Design, characterization and use of magnetophages, a new kind of MRI contrast agent

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Purpose : Largely used in therapeutic research and based on phage biology, the phage display technology [1] has been recently implemented in our laboratory to develop new specific contrast agents. This technique allows to identify, from a large pool of phages called library, peptides with high affinity for a given target. Specific MRI contrast agents are finally obtained by coupling the selected peptides, obtained by DNA sequencing of the isolated phages, to magnetically active species. In the present work, we have evaluated the possibilities to obtain new entities called magnetophages, by direct coupling of the isolated phages to superparamagnetic particles (USPIO).

Methods : Magnetophages were obtained by reaction of the dextran coating of USPIOs with epichlorhydrin and then with phages. Magnetically labeled phages were isolated by selective precipitation with PEG/NaCl. Magnetophages were characterized by NMRD (proton nuclear magnetic relaxation dispersion) using a Stelar Spinmaster relaxometer (Mede, Italy). Iron content was determined by ICP (Jobin Yvon JY70+, Longjumeau, France). The affinity of magnetophages was tested by comparing their K_d towards phosphatidylserine (PS) to the K_d of the corresponding non-magnetic phages. This evaluation was performed by ELISA and by competition with annexin V, a marker of phosphatidylserine [2]. The value of K_d corresponds to the observed IC_{50} in the fixation curve. Competition curve was obtained by incubating PS with serial dilutions of annexin V prior to phage addition at a concentration corresponding to the IC_{50}. Apoptosis was induced in JURKAT cells by adding camptothecin in the culture media. Magnetophages were incubated with treated and non-treated JURKAT cells for 60 mins and the suspensions were centrifuged to eliminate unbound magnetophages. Pellets were finally suspended in 100 µl of gelatin 2 % in PBS.

Results : Magnetophages were obtained by coupling phages and USPIOs. NMRD profiles demonstrate the reproducibility of the method. r_1 and r_2 relaxivities of magnetophages were higher than those of uncoupled USPIOs. As seen from their fixation curves, the specific phages and the magnetophages have an almost equivalent affinity for PS (K_d = $6.173.10^{-12}$ M and $1.527.10^{-11}$ M respectively). In the competition experiment with annexin V, the binding of magnetophages to PS decreases with the increase of the competitor, indicating that magnetophages keep their specificity for PS. Preliminary images indicate a larger uptake of the magnetophages by camptothecin activated JURKAT cells.

Conclusion : Binding assays show that magnetophages retain their specificity, in the present case for the markers of apoptosis. Considering their strong T_2 effect at high field these systems are potentially usable as specific MRI contrast agents. *In vivo* imaging and possibility to use the magnetophages in applications such as magnetophoresis or *in vitro* tests are under investigations.

References

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