Stealthy magnetophages, a new tool for molecular imaging

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INTRODUCTION

Widely used in therapeutic research, phage display technology has been implemented in our laboratory to design MR contrast agent (CA) for molecular imaging[1,2]. Phage display allows the identification of high affinity binding peptide (i.e. for a molecule diagnostically relevant) from a heterogeneous mixture of bacteriophages called random library. These phages are different from each others by the sequence of the oligopeptide carried by a protein of the phage wall in such way that all the possible peptide sequences are statistically represented. The sequence of the peptide is determined by sequencing the genome of the corresponding binding phage isolated after several rounds of affinity selection. The MR CA is finally obtained by coupling the selected peptide to a magnetic center. In previous works[1,3], we have developed the concept of magnetophages, entities obtained by directly coupling USPIO to phages isolated after the phage display procedure. We have shown that magnetophages can be used as in vitro MR contrast agent. In this study, we evaluated the possibility to extend in vivo the use of magnetophages. Nevertheless, the major problem is their non specific phagocytosis by the cells of the reticulo-endothelial system (RES), mainly the Kupffer cells. Consequently, magnetophages are rapidly accumulated into the liver, making them unavailable in the blood pool to reach its target. This problem was abrogated by surface modifications of USPIO by PEG, designing stealthy magnetophages characterized by a much longer circulation time. For this purpose, we have chosen a phage (called E3) specific for phosphatidylserine (PS), a marker of apoptosis[2], as prototype. It will be referred in this poster as magnetophage E3 if magnetically labeled.

METHODS

Synthesis of magnetophages : 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. Stealthy magnetophages were obtained by the same way but using pegylated USPIO.

Saturation and inhibition curves : Magnetophages (stealthy or not) would be superfluous if the linking of USPIO to the phage wall did alter the interaction properties of the corresponding non-magnetic phages. The affinity of magnetophages E3 was tested by comparing their Kd towards PS to the Kd of the corresponding non-magnetic phages. This evaluation was performed by ELISA and by competition with annexin V, the natural ligand of PS. The value of Kd 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}.

In vivo studies : Stealthy magnetophages and magnetophages, specific or not of the apoptotic marker phosphatidylserine, were injected via the tail vein of anesthetized male mice bearing apoptotic liver or not. Apoptosis was induced by intraperitoneal injection of 10 µg of anti-Fas antibody. MRI images were acquired using a T_2-weihted spin-echo sequence (TR/TE = 2000/20 msec, NE = 4, matrix 128x128, slice thickness = 2,5 mm, FOV = 6 cm) and intensities measured in regions of interest defined in the liver. Analysis of the images is based on the modifications of the signal compared to the pre-contrast image and intensities were expressed as relative enhancement in percentage (RE%). Images were acquired on a 4.7 Tesla Avance 200 system (Bruker, Karlsruhe, Germany) equipped with a vertical magnet.

RESULTS AND DISCUSSION

Fixation curves of magnetophages E3 and corresponding phages (bearing the same peptide) are shown in figure 1. As seen from these fixation curves, they have an almost equivalent affinity for PS. K_d=6.2 10^{-12} M and 1.5 10^{-11} M, respectively for specific phages and magnetophages.

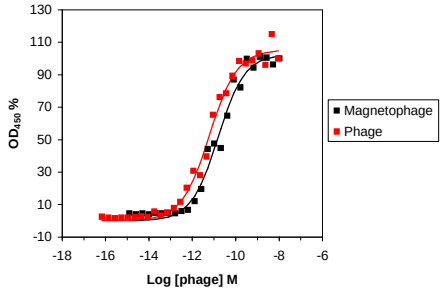

Fig 1 : Fixation curves of phages and magnetophages bearing a PS specific peptide.

In the competition experiment with annexin V, the natural binder for PS (figure 2), the binding of magnetophages E3 to PS decreases with the increase of the competitor, indicating that magnetophages keep their specificity for the target.

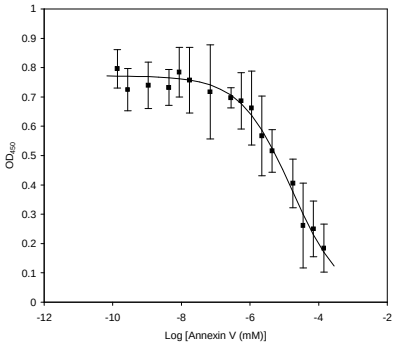

Figure 2. Competition curve of magnetophages bearing a peptide specific for PS with annexin V.

From these experiments it can be emphasized that USPIO do not alter the binding properties and so magnetophages can be employed as MR CA.

Figure 3 shows that intravenous injection of magnetophages, specific or not of PS, induce rapid darkening of the normal or apoptotic liver, meaning that they are rapidly accumulated by the Kupffer cells of this organ. Figure 4 shows that injection of stealthy magnetophages E3 (specific for PS) to mice bearing normal liver do not darken the normal liver. In the contrary, these same stealthy magnetophages injected to mice bearing apoptotic liver induce a decrease of the apoptotic liver intensity. This means that stealthy magnetophages are able to reach its target and so that their circulation time has been improved by making them invisible to the RES.

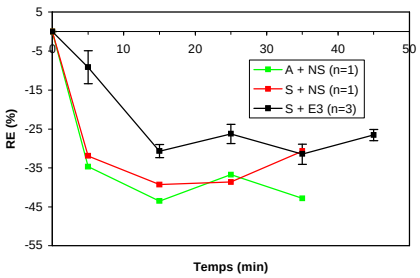

Figure 3 : Non specific magnetophages (NS) and magnetophages specific of PS (E3) injected in healthy (H) and apoptotic liver (A). Decrease of RE shows that magnetophages are accumulated in the liver.

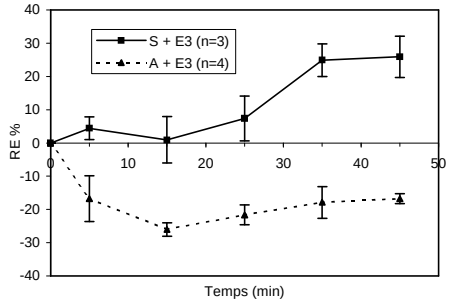

Figure 4 : Injection of stealth magnetophages specific of PS (E3) in apoptotic liver (A) and in the healthy one (S). Decrease of RE in the first case suggest that stealthy magnetophages E3 are accumulated in the apoptotic liver only.

CONCLUSION

In the present study, we have shown that labeling phages with USPIO did not alter the binding properties with the target. This together with the fact that magnetophages exhibit strong T_2 properties authorize their use as MRI contrast agent. Nevertheless, when injected *in vivo*, magnetophages are rapidly accumulated by the liver, becoming insufficiently concentrated in the vascular compartment to interact with its target. PEG is known to drastically reduce adsorption of plasmatic proteins at the surface of circulating particles. Consequently, pegylation of USPIO reduces its opsonisation and consequently accumulation by phagocytic cells. Coupling of such pegylated USPIO to phages has designed entities we called stealthy magnetophages. We have shown in this work that stealthy magnetophages specific of PS can discriminate apoptotic liver from healthy one. They are became invisible to the RES and can therefore be vectorized to their target. Stealthy magnetophages can so be used as *in vivo* molecular contrast agent. Also, we emphasize that a biodistribution map of the target can be drawn up by the use of stealthy magnetophages.

REFERENCES

1. Segers J.,Applications of phage display technology to molecular imaging by MRI, Ph D. thesis, Mons, 2005.
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3. Segers Jerome et al., ISMRM, May 2004, Kyoto.