

論 文 内 容 要 旨

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学位論文題目	Impact of anti-PEG antibodies on the blood clearance of some clinically approved PEGylated protein therapeutics (PEG化タンパク質製剤の血液クリアランスに対する抗PEG抗体の影響)		
<p>Modification of proteins with amphiphilic polymer polyethylene glycol (PEG) has been regarded as a breakthrough in the therapeutic application of proteins, to improve the pharmacokinetic characteristics of encapsulated drugs, and to achieve better therapeutic outcomes with fewer side effects. However, some PEGylated proteins are reported to induce what is referred to as the “accelerated blood clearance (ABC) phenomenon” resulting in the increased clearance and concomitantly compromising their effectiveness as well as unwanted adverse reactions. Accordingly, in 2014, the Food and Drug Administration (FDA) recommended the screening of therapeutic PEGylated proteins in the clinical phases for their ability to induce anti-PEG antibodies. The aim of this research was, therefore, studying the competence of some therapeutic PEGylated proteins, which are clinically tested or approved before 2014, to induce anti-PEG antibodies, and consequent ABC phenomenon as well as investigating the mechanism behind anti-PEG antibodies production. G-LASTA[®] (PEG-G-CSF) is a recombinant PEGylated human granulocyte colony-stimulating factor (G-CSF) that induces the production of neutrophils and used for treatment of chemotherapy-induced neutropenia. In clinical settings, G-LASTA[®] is administered to cancer patients via both the subcutaneous and intravenous routes. In a murine study, I showed that, regardless of administration route, initial doses of G-LASTA[®] elicited anti-PEG immune responses in a dose-dependent manner. Intravenous administration elicited higher levels of anti-PEG IgM than the subcutaneous route. Initial doses of G-LASTA[®] (6 mg/kg) that were high enough to trigger production of anti-PEG IgM, did not trigger the accelerated clearance of a lower subsequent dose (0.06 mg/kg). However, when the subsequent dose of G-LASTA[®] was raised to (6 mg/kg), the initial dose triggered the accelerated clearance of the second dose via an anti-PEG IgM-mediated complement activation. These results indicate that anti-PEG antibodies might compromise the therapeutic activity and/or reduce tolerance of PEGylated formulations when the dose is high enough to trigger complement activation. Next, I examined anti-PEG immune responses in mice for peginterferon alfa-2a (Pegasys[®]), a clinically approved PEGylated protein therapeutic for hepatitis C. Compared with G-LASTA[®], clinical and higher dose Pegasys[®] barely elicited anti-PEG immune responses. Repeated high doses of Pegasys[®] elicited anti-PEG IgM production, dependent on dose frequency, and triggered the rapid clearance of subsequent doses. In addition, pre-existing anti-PEG IgM, elicited by a different PEGylated protein, PEGylated ovalbumin (PEG-OVA), accelerated clearance of Pegasys[®]. After that, I studied the anti-PEG immunity for PEGylated Factor VIII (Jivi[®]), which is used for the treatment of hemophilia A, in which treatment is reported being associated with hyper-sensitivity reactions (HSRs) and loss of efficacy in some cases. Jivi[®] could efficiently induce the production of both anti-PEG and anti-Factor VIII antibodies, where anti-PEG antibodies are found to be the responsible for these HSRs. Two mouse hybridoma models secreting anti-PEG IgM (namely HIK-M09 and HIK-M11) were developed and employed to screen the effect of pre-existing anti-PEG antibodies on the serum concentration of Jivi[®], where both models could efficiently activate the complement system and accelerate the clearance of Jivi[®]. Later on, I investigated the cells contributing to anti-PEG IgM production. Anti-PEG IgM induction by G-LASTA[®] was diminished in athymic nude mice lacking T cells and in splenectomized mice. In addition, anti-PEG IgM production was significantly diminished in the cyclophosphamide-treated mice for depletion of B cells. These results indicate that anti-PEG IgM production by G-LASTA[®] occurs in spleen in a T cell-dependent manner, which differs from PEGylated liposomes (T cell-independent antigen). However, B cells, both marginal zone and follicular, are essential for anti-PEG IgM production in both PEGylated preparations. Overall, the obtained results indicate that the anti-PEG IgM production and its ability to induce complement activation and ABC phenomenon are complicated and differ between PEGylated proteins. However, results generally underscore the importance of screening for both pre-existing and treatment-induced anti-PEG antibodies together with complement activation in patients, prior to, and during treatment with PEGylated proteins and provide a step toward understanding the mechanism of the anti-PEG immune response.</p>			