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Gene Targeting to the Uteroplacental Circulation of Pregnant Guinea Pigs

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Abstract

Our study aimed to target adenoviral gene therapy to the uteroplacental circulation of pregnant guinea pigs in order to develop a novel therapy for fetal growth restriction. Four methods of delivery of an adenovirus encoding β-galactosidase (Ad.LacZ) were evaluated: intravascular injection using phosphate-buffered saline (PBS) into (1) uterine artery (UtA) or (2) internal iliac artery or external administration in (3) PBS or (4) pluronic F-127 gel (Sigma Aldrich). Postmortem examination was performed 4 to 7 days after gene transfer. Tissue transduction was assessed by X-gal histochemistry and enzyme-linked immunosorbent assay. External vascular application of the adenovirus vector in combination with pluronic gel had 91.7% success rate in terms of administration (85% maternal survival) and gave the best results for maternal/fetal survival and local transduction efficiency without any spread to maternal or fetal tissues. This study suggests an optimal method of gene delivery to the UtAs of a small rodent for preclinical studies.

Keywords

prenatal gene therapy, uteroplacental circulation, guinea pig, adenovirus vector, pluronic gel, VEGF-A_{165}

Introduction

Fetal growth restriction (FGR) is an obstetric complication in which the fetus does not achieve its genetically determined growth potential. Pregnancies complicated by FGR have an increased rate of perinatal complications and mortality, and in the long term, there is an increased risk of cardiovascular and neurological disorders.[1-6] It has been established that reduced uteroplacental perfusion can cause FGR, and to the best of our knowledge, treatments or management strategies remain elusive.

We have shown previously that in pregnant sheep, there was a significant increase in uterine blood flow until 1 month after injection of adenovirus vectors containing the vascular endothelial growth factor (VEGF)-A_{165} gene when compared with uterine arteries (UtAs) injected with a control adenovirus containing the β-galactosidase gene.[7,8] Moreover, we have demonstrated a reduction in UtA contractility and adventitial neovascularization 30 to 45 days after local administration of Ad.VEGF-A_{165} to the UtAs of pregnant sheep.[7,8] There was no undesirable vector expression in other maternal/fetal tissues as determined by semi-nested reverse transcription polymerase chain reaction. Recent data from sheep pregnancies affected by FGR demonstrate that mid-gestation maternal UtA injection of Ad.VEGF-A_{165} vector led to fewer instances of marked FGR, defined as fetal weight more than 2 standard deviations from the contemporaneous nongrowth restricted control mean, at term.[9] There was also a fall in ratio of the head (biparietal diameter [BPD]) to abdominal size (abdominal circumference) over gestation, which was significantly lower at term than control untreated FGR fetuses and no different from nongrowth restricted control fetuses. There was no significant difference in the BPD measurements, suggesting that the ''brain sparing'' that is seen in this paradigm of FGR had been ameliorated.[9]

The guinea pig is an appropriate model for human FGR and normal pregnancy as compared with other rodents, since their

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placentation is more similar to that of human, having a homologous process of trophoblast invasion[10] and trophoblast cell proliferation.[11] In addition, they have a hemochorial type of placenta, as in humans.[12] Interventions such as periconceptual maternal nutrient restriction lead to asymmetric growth restriction with similar long-term consequences to human FGR.[13] The long gestational age of 65 to 68 days in the guinea pig allows time for therapy to be administered in mid- or late gestation and for an effect on fetal growth to be realized.

Therapeutic adenoviral vector delivery to adult guinea pigs has been described via the following injection routes: intradermal,[14] subcutaneous,[15] intramuscular,[16] intra-articular,[17] heart (left ventricle),[18] and intracochlear.[19] Senoo et al detected the adenovirus vector in several fetal organs, after injecting vectors into the umbilical vein of late gestation guinea pig fetuses at laparotomy[20]; our aim however was to test different methods to achieve localized maternal gene delivery to the UtAs without spread to the fetus as a therapeutic approach to prevent FGR. We propose that maternal gene therapy localized to the uterine vasculature, producing an increase in uteroplacental perfusion, represents a solution to treating FGR, and this is currently being translated into the clinic in the EVERREST program (www.everrest-fp7.eu). Our long-term goal is to first evaluate whether local maternal VEGF gene transfer to the uterine and radial arteries improves fetal growth in an FGR guinea pig model and later in human pregnancies complicated by FGR. For this purpose, the experiments described were conducted with an adenovirus encoding a reporter gene β-galactosidase rather than VEGF, in order to be able to quickly stain the blood vessels and evaluate the extent of transgenic protein overexpression. We tested 2 local uteroplacental intravascular routes of administration and an external vascular transduction method combining vector with either phosphate-buffered saline (PBS) or pluronic gel. Pluronic gel is a thermolabile gel that is liquid when cold but solidifies at body temperature. It has commonly been used for drug and viral delivery to a variety of vascular beds in preclinical and clinical studies and shown to be safe to administer, nonirritant, and cytocompatible.[21-24]

