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Ultrasound guided AAV9-GFP delivery in fetal pigs for in utero fetal gene therapy

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Objective

Over the last 20 years, swine (Sus scrofa) has been increasingly used as a suitable animal model in various biomedical research programs. Pigs share several anatomic, physiologic, genetic and metabolic features with humans that make them a promising animal model for translational medicine. New genome editing technologies combined with somatic cell nuclear transfer, have recently increased the use of pigs in biomedical research as a valid alternative to non-human primates which have high costs and ethical limitations. We aimed to develop an In Utero Fetal Gene Therapy approach in pigs using ultrasound guided delivery of an adeno associated virus carrying the green fluorescent protein transgene (AAV9-GFP).

Methods

A pregnant sow at 80 days of gestation (term = 115 days) was anesthetized and prepped for ultrasound imaging. A Voluson S8t BT22 ultrasound system was first used to identify the location and orientation of fetal piglets in the litter and to determine the most straightforward route of injection. Three fetuses allocated in the right horn of the uterus presented a good orientation for the procedure and received 1 ml of saline via ultrasound guided intracardiac injection using a 20-gauge spinal needle. After the procedure, cardiac activity of each fetus was monitored, and the sow was supported through recovery from anesthesia. At 110 day of gestation the sow was anesthetized again for the vector injection. Two fetuses located in the left horn and one of the previous saline injected fetus, received an ultrasound guided intracardiac injection of scAAV9-GFP (1.2 x 10^12 vg copies). The total procedure (from sow sedation to resuscitation) required less than 30 minutes. The sow recovered quickly, without complications from surgery or preterm delivery.

Results

A total of 16 live piglets were naturally delivered at 115 day of gestation (no mummified or stillborn were obtained). Birth weight, temperature and the general health status of the piglets was normal. The day after delivery, DNA was extracted by tail biopsy and analysed by standard PCR for GFP sequence detection. Three out sixteen piglets resulted positive for GFP and were daily monitored for different health parameters. Growth curves and behaviours were comparable to control sibling. Non sign of distress, immunity reactions or lethargic phenotype were observed. Animal were culled 30 days after birth and different tissues were collected for molecular and histological analysis. PCR analysis using GFP primers revealed widespread of virus in liver, hearth, muscle and brain. Histological analysis to assess the GFP expression are still under evaluation.

Conclusion

For inherited genetic diseases, In-Utero Fetal Gene Therapy (IUFGT) offers the potential of prophylaxis, allowing the correction of a genetic defect before early irreparable tissue damage has occurred and, overall, improving postnatal clinical outcomes. Here we used a pig model to show the feasibility of an ultrasound guided procedure to systemically deliver a reporter vector (scAAV9-GFP) by intracardiac injection. Despite this pilot experiment, allowed us to set up several surgical details and understand the virus biodistribution, further experiments aimed at studying the AAV9-GFP dose-response are needed. Our final goal is to use this protocol to demonstrate safety and efficacy of IUFGT in a pig model of Leigh Syndrome.