Biodistribution of Polymeric Nanoparticles following in utero Delivery to a Nonhuman Primate

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Abstract

Introduction: Monogenic diseases can be diagnosed before birth. Systemic fetal administration of nanoparticles (NPs) grants therapeutic access to developing stem cell populations impacted by these classes of disease. Delivery of editing reagents in these NPs administered before birth has yielded encouraging results in preclinical mouse models of monogenic diseases. ***Methods:*** To translate this strategy clinically, the safety and efficacy of this strategy in larger animals will be necessary. We performed a pilot biodistribution study in 3 fetal nonhuman primates (NHPs) in mid-gestation examining systemic delivery of polymeric NPs loaded with fluorescent dye. ***Results:*** We found several similarities in distribution to our experience in mice, namely, extensive uptake in fetal liver and spleen. A striking finding that is not recapitulated in the mouse was the accumulation of NPs in the zones of proliferation and ossification of the fetal bone. Of great importance, there did not appear to be NP accumulation in the fetal male or female germline zones or maternal tissue. ***Conclusion:*** These studies were vital to the next step of testing editing reagents in the fetal NHP with a goal of treating monogenic diseases before birth.

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Published by S. Karger AG, Basel

Introduction

Through prenatal genetic testing, clinicians can diagnose many monogenic diseases before birth [1, 2]. And with advanced imaging techniques, we can evaluate for

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and prognosticate fetal structural disorders [3, 4]. With this clinical information, we can treat a broad range of diseases earlier in development [5–7]. There are ongoing efforts to do so in a minimally invasive fashion. This study sought to understand the biodistribution of polymeric nanoparticles (NPs) in a fetal nonhuman primate (NHP) with these translational goals in mind.

Fetal therapy carries advantages over postnatal therapy for several disease categories. For diseases like myelomeningocele or cystic fibrosis, where there is irreversible tissue damage before birth, fetal therapy is the optimal, and perhaps only, window to allow normal tissue development [8]. For diseases in which babies are born with respiratory failure, like pulmonary hypoplasia and congenital diaphragmatic hernia, fetal therapies that improve lung structure and function before the baby takes its first breath are the best treatments. For genetic diseases, where gene and stem cell therapies are possible, the prenatal period offers unique access to proliferating and migrating stem cell populations [9–13]. Additionally, therapy in the fetus carries a stoichiometric advantage as humans increase in size by 3- to 10-fold from the time of midgestation to birth; to give the same dose per weight ratio of therapeutic reagents to stem cells, smaller volumes and overall amounts of agents are needed. Furthermore, in utero interventions take advantage of the developing fetus's immature (and therefore immune-tolerant) immune system, which can be a barrier to the efficacy of gene therapy in the postnatal period [14].

NPs, small spherical particles that are typically 50–500 nm in size, can be synthesized from natural or artificial polymers and can be loaded with a variety of drugs and therapeutic agents. Most of our laboratory experience with fetal delivery of NPs has been with particles synthesized from poly(D,L-lactic-co-glycolic acid) (PLGA) polymer, which is biodegradable and biocompatible and found in many FDA-approved products. PLGA NPs can be loaded with hydrophobic drugs [15, 16], water-soluble drugs [17, 18], and nucleic acids of various chemistries [19–23]. NPs enter cells by endocytosis and release cargo inside the cytoplasm after endosomal escape [24]. This allows for intracellular delivery of agents that otherwise have low cellular uptake. Our laboratory and others have demonstrated safe and effective prenatal intravenous and intra-amniotic delivery of PLGA NPs in mice [25–28]. For example, in preclinical mouse models of hemoglobinopathy and surfactant deficiency, NP-mediated delivery of therapeutic agents to fetuses resulted in phenotypic improvement [26, 29]. Our hypothesis for these experiments in NHP was that NP distribution would be similar to that in fetal mice, but these interactions are largely unstudied and there may be biological differences in these species that could have significant impact on clinical translation.

From a therapeutic standpoint, the translational potential of our approach is clear: since the 1980s, umbilical vessels have been cannulated under ultrasound guidance to deliver fluid, blood transfusions, and drugs in midgestation with a risk of fetal loss that is less than 1% [30]. A necessary step in the clinical translation of other agents is an understanding of their behavior in a large animal model. Distribution to target organs is necessary for efficacy, and it is important to monitor distribution to the fetal gonadal tissue and maternal tissues to minimize off-target effects. In our prior experience, we have not seen uptake or editing in the fetal germline or maternal tissue, but this needs to be monitored to assess the safety of this therapeutic strategy [29].

