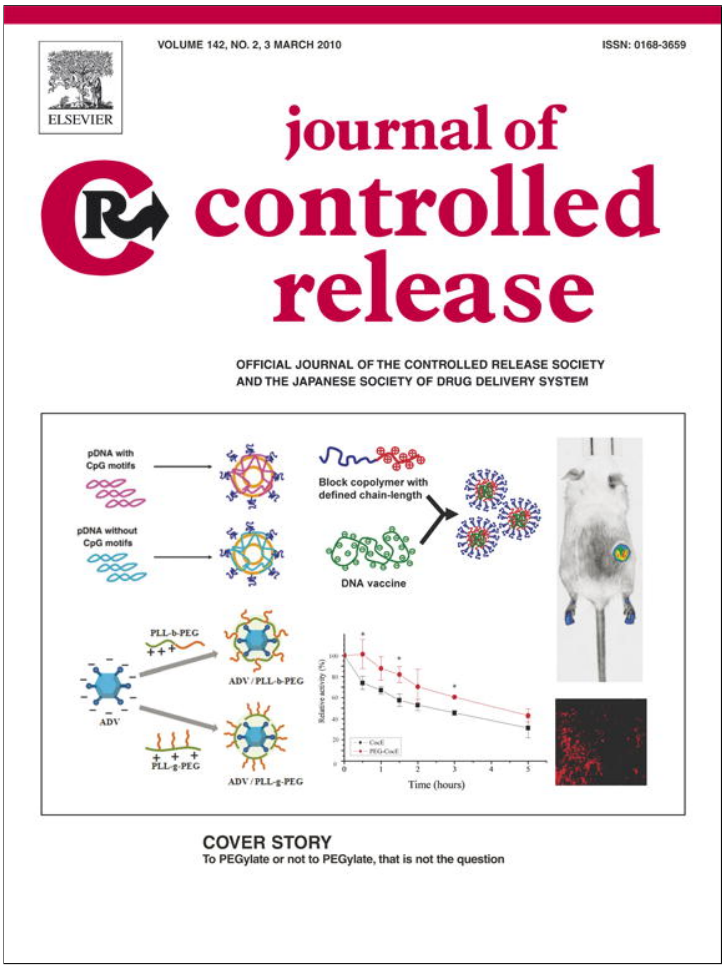

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Cover Story

To PEGylate or not to PEGylate, that is not the question

Systemic drug delivery using nano/micro vehicles has been limited by various reasons, but one of the important factors is a short blood circulation time of an intravenously (i.v.) administered drug delivery system. It has been known for more than a few decades now that surface modification of the drug delivery systems with poly(ethylene glycol), known as PEGylation, results in prolonged circulation times in blood. Naturally, PEGylation has been used widely in the development of various i.v. formulations for delivery of various active pharmaceutical ingredients, including peptides, proteins, and genes.

Despite extensive applications of PEGylation, the exact mechanisms how PEGylation affects the systemic circulation time and how it enhances bioefficacy are not clearly understood. Sometimes, PEGylation resulted in unexpected outcomes. It has been shown that the effect of prolonged circulation of an i.v. injected PEGylated liposome disappears, if the second dose is injected in a few days later, due to the production of anti-PEG IgM [1]. This "accelerated blood clearance (ABC)" phenomenon has been a concern for treating diseases which require repeated delivery of the PEGylated formulations for long-term treatment. Clearly, better understanding is required for more effective use of PEGylation. In this issue we have five articles dealing with various aspects of PEGylation. The articles collectively present better understanding on the role of PEGylation, development of new polymer structures for PEGylation, and its new applications.

1. The ABC phenomenon

Professor Kiwada and his colleagues solved a puzzle of the ABC phenomenon by demonstrating that the presence of specific CpG motifs in the PEGylated lipoflex is responsible for production of anti-PEG IgM [2]. Production of anti-PEG IgM was not observed with PEGylated "empty" cationic liposome or PEGylated lipoplex containing less immunostimulatory pDNA, i.e., non-CpG pDNA, at same doses. These findings indicate that the CpG motif in pDNA is a major cause of enhanced anti-PEG IgM production. Their current findings suggest that PEGylated lipoplex containing non-CpG pDNA may achieve efficient gene expression levels in solid tumor upon repeated injection.

