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Fetal uptake of intra-amniotically delivered dendrimers in a mouse model of intrauterine inflammation and preterm birth

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Abstract

Intrauterine inflammation is associated with preterm birth and can lead to fetal neuroinflammation and neurobehavioral disorders in newborns. Dendrimers can intrinsically target and deliver drugs for the treatment of neuroinflammation. We explore whether hydroxyl polyamidoamine (PAMAM) dendrimer (G4-OH)-based nanomedicines can be delivered to the fetus by intra-amniotic administration, in a mouse model of intrauterine inflammation. The time-dependent accumulation of G4-OH-fluorophore conjugate was quantified by fluorescence. These studies suggest that, after intra-amniotic administration, there is significant accumulation of dendrimer in the fetus gut and brain. In addition, there is some fetal–maternal transport of the dendrimer. Confocal microscopy confirmed the presence of G4-OH in the fetal brain, with a large accumulation in the brain blood vessels and the brain parenchyma, and some microglial uptake. We believe that intra-amniotic administration of G4-OH-drug nanomedicines may enable the treatment of diseases related to intrauterine inflammation and fetal neuroinflammation.

Key words: Intrauterine inflammation; PAMAM dendrimer; Intra-amniotic drug delivery; Biodistribution; Fetal brain

Background

In the United States, approximately 12% of all live births are preterm.[1] Although mechanisms underlying spontaneous preterm birth are not fully understood, intrauterine inflammation is associated with most cases. The presence of intrauterine inflammation has been linked to a devastating spectrum of neurobehavioral disorders in these children, ranging from cognitive and learning disability to motor dysfunction such as cerebral palsy,[2] though not all cases of cerebral palsy are caused by inflammatory injury.

Various models of systemic and local maternal inflammation have been used to simulate preterm birth and perinatal morbidity associated with prematurity and intrauterine inflammation.[3-10] Our team has utilized a mouse model of intrauterine inflammation (produced by localized intrauterine lipopolysaccharide [LPS] infusions) that recapitulates clinical features present in human disease to study mechanisms of fetal brain injury and to understand and develop therapeutic interventions. In this mouse model, we demonstrated that in addition to causing preterm birth, exposure to intrauterine inflammation leads to neuroinflammation and neurotoxicity in the fetal brain, making this model a useful tool for evaluation of therapeutic interventions for the mother and fetus.[5,6,11] Development of successful therapeutic interventions that address both preterm birth and prematurity-related perinatal morbidity has been a challenge. Nanotechnology

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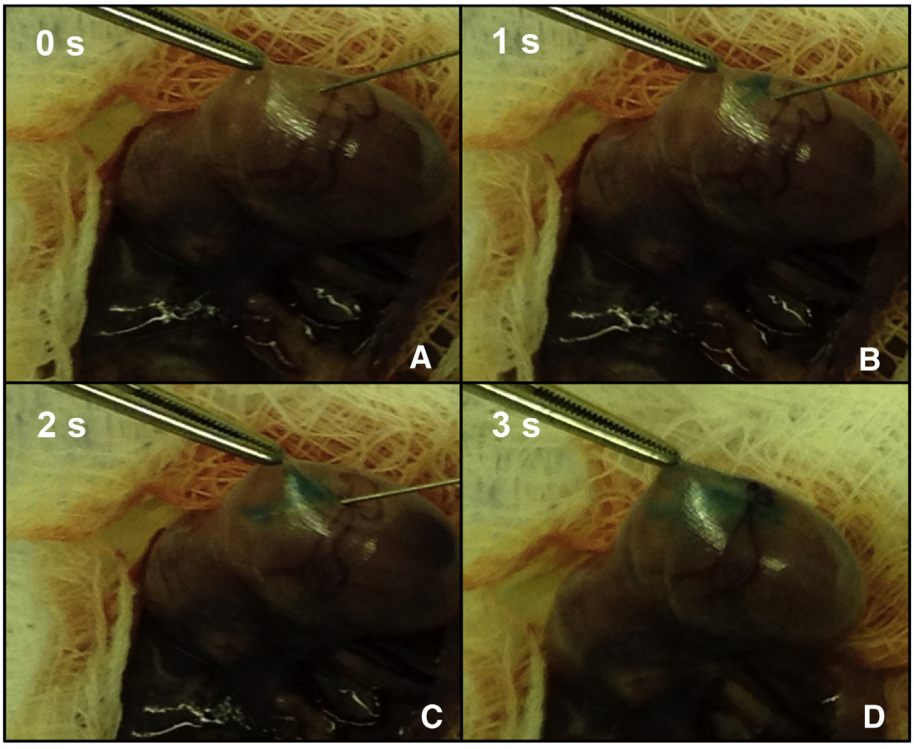

