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. The main research projects of the division have been focused on polymer drug carrier systems, or polymeric micelle drug carrier systems, for efficient therapeutic and diagnostic treatments. One project aims to develop a polymer-based magnetic resonance imaging (MRI) contrast-agent carrier system for the precise diagnosis of disease states. Polymer-based MRI contrast agents have great potentials to improve diagnostic accuracy and to provide pathophysiological states of diseases, which general low-molecular-weight MRI contrast agents cannot exhibit. A specific characteristic of polymer-based MRI contrast agents is their long blood half-live, which is a main role in polymer-based MRI contrast agents' specific features described above. However, the long half-life might increase the risk of free gadolinium ions (Gd3+) being released from gadolinium (Gd) chelates. Therefore, we have developed new stable polymer-based MRI contrast agents exhibiting appropriate blood half-lives. Furthermore, a comparison method to evaluate the stability of Gd chelates and an established high-performance liquid chromatography (HPLC) method are needed. We have confirmed that prepared polymer-based MRI contrast agents show greater stability than low-molecular-weight MRI contrast agents. The other project aims to develop a new poly(ethylene glycol) (PEG) conjugation method (PEGylation) for biopharmaceutic agents. Because the characteristics of PEG, which are safety, nontoxicity, and very low immunogenicity, have been commonly accepted, PEG has been widely used for biopharmaceutics, as well as cosmetics. However, inductions of antibodies against PEG have been found in patients who have been treated with PEGylated biopharmaceutics, and anti-PEG antibodies significantly affect the therapeutic efficacy of PEGylated biopharmaceutics. We have studied PEG-related immunological issues and, as a result of the study, have suggested a new PEGylation for therapeutic proteins to reduce antibody responses against both the proteins and PEG.

Research Activities

Development of polymer-based Gd chelates for the safety of MRI contrast agents

A common clinical method for diagnostic purposes is MRI, and contrast agents for MRI have been widely used to visualize blood vessels. Paramagnetic metal ions, such as a Gd ion, have been used for MRI contrast agents and need chelate groups, such as the diethylenetriamine pentaacetic acid (DTPA) group and the 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacatic acid (DOTA) group, to form a stable metal ion complex, and Gd chelate complexes are, in general, low-molecular-weight complexes. Normally, Gd chelates

exhibit short blood half-lives and are immediately diffused into the bloodstream after intravenous injection. Therefore, high volumes of Gd chelates, as the MRI contrast agent, are administered with a single injection, and we repeatedly inject MRI contrast agents for each diagnostic examination. Therefore, the risks of Gd-based MRI contrast agents are increased, as is the incidence of nephrogenic systemic fibrosis, in patients who have decreased renal function. The risks of Gd chelates are caused by the release of free Gd3+ from Gd chelates (Gd toxicity), which we believe are in stable chelate complexes. Furthermore, although their clinical significance has not been understood, repeatedly injected Gd chelates have enhanced signals in both the nucleus dentatus and globus pallidus. These facts indicate that an appropriate design is necessary for stable Gd chelates.

We have shown attractive functions of polymer-based MRI contrast agents for precise MR images which are not generally provided by low-molecular-weight Gd chelates. Although attractive functions have been achieved by a characteristic of polymer-based MRI contrast agents, they have long blood half-lives. These long half-lives will be an issue regarding Gd toxicity, and the stability of polymer-based MRI contrast agents must be proved.

Long-term exposure of the body to polymer-based MRI contrast agents increases the likelihood of interactions between Gd chelates and endogenous substances; therefore, also increased is the risk of Gd3+ release from Gd chelates. What must be developed are new polymer-based MRI contrast agents exhibiting stable Gd chelates and methods for evaluating chelate stability. We have noticed that Gd chelates with low stability have been used as linear-type chelates and in 7-coordinated Gd chelate groups. Therefore, we have focused on macrocyclic chelate ligands, which have a chemical structure more rigid than liner-type chelates. In addition to the chemical structure of chelate groups, we also considered the number of chelate sites. We selected a 1,4,7,10-tetraazacyclododececane,1-(glutaric acid)-4,7,10-triacetic acid (DOTAGA) chelate group, which is a macrocyclic and 8-coordinated Gd chelate group, as a stable polymer MRI contrast agent. The 8-coordinated DOTAGA group is expected to have greater stability than the 7-coordinated DOTA group.

