Tuftsin-Gd-DOTA Conjugates as Potential MRI Reagents for Macrophage Imaging

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Introduction

MRI is an established tool to detect excitotoxic oedema associated with acute cerebral ischaemia as well as to delineate mature infarction *in vivo*. It is well established that inflammation contributes to cell damage caused by brain ischaemia [1]. Macrophages have been shown to play a role in the cerebral inflammatory process [1]. To assess the role of macrophages in development of ischaemic infarction we are developing cell-specific optical and MR imaging reagents. The lead molecule for the MRI reagents is a peptide tuftsin. Tuftsin is known to be responsible for activation of macrophage cell lines and it is internalized through a receptor-mediated mechanism by macrophages [2]. We have used both tuftsin tetrapeptide (Thr-Lys-Pro-Arg) and pentapetide (Thr-Lys-Pro-Pro-Arg) as lead molecules to tag the probe for macrophage imaging. The pentapeptide shows greater affinity to the tuftsin receptor than the tetrapetide [3]. Biological activity of tuftsin is not influenced by the presence of N-terminal substituents such as fluorescein (FITC) [4], suggesting that such conjugates can be targeted for labelling of macrophages.

Methods

'Bifunctional' phosphorescent and fluorescent tetrapeptide and pentapeptide with DOTA derivatives have been obtained by stepwise synthesis and solid phase peptide synthesis. Both terbium and gadolinium conjugates of tuftsin were produced and products were chemically characterised by mass spectrometry, NMR and fluorometry. Binding and/or uptake of terbium complex of tuftsin (both tetrapeptide and pentapeptide) by macrophages was investigated. Macrophages were isolated from the peritoneal fluid of C57BL6 mice and incubated at 37˚C in RPMI 1640 medium (Gibco) supplemented with 25 mM HEPES (pH 7.3), 2 mM glutamine, 5% foetal calf serum and penicillin/streptomysin overnight in atmosphere containing 5% CO_2. Macrophages were incubated with the conjugate in phosphate buffered saline (PBS) supplemented with 1.2mM $MgSO_4$, 1.2mM $CaCl_2$, 5mM KCl and 10mM D-glucose for 30 minutes at 37˚C, and unbound fluorescein- and terbium-conjugates were removed by centrifugation and washing with fresh PBS. To prime the isolated macrophages, cells were incubated with lipopolysaccharide (LPS, 1µg/100ul) for 15 min before adding tuftsin conjugates. Fluorescence emission spectra were obtained on a Perkin-Elmer LS55 fluorometer with excitation set at 366 nm and confocal microscope images using a Leica laser scanning microscope equipped with an ArKr laser.

The relaxivity of the Gd-tetrapeptide was measured in PBS without and with added bovine serum albumin (BSA, 8%). Gd-tuftsin was added to a suspension of macrophages (5×10^5) to give final concentrations of 0, 100, 200 and 500 µM. 200 µM of Gadodiamide was used to assess possible binding/uptake of the Gd-chelate. Following incubation at 37˚C for 60 min cells were washed twice with fresh PBS and immobilized in gelatine (4%) and imaged using a T_1-weighted spin-echo MRI at 7T (a smis console interfaced to a horizontal Magnex Magnet).

Results

The fluorescence emission spectra from cells incubated in the presence of varying concentrations of Tb-tetrapeptide are shown (Fig. 1). A good dose response is evident from these spectra, suggesting that the conjugate is internalized/bound through the tuftsin receptor mechanism. It should be also pointed out that the time-gated fluorescence spectra have very flat baseline and low signal intensity from endogenous compounds. Confocal microscope images of native macrophages show autofluorescence (Fig. 2, left panel). Incubation with 5 µM FITC-tuftsin substantially increased fluorescence in cells (Fig. 2, middle panel) and a further increase was evident with LPS spiking (Fig. 2, right panel).

