Mechanistic study of PEG-related IgM response and importance of an interface between PEG and hydrophobic blocks,

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ABSTRACT SUMMARY

Injections of PEGylated liposomes cause rapid blood clearance of the second dose of PEG-liposomes, which is known as the accelerated blood clearance (ABC) phenomenon. The role of PEG main chain specific IgM (anti-PEG IgM) has been suggested in the ABC phenomenon. In this study, we compared PEG-liposome with PEG-PBLA micelles, in terms of the ABC phenomenon induction, as well as the IgM recognition. In our mechanistic study of the ABC phenomenon, we have yielded new findings regarding the ABC phenomenon. Taken together with our results, we concluded the induced IgM binds to an interface between PEG and hydrophobic blocks rather than PEG main chain.

INTRODUCTION

Poly(ethylene glycol) (PEG) has been widely used to modify protein drugs or liposomes, known as PEGylation, and enhance their therapeutic efficacy.

Recent studies have reported that antibodies against PEG have been generated by injection of PEGylated proteins and PEGylated liposomes (PEG-liposomes) in animal models. This immune responses by the PEG-liposomes exhibited the faster clearance of the subsequent PEG-liposomes from blood circulation is known as the accelerated blood clearance (ABC) phenomenon [1,2]. The produced IgM antibody binds to the second dose of PEG-liposomes, leading to enhanced uptake in the liver and thus, eliminating the intended long-circulation property. Studies on PEG-liposomes regarding the ABC phenomenon concluded that the produced IgM was PEG-main chain specific antibody, i.e., anti-PEG IgM. However, our previous results of PEG-containing polymer micelle exhibited no relation to the ABC phenomenon [3]. The main proof of the anti-PEG IgM antibody has based on the results of conventionally performed ELISA.

This study presents more information on the mechanistic differences between PEG-liposomes and PEG possessing polymeric micelles relative to the ABC phenomenon. ELISAs and in vivo experiments involving the polymeric micelle carrier systems have yielded new findings regarding the ABC phenomenon.

The findings should significantly strengthen our understanding of the role played by produced IgM in the ABC phenomenon.

EXPERIMENTAL METHODS

Preparation of PEGylated polymeric micelles and PEG-liposomes are described in previous reports [3,4]. For induction of the ABC phenomenon, we injected these carriers at the dose of 2.0 µmol lipid/kg (PEG-liposome), or 0.0 – 30 mg/kg (PEG-PBLA micelle). The second dose of carriers was injected at the dose of 5.0 µmol lipid (4.0 mg)/kg for PEG-liposome or 4.0 mg/kg for PEG-PBLA micelle.

Binding assay of the sera obtained after the ABC phenomenon induction was determined by conventionally performed PEG-DSPE coated ELISA or anti-PEG IgG antibody coated sandwich ELISA.

RESULTS AND DISCUSSION

From our previous study, PEG-P(Lys-DOTA-Gd) micelle did not exhibit the ABC phenomenon induction. Contrary to PEG-P(Lys-DOTA-Gd) micelle, the injections of PEG-PBLA micelle did induce the ABC phenomenon which exhibited the rapid clearance of PEG-
liposome in blood. ELISA experiment revealed the IgM response of carrier-injected sera to PEG-DLPE coated plate. As shown in Figure 1, no response was found in case of PEG-P(Lys-DOTA-Gd) micelle, while PEG-PBLA micelle did exhibit the IgM response. Both polymeric micelles have outer PEG shell layer, however, only PEG-PBLA micelle exhibited the induction. We performed the second dose of PEG-PBLA micelle in the wake of the ABC phenomenon which was induced by the first dose of PEG-PBLA micelle. PEG-liposome exhibited the rapid clearance from the blood, however, the second dose of PEG-PBLA micelle did not exhibit a change in PEG-PBLA micelle's plasma concentration. This difference between PEG-liposome and PEG-PBLA micelle is new finding in the ABC phenomenon. Due to the different observations of behavior of PEG-PBLA micelle, compare with PEG-liposomes, we performed a sandwich ELISA. The result of sandwich ELISA indicated no difference between sera from the ABC phenomenon and naïve sera, as shown in Figure 2. Interestingly, the sandwich ELISA exhibited no binding of PEG-OH to anti-PEG IgG antibody on the plate, whereas PEG-DSPE and PEG-liposome bound to the surface as positive controls. From conventional and sandwich ELISA experiments, we have concluded our results. The IgM induced in the ABC phenomenon does not bind to PEG main chain, but does bind to an interface between PEG and hydrophobic blocks. In the conventionally performed ELISA, the IgM bound to PEG-DSPE and other PEG-hydrophobic conjugates, while sandwich ELISA did not exhibit specific binding of the IgM. The in vivo behavior difference between PEG-liposome and PEG-PBLA micelle is explained as follows. PEG chain length and densities on the surface are different. The IgM might bind both carriers, however, macrophage can easily capture IgM-PEG-liposome bound complex than that of PEG-PBLA micelle.

CONCLUSION

The results in this study have yielded important insights into the ABC phenomenon's underlying mechanism. From our experiments, we have noted that the IgM seemed to recognize an interface between hydrophilic PEG chain and hydrophobic block of PEG-conjugates, rather than recognize PEG main chain

REFERENCES


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