A novel system for removing etiologic factors in the blood: Drug-Navigated Clearance System (DNCS)

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SUMMARY
Some cytokines and inflammatory mediators are considered to be pathogeneses of rheumatoid arthritis (RA), which are referred to as rheumatoid factors (RF). We have developed a novel therapeutic strategy, drug-navigated clearance system (DNCS), which leads the autoantibody, RF, toward the hepatic LDL receptors (LDLRs) by use of a newly designed drugs, “navigator”. Protein A that bind to the target antibody (RF) was chemically conjugated to apolipoprotein E (ApoE) which directly bind to the hepatic LDLR and dextran sulfate (DexS) which binds to LDL and then indirectly accumulate to the liver. The conjugation reaction was monitored on SDS-PAGE. In vitro uptake of fluorescently labeled IgG by HepG2 was greatly increased in the presence of navigator molecule. In addition, the elevated RF level of the RA model rat was reduced immediately after the navigator injection and last for about 2 days.

INTRODUCTION
A variety of deceases are well known to be resulted from the increasing of etiologic factor in the blood due to the metabolic abnormality. To remove the etiologic factors from the patients, an extracorporeal circulation is one of the most direct and effective strategies. However, long-term suffering and the enormous costs are the big issues. Various medications suppressing its production or synthesis are the alternatives, but it is not easy to regulate only the target substrate and any change of the other factors may lead to the side effect. Low density lipoprotein (LDL), beta-2 microglobulin, various autoantibodies are the examples of the factors to be removed from the blood stream.

We have been developing a novel strategy for removing these factors, named drug-navigated clearance system (DNSC). The concept is that the target etiologic substance is navigated from an original abnormal metabolic pathway to the other normal metabolic pathway by using “navigator” molecules composed of a moiety capturing the target molecule and a navigating moiety which leads the target molecules to the normal metabolic pathway (Figure 1).

In the present paper, rheumatoid arthritis (RA) is the target decease, and then the antibody in RA, which is referred to as rheumatoid factors (RF), is the target etiologic molecule. LDL receptor (LDLR) mainly expressed on the hepatocytes was selected as the normal metabolic pathway to which the RF is lead. Tow target-capturing moieties was tried. One is dextran sulfate (DexS), which has high affinity to circulating LDL. DexS attached to the LDL is lead to the LDLR in the liver. The other one is apolipoprotein E (ApoE) which has the direct affinity to the LDLR. These navigating
moieties were chemically conjugated to protein A (ProA) that capturing the antibody Fc region. The navigating efficacy of the two types of navigators was investigated in vitro by using HepG2 cells up-regulated in the LDLR expression and also in vivo by using the mouse RA model.

EXPERIMENTAL METHODS

The navigators were prepared by three synthetic routes. First, ProteinA-biotin and navigating moiety-biotin was conjugated via avidin molecules. Second, ProteinA-biotin was bound with navigating moiety-avidin prepared using sulfo-succinimidyl-4-[N-maleimidomethyl] cyclohexane-1-carboxlate (sulfo-SMCC). Third, diazirine labeled navigated moiety was reacted.

In order to evaluate the cellular uptake of the target AB by the action of the navigator molecules, Alexa 647 (red) labeled target AB was incubated with HepG2 cells or control HUVECs in the presence or absence of fluorescein labeld navigator for 24 hs. The amount of taken up AB and their intracellular distribution was evaluated by FACS and CLSM.

In addition, collagen-induced arthritis mouse was prepared by collagen emulsion injection using Freund's Complete Adjuvant (FCA) with a modified protocol. After checking the RA level, the prepared navigator was injected intravenously and the RA level was monitored as time using the detection kit.

RESULTS AND DISCUSSION

The band of ApoE was slightly changed on the SDS-PAGE after the biotinylation. In the first scheme, it was possible to prepare the conjugation easily, but the band derived from the multi-molecular complex was observed when the biotin labeled molecules was mixed with avidin. The preparation method strongly affected the conjugation number of the moieties.

In the HepG2 uptake experiment, the amount of the complex localized in the cells was increased with the incubation time. On the other hands, this tendency was not observed on the control experiments. Moreover when the non-labeled ApoE was used as the competitor, cellular uptake was specifically inhibited.

The intracellular distribution of the target RF (red) and the navigator (green) was found to be very similar, indicating that the RF was lead into HepG2 via the navigator molecules.

Figure 2 show the RF level in the blood after injecting collagen. The RF level was successfully increased 6 weeks after stepwise twice collagen injection. After 8 weeks post RA induction the navigator molecules was intravenously injected once. Immediately after the injection the RA level was reduce and the lowered level was last about two days.

CONCLUSION

Although the reduction level was not necessarily satisfactory, our new strategy DNCS was proved to work in vitro and in vivo and to be effective in removing an etiologic factor from the blood stream. We are now designing more effective navigators and are going to evaluate them.

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