For this study, an NHP was chosen as it is evolutionarily close to humans in terms of placentation and fetal development. At the outset of our study, it was unknown how similar the behavior of polymeric NPs in NHPs matched our experience in mice. We sought to answer these questions by performing a biodistribution study of PLGA NPs following in utero systemic delivery to NHPs.

Methods

NP Preparation
PLGA NPs were formulated using a solvent evaporation technique. Ester-terminated PLGA polymer with 50:50 lactide:glycolide ratio and molecular weight 54,000–69,000 was purchased from Sigma Aldrich (CAS #: 26780-50-7). One-hundred (100) mg of the polymer was dissolved in 1,950 µL of dichloromethane (Millipore Sigma, CAS #: 75-09-2) overnight. To trace the NPs in tissues, 50 µL of DiI dye (Biotium, CAS #: 41085-99-8) at 10 µm in dimethyl sulfoxide (Millipore Sigma, CAS #: 67-68-5) was added to the dissolved polymer immediately prior to formulation (0.5% [w/w]). To simulate the conditions used to create optimal loading of gene editing reagents [29], 200 µL of ultrapure water was added dropwise under vortex into the polymer/dye solution and sonicated with a probe tip sonicator. This water-in-oil emulsion was then added dropwise under vortex into 4 mL of 5% (w/v) polyvinyl alcohol (Sigma Aldrich, CAS #: 9002-89-5, molecular weight 13,000) solution and sonicated with a probe tip sonicator to form a water-in-oil-in-water emulsion. The resulting double emulsion was diluted into 30 mL of 0.3% (w/v) polyvinyl alcohol

solution while mixing on a stir plate. The remaining dichloromethane was evaporated using a rotary evaporator. The NPs were centrifuged at 16,000 g and 4°C for 15 min and then washed in ultrapure water twice by centrifugation to remove excess polyvinyl alcohol. After the final centrifugation, the supernatant was removed, and the NPs were resuspended at an estimated 1:1 w/w ratio of NPs:trehalose (MP Biomedicals, CAS #: 6138-23-4).

NPs were aliquoted, flash frozen in liquid N_2, and lyophilized for 24 h. An aliquot was resuspended at 10 mg/mL in phosphate-buffered saline, and the size and zeta potential were measured via dynamic light scattering (Malvern Instruments). The NPs used for the study were 292+/−5 nm in size with a low polydispersity index of 0.11 and a zeta potential of −3 mV. NPs were stored at −20°C, and aliquots were shipped on dry ice to St. Kitts Biomedical Research Foundation for the study, and were maintained in a frozen state until use.

In utero NP Administration in Mice

All animal use was in accordance with the guidelines of the Yale Animal Care and Use Committee. C57BL/6J mice were obtained from the Jackson Laboratory. Female mice (8–20 weeks old) were housed overnight with male breeders on a predetermined date, and pregnancies were confirmed by palpation 2 weeks later. Pregnant mice at day 15 postconception were used for the study. At the time of the procedure, the mice were anesthetized with vaporized isoflurane (2% for induction and maintenance) and injected subcutaneously with 3.25 mg/kg of extended-release buprenorphine (Ethiqa XR) and 5 mg/kg of meloxicam. Lidocaine 0.5% was used for local analgesia. Prior to surgical incision, the abdomen of the pregnant mouse was prepped with betadine solution. A midline laparotomy incision was performed to exposure the gravid uterus. NPs were resuspended at 10 mg/mL in phosphate-buffered saline and drawn up into a glass micropipette (tip diameter ~50 μm). Each fetus received a 15 μL volume of NPs (150 μg) intravascularly via the vitelline vein. The female's abdomen was closed, and she was closely monitored until she woke up. At 4 h post-NP injection, the pregnant mice were killed via overdose of vaporized isoflurane. The fetuses were delivered via cesarean section and washed in phosphate-buffered saline. Representative samples of maternal tissues and placenta were also collected.

In utero NP Administration in NHPs

Three social breeding enclosures housing approximately 24 female African green monkeys (*Chlorocebus sabaeus*) from the St. Kitts Biomedical Research Foundation facility were designated as timed breeding colonies for this study. Males were released into the social enclosures on a predetermined date for a period of 14 days. Pregnancies were confirmed by ultrasound scans at 5 weeks after the breeding period. The date of conception was assigned as the midpoint day of exposure to the male. All work was approved by the Yale Animal Care and Use Committee and conducted in compliance with test facility standard operating procedures.

Five pregnant monkeys were available for the study. Three of the pregnant female monkeys were randomly assigned to the treatment group, while the remaining two served as controls. The monkeys in the control group did not receive any injection. All three monkeys in the treatment group were administered 30 mg of PLGA NPs dissolved at 10 mg/mL in phosphate-buffered saline (3 mL total volume). The dose was estimated to be 300–400 mg/kg, which was the dose shown to be efficacious and safe in our small animal studies [29]. The estimated gestational age at time of injection was 80 days, but there is some uncertainty and variability as the breeding period was 14 days.