Methods

Time-mated female Dunkin Hartley guinea pigs (Harlan Animal Research Laboratory, United Kingdom) were scanned at 20 to 30 days gestational age (DGA) to confirm pregnancy using a 7 to 10 MHz probe (Siemens, Bracknell, United Kingdom). A midline laparotomy was performed under general anesthesia (diazepam 5 mg/kg, atropine 0.5 mg/kg, ketamine 40 mg/kg; Hameln Pharmaceuticals, Gloucester, United Kingdom) at either ~30 DGA or ~45 DGA maintained by isoflurane inhalation (1.5%-2.0%), and an adenovirus vector encoding for a reporter β-galactosidase gene (Ad.LacZ, 1×10^{10} viral particles per animal) was administered to the uterine and radial arteries by 4 different methods described subsequently.

Intravascular Injection of the Vector Into the UtAs

For direct injection into UtAs, the vessel (200-350 μm diameter) was located as it ran in the posterior surface of the mesometrium lying parallel and lateral to each uterine horn and visualized using a light (KL 1500 LCD, Schott, Mainz, Germany) shone through the mesometrium from behind. A 3-cm section of the UtA within the posterior surface of the mesometrium was gently cleared of surrounding fat and exposed using a small straight vannas scissors (103102; John Weiss and Company, Milton Keynes, United Kingdom). The vessel proximal to the injection site was temporarily occluded using a microvascular clip (610195; Harvard Apparatus, Kent, United Kingdom), and the vector (1×10^{10} viral particles of Ad.LacZ in a volume of 0.5-1.3 mL PBS) was then administered over approximately 1 minute using a custom-made 34 G needle mounted on a short 5-cm catheter attached to a 1-mL syringe. Once the needle was removed, a second microvascular clip was placed over the injection site to compress the injection site for a minimum of 3 minutes after which both occluders were removed and the vessel was carefully observed for bleeding. If necessary to achieve hemostasis, adrenaline (10 μL; Hameln Pharmaceuticals, Gloucester, United Kingdom) was applied to the vessel for vasoconstriction. The injection procedure was then attempted in the UtA on the other horn.

Intravascular Injection of the Vector Into the Internal Iliac Arteries

The internal iliac artery has the UtA as its most distal branch situated approximately 3.5 cm from the origin of the internal iliac artery (Figure 1). For direct injection of the internal iliac artery, the vessel was identified as it runs with a neurovascular bundle along the pelvic side wall beneath the posterior aspect of the peritoneum at the level of the cervix. Careful dissection of the parietal peritoneum over the neurovascular bundle with a small straight vannas scissors revealed the prominent internal iliac vein and the smaller-sized artery (200-250 μm in diameter) behind it, as it coursed transversely from its origin at the common iliac artery towards the cervical parametrium. A 1.5 cm portion of the artery was exposed, and the vessel was temporarily occluded using a microvascular clip, and the vector was administered distal to the occlusion over approximately 1 minute using a 34 G needle as described previously (1×10^{10} viral particles in 1.0-1.5 mL of PBS). Following vector injection and removal of the needle, a second microvascular clip was placed over the injection site for 3 minutes. Both clips were then removed, and the vessel was carefully observed for bleeding. Adrenaline was applied externally to the vessel if necessary, as described previously, to achieve hemostasis. The injection procedure was then attempted on the opposite side of the internal iliac artery.

