MRI targeting of apoptotic cells in injured areas of brain in a mouse model of Parkinson's disease

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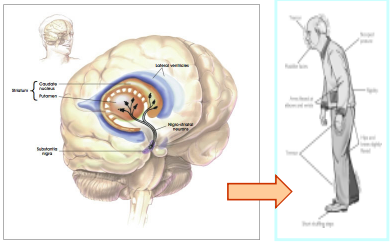
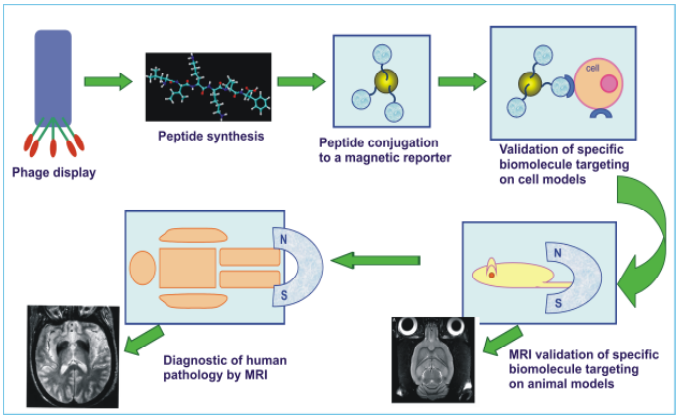
INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a massive loss of dopaminergic neurons (DN) located in the basal ganglia, both by apoptosis and by neuroinflammation [1]. The consequent dopamine defficiency in the striatum are responsible for most of the movement disorders (e.g., muscle rigidity, tremor, bradykinesia or akynesia, postural instability) called parkinsonism.

AIM OF THE WORK

- Develop a molecularly targeted contrast able to detect DN dead by apoptosis in midbrain of PD patients and help in this way to it's early diagnosis.
- Peptide specific to apoptotic cells (PPS) identified by phage display [2] was conjugated to USPIO (PEG-USPIO-PPS) and used to image injured areas in a PD mouse model by MRI.





1) DETECTION OF APOPTOSIS IN DOPAMINERGIC CELL CULTURE

- The affinity for apoptotic cells of USPIO-PPS was first validated on DN cultures by MRI, followed by the measurement of R_2 and of the Fe concentration.
- DN were isolated from postnatal rat brain (P 0-16) [3].
- Apoptosis was induced in cell cultures with MPP+ (1-methyl-4-phenylpyridinium) and then was confirmed by the immunostaining of caspase 3 (**Figure 1**: cells treated with MPP+ present a brown staining in opposition to healthy cells) and the measurement of it's enzymatic activity (**Figure 2**: MPP+ neurons showed higher enzymatic activity than healthy neurons demonstrating the apoptotic effect of MPP+).
- MPP+-treated cells incubated with PEG-USPIO-PPS showed lower signal in T_2-weighted images acquired by MRI (Bruker AVANCE-200, 4.7T) as opposed to healthy cells and MPP+ cells incubated with USPIO-PEG (**Figure 3**).
- The normalized relaxation time (R_2^{Norm}) was measured on cell samples at 60 MHz on a Bruker minispec (**Figure 4**), while Fe concentration was measured after cell samples digestion with 5N HCl (**Figure 5**). MPP+ cells presented the highest R_2^{Norm} and iron concentration proving the binding of PEG-USPIO-PPS to apoptotic cells.

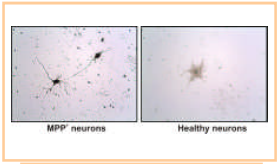


Figure 1. Immunostaining of caspase 3.

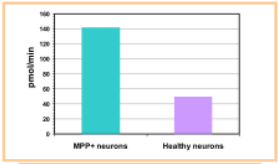


Figure 2. Enzymatic activity of caspase 3.

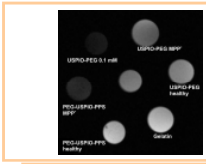


Figure 3. Detection of apoptotic cells by MRI.

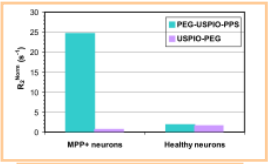


Figure 4. R_2^{Norm} of MPP+ and healthy cells.

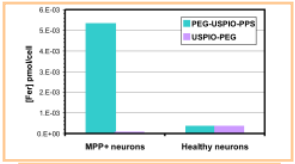


Figure 5. Fe concentration in MPP+ and healthy samples.

2) IN VIVO MRI AND IMMUNOHISTOCHEMISTRY

- MRI was carried out on male C57Bl/6J mice treated with MPTP (1-methyl-4-phenyl-1.2.3.6-tetrahydropyridine) (2 i.p. injections per day during 5 days) [4] and injected with 30 µmoles/kg of PEG-USPIO-PPS or of USPIO-PEG. The images were acquired during the MPTP treatment (the 4th and the 5th days), as well as one day and one and three weeks after the last injection.
- The MR images acquired on MPTP mice injected with PEG-USPIO-PPS showed that the targeted areas in brain correspond to the injured region in parkinsonian mice. This specific contrast of injured areas was evident after 4 days of treatment, it reached a peak the 5th day (**Figure 6**), and decreased slowly after MPTP treatment.
- Contrast/noise ratio (C/N) measured on MR images during and after MPTP exposure (**Figure 7**) confirms that targeting of apoptosis was the most important on 5th day of MPTP treatment and decreased next days.
- MRI targeting of apoptosis was then compared to the immunodetection of DN in areas injured by MPTP treatment (**Figure 8**). DN were detected by immunohistochemistry with an anti-TH (tyrosin hydroxylase, a characteristic enzyme of DN) antibody. Apoptotic areas in MRI correspond with TH location in brain.

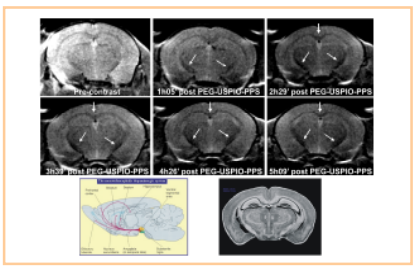


Figure 6. MRI 5 (Bruker AVANCE-200, 4.7T, RARE, TR/TE = 3045/21.3 ms, FOV = 2.5 cm, slice thickness = 0.7 mm, spatial resolution = 98 µm) of brain in mice injected with PEG-USPIO-PPS the 5th day of MPTP treatment.

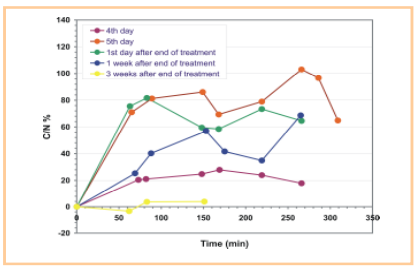


Figure 7. Evolution of C/N during MPTP treatment.

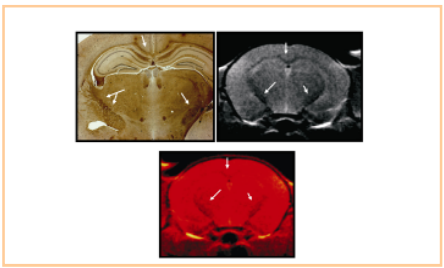


Figure 8. Colocalisation of MRI targeted areas and TH detection by immunohistochemistry.

CONCLUSION

Affected areas in PD were imaged in MPTP mice by targeting of apoptotic cells with USPIO vectorized by a phosphatidylserine-specific peptide. This new contrast agent could assist in early diagnosis of PD patients.

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