Prior to NP administration, the monkeys were anesthetized using intramuscular ketamine (8 mg/kg) and xylazine (1.6 mg/kg). Vital signs (temperature, heart rate, and respiratory rate) were monitored for the pregnant females while anesthetized. For the injection procedure, the abdomen of the pregnant monkey was prepped with betadine solution and draped in a sterile fashion. An ultrasound probe was placed in a sterile sheath and used to identify the umbilical vessels and fetal heart. A spinal needle (27 gauge) was then inserted into either the umbilical vein or fetal heart under ultrasound guidance (Fig. 1a, b). During the harvest of tissues, the umbilical vein is about 1 mm and the spiral course of the vessel can be appreciated (Fig. 1c). The NPs were injected, and the needle was withdrawn. Pressure was held at the skin site as needed, and vital signs were closely monitored as the female woke from anesthesia.

At 4 h after NP injection, monkeys were euthanized by administering sodium pentobarbital (100 mg/kg) and perfusing the cephalic circulatory system with chilled 0.9% saline. All study animals (adult females and respective fetuses) were subjected to a complete necropsy examination, including evaluation of the musculoskeletal system; external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. Representative samples of the tissues were collected from all fetuses and gravid females.
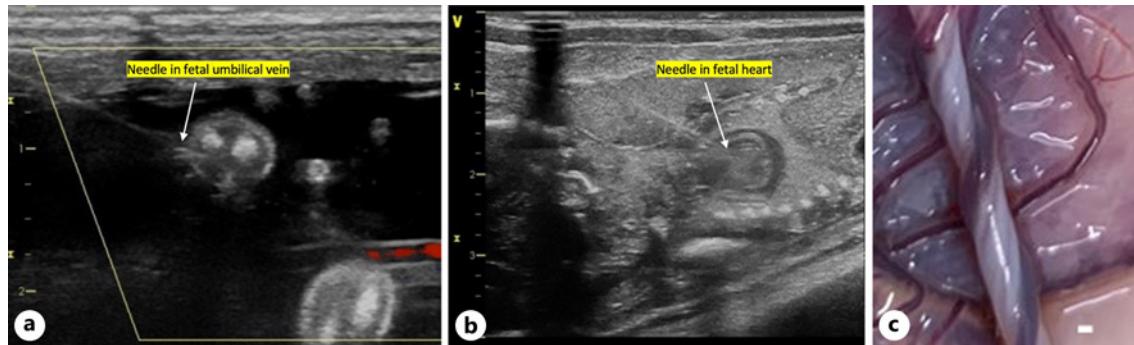
Fig. 1. Ultrasound images of NP injection into NHP umbilical vein (**a**) and fetal right ventricle (**b**). **c** Image of the spiraling umbilical vein (white bar = 1 mm).

Tissue Processing and Biodistribution Analysis

The tissue specimens were fixed in 4% paraformaldehyde (Electron Microscopy Sciences, CAS #: 30525-89-4) for 24 h at 4°C and then dehydrated in 20% (w/v) sucrose solution in phosphate-buffered saline for 48 h at 4°C. The tissues were then embedded in Optimal Cutting Temperature compound (Fisher Scientific, Catalog No.23-730-571) and frozen in 2-methylbutane (Sigma Aldrich, CAS #: 78-78-4) on dry ice. The frozen tissues were stored at −80°C until further processing. Each tissue was sectioned at a 15 μM thickness using a Leica Cryostat (CM1860). The tissue sections were mounted on glass slides, air dried, and then stored at −20°C with desiccant. Prior to imaging, the tissue sections were incubated in phosphate-buffered saline to rehydrate the tissues and remove excess Optimal Cutting Temperature compound. The slides were mounted onto glass coverslips (0.13–0.16 mm thickness) with a glycerol-based medium containing a DAPI nuclear dye (Vector Laboratories, Catalog No. H-2000).

Tissues were imaged using a spinning disk confocal microscope (Zeiss Axio Observer Z1, AxioCam MRm). Laser powers and exposure times were set individually for DAPI, Cy2, and Cy3 and kept the same for all NHP and mouse tissues. Three 40×, single-plane images were captured for each tissue. The focal plane was chosen by scanning the specimen in "live" mode in Zen for sharp morphological features in the DAPI channel.