The relaxivity of Gd-tuftsin at 400 MHz was found to be 2.52 $mM^{-1}\cdot s^{-1}$ in PBS solution. This is comparable with that observed for other DOTA-monoamide complexes of gadolinium. The relaxivity of Gd-tuftsin in PBS with 8% BSA solution was 2.64 $mM^{-1}\cdot s^{-1}$. This suggests that a very low percentage of Gd-tuftsin integrate noncovalently with BSA possibly in form of (Gd-tuftsin)·BSA. The *in vitro* MRI experiments with macrophages showed that there was a marked increase in T_1-weighted MRI signal intensity in samples containing cells labelled with either 200µM or 500µM of Gd-tuftsin. In all other samples, including those containing macrophages incubated in the presence of 200µM of Gadodiamide, minimal changes in signal intensity were evident. These data show that tuftsin tetrapeptide retained its binding activity at the tuftsin receptor after conjugated with Gd-complex.

Conclusion

These results suggest that tuftsin conjugates obtained are useful as fluorescence probes to detect macrophage cells. Owing to long fluorescence lifetime, time-gated fluorescence imaging can be exploited in vivo to detect signal from Tb-tuftsin complex with low interference by background signal. The Gd-tuftsin conjugates bear a potential as MRI contrast reagents to be used to image, for instance brain injuries and involvement of macrophage-mediated inflammation. It is expected that macrophage-specific MRI reagents will aid assessment of the role of macrophages both in damage and repair processes in a variety of cerebral disorders.

Acknowledgements

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References

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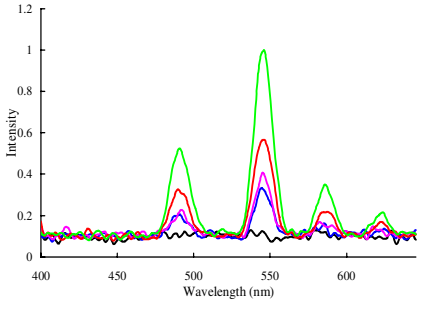

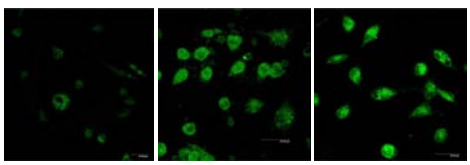

Fig. 2. Confocal microscope images of native macrophages (left panel), macrophages incubated with 5 µM of fluorescein-labelled tuftsin tetrapeptide conjugate (middle panel) and LPS-spiked macrophages incubated with 5 µM of fluorescein-labelled tuftsin conjugate (right panel)

Fig.1. Fluorescence emission spectra of macrophages incubated with varying concentrations of the Tb-tetrapeptide complex. Spectra are shown for five different concentrations: 0µM (black trace), 10µM (blue trace), 20µM (pink trace), 50µM (red trace), 100µM (green trace) and were recorded using a time delay of 0.1ms and a gate time of 10ms with excitation at 366 nm.

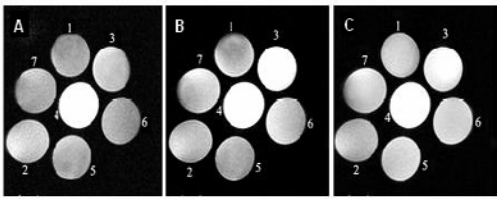

Fig. 3. T_1-weighted MRI of macrophages labelled with different concentrations of Gd-tuftsin. 1: 0µM; 2: 100µM; 3: 200µM; 4: 500µM of Gd-tuftsin; 5: 200µM of Gadodiamide; 6 and 7: 4% of gelatine. Images of 2-mm thick slices were acquired with a spin-echo pulse sequence (left panel: TR=300ms, and TE=10ms, middle panel: TR=500ms, and TE=10ms, right panel: TR=1000ms, and TE=15ms). FOV of $35\times35mm^2$ and matrix of 256×256 were used for all panels.