MRI follow-up of monocyte/macrophages colonisation of a rat brain tumour

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Introduction

Brain tumours represent a chemoattractive environment which has been shown to be highly colonized by microglia and by monocyte-derived macrophages [1]. Therefore, macrophages may represent a potential vector to bring anti-tumoral therapy into the tumour. Although cellular therapy has been used extensively for the treatment of different brain pathologies, many studies were based on the local implantation of cells directly in the brain [2]. A more clinically relevant approach would be the use of intravenously administrated cells. Since few years, multimodal contrast agents have been developed to follow-up the cells both by MRI and by histological techniques [3]. The objective of our study was to evidence that labeled monocyte/macrophages can be visualised by MRI after intravenous injection and can be used to target brain tumour area.

Methods

The P388-D1 cell line was used as monocyte/macrophage. Cells were incubated overnight at 37°C with micron-sized fluorescent iron-oxyde particles (Bangs Laboratory; 63.4µg of iron per $2.5.10^6$cellules) then washed twice with saline buffer and replaced in the incubator. Cell labelling was confirmed by flow cytometry (Ex : 488nm, Em : 520nm). For *ex vivo* experiments, labeled P388-D1 cells were administrated intravenously (2.10^6 cellules/rat) in rat bearing C6-glioma on day 3 after glioma implantation. On day 7, animals were PFA-fixed and brains were removed for subsequent *ex vivo* MRI. For *in vivo* analysis, rat received C6 cells implantation on the right and saline on the left striatum, monocytes were injected on day 3 and rats were subjected to MRI at different time points (day 4, 5, 6, 7, 8, 10, 12, 13). On day 13, rats were also PFA-fixed and analysed *ex vivo*. All MRI experiments were done at 2.35T (SMIS console) with gradient-echo sequences (TR/TE=400/25ms; flip angle = 30°). After MRI experiments, brains were sliced and Prussian blue staining (iron detection) or isolectin staining (BS-I B4; monocyte/macrophages detection) were performed.

Results

Although cells stopped to proliferate in the presence of iron, following the washes, they recovered well and restarted to proliferate (Doubling time: unloaded cells; 19.2h; loaded cells: 20.8h). Flow cytometry revealed that 99.9% were loaded; that the cells remained loaded at least for 4 days (Fig 1) and that no morphological changes were observed. *Ex vivo* imaging on rat bearing tumours show that, when the monocytes/macrophages are intravenously administrated, they present the capacity of migrating in the brain and more especially in the vicinity of the tumour. This is visualised by a strong signal loss on the tumour: reduction of 87% of signal as compared to the contralateral striatum (Fig 2a-b). The origin of the signal loss was finally confirmed with histology: iron containing cells are actually found in the tumor (Fig 2c-d). *In vivo* experiments show that the maximum signal loss was obtained on day 7 after tumor implantation (day 4 after monocytes injection) and also revealed that monocytes remained in the tumor vicinity few days (at least until day 13). Moreover, as rats were implanted with C6 glioma cells in the right striatum and with saline in the left striatum (Fig 3a), we demonstrate that although some monocytes are found in both cortex (Fig 3c), we observed a loss of signal only in the right (tumoral) striatum (Fig 3b-d) suggesting that intravenously injected monocytes are attracted mainly by the tumor itself.

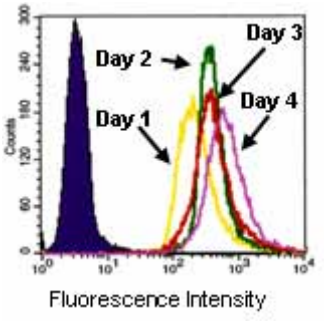

Fig 1: Facs analysis of cel labeling as function of time.

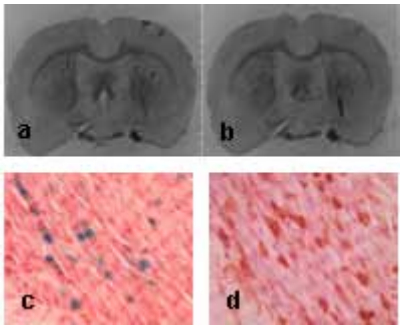

Fig 2: Ex vivo detection of intravenously injected monocytes by MRI : **a-b** (resolution 78*78*375µm) or by histology : **c**: Prussian blue coloration; **d**: Isolectin histochemistry.

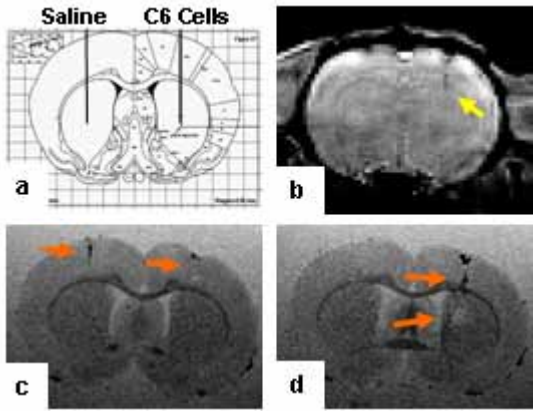

Fig 3: *In vivo* detection of intravenously injected monocytes by MRI. **a** : rat received C6-cells on right striatum and saline on left striatum. **b**: MRI on living rat on day 7 post-implantation (273*273*930µm); **c-d**: *ex vivo* MRI on day 13 (resolution 97*97*150µm).

Discussion

Our results show that MRI is a useful non-invasive methodology to follow cell entry in the pathological brain. After verifying that the cell labelling doesn't affect the cell viability, we have shown that it is possible to detect the tumour colonization following intravenous administration of magnetically-labeled monocyte/macrophages. A cellular strategy using these cells as vectors might be used to specifically target the tumour to bring anti-tumoral therapy. MRI would be helpful not only to detect the cells but also to confirm the efficacy of the anti-tumoral therapy.

References

[1]-Neurosurgery (2000) 46: 957-961. [2]-Neuroimage (2004) 21: 311-317. [3]-PNAS (2004) 101: 10901-10906.