Raw images were used for quantitative analysis using Zen Microscopy software. The brightness histogram was analyzed in the red channel. Control images were used to determine the baseline brightness of a given tissue. Positive pixels above that threshold were quantified to estimate brightness over matched control, with 3–6 images used for each tissue. The distinction of high, moderate, and low uptake was subjective based on the appearance of the image,

which correlated with positive pixels over control. Each NHP animal is presented individually. Data presented for mice represent 3 animals. Brightness was compared by Student's *t* test using Prism statistical software. Our notation for statistical significance was as follows: *<0.05, **<0.01, ***<0.001, ****<0.00001.

Results

Due to differences in NHP gestational age from the 2-week breeding period, the three injected monkey fetuses had varying weight, leading to three different doses (Table 1). We estimated the fetal mouse doses to be between 300 and 400 mg/kg based on our previous studies [29].

Similarities in Systemic Fetal NP Biodistribution between Mouse and NHP

Within the dose range and gestational ages tested, there is robust uptake of particles in the fetal liver and spleen (Fig. 2). The E15 mice and mid-dose NHP both received approximately 400 mg/kg of NP and have similar uptake in fetal liver and spleen. The fetal NHP that received a relatively low dose per weight (170 mg/kg) demonstrated uptake but to a lesser degree in the fetal liver and spleen (Fig. 2d, h, i, j) and this appears to filter out most of the NP from going further systemically (Fig. 2, 3; online suppl. Fig. S1; for all online suppl. material, see https://doi.org/10.1159/000543138). In mice and the NHP that received the mid or higher NP doses, uptake can be appreciated in the adrenal gland (Fig. 3a, f).

In mice and the high- and mid-dose NHP, there is significant uptake in the renal glomerulus but less so in the renal parenchyma and tubules with more particles in the parenchyma at higher doses (Fig. 3b, g, h). In the low-dose NHP, very few particles are observed in the kidney (Fig. 3g,

Table 1. Dose and route of NPs delivered to the studied animals

Animal	Fetal sex	Mass of NPs administered, mg	Weight of fetus, g	NP dose, mg/kg	Delivery target
NHP A1	Female	30	42	700	Umbilical vein
NHP A2	Male	30	72	400	Right ventricle
NHP A3	Male	30	176	170	Right ventricle
NHP B1	Male	–	–	0	–
NHP B2	Female	–	–	0	–
Mouse	Male/female	0.18	0.450	400	Vitelline vein
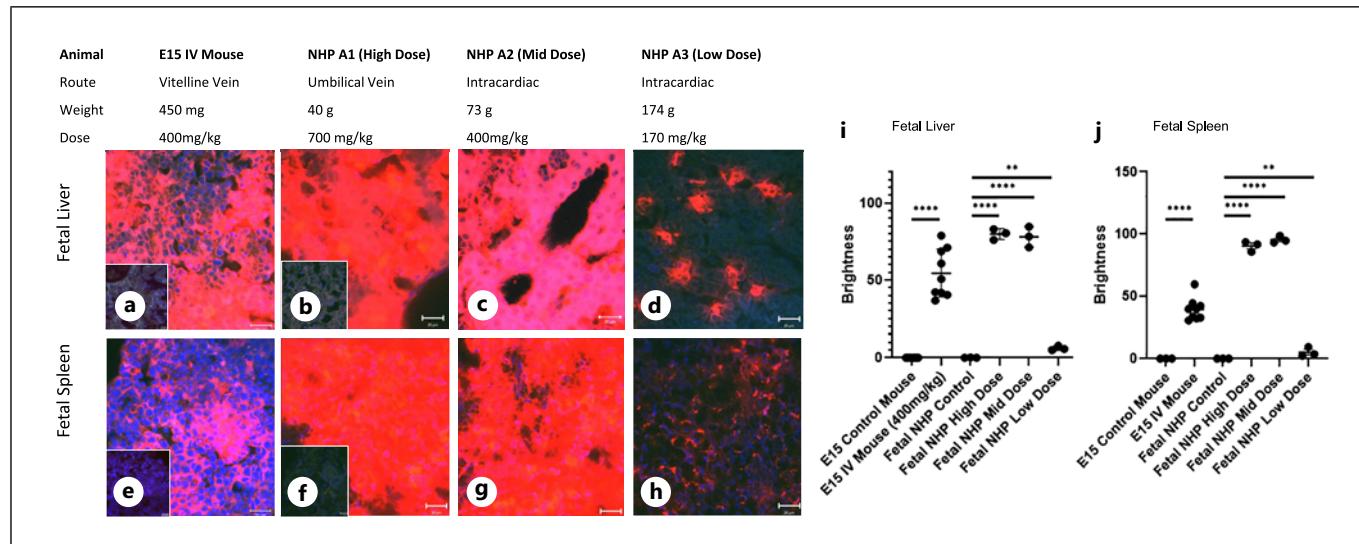
Fig. 2. Confocal microscopy images (40×) following intra-vascular injection of red fluorescent NPs with control images in the left lower corner. **a**, **e** E15 mouse fetal liver and spleen following a dose of 400 mg/kg. **b–d**, **f–h** Fetal NHP liver and spleen following a dose of 170, 400 and 700 mg/kg (blue = DAPI nuclear stain; red = DiI lipophilic dye; white bar = 20 μm). Brightness comparison of control and injected animals for fetal liver (**i**) and fetal spleen (**j**) ($n = 3$ for mice, $n = 1$ for each NHP, 3 images for each tissue analyzed). Brightness was compared by Student's t test (ns > 0.05, *<0.05, **<0.01, ***<0.001, ****<0.00001).