A successful injection of the uterine or internal iliac arteries was defined as the vector fluid seen moving down the vessel during injection. Failed injections were due to vector being inadvertently injected into the vessel wall, leaking out of the vessel, or coming out of the other side of the vessel, opposite

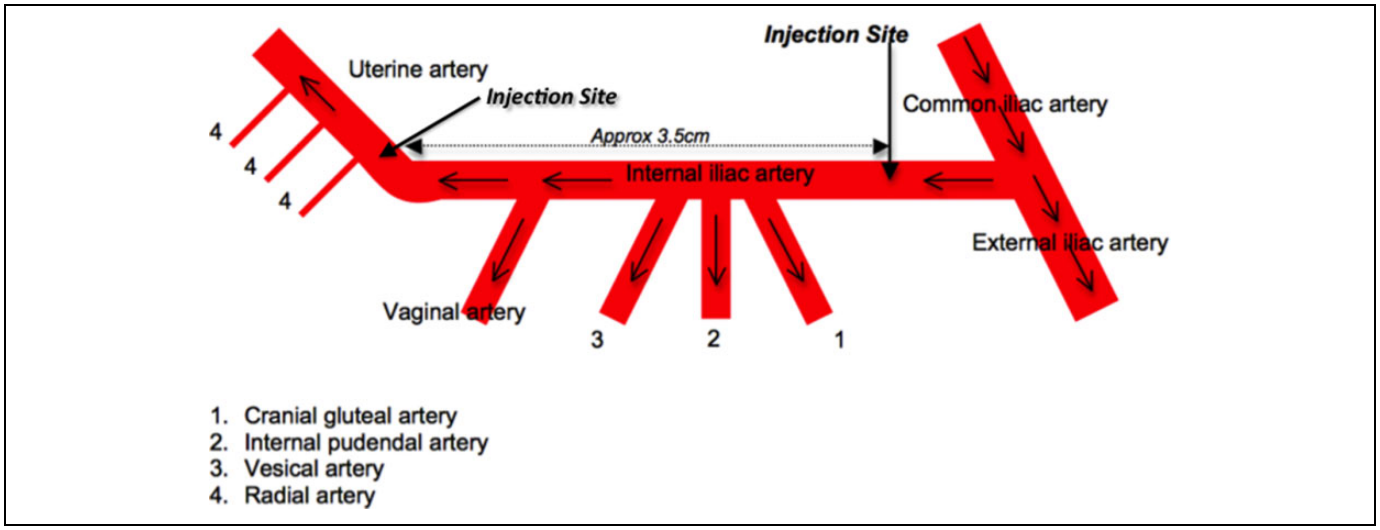

Figure 1. Diagram of the internal iliac artery and its branches in the guinea pig—cranial gluteal (supplying the dorsolateral wall of pelvis), internal pudendal artery (supplying the external genitalia), inferior vesical artery (supplying the lower bladder and also forming the middle rectal artery supplying the rectum), vaginal artery, and uterine artery, which is quite some way along the internal iliac artery. The injection sites on the uterine and internal iliac arteries have been marked.

the injection site. In case of failed injections, 1 to 2 further attempts to inject the artery were made. A dissecting microscope (Discovery V8 Stereomicroscope; Carl Zeiss Ltd, Hertfordshire, United Kingdom) was used to visualize vessels during dissection and intravascular injection.

External Administration of the Vector to the UtAs in PBS

For external vascular administration of the vector in PBS, the vector (1×10^{10} viral particles Ad.LacZ in a total volume of 0.5-1.0 mL PBS bilaterally) was dribbled onto the exposed UtA within the mesometrial fat, and the vessel was left still for 5 minutes before the uterus was subsequently mobilized. Previous experiments in sheep have demonstrated that a 5-minute contact period of the vector with the UtA is sufficient to result in significant transgenic protein expression.[7] During this 5-minute time period, if access to the UtA necessitated the uterus to be lifted out of the abdominal cavity, the uterine horn was kept warm and moist by wrapping it in a sterile gauze soaked with warm saline. No vessel occlusion was used in this technique. For radial artery gene transfer, the uterine horn on one side was moved across to expose the arteries as they ran within the uterine mesometrium. Sometimes, it was not possible to transduce the UtA and radials at the same time because of difficulties in exposing all these vessels simultaneously. In such instances, the UtA was transduced first, and after a 5-minute wait, the radials were exposed and transduced. The procedure was repeated on the contralateral side.

External Administration of the Vector to the UtAs in pluronic Gel

For external vascular administration of the vector in pluronic gel, the vector was combined with a freshly made thermosensitive polymer gel (pluronic F-127) that is liquid when cold but gelatinizes when brought to room temperature. A 25% (wt/vol) solution of pluronic F-127 (P2443; Sigma Aldrich, Missouri) was prepared by dissolving the powder in cold double-distilled deionized water the day before surgery. The solution was placed on a rotating platform overnight at 4°C to ensure the polymer was homogenously mixed. Immediately before administration, the vector (Ad.LacZ, 1×10^{10} viral particles) was reconstituted in 1 mL of pluronic gel and mixed by gently pipetting up and down on ice. After exposing the UtA as it ran within the mesometrium, the vector–pluronic gel combination was dribbled onto the external surface of the UtA using a 1-mL micropipette and left to solidify for 5 minutes. Similarly, the radial arteries were then transduced for a further 5 minutes. No vessel occlusion was used in this technique. The procedure was then repeated on the contralateral side.