Poly(glutamic acid)-based MRI contrast agents possessing DOTAGA chelate groups have been synthesized by our division, and we used the HPLC method to evaluate free Gd3+ released from Gd chelates. We have evaluated polymer-based MRI contrast agents and several low-molecular-weight Gd complexes, such as Gd-DTPA, and Gd-DTPA-bismethylamide (BMA). We evaluated the stability of Gd chelates in 50% serum conditions. High phosphate concentration (0.05 M) in a 50% serum condition increased in the release of Gd3+ from a low-molecular-weight Gd chelate. Poly(glutamic acid)-based Gd chelates exhibited Gd chelate stability similar to that of Gd-DOTA. Previously prepared polymeric micelle MRI contrast agents exhibited the greatest Gd chelate stability, although they have been used in a 7-coordinated 1,4,7-tris(carboxymethylaza)cyclododecane-10-azaacetylamide (DO3A) chelate group. We believe that the polymeric micelle structure is an important advantage for protecting Gd3+ from proteins, other metal ions, and other substances. We have started to prepare new polymeric micelle MRI contrast agents, which possess 8 coordinated chelates.

Development of a new PEGylation technology

The most widely used polymer for biopharmaceutics is PEG. The method of PEG conjugation called PEGylation is a simple, versatile way to produce therapeutic proteins. In addition, PEGylation has been used to cover the surfaces of drug carriers. PEGylation has various merits for the development of biopharmaceutics, such reducing protein immunogenicity and improving protein pharmacokinetics. However, because of the usefulness of PEGylation, a significant issue is immunogenicity, namely antibody responses against PEG. For one example, PEGylated uricase exhibited anti-PEG antibody responses in patients. Antibodies against PEG has been clinically reported and found in patients who had repeatedly received PEGylated biotherapeutic agents. In fact, strong anti-PEG antibody responses have been found in nonresponsive patients, whereas weak anti-PEG antibody responses have been found in responsive patients. To date, more than 10 PEGylated therapeutic proteins have been under clinical trials. The above example, as well as other clinical reports regarding anti-PEG antibodies, indicate the importance of anti-PEG antibodies for therapeutic efficacy; therefore, the generation of anti-PEG antibodies has attracted much attention. Although the standard belief against PEG is thought to be a non-immunogenic, or weakly immunogenic polymer, researchers have recently noticed that PEGylated agents become, somehow, immunogenic.

We have examined the immunogenicity of synthetic PEG-block copolymers and have suggested a concept that reduces antibody responses against PEG by interfering interactions between a PEG chain and PEG-specific molecules. We have started a new project, which has been funded by the Japan Society for the Promotion of Science (funded for the promotion of Joint International Research), to develop a new PEGylation method and have collaborated with researchers at Utrecht University. We have succeeded in preparing several examples of PEGylated proteins, which native proteins are highly immunogenic proteins. Initially, we optimized conditions to activate lysine terminal amine groups in proteins and coupled PEG derivatives to synthesize a series of new PEGylated proteins. Coupling reactions were followed by gel permeation chromatography, and an excess amount of PEG derivatives was removed by ultracentrifugation. We characterized the new form of PEGylated proteins derivatives by means of ^{1}H nuclear magnetic resonance spectroscopy and gel permeation chromatography. Further characterization and *in vivo* experiments will be performed next year.

Publications

Yokoyama M, Shiraishi K. Stability evaluation of Gd chelates for macromolecular MRI contrast agents. *MAGMA.* 2020 Aug; **33**(4): 527-536. doi: 10.1007/s10334-019-00805-8. Epub 2019 Dec 10. PubMed PMID: 31823277.