h). In mice and NHP, there are small amounts of particles in the developing villus of the intestine; in NHP at low dose, there are no appreciable particles, but the uptake increases with increased dosage (online suppl. Fig. 1A, G). For the size and composition of NPs tested, there is minimal NP uptake in fetal brain, skin, or skeletal muscle in both mice and NHP (online suppl. Fig. 1 B–D, H–J).

Differences in Systemic Fetal NP Biodistribution between Mouse and NHP

One of the most striking findings in this NHP study is the uptake of NPs into the fetal long bone zones of ossification. This is not appreciable in the fetal mouse long bones (Fig. 3c, d, i). At the highest dose, NPs can be appreciated at high levels in the fetal pancreas (online suppl. Fig. 1K); we have not tested this dose in mice. At the more moderate doses, there are few particles in fetal pancreas (online suppl. Fig. 1F, K).

In previous studies and these experiments, we have seen robust NP uptake in fetal mouse lung after systemic administration. In the NHP, there are NPs appreciable in the fetal lung, but even at high doses there is less uptake in the fetal NHP lung than in the E15 mouse (Fig. 3e, j). Finally, despite intracardiac injection for two of the NHP animals, there is minimal particle accumulation in the cardiac muscle in contrast to our experience in the E15 mouse (online suppl. Fig. 1F, L).
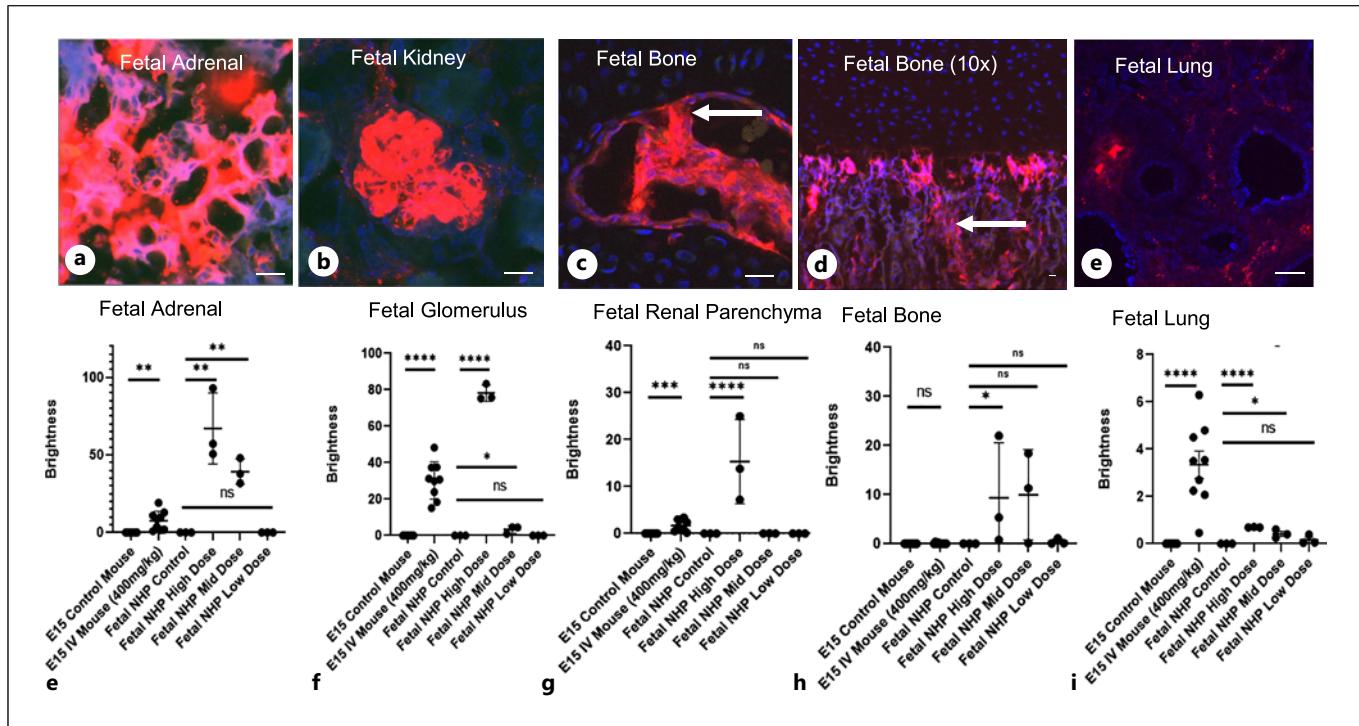
Fig. 3. Confocal microscopy images of fetal NHP tissues with moderate uptake of red fluorescent NPs (mid dose, 400 mg/kg) following IV injection. **a** Fetal adrenal gland (40×). **b** Fetal kidney. **c, d** Fetal bone with NPs in zones of proliferation (white arrow) (40×) and ossification (white arrow) (10×). **e** Fetal lung (blue = DAPI nuclear stain; red = DiI lipophilic dye; white bar = 20 μm). Brightness comparison of control and injected animals for fetal adrenal gland (**f**). **g** Fetal kidney glomerulus. **h** Fetal renal parenchyma. **i** Fetal bone. **j** Fetal lung ($n = 3$ for mice, $n = 1$ for each NHP, 3 images for each tissue analyzed). Brightness was compared by Student's t test (ns > 0.05, *<0.05, **<0.01, ***<0.001, ****<0.00001).