After vector administration (by either of the 4 methods described previously), the abdomen was closed in layers. The rectus sheath was closed with continuous 2-0 Vicryl with tapercut needle (Ethicon, Sint-Stevens-Woluwe, Belgium) to prevent herniation of the abdominal contents. The subcutaneous tissue and skin were closed with continuous 2-0 Vicryl with tapercut needle (Ethicon) and continuous 2-0 Vicryl with cutting needle (Ethicon), respectively. A 10% solution of lidocaine hydrochloride (0.5 mL) was administered subcutaneously just before closing the skin to provide local anesthesia.

Because there are only few antibiotics that are well tolerated in guinea pigs and many of those commonly used ones such as penicillin–streptomycin kill their symbiotic gut flora, no antibiotics were administered intraperitoneally prior to closing the incision. Following surgeries and recovery, animals were monitored for signs of miscarriage.

Postmortem Examination

Scheduled postmortem examination was performed 4 to 7 days after the administration of the vector, unless necessitated earlier by miscarriage or maternal distress. Survival data are presented on each route of injection. All procedures on animals were conducted in accordance with United Kingdom Home Office regulations and the Guidance for the Operation of Animals (Scientific Procedures Act 1986).

The fetuses were carefully evaluated for signs of miscarriage. Widespread sampling of maternal and fetal tissues was performed (maternal organs including uterine and radial arteries, uterus, cervix, placentae, bladder, pelvic muscles, as well as maternal and fetal liver, heart, kidney, lung, adrenal, spleen, and gonad). A detailed postmortem examination and microbiological culture of tissues was performed on tissues of animals that died after surgery to determine the cause of death.

Transgenic Protein Expression

For X-gal histochemistry, tissue samples were placed in 100% ethanol at postmortem examination and fixed for at least 2 hours before washing with PBS. Samples were then incubated in X-gal staining solution overnight in a rotary shaker protected from light, as described earlier.[7] Stained samples were then washed in PBS and photographed with a camera attached to a microscope (Discovery V8 Stereomicroscope; Carl Zeiss Ltd and Canon Powershot A620 digital camera, Tokyo, Japan).

Enzyme-Linked Immunosorbent Assay

β-Galactosidase levels in the UtAs, radial arteries, placentae, uterus, maternal and fetal liver, heart, kidney, brain, spleen, and gonads were determined by enzyme-linked immunosorbent assay (ELISA) using a commercially available assay kit (Boehringer Mannheim, Mannheim, Germany) as described previously.[7]

Statistics

Results are presented as means ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism version 5 using one-way analysis of variance (ANOVA [Kruskal-Wallis test]) to compare β-galactosidase levels observed using the 4 different routes of administration.

Results

The success rate of the 4 different methods of administration of adenoviral vectors to the uterine and radial arteries of pregnant guinea pigs is shown in Table 1. Maternal survival was described in terms of survival to scheduled postmortem examination 4 to 7 days after surgery.

Uterine Artery Injections

Injection of the UtAs of pregnant guinea pigs at 30 DGA was hampered by technical difficulties due to the small size of the vessels at mid-gestation (150-250 μm), preventing reliable and safe injection (n = 2 of 8; 25% success rate, 50% survival). Deferring injection until ¾ through gestation (45 DGA) was more feasible with successful injection seen in 44% of attempts (7 of 16 UtAs, n = 8 dams), even though the vessels were still small (300-400 μm diameter). The low success rate is attributed to difficulties dissecting out and injecting the UtA, which is deeply embedded in fat and is unsupported by the surrounding tissue. In addition, it is likely that even in those apparently successfully injected vessels, much of the vector leaked out of the vessel and behaved similar to the external administration of vector with PBS leading to poor local gene transfer. There were 2 maternal deaths, one during surgery that was ascribed to the long anesthetic time (>4 hours) and one within 24 hours of the surgery that was due to sepsis. There was no fetal loss in mothers who survived the procedure.

