Polyethyleneglycol: A Classical but Innovative Material

Antibody Response to PEGylated Substances

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Received February 3, 2013

In contrast to the general assumption that polyethyleneglycol (PEG)-conjugated substances lack immunogenicity and antigenicity, it has been reported that they can elicit antibodies against PEG (mainly anti-PEG immunoglobulin M (IgM)). In patients, the presence of anti-PEG antibodies may limit therapeutic efficacy of PEGylated substances as a consequence of inducing rapid clearance of and neutralizing biological activity of the substances. Here, we introduce specific examples of PEGylated substances including several PEGylated proteins and PEGylated particles (PEGylated nanocarriers) which induce anti-PEG antibody responses. Finally, we emphasize that the immunogenicity of PEGylated substances should be tested in the development stage and that the titer of anti-PEG antibodies in patients should be pre-screened and monitored prior to and throughout a course of treatment with a PEGylated substance.

Key words polyethyleneglycol; PEGylation; anti-polyethyleneglycol antibody response; protein; liposome; nanocarrier

1. INTRODUCTION

Polyethyleneglycol (PEG) is one of the most versatile synthetic polymers and widely believed to lack immunogenicity and toxicity. For instance, proteins modified with PEG have increased biocompatibility due to the steric hindrance of grafted PEG. Consequently, the pharmacokinetics of substances including proteins, nanoparticles (nanocarriers), adenovirus, are improved and thereby the PEGylated substances become useful for therapy. To date, several PEGylated proteins and nanocarriers have been approved by the U.S. Food and Drug Administration (FDA). However, naturally occurring anti-PEG antibodies and the anti-PEG antibodies derived by PEGylated substances have been observed in animal models and humans, including patients and healthy donors. This suggests that PEG is both immunogenic and antigenic. In addition, surprisingly, in healthy blood donors with no known prior exposure to PEG, the prevalence of anti-PEG antibodies has been detected up to 25%, which contrasts with a 0.2% occurrence reported over 20 years ago. This increase may be due to greater exposure to PEG in cosmetics, pharmaceuticals and processed foods. There is concern that anti-PEG antibodies limit the efficacy of PEGylated substances because they lead to enhanced blood clearance of PEGylated substances and decreased biological activity of the substances by neutralization.

2. ANTI-PEG IMMUNOGLOBULIN M (IGM) RESPONSE TO PEGYLATED PROTEINS

Biomedicines, such as protein drugs, have attracted considerable attention due to their high biological activity and specificity against target molecules. However, their application is sometimes limited due to their insufficient clinical effects as a result of susceptibility to destruction by proteolytic enzymes, a short circulating half-life, low solubility, rapid kidney clearance, and propensity to generate neutralizing antibodies. Covalent conjugation of PEG to bioactive molecules, by a process called “PEGylation,” is one of promising strategies being used to overcome these limitations.

Following the pioneering studies in the 1970s, the procedure for PEGylation was extensively expanded and developed to prolong the half-life of bioactive PEGylated protein and peptide. In most of these studies, attention was only paid to the immunogenicity of bioactive proteins and peptides, and not to the immunogenicity of PEG itself.

In animal studies, PEG is generally considered to be non-immunogenic, which may in part be due to rapid renal clearance of PEG. In fact, Richer and Akerblom demonstrated that subcutaneous administration of free PEG to mice elicits a weak and transitory immune response. In addition, they showed that a stronger anti-PEG immune response was found with PEGylated proteins, particularly ovalbumin (OVA). We have also very recently shown that a single intravenous administration of PEGylated bovine serum albumin (BSA) and OVA elicits an anti-PEG antibody response, similar to that from PEGylated liposomes, although the administration does not elicit specific neutralizing antibodies to BSA and OVA. It seems that the PEG on anchoring protein functions as a hapten to generate anti-PEG antibody formation. A hapten is a small molecule that can elicit an immune response only when attached to a large carrier such as a protein. It appears that the haptenic character of PEG depends on its structure and molecular weight, immunogenicity of the anchoring protein, and the presence of adjuvant.

Several reports have demonstrated that PEGylated protein products elicit anti-PEG antibodies (mainly anti-PEG IgM) in animals and humans. Among these studies, only a...
limited number showed that the elicited anti-PEG antibodies result in rapid clearance of a subsequent dose of PEGylated protein products: such a phenomenon was observed with PEGylated interferon (IFN) β-1a in Rhesus monkeys,20) PEGylated urate oxidase in humans,19) and PEGylated asparaginase in humans.18) This might in part be due to weak immunogenicity of the anchoring protein for PEG and no attempt to discriminate between anti-PEG antibodies and anti-anchoring protein antibodies.

3. ANTI-PEG IGM RESPONSE TO PEGYLATED NANOPARTICLES

We and others have reported that PEGylated liposomes lose the expected long circulating property when they are injected repeatedly in the same animal (the so-called accelerated blood clearance (ABC) phenomenon).24,25) On the basis of our earlier results,26,27) we proposed the following tentative mechanism for the cause of this phenomenon: anti-PEG IgM, which is produced in the spleen in response to the first dose, selectively binds to the PEG upon the second dose of liposomes injected several days later and subsequently activates the complement system, and, as a consequence, the liposomes are taken up by Kupffer cells in the liver. A similar phenomenon was observed with polymeric micelles28,29) and PEG-modified PLA-nanoparticles.30) These findings suggest that PEG on nanoparticles (nanocarrier) acquires immunogenicity and elicits an anti-PEG IgM and/or IgG response.

Doxil®, doxorubicin (DXR)-containing PEGylated liposomes, has been approved for clinical use.31) Initial studies with Doxil® showed no ABC phenomenon,32,33) although empty PEGylated liposomes induced enhanced clearance of a subsequent dose of Doxil®.34) This finding indicates that the cytotoxic drug DXR delivered by the PEGylated liposomes can inhibit the secretion of anti-PEG IgM from B cells presumably via suppression of function of the B cells, and consequently attenuates the ABC phenomenon. However, we recently reported that Doxil® induces the ABC phenomenon in dogs when it is given with 2 mg DXR/m² or lower, which is much lower than the conventional dose.34) It is likely that the lower dose of Doxil® rather activates the B cells and thereby enhances the secretion of anti-PEG IgM. This suggests that the suppression of ABC phenomenon by Doxil® is dose-dependent, and an increased Doxil® dose similar to the conventional treatment might inhibit the activation of B cells. Nevertheless, there has been no report of the ABC phenomenon with Doxil® in human patients. La-Beck et al. demonstrated that the clearance of Doxil® from patients does not change over the Doxil® dose range of 10 and 60 mg/m². In addition, Gabizon et al. have reported that Doxil® clearance from patients given 30–60 mg/m² was reduced by 30% after repeated treatments. This might be due to a lipid dose that was high enough to induce immunological tolerance and encapsulated DXR high enough to give damage on splenic B cells at the clinical dose of Doxil®.

4. CONCLUSION

It appears that anti-PEG IgMs can be produced by systemic administration of PEGylated substances such as proteins and nanoparticles. The cross-reactivity of anti-PEG antibodies may limit the efficacy of the PEGylated substances through enhanced clearance of the substances from the circulation and neutralizing the biological activity of the substances. The immunogenicity of PEGylated substances should be tested in the development stage and the titer of anti-PEG antibodies in patients should be pre-screened and monitored prior to and throughout a course of treatment with a PEGylated substance.

Acknowledgement This work was supported in part by a Grant-in-Aid for Scientific Research (B) (23390012) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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