Research Center for Medical Sciences
Division of Medical Engineering

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General Summary

The Division of Medical Engineering aims to provide new and essential techniques for developing medical treatment. We have focused on 2 projects. One project is to develop polymer or polymeric micelle drug carrier systems for efficient therapeutic and diagnostic treatment. An example of our drug carrier systems is a polymer-based magnetic resonance imaging (MRI) contrast agent carrier system for the precise diagnosis of diseases. The polymer-based MRI contrast agents possess great potentials for disease-specific MRI. However, a drawback of the polymer-based MRI contrast agents is a risk of the release of free gadolinium (Gd) ions from Gd-chelates, which is due to the long half-lives of the polymer-based MRI contrast agents. Therefore, we designed a new stable polymer-based Gd-chelate for disease-specific MRI and have synthesized the polymer-based Gd-chelate. The other project has focused on immunogenicity issues of poly(ethylene glycol) (PEG), which has been the most commonly used polymer for biopharmaceutics, cosmetics, and foods. Because PEGs are considered safe, nonimmunogenic or weakly immunogenic, and biocompatible, PEG conjugation (PEGylation) is the most common technique for therapeutic proteins. However, PEGylated materials reportedly induce anti-PEG antibodies. We have examined PEG-related antibody responses in terms of the chemical structures of PEG-conjugates and have suggested an interfering concept. The study revealed the immunogenic characteristics of PEGs.

Research Activities

Development of Gd-chelates for the safe use of polymer MRI contrast agents

The MRI contrast agents are diagnostic agents that enhance MRI signals of water by paramagnetic metal ions, such as Gd ions, and iron ions and have been used clinically. At present, MRI contrast agents are low-molecular-weight Gd-chelates, which have short half-lives. On the other hand, in several recent studies, repeated injections of specific Gd-chelate-based MRI contrast agents have caused nephrogenic systemic fibrosis in patients, who exhibited low renal function. A possible reason this fibrosis develops is the fibrillization of free Gd ions, which are released from unstable Gd-chelates.

Regarding the functions of MRI contrast agents, we have shown attractive functions of polymer-based MRI contrast agents for the precise diagnosis with MRI. However, polymer-based MRI contrast agents have much longer half-lives than do low-molecular-weight Gd-chelates. Although the long half-lives are a reason these polymer-based MRI contrast agents have attractive functions, they also cause our bodies to be long exposed to these agents. Therefore, the Gd stability in our body is an issue we consider important.

To avoid the release of free Gd ions from Gd-chelates, we have developed new polymer-

based MRI contrast agents exhibiting stable Gd-chelates. We used a 1-(1-carboxy-3-carbo-*tert*-butoxypropyl)-4,7,10-(carbo-*tert*-butoxymethyl)-1,4,7,10-tetraazacyclododecane group for conjugation to polymers, which were deprotected to form a 1,4,7,10-tetraazacyclododececane, 1-(glutaric acid)-4,7,10-triacetic acid (DOTAGA) chelate group. The DOTAGA chelate group possesses 8-coordination after conjugation, whereas the 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacatic acid (DOTA) chelate group possesses 7-coordination after conjugation. We synthesized poly(glutamic acid)-based MRI contrast agents possessing the DOTAGA chelate group. After we prepared new MRI contrast agents, we have developed a method to evaluate the stability of Gd ions by means of a high-performance liquid chromatography system. We compared several low-molecular-weight Gd-chelates and poly(glutamic acid)-based Gd-chelates. We found no significant differences of Gd release from Gd-chelates in a 50% serum condition. In contrast, a possible reason for the release of Gd ions is the interaction of Gd ions with phosphate ions. Therefore, we examined the mixture containing 50 mM phosphate buffer and examined the stability of Gd-chelates. We observed the significant release of Gd ions from a low-molecular-weight Gd-chelate. The poly(glutamic acid)-based Gd-chelates exhibited no significant differences in the release of Gd ions, as compared with diethylenetriaminepentaacetic acid gadolinium (Gd-DTPA). On the other hand, a previously prepared polymeric micelle MRI contrast agent, which possesses a 7-coordinated DOTA chelate group, exhibited the highest stability of Gd ions. We have started developing a new polymeric micelle MRI contrast agent possessing an 8-coordinated DOTAGA chelate group.

Immunogenicity issues of PEG

We have studied the immunogenicity of synthetic polymers called PEG-conjugates. A conjugation technique of PEG to proteins, nanoparticles, and bio-surfaces is a simple technique known as PEGylation. PEGylation to proteins produces bio-inert surfaces, and as a result, reduces the immunogenicity of proteins and improves their pharmacokinetics. However, in patients to whom PEG-protein drugs have been repeatedly administered, anti-PEG antibodies have reportedly been induced and have become a serious issue for medical treatments. Because PEGylated proteins are captured by anti-PEG antibodies, the therapeutic efficacy of PEGylated proteins has been decreased. In fact, nonresponsive patients, who have been treated with PEGylated uricase, exhibited strong anti-PEG antibodies' responses, whereas responsive patients exhibited very weak anti-PEG antibodies' responses. Many PEGylated therapeutic proteins have been examined in clinical trials. However, because PEGylated therapeutic proteins induce anti-PEG antibodies, each PEGylated protein has immunogenic potential. As by PEGylated proteins, anti-PEG antibodies are reportedly induced by PEGylated nanoparticles (liposomes, micelle, and other nanoparticles). The antibodies in reported cases are most often anti-PEG immunoglobulin M (IgM) antibodies. Although PEGs are thought to be nonimmunogenic or weakly immunogenic polymers and are used to create bio-inert surfaces for research, these facts indicate that PEGs induce specific immune responses.

We have used various PEG-block copolymers to reveal PEG-related immunogenicity and have optimized anti-PEG IgM responses by the use of PEG-poly(β-benzyl L-aspartate)

block copolymer (PEG-PBLA), and derivatives of PEG-PBLA (PEG-P(Asp-Bzl)). From our mechanistic study of anti-PEG IgM behaviors, we have suggested an interfering concept for reducing anti-PEG IgM responses and designed new PEG-block copolymers possessing an intermediate block. The prepared new PEG-block copolymer micelles have shown in various doses a weak or nonexistent anti-PEG IgM response in vivo. The result indicates that our interfering concept works under in-vivo conditions.

We have started a new project, which is funded by the Japan Society for the Promotion of Science (Fund for the Promotion of Joint International Research). The project is based on an earlier finding that the interfering concept helps to reduce specific antibody responses; the project focuses on the immunogenicity of PEGylated proteins. We collaborate with 2 international researchers to promote our project. In the first year of the project, we started to prepare new PEGylated proteins, which exhibit nonexistent or extremely weak anti-PEG antibody responses.

Reviews and Books

Shiraishi K, Yokoyama M. Toxicity and immunogenicity concerns related to PEGylated-micelle carrier systems: a review. *Science and Technology of Advanced Materials.* 2019; **20:** 324-36.

Yokoyama M, Shiraishi K. Chapter 5: Clinical Diagnostic Imaging. In: Ito Y, editor. *Photochemistry for Biomedical Applications.* p. 107-30.