Uterine artery injection (500 μL vector) resulted in gene transfer to the cervical end of the UtAs and the cervical end of the radial arteries. Increasing the injected vector volume from 0.5 mL to 1.0 mL resulted in gene transfer also to the radial arteries supplying the middle fetus (of 3 on one side). However, even the maximum volume that was injected (1.3 mL) was not successful in transducing the ovarian end of the UtA and radial arteries supplying the fetus at the ovarian end of the uterine horns. The maternal liver and uterus were marginally stained. No staining of the fetuses or placentae was seen.

Table 1. Surgical Parameters Using the Different Gene Delivery Techniques.[a]

Method of Ad.LacZ Vector Administration	Maternal Survival	Time Taken to Inject, hours	Total Number of Injection Attempts Required (Average)
Uterine artery injection	6/8 (75%)	2-2.5	4
Internal iliac injection	4/6 (66%)	2-2.5	5
External administration in PBS	5/6 (83.3%)	<1	1
External administration in pluronic gel	5/6 (83.3%)	<1	1

Abbreviation: PBS, phosphate-buffered saline.
[a]Ad.LacZ was administered to the uterine and radial arteries of pregnant guinea pigs at 40 to 45 days of gestation by one of the methods described in the table. The outcome of the surgery (in terms of maternal survival to scheduled postmortem examination 4-7 days after surgery) and the number of injection attempts required are summarized.

Injection of Internal Iliac Arteries

Internal iliac artery injection was successful in all 12 vessels (n = 6 dams, 45-47 DGA), although multiple attempts were required in each of the 12 vessels. Maternal survival was 66% (4 of 6) and fetal survival was 100% in those dams that survived. The deaths occurred only in the dams that received