It seems that the ABC phenomenon is not a universal one, i.e., it may not be observed with all PEGylated formulations. Interestingly, one of the studies by Professor Ishida and his group did not show the ABC phenomenon [3]. Their study indicated that a PEGylated cationic liposome formulation resulted in an efficient antitumor activity in a murine tumor model after three sequential injections without the ABC phenomenon. They demonstrated that two successive injections of liposomal oxaliplatin (l-OHP) allowed enhanced accumulation and broader distribution of subsequently injected (third dose) PEGylated liposomal l-OHP in solid tumor. On the other hand, a single injection of PEGylated liposomal l-OHP did not enhance the intratumoral accumulation of the second dose. After a single injection, the fraction of PEGylated liposomal l-OHP that reached the tumor tissue seemed to become almost entirely bound to tumor endothelial cells, and only a small proportion remained available for extravasation into the tumor interstitium. This relatively small fraction of liposomal l-OHP extravasating might be insufficient to exert a potent cytotoxic effect against the tumor cells and thus failed to enhance the intratumoral accumulation of second dose. In contrast, the cumulative cytotoxic effect of l-OHP imparted by two successive injections of PEGylated liposomal l-OHP allowed further extravasation and deeper penetration of subsequent third dose by decreasing tumor interstitial pressure as a result of a decrease in the number of tumor cells. This study indicates that we should focus on cumulative alteration in tumor microenvironment to obtain the sufficient outcome on cancer therapy by using a nanocarrier system via enhanced permeability and retention (EPR) effect.

The lack of the ABC phenomenon in the study by Professor Ishida and his colleagues was probably due to the toxic effect of encapsulated l-OHP on the splenic B-cells which produce anti-PEG IgM. Similar result was obtained in a previous study of Professor Ishida and Kiwada's group [4]. In addition, a relatively higher lipid dose (>16 μmol phospholipids/kg) was administered in this study. It has been reported that such higher dose did not cause anti-PEG IgM production presumably due to induced anergy in the splenic B-cells [4]. These observations indicate that the lipid dose and payloads of PEGylated liposome are critical to the outcome whether the formulation induces anti-PEG IgM production and thus, the ABC phenomenon.

2. Importance of the PEG structure on the effect of PEGylation

The effect of PEGylation is known to be affected by the structure of the PEG molecules. There are three examples on the effect of PEG structure on its bioefficacy in this issue. First, Professor Yang and his group applied a branched PEG, rather than a linear PEG, to protecting cocaine-induced toxic effect. PEGylation of cocaine esterase (PEG-CocE), as expected, increased its thermo-stability, reduced degradation of the enzyme by circulating proteases, and lowered the immunogenicity [5]. Their results showed the practicality of utilizing PEGylated CocE in protecting animals from lethal cocaine insult. It is interesting to note that using large, branched PEG helps offset the low density PEGylation. The use of a branched PEG results in the higher grafting density of PEG as compared with the linear PEG.

Second, Professor Wang and his group studied the structure–function relationship by synthesizing PEG-poly(aminoethyl methacrylate) (PAEM) diblock copolymer with well-defined chemical composition and nearly uniform distribution of chain-length [6]. The diblock copolymer may serve as a model system for probing the interaction

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between synthetic DNA vaccine carriers and dendritic cells. They showed that, with a PEG block of 2000 Da, the chain-length of the cationic PAEM block had a profound impact on the physico-chemical properties of the polyplexes, including average particle size and stability, as well as biological properties such as cytotoxicity, gene transfection, cellular uptake, and dendritic cell maturation. The optimum chain-length of the PEGylated PAEM block was 75 repeating units with negligible cytotoxicity and the highest gene transfection efficiency among the series of polymers tested. With further optimization of the PEG block size, these diblock copolymers may become clinically useful carriers for delivering DNA-based vaccines and therapeutics to dendritic cells.

Third, Professor Tae Gwan Park and his group have demonstrated that adenoviruses encoding an exogenous gene could be surface coated with block and graft copolymers of poly(L-Lysine) and PEG via ionic adsorption, resulting in several fold higher gene transfection efficiencies than the naked ones [7]. The physical PEGylation approach using PEG based cationic copolymers, as compared to chemical PEGylation, is a less destructive, versatile, and simple method for surface modification of various nanoparticles for prolonged circulation.

3. Future of PEGylation

Despite extensive research and development of clinically useful PEGylated products to date, it seems that PEGylation is still not clearly understood. Understanding the detailed mechanisms of PEGylation is necessary for further advances in the PEGylation technology. Furthermore, PEGylation is not limited to just grafting linear PEG molecules or to covalent grafting. The desired function of PEGylation will undoubtedly be enhanced by using new polymer structures, such as branched PEG and PEG-containing block copolymers, and also by using non-covalent methods of PEGylation. As shown by the studies published in this issue, the science of PEGylation has passed its infancy into the teenage stage (expect difficulties although exciting!), and more innovative studies are expected in the months to come. There is no question whether PEGylation is beneficial or not. Rather the question is how to PEGylate.

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