Lipid Nanoparticle Pre-Treatment Improves rAAV Diffusion in the Primate Liver and Enables an Increase of Therapeutic Transgene


uniQure B.V., Amsterdam, The Netherlands

Introduction / Background

Recombinant adeno-associated virus (rAAV)-vectors have been successfully used in preclinical studies and in clinical trials to express therapeutic proteins in the liver. Transduction of a limited percentage of hepatocytes can be sufficient for expression of secreted proteins. However, most hepatic metabolic monogenic disorders caused by the deficiency of an intracellular enzyme or membrane transporter would require a high percentage of hepatocytes to be transduced to achieve a therapeutic effect. This can be routinely achieved in rodents, but has proven to be a significant hurdle in large animals hampering the feasibility of such liver directed AAV based gene therapies.

Approach

The liver has a very high capacity to remove particles from the circulation. The cells from the reticuloendothelial system (RES) play a central role in this clearance process. Those cells can ingest and destroy foreign material and therefore constitute the first cellular barrier between the blood flow and the liver tissue. The saturation of the hepatic reticuloendothelial cells by nanoparticles or lipids has been shown to block uptake of particles from the circulation. We hypothesized that saturation of the RES could increase AAV-vector transduction and distribution in the liver, subsequently improving transgene expression, in large animals. Therefore, we explored the potential of pre-treatment with Intralipid, an FDA approved emulsion of soy bean oil, egg phospholipids and glycerin in non-human primates.

Study design

Non-human primates (NHPs, n=2) tested negative for the presence of anti-AAV5 neutralizing antibodies were injected intravenously with Intralipid (2g/kg) one hour before intravenous administration of rAAV5-hFIX at a dose of 9.7x10^{12} gc/kg (NHP3, NHP4). A control group (n=2) was injected with rAAV5-hFIX at the same dose after prior treatment with PBS (NHP1, NHP2). The animals were followed for 8 weeks before sacrifice. The levels of hFIX transgene in the plasma were analyzed by ELISA and the rAAV-vector DNA and transgene RNA copies numbers in liver tissue (8 liver lobes per animal) were determined by qPCR. The presence of AAV-vector DNA and transgene RNA was detected in liver tissue samples by fluorescent in situ hybridization (FISH) with a probe that hybridizes to both rAAV5 vector DNA and hFIX mRNA on 3 different liver lobes per animal. In addition to the probes, the tissues were stained with DAPI to visualize the cell nuclei and with an antibody against glutamine synthetase (GS) to visualize the central veins.

Results

Improved efficacy of rAAV-vector delivery after Intralipid pretreatment

After Intralipid pretreatment, an average increase of 3.5-fold in the levels of hFIX transgene expression was observed in the animals injected with rAAV5-hFIX when compared to the non-treated group (A). A similar increase was observed at the hFIX mRNA levels (data not shown). Accordingly, the vector-DNA copy numbers were 2.6-fold higher in the liver tissue of the animals treated with Intralipid than in the non-treated group (B). Furthermore, the H-score, a semi-quantitative scoring system based on the combination of the overall percentage and intensity values of cells positive for the signal, was applied. Based on the H score, the overall percentage of transduced hepatocytes was up to 4-fold higher when Intralipid was used (C).

Increased liver tissue transduction and distribution after Intralipid pretreatment

The distribution of rAAV-vector DNA/hFIX mRNA through the liver tissue and in relation to the portal and central veins was analyzed based on the overall percentage and scoring values of cells positive for the signal, was applied. Based on the H score, the overall percentage of transduced hepatocytes was up to 4-fold higher when Intralipid was used.

Conclusions

In summary, our data demonstrate that pretreatment with a lipid emulsion significantly improves the efficacy of rAAV-vector delivery to the liver and enables a broader hepatic cell targeting throughout the tissue. Intralipid is widely used clinically and safe, and therefore this approach can be easily employed to develop liver-targeted gene therapies for hepatic metabolic monogenic disorders that require a high percentage of hepatocytes to be transduced to achieve a therapeutic effect. More generally, the development of techniques that improve transduction efficacy independent of vector design will increase consistency between patients.