Serum anti-PEG IgM concentration is a determinant factor on hepatic accumulation of PEGylated liposome in the accelerated blood clearance phenomenon

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ABSTRACT SUMMARY

We have found that intravenous injection of PEGylated liposome induces production of anti-PEG IgM, which is responsible for clearance of subsequent dose of the liposomes (ABC phenomenon). However, it remains unclear how the amount of anti-PEG IgM associated with PEGylated liposome is related to clearance of PEGylated liposome from blood circulation in the ABC phenomenon. We here show the quantitative relationship between serum anti-PEG IgM concentration and strength of altered hepatic accumulation of PEGylated liposomes in the ABC phenomenon.

INTRODUCTION

PEGylated liposomes are frequently used as a carrier for anticancer drugs due to their longer circulating and higher tumor accumulating properties. However, we have reported that PEGylated liposomes lose their long circulating property when they are injected twice into the same body with certain interval (referred as the accelerated blood clearance (ABC) phenomenon¹). We elucidated that anti-PEG IgM, secreted in response to the first dose (the induction phase in the ABC phenomenon), is responsible for the rapid clearance of the second dose via complement activation and following complement-mediated endocytosis by Kupffer cells (the effectuation phase in the ABC phenomenon).

In addition to the anti-PEG IgM induced by PEGylated liposome, it has recently reported that naturally occurring anti-PEG IgM is observed in 4% of healthy blood donors². Since these anti-PEG IgM are considered as a causative factor upon the effectuation phase of ABC phenomenon, it will be important to determine serum anti-PEG IgM concentration prior to administration of PEGylated liposomes through the treatment course. To predict clearance of PEGylated liposome in the presence of anti-PEG IgM, quantitative evaluation between serum concentration of anti-PEG IgM and blood clearance of PEGylated liposome is important.

In this study, we studied the influence of induced anti-PEG IgM concentration on the hepatic accumulation of the second dose PEGylated liposome in the effectuation phase of ABC phenomenon.

EXPERIMENTAL METHODS

Preparation of PEGylated liposome:

PEGylated liposome composed of HEPC:Chol:mPEG2000-DSPF=1.85:1.00:0.15 (molar ratio) was prepared by thin film hydration followed by membrane extrusion. To follow their biodistribution, PEGylated liposome was labeled with a trace amount of ³H-CHE (40 μCi/μmol phospholipids (PL)). The mean particle size was 102.5 ± 3.9 nm.

Animal treatment:

To induce anti-PEG IgM production, each dose (0.01, 0.1 and 5.0 mmol PL/mouse) of PEGylated liposomes was intravenously injected into Balb/c mice. At day 5 after a single injection of PEGylated liposomes, following studies were carried out.

Quantification of anti-PEG IgM in serum:

To determine concentration of anti-PEG IgM in serum, blood was collected from tail vein. A simple ELISA procedure by means of a mPEG2000-DSPF coating 96-well plate with HIK-M09 monoclonal anti-PEG IgM as a standard was employed.

Biodistribution study:

To evaluate biodistribution of PEGylated liposomes in each first dose received mice and non-pretreated mice, ³H-CHE labeled test PEGylated liposome (0.1, 0.2 and 0.5 μmol PL/mouse) was intravenously injected. Samples (blood and liver) were collected at 2, 15, 30 and 60 min following injection and radioactivity in the samples was determined. Hepatic clearance (CLh) and amount of PEGylated liposomes accumulated in liver through ABC phenomenon (XhA) were calculated as follows:

\[ \text{CLh} = \frac{X_{h(60\text{ min})}}{AUC_{(0\text{→}60\text{ min})}} \]
\[ \text{XhA} = (X_{h(15\text{ min})} \text{ under the ABC phenomenon}) - (X_{h(15\text{ min})} \text{ under the normal condition}) \]

where \( X_{h(60\text{ min})} \) and \( X_{h(15\text{ min})} \) are the amount of PEGylated liposomes accumulated in liver at 60 and
15 min post-injection, respectively. AUC\((0\to60 \text{ min})\) is the area under the blood concentration–time curve from time 0 to 60 min post-injection.

RESULTS AND DISCUSSION

The serum anti-PEG IgM concentrations after injection of 0.01, 0.1 and 5.0 nmol PL of PEGylated liposomes were 11.8 ± 5.2, 34.6 ± 8.1 and 88.3 ± 11.9 μg/mL, respectively. Consistent with our earlier study\(^3\), PEGylated liposomes induced anti-PEG IgM production in a dose dependent manner.

The strength of ABC phenomenon during the effectuation phase is defined by the rate or amount of hepatic accumulation of PEGylated liposome (CLh or XhA, respectively). Under the normal condition, rate of hepatic accumulation of PEGylated liposome was independent of the injected dose (about 0.0035–0.0038 mL/min at any injection dose) and amount of hepatic accumulation of PEGylated liposomes increased linearly with increasing injected dose. Under the ABC phenomenon, the CLh increased logarithmically with increasing serum anti-PEG IgM concentration (Fig. 1). But the CLh declined to near spontaneous rate at the higher second dose, indicating that limited amount of PEGylated liposomes accumulated in liver through ABC phenomenon. In other words, the rate of hepatic accumulation under the ABC phenomenon is normalized at the higher dose.

These results clearly showed that there was a good relationship between serum anti-PEG IgM concentration and strength of ABC phenomenon in the effectuation phase.

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CONCLUSION

In this study, we showed that CLh and XhA in the effectuation phase of ABC phenomenon increased logarithmically with increasing the serum anti-PEG IgM concentration. Particularly, we observed that serum anti-PEG IgM concentration is a determinant factor of the value of XhA of second dose, regardless of any injection dose.

REFERENCES


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