Distribution to Gonads and Maternal Tissue

The NHP that received the highest NP dose was female. Scant particles are visible in the parenchyma of the ovary, but none are appreciated in the ovum (Fig. 4a, e). The fetal NHPs that received the mid and low dose were male. In the male who received 400 mg/kg of NPs, particles are appreciable in the interstitium of the testicle, but none are identified in the seminiferous tubule; in the NHP that received 170 mg/kg of NPs, no particles were appreciated in the testicle (Fig. 4b, f). The livers of all three injected mothers were analyzed and no particles were appreciated in the maternal livers (Fig. 4c, g). Finally at the highest dose, scant particles could be appreciated in the placenta, but at 400 mg/kg of NPs and under, no NPs could be appreciated in placental tissue (Fig. 4d, h).

Discussion

Particle-based fetal therapy has the potential to treat a variety of genetic and structural diseases as these particles can be loaded with gene editing reagents, growth factors, or small molecule drugs. To date, preclinical, proof-of-concept studies have been completed in rodent models of disease [26–29, 31, 32]. For clinical translation, validation in larger animal models will be needed and NHP is the closest to human.

There were several clear findings in this study. The first finding was technical that for mid-gestation NHP, intracardiac injection is less technically challenging and more reproducible than injection into the small ~1-mm umbilical vein (Fig. 1). Prior work describing cell and viral vector delivery has frequently used an intraperitoneal route [33–35] and has described vasospasm of the umbilical vein and fetal bradycardia [36]. We did not observe fetal bradycardia or cardiovascular hypodynamics during the course of our injections for the umbilical vein or intracardiac route. The volume of injection is another important safety consideration in terms of fetal intravascular volume status and hemodynamics. In the mouse, an IV injection of 15 mL in a 450 mg fetus represents approximately 40% of the circulating volume (this is likely an overestimate as it

