Potential use of NF-κB Decoy Complex with Fucose-appended Dendrimer/α-cyclodextrin Conjugate for Treatment of Fulminant Hepatitis

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ABSTRACT SUMMARY
To reveal the potential of NF-κB decoy complex with fucose-appended dendrimer/α-cyclodextrin conjugate (Fuc-S-α-CDE, generation 2; G2) for the treatment of fulminant hepatitis, we evaluated the inhibitory effect of Fuc-S-α-CDE (G2)/NF-κB decoy complex on nitric oxide (NO) production in NR8383 cells, rat alveolar macrophages, stimulated with lipopolysaccharide (LPS). The NF-κB decoy complex with Fuc-S-α-CDE (G2) significantly suppressed NO production in NR8383 cells, fucose receptor (Fuc-R) expressing cells, stimulated with LPS. Furthermore, the complex suppressed the fulminant hepatitis caused by LPS after intravenous administration to mice, due to the Kupffer cell-selective NF-κB decoy delivery. These results suggest that Fuc-S-α-CDE (G2)/NF-κB decoy complex has the potential for the treatment of fulminant hepatitis.

INTRODUCTION
Fulminant hepatitis is a serious, life-threatening disorder. However, a number of clinical trials for the treatment of fulminant hepatitis did not show enough substantial benefits. Thus, a new paradigm is needed for the future regimen.

Previously, we prepared that the starburst polyamidoamine (PAMAM) dendrimer/α-cyclodextrin conjugates bearing mannose (Man-α-CDE (G3)) as antigen presenting cell (APC)-selective gene carrier. However, Man-α-CDE (G3) did not show the APC-selective gene transfer activity, probably due to the inappropriate spacer to recognize the receptor. Therefore, we newly synthesized fucosyl-oxypropyl-thio-propionylate group as a spacer to recognize fucose receptor (Fuc-R), which is highly expressing in APC. In the present study, we newly synthesized Fuc-S-α-CDE (G2) (Fig. 1) and evaluated the potential of Fuc-S-α-CDE (G2)/NF-κB decoy complex for the treatment of fulminant hepatitis.

Fig. 1. Chemical Structure of Fuc-S-α-CDE (G2)

EXPERIMENTAL METHODS
Nitrite determination
NR8383 cells (1 x 10⁵ cells/well) were treated with LPS (100 ng/mL) in the presence of Fuc-S-α-CDE (G2, DSF2)/NF-κB decoy complex for 8 h, followed by the incubation with culture medium for 12 h. NO production was estimated by measurement of nitrite in the culture supernatant using Griess reagents. Nitrite levels were determined using NaNO₂ as a standard.

Cellular uptake of FITC-NF-κB decoy complex with Fuc-S-α-CDE (G2, DSF2)
NR8383 cells (1 x 10⁵ cells/well) were treated with Fuc-S-α-CDE (G2, DSF2)/FITC-NF-κB decoy complex for 1 h. Then, the fluorescence intensity of FITC-NF-κB decoy in cells was determined by a flow cytometer.

In vivo study
Murine fulminant hepatitis was induced by intraperitoneal injection with a solution containing 100 ng of LPS and 25 mg of D-galactosamine. After 1 min, Fuc-S-α-CDE (G2, DSF2)/NF-κB decoy complex was administered.
intravenously. The survival rate was monitored over the next 60 h. The plasma TNF-α levels were assayed by ELISA. The levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in mice were measured using diagnostic kits. Accumulation of FITC-NF-κB decoy complex in hepatic non-parenchymal cells was determined by fluorescence microplate reader.

RESULTS AND DISCUSSION

To evaluate the Fuc-R expressing cell-selective anti-inflammatory activity of Fuc-S-α-CDE (G2, DSF2)/NF-κB decoy complex, we evaluated the inhibitory effect of the complex on NO production in NR8383 cells (Fuc-R (+)) and Colon-26 cells (Fuc-R (-)). Fuc-S-α-CDE (G2, DSF2)/NF-κB decoy complex significantly suppressed the NO production in NR8383 cells, but not in Colon-26 cells. These results suggest the potential of Fuc-S-α-CDE (G2, DSF2) as a Fuc-R expressing cell-selective NF-κB decoy carrier.

Next, to examine the effect of Fuc-R on cellular uptake of Fuc-S-α-CDE (G2, DSF2)/NF-κB decoy complex, we determined the fluorescence intensity of FITC-NF-κB in NR8383 cells with or without fucose, a competitor of Fuc-R by a flow cytometer. The fluorescent intensity of FITC-NF-κB transfected with Fuc-S-α-CDE (G2, DSF2) was lowered by the addition of fucose in a dose-dependent manner (Fig. 2). These results suggest the Fuc-R-mediated cellular association of FITC-NF-κB decoy complex with Fuc-S-α-CDE (G2, DSF2).

To investigate the inhibitory effects of Fuc-S-α-CDE (G2, DSF2)/NF-κB decoy complex on fulminant hepatitis, we evaluated the survival rate and plasma TNF-α, AST and ALT levels. Furthermore, we determined the accumulation of FITC-NF-κB decoy complex in hepatic non-parenchymal cells. The mice treated with Fuc-S-α-CDE (G2, DSF2)/NF-κB decoy complex significantly survived, compared to LPS alone and naked decoy alone (Fig. 3). Additionally, TNF-α, AST and ALT levels in blood were reduced after treatment with the complex, compared with LPS alone. Importantly, FITC-NF-κB decoy was significantly accumulated in hepatic non-parenchymal cells after treated with the complex with Fuc-S-α-CDE (G2, DSF2). Taken together, these results strongly suggest that Fuc-S-α-CDE (G2, DSF2)/NF-κB decoy complex inhibits fulminant hepatitis caused by LPS.

Fig. 2. Effects of Fucose on Cellular Uptake of FITC-NF-κB Decoy Complex with Fuc-S-α-CDE (G2, DSF2) into NR8383 Cells

Fig. 3. Effects of Fuc-S-α-CDE (G2, DSF2)/NF-κB Decoy Complex on Survival Curve of Fulminant Hepatitis Mice Induced by LPS and D-Galactosamine

Each line represents the survival of 4-10 mice.

*p < 0.05 versus LPS alone.

CONCLUSION

These results suggest that Fuc-S-α-CDE (G2)/NF-κB decoy complex has the potential for the treatment of fulminant hepatitis, through Fuc-R-mediated cellular uptake in cells.

REFERENCE