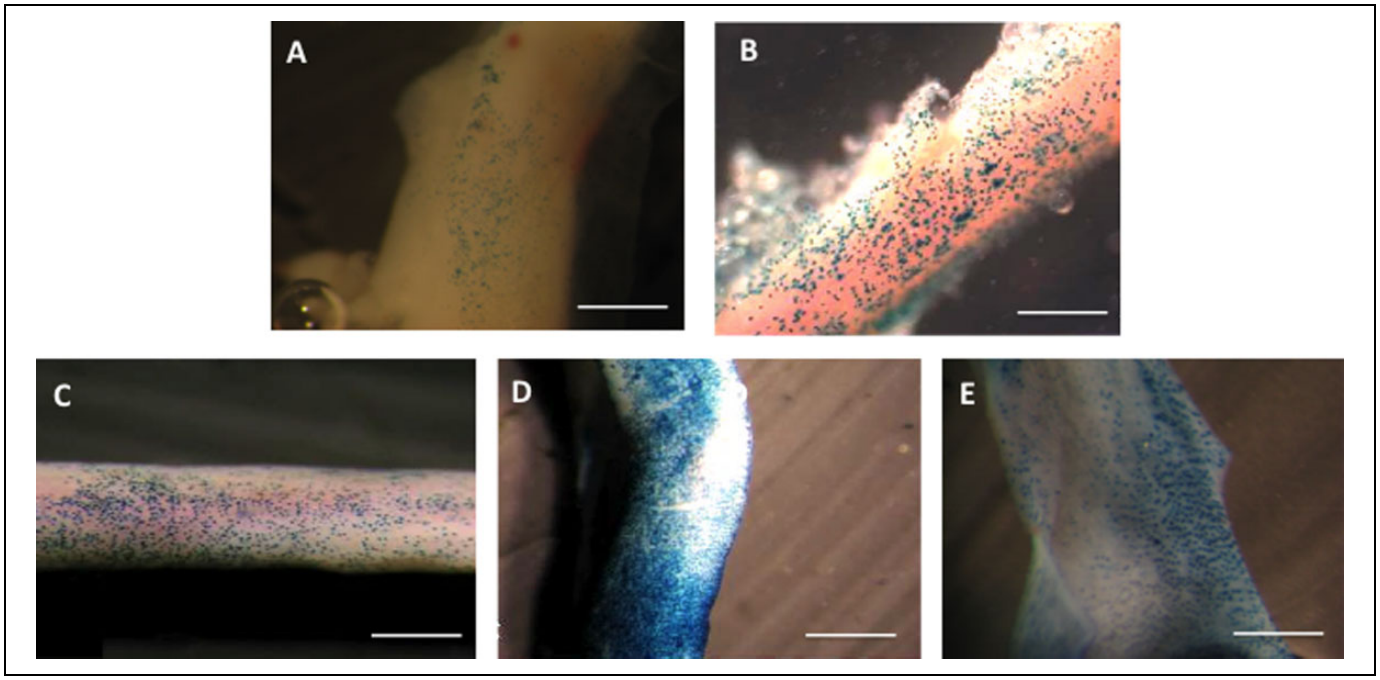

Figure 2. X-gal staining of guinea pig uterine and radial arteries 4 to 7 days after Ad.LacZ administration by one of the following different routes: (A) direct injection into the uterine artery; (B) external administration in phosphate-buffered saline (PBS); (C) external administration in pluronic gel—uterine artery; (D) external administration in pluronic gel—radial artery; (E) external administration in pluronic gel—uterine artery (luminal/inside view). Even though direct injection into the uterine artery led to some expression of β-galactosidase, it was less than that achieved via external administration of the vector. Maximum local tissue transduction was achieved when Ad.LacZ was administered using pluronic gel as a vehicle. Scale bar = 150 μm.

1.5 mL vector, which was probably related to bleeding following the multiple (5-6) attempts that were needed on each side to inject the full volume of vector, rather than vector toxicity.

Administration of Ad.LacZ into the internal iliac artery resulted in positive X-gal staining in the internal iliac artery, maternal liver, pelvic muscles, bladder, and cervix. There was no expression in the uterine/radial arteries, the fat around the UtAs, the placentae, or fetuses. Increasing the volume of vector injected did not lead to positive expression in the UtA.

External Transduction of Uterine and Radial Arteries With Vector in PBS

External transduction of the exposed UtA by the vector in PBS was technically easy to perform and less traumatic compared with the intravascular injection methods with a good survival rate (5 of the 6 dams survived and no fetal losses in surviving dams). The only maternal death happened during a long 4-hour surgery where exposure of the UtA was technically difficult and led to bleeding.

External administration of the vector to the uterine and radial arteries resulted not only in positive expression in all the vessels on which the vector had been applied but also in the fat surrounding the UtAs. All radial arteries stained positively, as well as the maternal liver and uterus. Although there was slight staining in the placentae, there was none detected in the fetuses.

External Administration of Vector–pluronic Gel Combination

Administration of the vector in combination with thermolabile pluronic gel was technically straightforward, shortening the duration of the surgery by more than half (from 3-4 hours to only 1 hour) with successful gene transfer in 91.7% of the cases (n = 11 of 12 vessels, n = 6 dams). Fetal survival was 100% in the dams that survived successfully. In one case, it was not possible to administer the vector to the right UtA because the vessel was very small and consequently difficult to visualize within the fat pad. This dam miscarried the next morning, probably due to excessive manipulation of the uterine horn, which had to be exposed for long periods of time during surgery. There was no evidence of peritoneal inflammation, bleeding, or infection at postmortem examination.

Administration of Ad.LacZ using pluronic gel as a vehicle led to very high levels of local transgenic protein expression in the uterine and radial arteries to which the vector had been applied, not only on the external surface but on the inner luminal surface as well. The liver and uterus had minimal β-galactosidase expression in 1 animal and did not stain positively in any of the other animals, when incubated in the X-gal staining solution overnight. All other maternal and fetal tissues sampled (placentae, heart, kidney, lung, gonad, bladder, brain, and adrenal) did not show any positive staining with the X-gal staining solution. Representative pictures of X-gal staining in the UtAs, after the different methods of administration, are shown in Figure 2.

Table 2. Efficiency of Gene Transfer After Administration of the Vector Using Different Routes.[a]

Method of Administration	Efficiency of Gene Transfer					
	Uterine Artery	Radial Artery	Maternal Liver	Maternal Lung	Uterus	Placenta
Uterine artery injection	++	−	+++	−	+	−
Internal iliac injection	−	−	+++	−	−	−
External administration in PBS	++	+++	+++	−	++	+
External administration in pluronic gel	++++	++++	−	−	−	−

Abbreviation: PBS, phosphate-buffered saline.
[a]Tissue samples were collected at postmortem examination and stained overnight in X-gal staining solution. Photomicrographs of the stained samples were carefully examined by a single observer who was blinded to the route of administration. The degree of blue staining, if any, was compared among all stained images and denoted as '+' indicating least staining and '++++' denoting maximum staining.