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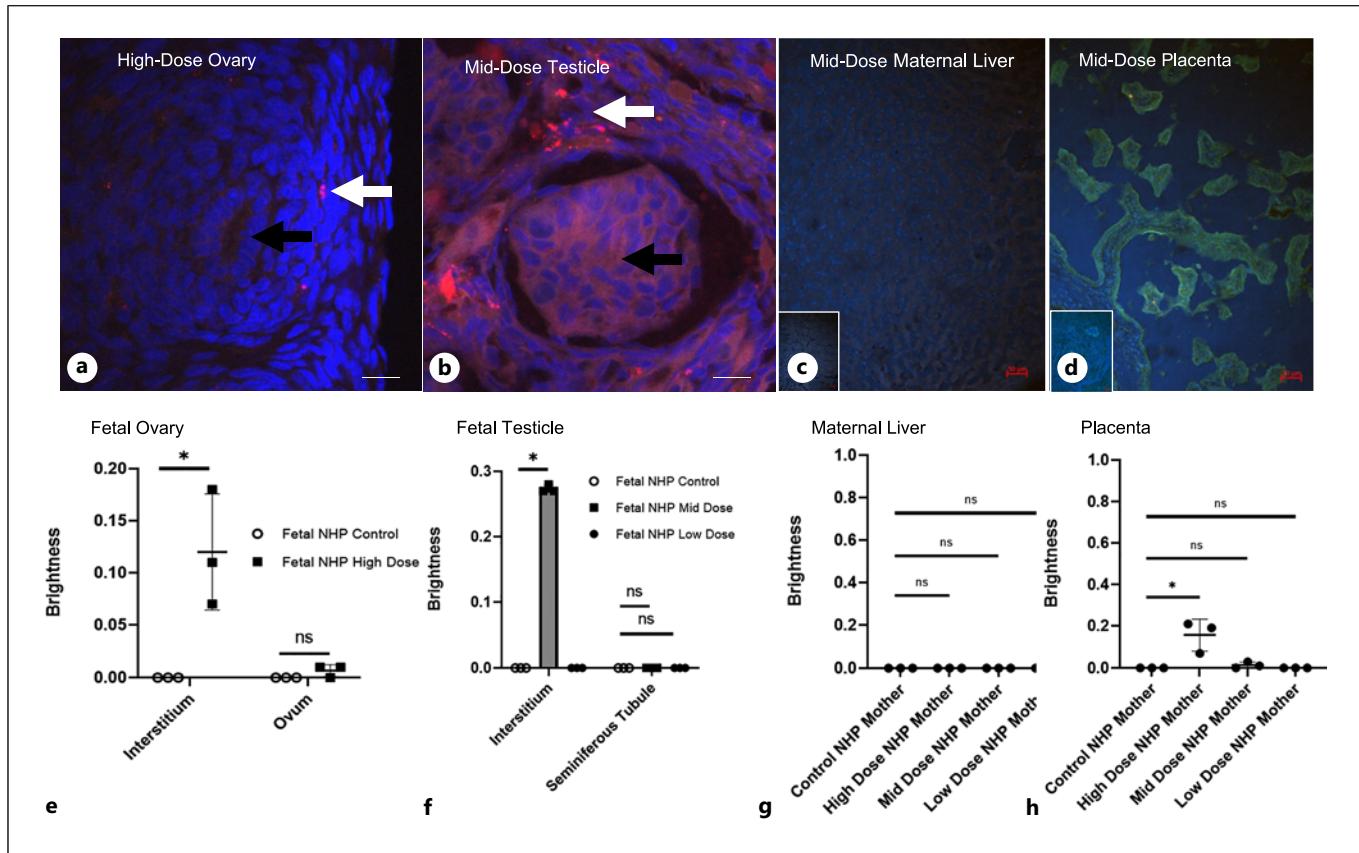
Fig. 4. Confocal microscopy images of fetal NHP reproductive tissues, maternal liver, and placenta following fetal IV injection of red fluorescent particles. **a** Fetal ovary (700 mg/kg) (white arrow = interstitium; black arrow = ovum) (40×). **b** Fetal testicle (mid dose, 400 mg/kg) (white arrow = interstitium; black arrow = seminiferous tubule) (40×). **c** Maternal liver (400 mg/kg) (10×) (uninjected control in lower corner). **d** Placenta (mid dose, 400 mg/kg) (10×) (uninjected control in lower corner) (blue = DAPI nuclear stain; red = DiI lipophilic dye; white bar = 20 μm; red bar = 50 μm). Brightness comparison of control and injected animals for fetal ovary (**e**). **f** Fetal testicle. **g** Maternal liver. **h** Placenta ($n = 1$ for each NHP, 3 images for each tissue analyzed). Brightness was compared by Student's t test (ns > 0.05, *<0.05).

does not take circulating placental volume into account). We have seen excellent survival to full term in mice after this volume of NPs is administered in isotonic fluid. In future studies, we would use fetal measurements to more accurately judge the dose and volume of delivery.

Due to biological variation and slight differences in gestational age, the animals in the study varied in weight (40–170 g) and therefore our dose range was variable (170–750 mg/kg). Overall, we see that dose is an important variable that affects the tissue accumulation of NPs, which we have seen in studies in rodents [37]. Our findings in NHP are more likely related to the broad range of doses rather than the differences in gestational age. As there are significant differences during gestation (relative number and migration of stem cells population and

permissiveness of vasculature), we are in the process of studying the impact of gestational age on gene editing in the mouse model. This study also demonstrated that below a certain dose, minimal particles make it beyond filtration by the liver and spleen [38]. This has safety and effectiveness implications as with low doses, very few particles make it to other organs (Fig. 3, 4; online suppl. Fig. 1). This has been an established observation in postnatal animals and there are described strategies to use nontherapeutic "priming" of particles to saturate the Kupfer cells of the liver followed by delivery of therapeutic particles [39].