Figure 1. **(A-D)** The process of intra-amniotic injection of dendrimer in one of the fetuses.

may offer opportunities to address this challenge, provided it is safe and efficacious in the perinatal period.

Nanomedicine-based delivery strategies are emerging in the treatment of inflammatory disorders. These approaches can improve drug bioavailability, targeting, and sustained efficacy.[12-15] Specifically, systemic administration of hydroxyl-terminated poly(amidoamine) (PAMAM)(G4-OH) dendrimers was reported to target activated microglia and astrocytes in the brain of newborn rabbits with cerebral palsy. *N*-acetylcysteine (NAC), an anti-inflammatory and antioxidant drug, was conjugated to the dendrimer, and the dendrimer–NAC conjugate was shown to significantly improve motor function and reduce neuronal injury and inflammation in the rabbit model of cerebral palsy.[15] In a guinea pig model of chorioamnionitis, cervical administration of hydroxyl-terminated PAMAM dendrimer, by itself, was shown to inhibit bacterial growth and prevent preterm birth. This finding indicates that hydroxyl-terminated PAMAM dendrimers can be used as a potential topical microbicide for the treatment of ascending uterine infections[16] and may be good candidates for intra-amniotic therapy. Thus, fetal uptake studies may offer valuable insights into their effectiveness as drug delivery agents.

If neonatal attenuation of neuroinflammation with dendrimers can produce significant motor function improvement in cerebral palsy, fetal dendrimer therapies may have a significant impact on preventing cerebral palsy and preterm birth. A lack of studies on drug efficacies and trans-placental transport has limited the use of fetal nanotherapies in the perinatal period. This study seeks to ascertain whether dendrimers, when delivered intra-amniotically, can be taken up by the fetus.

Methods

Animals

We used a mouse model of intrauterine inflammation and preterm birth as described in an established protocol.[6,5,17] Briefly, we anesthetized pregnant CD1 mice (Charles River) with continuous isoflurane in oxygen and performed intrauterine injections of LPS (100 μg/dam in 100 μL phosphate-buffered saline solution; from *Escherichia coli*, 055:B5; (Sigma Chemical Co., St. Louis, MO) or phosphate-buffered saline solution (PBS) on day 17 of gestation (19 days is full-term gestation). A mini-laparotomy was performed in the lower abdomen.

Either LPS (n = 6 dams for 24 h; n = 3 dams for 6 h) or an equal volume of vehicle (normal saline (NS); control) (n = 4 dams for 24 h; n = 3 for 6 h) was infused into the uterus between the 1st and 2nd gestational sacs closest to the cervix. Using a Hamilton syringe, we injected dendrimer-Cy5 conjugates (D-Cy5; 10 mg/kg at a concentration of 2 μg/mL) intra-amniotically into each gestational sac on the right side immediately after LPS or vehicle injection (Figure 1). We chose this dose based on one study of intravenous dendrimer injection in newborn rabbits[15] and after taking into account that intra-amniotic injection is somewhat similar to oral administration in that the fetus swallows the amniotic fluid. The left side of the uterus was not injected and served as an internal control. Surgical incisions were closed, and the dams were recovered in individual cages.

As depicted in Figure 1, the D-Cy5 solution spread quickly into the amniotic fluid within 1 to 3 sec. No leakage of D-Cy5 was seen from the picture during or after injection.