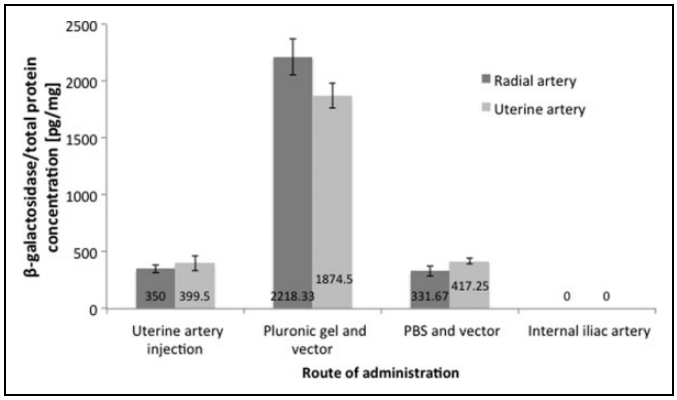

Figure 3. Quantification of protein levels of β-galactosidase by enzyme-linked immunosorbent assay (ELISA) in the uterine and radial arteries following the different routes of administration.

Measurement of β-Galactosidase Levels by ELISA

Quantitative analysis of β-galactosidase expression in tissues using ELISA found that the most effective route of administration was the combination of pluronic gel and vector, yielding the highest mean levels in samples collected from UtA ($P = .0048$, n = 4 vessels for each route of administration, one-way ANOVA). Administration of Ad.LacZ via UtA injection yielded similar results to external route of administration using PBS and Ad.LacZ vector combination. After internal iliac artery injection, no β-galactosidase expression was detected in uterine or radial arteries (Table 2 and Figure 3).

β-Galactosidase expression was also quantified in the fetal tissues (kidney, gonad, muscle, bone marrow, thymus, and amniotic fluid). The highest β-galactosidase concentration was observed in amniotic fluid (45 pg/mg) after administering vector externally to the UtA and radial arteries in PBS. The second highest value of β-galactosidase was observed in bone marrow (14 pg/mg) after UtA injection with the vector. Placental β-galactosidase expression was detected only in the animals wherein external transduction of Ad.LacZ was performed using PBS as a vehicle and was quantified to be 57 pg/mg. None of the other tissues showed positive β-galactosidase expression by any of the routes of administration.

Discussion

In this study, we have demonstrated that the most effective method of gene transfer to the pregnant guinea pig uterine and radial arteries is external administration of the vector in combination with pluronic gel as a vehicle. Moreover, application of the vector by this technique is technically straightforward and atraumatic as an intravascular injection is not required. The delivery method achieved localized gene transfer restricted only to the uterine and radial arteries, with no spread of vector to other maternal and fetal tissues. Since pluronic gel becomes solid immediately after administration, due to its characteristic property of reversible thermogelation, there is minimal spread of the vector to other organs. In particular, with relevance to maternal uteroplacental gene transfer, external vector administration using pluronic gel was safe for the developing fetus as there was no vector spread to fetal tissues. In addition, external application of adenovirus either in pluronic gel or PBS achieved 84% to 100% fetal survival, in contrast to direct intraplacental adenoviral administration that only achieved fetal survival rates of 30% to 42%.[25]

Our results indicate that using a localized pluronic gel-based depot of slow viral vector release facilitates greater transgene expression in comparison to other techniques, when direct vascular injection may be technically challenging. Pluronic gel has been used to deliver Ad.LacZ to the carotid artery of rats. Five days postinfection, the presence of the β-gal transgene was visualized throughout the arterial wall.[21] The combination of vector with pluronic gel has been used as a vehicle for virus-mediated gene delivery for a number of indications but not previously to the vessels of the uteroplacental circulation. Poloxamer gels display low toxicity at therapeutically beneficial doses and do not increase serum triglycerides and cholesterol in animal models.[26] Clinical uses of pluronic F-127 have included the controlled delivery of drugs to the eye,[27] nasal passage, as well as parenteral and subcutaneous administration.[28] pluronic gels had been used previously as a vehicle for local adenoviral gene delivery in preclinical and clinical studies[21,23,29] and shown to be safe to administer. Depending on the concentration of the gel, it may last from a few hours to a few days, undergoing slow dissolution in the body fluid. Pluronics have been shown to be nonirritant, noninflammatory, and

cytocompatible with many different cell types.[30] They are primarily cleared from the body via the renal route in the form of unimers.[31] They increase the transduction efficiency of adenoviral vector delivery to vascular cells in vivo compared to PBS,[24] probably because of the slower kinetics associated with the longer term vector release from the gel or the closer proximity of viral vector to the cell surface. Because of the thermoresponsive behavior of pluronic F-127, the gel is delivered in vivo over a short period of time before solidification.

