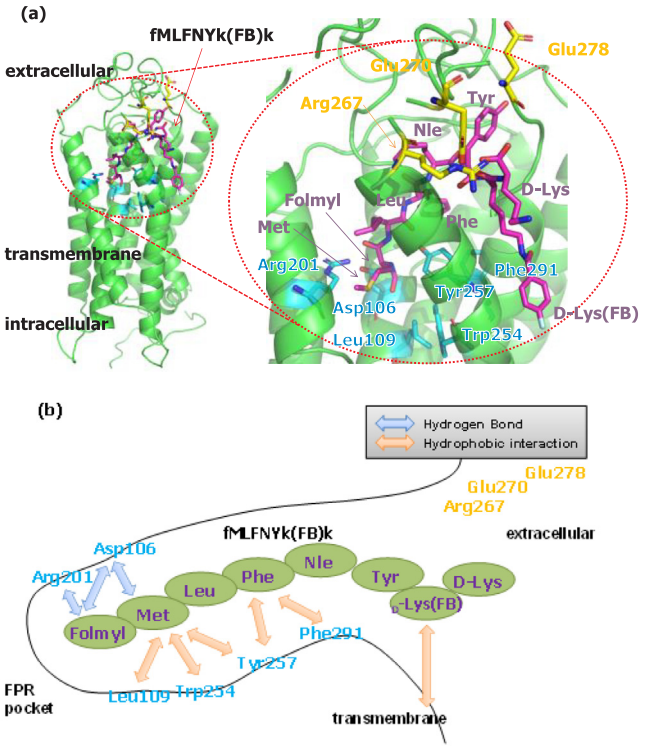
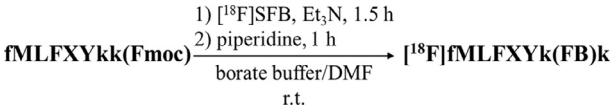


Fig. 2. (a) Docking mode prediction for fMLFXYk(FB)k in the binding site. (b) Conception of the interaction between fMLFXYk(FB)k and FPR.

[^{18}F]fMLFXYk(FB)k was obtained by conjugating *N*-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB) and precursor fMLFXYkk(Fmoc) and removing the Fmoc protecting group of the Lys linker using piperidine (Scheme 1). [^{18}F]SFB was synthesized according to a previously reported method.[22,23] [^{18}F]fMLFXYk(FB)k was purified from unlabeled precursors and other reagents by reverse-phase HPLC with a radiochemical yield of 16% from [^{18}F]fluoride ions (specific activity > 30.0 GBq μmol^{-1}) and a radiochemical purity of > 96%.



Scheme 1. Synthesis of [^{18}F]fMLFXYk(FB)k.