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Developing A Formyl Peptide Receptor Targeted Tracer For SPECT Imaging Of Leukocyte Recruitment

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Leukocyte recruitment is a hallmark of the inflammatory response, thus non invasive imagining of leukocyte infiltration may provide a dynamic and localised read out of inflammatory events, aiding diagnosis of disease and drug discovery. Single Photon Emission Computed Tomography (SPECT) is a sensitive and versatile tomographic imaging technique allowing 3D localisation of a desired target or process, through the detection of a gamma emitting tracer. Current methodologies for imaging leukocytes are hampered by lack of specificity or require laborious and potentially hazardous *ex vivo* labelling of cells. Developing radio labelled ligands with high affinity for leukocyte receptors may provide a novel method for labelling leukocytes *in situ.* We have thus targeted the Formyl Peptide Receptor (FPR)-1, which is highly expressed on leukocytes, using an antagonist, cFLFLFK, conjugated to a tetraglycine chelate to allow for incorporation of ^{99m}Tc, a widely used radioisotope. An ideal radiotracer should show high receptor affinity and specificity while also having limited physiological effects on the target cells. Here in we test the receptor binding and *in vitro* effects of this prospective tracer on human leukocytes to assess its suitability for *in vivo* imaging of inflammation.

In vivo experiments were performed using a 'cold' chemistry alternative to the tracer where by rhenium (Re) was chelated to the ligand to mimic ^{99m}Tc. Competitive ligand binding assays were performed using HEK293 cells transfected to stably express FPR1. Cells were incubated for 90 min with various concentrations of Re.cFLFLFK ($1x10^{-5}$–$1x10^{-10}$M) and a fixed concentration of [125I]-fNleLeuPheNleTyrLys. Following three wash steps the remaining ^{125}I signal was measured on a gamma counter and IC50 determined from binding curves. Effects on Leukocyte function were assessed in human neutrophils isolated from healthy adults. Chemotactic responses of cells were investigated using neuroprobe^{TM} chemotaxis plates. Neutrophils ($4x10^6$ cells/ml) were pre treated with Re.cFLFLFK or the native peptide (0.1- 300nM) before being exposed to a chemoatractant gradient IL-8 (100ng/ml) for 90 min. The number of cells migrated across the membrane were then counted manually on a haemocytometer. Neutrophil inflammatory cytokine release was assessed in terms of TNF-α stimulated IL-1B release. $1x10^6$ cells/ml were incubated with RecFLFLFK, or native peptide before being exposed to 10nM TNF-α and incubated for 18h. Supernatants were collected and IL-1β protein levels determined using an ELISA MAX^{TM} IL-1β ELISA kit (BioLegend). Statistical evaluation was performed using ANOVA with Bonferroni post hoc analyses N = 4 for each group. $P<0.05$ was considered significant

Receptor binding assays revealed an IC50 of 18nM for Re.cFLFLFK which was not dissimilar from the native peptide, suggesting tetraglycine chelation does not significantly affect receptor binding. Many FPR ligands exhibit potent effects on

neutrophil inflammatory function, thus we investigated our proposed FPR antagonist based tracer in chemotaxis and IL-1B release assays. Re.cFLFLFK showed no intrinsic chemoattractive activity, not inducing greater migration that vehicle alone. While exposure to an IL-8 gradient caused a 10 fold increase in migrated cells, pre incubation with Re.cFLFLFK had no effect on this response. Furthermore none of the Re.cFLFLFK concentrations tested caused IL-1β release above vehicle or affected TNF-α stimulated IL-1β release. The proposed SPECT tracer shows promise in that it displays high receptor affinity while having no effect on inflammatory function. Preliminary *in vivo* SPECT scans in a murine LPS model of inflammation also show increased signal at the site of LPS injection.