Research Center for Medical Sciences
Division of Medical Engineering

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General Summary

The division of Medical Engineering provides new and essential techniques for development of medical treatment. We have developed polymeric micelle drug carrier systems for therapeutic and diagnostic treatment. One example of the polymeric micelle drug carrier system for the medical treatment is a polymeric micelle carrying MRI contrast agent for diagnosis of acute ischemic stroke. We have collaborated with a division of basic science in our university. Recombinant human tissue-type plasminogen activator (rt-PA) is an only therapeutic drug for the thrombolysis therapy in acute phase of ischemic stroke, and the thrombolysis therapy is allowed within 4.5 h of the onset of ischemic stroke. However, the use of rt-PA accelerates risk of symptomatic hemorrhages. Therefore, a novel diagnostic concept for the safety thrombolysis therapy, which reduces risk of symptomatic hemorrhage, is highly desired. We have succeeded in obtaining specific areas, where only the polymeric micelle MRI contrast agent can exhibit, in ischemic hemisphere in a rat ischemia-reperfusion injury model by the use of our MRI contrast agents. We have examined immunogenicity of synthetic polymers. Poly(ethylene glycol) (PEG) have been widely used as safe, non or very weak-immunogenic, and biocompatible materials in pharmaceutics. Therefore, PEGylation, which is a PEG conjugation technique to proteins, and lipids, is the most common technique for therapeutic proteins. However, there are number of reports that anti-PEG antibodies have been induced by PEGylated proteins, and PEGylated nanoparticles. We have studied the PEG-related antibody responses in terms of chemical structures of PEG-conjugates. The study reveals immunogenic characteristics of PEG and suggests how we can avoid the PEG-related antibody responses.

Research Activities

Evaluation of Gadolinium (Gd)-chelates for the safety use of polymer MRI contrast agents

A diagnostic agent, MRI contrast agents, have been used in clinics. MRI contrast agents have to be used safely, however, there are number of reports that repeated injections of MRI contrast agents caused nephrogenic systemic fibrosis (NSF) in patients, who exhibited low renal functions. The reason of MRI contrast agent-related NSF is due to release of free Gd ions from Gd-chelates. In the present, MRI contrast agents are low-molecular-weight Gd-chelates, therefore, half-lives of those MRI contrast agents are short. In contrast, half-lives of our polymer-based MRI contrast agents are much longer than those low-molecular-weight Gd-chelates. This indicates that our polymer-based MRI contrast agents exhibit long time exposure in body. To avoid free Gd ions-related NSF, we must use very stable gadolinium-chelates and evaluate Gd release behaviors. We started to

evaluate stability both of low-molecular-weight Gd-chelates and macromolecular Gd-chelates by means of HPLC. The reason of free Gd ion release is mostly due to interaction between Gd-chelates and serum proteins, therefore, we incubated each Gd chelate with serum proteins, and we detected amount of free Gd ions. So far, our polymer-based MRI contrast agents exhibited good stability in the experiments, which we have observed no free Gd ions, whereas some low-molecular weight Gd chelates released free Gd ions. This indicates our polymer-based MRI contrast agents are stable in the condition, but, we need further experiments to evaluate stability of our polymer-based MRI contrast agents in severe conditions.

Study of synthetic polymers' immune responses

We have been studying immunogenicity of synthetic polymers, poly(ethylene glycol)-conjugates (PEG-conjugates). A very simple, and versatile technique of PEG conjugation to proteins, which is called PEGylation, reduces proteins' immunogenicity and improves proteins' pharmacokinetics. As well as PEGylation on to proteins, PEGylation has been widely used for drug carriers. However, induction of antibodies against PEG (anti-PEG Abs) have observed in patients who received repeated administrations of PEG-protein drugs and have become serious issues for medical treatments. In the presence of anti-PEG Abs, therapeutic efficacy of PEGylated proteins' treatment will be lost. In fact, non-responder patients, who treated PEGylated uricase treatment, exhibited strong anti-PEG Abs' responses, whereas responder patients exhibited weak anti-PEG Abs' responses. So far, many PEGylated proteins have been studied for next generations of protein drugs, however, these facts indicate immunogenic potential of any PEGylated proteins. Furthermore, there are number of reports that PEGylated nanoparticles, such as liposomes, micelle, and other nanoparticles, clearly induced anti-PEG IgM antibodies (anti-PEG IgM). Although PEG has been thought to be no or very weak immunogenic material, these facts indicate that a specific immune response against PEG exists. We have confirmed PEG-related immunogenicity by the use of PEG-poly(β-benzyl L-aspartate) block copolymer (PEG-PBLA) and optimized anti-PEG IgM induction. To reduce PEG-related immunogenicity, we designed new PEG-block copolymers possessing an intermediate block chain. We succeeded in preparation of new PEG-block copolymers, which were confirmed by means of ^{1}H NMR. We used a solvent-evaporation method for polymeric micelle preparation. We succeeded in preparation of those polymeric micelles, and diameters of those micelles were in a range of 60-120 nm. We used those polymeric micelles to examine anti-PEG IgM induction in vivo. Several dose amounts of those polymeric micelles were intravenously injected, and sera were collected for anti-PEG IgM detection. ELISA was performed for detection of anti-PEG IgM, and we found that the anti-PEG IgM induction was intermediate block chain-length-dependent. We observed new PEG-block copolymers possessing long intermediate chain lengths exhibited very low anti-PEG IgM responses, whereas both PEG-PBLA and PEG-block copolymer possessing a short intermediate chain length exhibited high anti-PEG IgM responses. We will perform further experiments to make a firm conclusion for correlation of chemical structures and the anti-PEG IgM response.

To modulate PEG-related immune responses, we examined PEG-PBLA's antibody

responses in combination with an adjuvant. Adjuvants are known to enhance immune responses via the innate immune system, therefore, we examined to modulate the antibody response of PEG-PBLA. At first, we separately injected both the adjuvant and PEG-PBLA, and the anti-PEG IgM response was same as PEG-PBLA without adjuvant. In contrast, an injection of adjuvant-included PEG-PBLA exhibited drastic differences in the anti-PEG IgM response. Initially the anti-PEG IgM response exhibited significantly high responses, however, we observed dramatic decrease in the anti-PEG IgM response at two weeks after the injection. Furthermore, the mouse, which was injected adjuvant-included PEG-PBLA, exhibited no more anti-PEG IgM responses against 2nd PEG-PBLA injection. These results suggest cause of anergic or torelant antibody responses. We will examine further anti-PEG IgM responses against adjuvant-included PEG-PBLA in terms of cytokine relations.

Those above two topics suggest possibilities to reduce/modulate against PEG-related immune responses. To prove PEG-related antibody responses is to prove T-cell independent antibody responses of antigens. Owing to PEG's specific features, PEG-related antibody responses may uncover the T-cell independent antibody response.

Publications

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