Accelerated blood clearance phenomenon upon PEGylated protein

Yu Mima¹, Yosuke Hashimoto¹, Taro Shimizu¹, Tatsuhiro Ishida¹, and Hiroshi Kiwada¹

¹Department of Pharmacokinetics and Biopharmaceutics, Institute of Health Biosciences, The University of Tokushima, 1-78-1 Sho-machi, Tokushima, 770-8505, Japan
mmyuummm@yahoo.co.jp

ABSTRACT SUMMARY

In this study, we showed that PEGylated protein can induce anti-PEG IgM production and the induced anti-PEG IgM is responsible for accelerated clearance of subsequent dose of PEGylated protein. Interestingly, anti-PEG IgM induced by PEGylated liposome did not react with PEGylated protein, but the one induced by PEGylated protein did react another PEGylated product. This indicates that the induced anti-PEG IgM do not necessarily react with entire PEGylated materials. Our study gives a new insight in designing PEGylated formulation and developing therapeutic regimen with combination of PEGylated formulations.

INTRODUCTION

We have reported that PEGylated liposomes lose their long circulating properties when they are injected subsequently into the same body with certain conditions (the accelerated blood clearance (ABC) phenomenon)¹. We elucidated that anti-PEG IgM, secreted from spleen in response to first dose of PEGylated liposome, is responsible for this phenomenon. This suggests that PEG on PEGylated liposome is immunogenic.

We have also reported that a single intravenous administration of PEGylated bovine serum albumin (PEG-BSA) and PEGylated ovalbumin (PEG-OVA) elicited an anti-PEG IgM response². In addition, it has been recently reported that rapid loss of enzymatic activity of PEGylated uricase was induced by its repeated injection³.

However, it is not yet clear whether the rapid loss of enzymatic activity of PEGylated protein is caused by accelerated blood clearance or another mechanism, for instance, production of neutralizing antibodies. In this study, therefore, we investigated whether blood concentration of PEG-OVA as a model PEGylated protein is decreased during its repeated injection. In addition, we examined if anti-PEG IgMs, induced by either PEGylated protein or PEGylated liposome, have similar property and affect the biodistribution of subsequent each dose.

EXPERIMENTAL METHODS

Preparation of PEG-OVA: Twenty molar excess of activated PEG_2000 was reacted with OVA for 4 h at room temperature. Unreacted PEG was removed by the cation exchange column.

Preparation of PEGylated liposome: PEGylated liposome composed of HEPC:mPEG_2000:DSPE:Chol (1.85:0.15:1 molar ratio) was prepared by Bangham method. To follow their biodistribution, PEGylated liposome was labeled with a trace amount of ³H-CHE (40 µCi/µmol of phospholipids). The mean diameter was 96.2 nm.

Animal experiments: To induce anti-PEG IgM production, PEG-OVA (500 µg/kg) or PEGylated liposome (0.1 µmol phospholipids/kg) was intravenously injected into BALB/c mice. At day 5 after the injection, anti-PEG IgM in serum and biodistribution of subsequent doses of test PEG-OVA or test PEGylated liposome were assessed.

ELISA: To detect anti-PEG IgM or anti-OVA IgM, a simple ELISA procedure with mPEG2000-DSPE or OVA coating 96-well plate was employed.

Biodistribution study: To evaluate blood concentration of 2nd dose of test PEG-OVA (500 µg/kg), sandwich ELISA with anti-PEG monoclonal antibody-coating plate and HRP-conjugated anti-PEG monoclonal antibody was employed.

To evaluate biodistribution of PEGylated liposome in each mice, ³H-CHE labeled test PEGylated liposomes (5 µmol phospholipids/kg) were intravenously injected. Then, samples (blood, liver and spleen) were collected at 1 h following injection and radioactivity in the samples was determined.

RESULTS AND DISCUSSION

The level of anti-PEG IgM production was assessed on day 5 after a single injection of PEG-OVA (Figure 1a). PEG-OVA (500 µg/kg) induced anti-PEG IgM production as PEGylated liposome (0.1 µmol phospholipids/kg) induced. In addition, anti-OVA IgM production was hardly induced on day 5 after a single injection of PEG-OVA (Figure 1b).

Figure 1. Anti-PEG IgM (a) and anti-OVA IgM (b) induction by a single injection of PEGylated liposome.
or PEG-OVA. Each value represents the mean ± S.D. (n=3).

To evaluate whether anti-PEG IgM affects the circulating property of PEG-OVA, blood concentration of test PEG-OVA was determined in the mice had received the PEG-OVA or PEGylated liposome 5 days before (Figure 2). Non-treated mouse was served as a control. The blood concentration of test PEG-OVA was reduced rapidly in the mice, had received PEG-OVA. In the serum obtained from PEG-OVA treated mice, anti-OVA IgM was not detected (Figure 1b). These results suggest that the accelerated blood clearance of test PEG-OVA is caused by the induced anti-PEG IgM. On the other hand, the mice had received PEGylated liposome and displayed large amount of anti-PEG IgM, did not show the accelerated blood clearance of test PEG-OVA. This suggests that the anti-PEG IgM induced by PEG-OVA or PEGylated liposome does not have same property.

Biodistribution of test PEGylated liposome was studied in the mice had received PEG-OVA or PEGylated liposome 5 days before (Figure 3). Non-treated mouse was served as a control. As shown in control mice (None), 55.3 %dose of test PEGylated liposome remained at 1 h after injection in blood and only 10.1 %dose accumulated in liver. By contrast, in the PEG-OVA pretreated mice, only 2.56 %dose remained in blood, 89.0 %dose was in liver. Similar rapid clearance and enhanced hepatic accumulation of test PEGylated liposome was observed in the mice had received 1st dose PEGylated liposome, which is consistent with our earlier study 1. To the contrary with PEG-OVA, PEGylated liposome is likely to have higher reactivity to anti-PEG IgM induced by both PEG-OVA and PEGylated liposome.

In addition, the anti-PEG IgM level in the PEG-OVA pre-treated mice was significantly decreased 24 h after the mice injected free PEG20000, but not in the PEGylated liposome pre-treated ones (data not shown). This result may suggest that the anti-PEG IgM induced by PEG-OVA recognizes simple repeating structure of soluble PEG, but the one induced by PEGylated liposome recognizes only the PEG exposed and fixed on PEGylated liposome. It is known that PEG on PEGylated liposome has unique conformation (mushroom or brush) 4, therefore, the anti-PEG IgM induced by PEGylated liposome may recognize such conformation of PEG on the liposomes.

Figure 2. Blood concentration of test PEG-OVA in either non-treated mice (control), the mice pretreated with PEG-OVA or PEGylated liposome. Data represent mean ± S.D. (n=3).

Figure 3. Biodistribution of test PEGylated liposomes at 1 h after injection in either non-treated mice (control), the mice pre-treated with PEG-OVA or the mice pre-treated with PEGylated liposome. Data represent mean ± S.D. (n=4).

CONCLUSION
In this study, we showed that PEGylated protein induces ABC phenomenon by its repeated injection as PEGylated liposome does. Interestingly, our results indicated that the induced anti-PEG IgM do not necessarily react with entire PEGylated materials. Our study suggests that as we design a therapeutic regimen with several types of PEGylated formulations, we should pay attention to anti-PEG IgM production and their reactivity to the PEGylated formulations with care.

REFERENCES

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