This study demonstrated that PLGA particle uptake was most robust in fetal liver and fetal spleen (Fig. 2). Delivery of particles to fetal liver may be a means to treat inborn errors of metabolism where gene replacement or

editing could correct diseases of synthetic liver function. The fetal hematopoietic stem cell resides in the fetal liver in mid-gestation [40]. The editing reagents that we have used in fetal mice have been validated in human cell lines, so we presume they will be effective in NHP cells [41]. As a next step, we need to optimize editing reagent for NHP tissue. Once optimized, given the tremendous uptake in fetal NHP liver, we are poised to perform experiments testing editing reagents in fetal NHP.

These experiments demonstrated that there are differences between distribution in mice and NHP (Fig. 3). Compared to fetal mouse, fetal NHP demonstrated higher uptake in adrenal gland and the zones of proliferation and ossification in the bone. The biologic basis for these differences is unknown and will be a focus of future studies testing NPs carrying editing reagents. This robust delivery may open potential therapeutic strategies for diseases such as congenital adrenal hyperplasia and osteogenesis imperfecta, respectively.

The delivery of NPs to fetal NHP lung was appreciable but less robust than our experience in the mouse. Our group has previously demonstrated that particle-based delivery of epigenetic therapy can alter lung development in a preclinical rat model of lung hypoplasia in the setting of congenital diaphragmatic hernia [25, 31]. For these studies, we had already demonstrated that particles with different chemistries have higher delivery to lung than the PLGA particles used in this NHP study. This would need to be established in a larger animal before clinical translation was considered. Similarly, systemic particle delivery to lung, bowel, and pancreas (targets for cystic fibrosis) was less impressive in NHP than mice (Fig. 3; online suppl. Fig. 1). Other particle modifications (size, charge, surface chemistry) need to be tested with this application in mind. Uptake in fetal NHP pancreas was notable at the highest dose delivered, but the long-term tolerance of such a dose has not been established. Delivery of therapeutic cargo to fetal brain has the potential to correct congenital disease of the central nervous system. Delivery to muscle could have implications for muscular dystrophy. Delivery to skin could have implications for congenital skin diseases such as epidermolysis bullosa. Unfortunately, delivery of PLGA NPs that are >200 nm to fetal brain, muscle, and skin in the mouse and NHP remain disappointing (online suppl. Fig. 1).

Finally, establishment and optimization of safety are of the utmost importance for delivery of fetal therapeutics. We noted the high degree of uptake of these particles in fetal kidney at the higher doses delivered. In mice, we see long-term postnatal survival and normal growth following systemic fetal NP delivery. Following particle-based fetal intervention in larger animals, kidney function should be monitored in long-term studies. If systemically delivered particles are used to deliver editing reagents, the gonadal tissue needs to be examined; germline correction, off-target edits, or disruption of developmentally vital genes would be unfavorable consequences of this line of therapy. In this study, the female fetal NHP was the animal that received the highest dose. In that ovary, particles can be appreciated at low levels in the parenchyma, but none can be appreciated in the ovum (Fig. 4). Similarly, in the testes of the two male fetal NHPs, particles could be appreciated in the interstitium, but not in the seminiferous tubules (Fig. 4), where sperm is made. In our experience using particle-based gene editing in fetal mice, we did not see editing of the germline, but this must be tested in subsequent experiments if this strategy is tested in larger animals. Finally, systemic fetal administration of PLGA NPs results in negligible uptake in maternal liver and placenta (Fig. 4), which is similar to our experience in mice [29]. It should be noted that the maternal dose in humans would only be a fraction of the dose to the fetus (500 g fetus/60,000 g mother), so significant placental crossing and distribution in maternal tissue remains unexpected. For any future NHP study where editing reagents are administered, on-target and off-target editing of maternal tissues and fetal germline tissues should be measured to quantify risk in the setting of clinical translation.

Despite the limited number of NHP used for this study, our group gained both technical insight and insight into the behavior of these particles in a larger animal system. A shorter mating window and pre-procedural ultrasound to better estimate fetal mass may help standardize the dose. We are now poised to test these agents in a longer-term study for efficacy and safety of our proposed systemic fetal gene editing strategy.

Statement of Ethics

All work was approved by the Yale Institutional Animal Care and Use Committee (2023-11632) and conducted in compliance with test facility standard operating procedures.

Conflict of Interest Statement

Funding Sources

Author Contributions

D.A.E., N.K.Y., W.M.S., and D.H.S. designed the work; D.A.E., A.Y.L., J.M.S., M.E.G., R.R., M.O., M.O.B., and D.H.S. performed research; D.A.E., A.Y.L., E.I.D., W.M.S., P.M.G., and D.H.S. analyzed and interpreted data; D.A.E. and D.H.S. wrote the paper; and all authors reviewed, revised, and approved the final paper.

Data Availability Statement

The data that support the findings of this study are openly available in figshare at 10.6084/m9.figshare.26035780.

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