In our study, a higher concentration of β-galactosidase expression was observed in the radial arteries compared with the UtAs. This might be due to the finding that the UtAs are deeply embedded in fat, whereas the radial arteries are only covered by a thin mesentery, allowing more effective diffusion of the viral vector through the vessel wall. External administration of Ad.LacZ vector combined with PBS led to low transgenic protein expression in the vessels, probably due to the low viscosity of PBS solution, which allowed it to spread from the target vessel to the surrounding tissues. Although the uterine and radial arteries were left still for 5 minutes after application of the PBS Ad.LacZ vector combination, the fluid was seen to dribble down into the pelvic cavity. Our previous work in sheep demonstrates that contact of the vector with the UtA for 5 minutes is sufficient to result in significant transgenic protein expression.[7]

Intra-arterial administration of vector into the UtAs of pregnant guinea pigs only led to partial transduction of the uteroplacental circulation that was limited to those vessels closest to the injection site. Increasing the volume of vector injected did not result in transduction of the furthest zones. Similarly, in an angiographic study to examine the vascular anatomy of guinea pig UtAs, contrast medium injected into the UtA in vivo failed to enter the most distal radial arteries and placentae,[32] probably because they are preferentially served by the ovarian arteries. Vector injection into the ovarian arteries was considered, but because they were of even smaller diameter than the UtAs, it was not attempted. Delivery into the UtA using interventional radiology was considered, since guinea pig UtAs have a sufficiently wide caliber at mid-gestation to be easily injectable by angiographic-guided catheter.[25] We were concerned however that trauma to the vessel internally would result in a high rate of miscarriage.

Although injection of the internal iliac artery was feasible, gene transfer to the UtAs was not achieved, and instead, there was gene transfer observed preferentially to the pelvis of the guinea pig. This was not unexpected since the arteries supplying the pelvic musculature and organs are the first 3 branches of the internal iliac artery, which suggests that the vector has been redirected to those vessels by hemodynamic forces. Moreover, the technique was associated with significant fetal and maternal loss, most probably because of the long anesthetic time required to complete the procedure.

We observed that intravascular administration of Ad.LacZ as well as external administration in PBS resulted in strong liver transduction. Previous studies of adenovirus gene transfer in the guinea pig found a strong liver tropism, most likely due to the high density of coxsackievirus and adenovirus receptor on hepatocytes.[33-36] We did not observe this liver tropism in dams that received Ad.LacZ in pluronic gel, most probably because the gel confined the viral particles to the site of administration with minimal spread to other tissues and organs.

The efficacy of maternal uteroplacental VEGF gene therapy to treat FGR has been tested in growth-restricted sheep where the large vessel size permits safe direct intravascular injection.[9] It was important for translation into the clinic to demonstrate efficacy for improved fetal growth in a second animal model of FGR, and we have chosen to use the guinea pig for reasons described previously. Safe and high-level local gene transfer was not achieved using direct intravascular injection due to the technical difficulties encountered but was demonstrated using external administration with pluronic gel. Current studies are investigating the efficacy of this treatment in the maternal nutrient-restricted guinea pig that has asymmetrical FGR and will include examination of the mechanism of action of the intervention. For human trials, we propose using an interventional radiology technique to deliver vector into the UtA, which is currently applied in the clinic for embolization of uterine fibroids[37] or prevention of postpartum hemorrhage.[38]

Direct adenovirus vector injection into the UtAs of pregnant sheep is safe and leads to high local transgenic protein expression with minimal maternal and fetal mortality or morbidity.[7-9,39] Injection of the UtA in the rabbit can be achieved using catheter-mediated injection under angiographic control.[40] This leads to high transduction efficiency in the UtA and placental trophoblastic cells. The much smaller diameter of the guinea pig UtA compared to the sheep UtA makes its injections more challenging and more prone to complications.

In summary, we have shown in the pregnant guinea pig that administration of recombinant adenoviruses using thermolabile pluronic gel as a vehicle leads to robust transgenic protein expression in the uteroplacental vessels, both on the adventitial and luminal surfaces. There is no vector expression in other maternal and fetal tissues. Surgery time was shorter when compared to alternative intravascular injection methods, thereby lowering the risk of morbidity and mortality. Our findings suggest that vector administration using pluronic F-127 as a vehicle is the optimum method of gene targeting to the uteroplacental blood vessels of pregnant guinea pigs.

Declaration of Conflicting Interests

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