Active Targeting of Perfluorocarbon Nanoemulsions for $^{19}$F MRI

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ABSTRACT SUMMARY

Novel Perfluorocarbon (PFC) emulsion particles were generated which combined PEGylation to reduce uptake by monocytes/macrophages and antibodies (AB) linked to the PEG-chain as specific targeting moiety. Specific targeting to J774A.1 macrophages and the Kelly cell line (human neuroblastoma cells) was achieved by attachment of anti-CD204-ABs or anti-GD2-ABs to the Chol-PEG anchor. Linking a non-specific isotype-matched IgG to the Chol-PEG anchor did not lead to cellular uptake by the Kelly cell line. Analysis of the biodistribution of PEGylated vs. non-PEGylated PFCs by non-invasive $^1$H/$^{19}$F MRI (magnetic resonance imaging) revealed that PEGylated PFCs displayed a longer circulation in the bloodstream and a delayed infiltration into spleen and liver within the first 4h after intravenous application. These results indicate that combining PEGylation with antibodies as specific targeting moiety for $^{19}$F emulsion particles is suitable to track distinct cell types in-vitro and possibly in-vivo.

INTRODUCTION

Perfluorocarbon nanoemulsions (PFC-NE) have been recently used to visualize inflammatory foci, to track stem cell homing and analyze the migration of dendritic cells non-invasively by $^{19}$F MRI$^1$. Due to the lack of any $^{19}$F background in the body, PFCs can be detected with a high degree of specificity by $^{19}$F MRI. PFCs are biochemically inert and can be administered in form of biocompatible nanoemulsions by using phospholipids (soy or egg lecithin) as emulsifiers. After intravenous administration, PFC nanoemulsion particles are taken up by monocytes and neutrophils which migrate into the inflamed area$^2$. However, a large fraction of the administered PFC emulsion is cleared by the spleen and liver.

PEGylation has been widely used to reduce the uptake by phagocytic cells in cell culture and to delay clearance by the reticuloendothelial system in-vivo. To target nanoparticles to distinct cell types, the attachment of antibodies or peptides has been performed. In the present study, we aimed to achieve a targeting of PFC nanoemulsion particles to the J774 macrophage cell line and the human neuroblastoma cell line Kelly. To this end, we linked specific antibodies to the PEG moiety of a cholesterol-based anchor. Furthermore we compared the biodistribution of PEGylated and non-PEGylated PFC-NE particles by non-invasive $^1$H/$^{19}$F MRI.

EXPERIMENTAL METHODS

Blank PFC-NE was manufactured via high pressure homogenization on a Microfluidizer M110-P (Microfluidics). Blank NE consist of perfluorooctylbromide (PFOB; 10-40 % m/m) as PFC, purified lecithin as emulsifying agent (2-4 % m/m) and a 10 mM phosphate buffer. Lissamine rhodamine B was used as a fluorescent marker for flow cytometry. It was added to the lecithin before the homogenization step. PEGylation of nanoemulsions was achieved by the use of distearoyl-phosphatidylethanolamine-PEG$_{2000}$ (DSPE-PEG$_{2000}$) or Chol-PEG$_{1300}$.

For active targeting approaches a cholesterol-based anchor molecule was used. It consists of a cholesterol molecule esterified with polyethylene-glycol (PEG$_{1300}$) which binds to a reactive N-hydroxy-succinimid (NHS) group. ABs can be bound to the anchor molecule which was inserted into the phospholipid monolayer of preformed PFC-NE. Ratio of anchor to AB is 50:1 giving the nanoparticles an additional PEGylation by excess anchor-molecules. Anti-CD204-AB has a high affinity to the scavenger receptor of J774 cells. In Kelly cell experiments an anti-GD2-AB was chosen for targeting and non-specific isotype-matched IgG as control.

To analyze cellular association, J774 and the Kelly cell line were incubated with rhodamine labeled nanoparticles for 120 min at 37°C. Cell viability was tested with a commercially available viability assay (CellTiterGlow; Promega).

For MRI experiments, 100 µl of PFC-NE was injected intravenously into the tail-vein and mice were analysed after 30, 120, 240 min and after 24h.
MRI measurements were performed at a vertical 9.4 T Bruker AVANCE III Wide Bore NMR spectrometer (Bruker; Rheinstetten, Germany) operating at frequencies of 400.21 MHz for $^1$H and 376.42 MHz for $^{19}$F measurements using Bruker Microimaging units (Micro 2.5) as described previously. Mice were anesthetized with 1.5 % isoflurane and were kept at 37 °C during the measurements. $^1$H MR reference images were acquired within 0.5-6 min. Corresponding $^{19}$F images were recorded from the same FOV with an acquisition time of 21 min. The full protocol for MRI studies including both $^1$H and $^{19}$F imaging took around 40 minutes and was well tolerated by all mice which recovered from anaesthesia within five minutes.

RESULTS AND DISCUSSION

First we tested whether the PFC nanoemulsion particles had any effect on cell viability. We used the CTG assay which determines the number of viable cells in culture based on quantitation of the ATP present. All PFC-NE used had no effect on cell viability.

We investigated whether PEGylation impairs the cellular association of the PFC-NE particles by J774 macrophages. Incorporation of 5 mol % Chol-PEG$_{1300}$ (figure 1, column B) resulted in a 6-fold reduced cellular association compared to a non-PEGylated control (column A). 10 mol % Chol-PEG$_{1300}$ nearly completely blocked it (column C).

Active targeting of a PFC-NE to J774 cells via anti-CD204-AB (figure 2, column B) showed an increase of 20 % in cellular association compared to an untargeted and non-PEGylated control (Column A).

Kelly cells showed no significant uptake of non-PEGylated PFC-NE (column C). Active targeting of a PFC-NE to Kelly cells, rich in GD2-receptors, with an anti-GD2-AB exhibited a cellular association of 70 % (column E). A control using non-specific isotype-matched IgG-PFC-NE showed only 5 % cellular association (column D).

Finally, we analyzed the $^{19}$F signal in blood, liver and spleen of PEGylated and non-PEGylated PFC-NE by $^{19}$F MRI in mice. $^{19}$F measurements were performed 30min, 2h, 4h and 24h after i.v. injection. Non-PEGylated particles showed a strong signal in liver and spleen 30 min, 2h and 4h after after injection. In contrast, PEGylated PFC-NE particles showed very weak signals in liver and spleen after 30 min which increased over time and reached the values for non-PEGylated particles after 4h. The blood signal was similar between the two nanoparticles after 30min but was stronger after 2h and 4h for the PEGylated particles. No blood signal could be detected after 24h.

CONCLUSION

Combining PEGylation with antibodies as specific targeting moiety for $^{19}$F nanoemulsion particles is suitable to track distinct cell types in vitro and possibly in vivo.

REFERENCES


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