

Prenatal Therapy in Developmental Disorders: Drug Targeting via Intra-Amniotic Injection to Treat X-Linked Hypohidrotic Ectodermal Dysplasia

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TO THE EDITOR

Pathologies associated with genodermatoses and other genetic disorders can irremediably affect fetuses, making early-stage therapies desirable. Prenatal maternal drug administration, however, exposes mothers to potential drug toxicity and is limited by the variability in transplacental drug delivery. Alternative approaches to fetal treatment should entail low-risk drug delivery with reproducible pharmacokinetics. X-linked hypohidrotic ectodermal dysplasia (XLHED), the most common inherited disorder of ectoderm development, is caused by a lack of the signaling molecule ectodysplasin A1 (EDA1), which is essential for ectodermal placode formation and subsequent development of various skin appendages, glands, and teeth (Mikkola, 2009). Patients with XLHED have less hair, fewer or no eccrine sweat, sebaceous and meibomian glands, and malformed or absent teeth. Insufficient thermoregulation can lead to perilous hyperthermic episodes during infancy (Blüschke *et al.*, 2010) and remains an important issue throughout life (Hammersen *et al.*, 2011). Many affected individuals suffer from recurrent airway and eye problems (Dietz *et al.*, 2013). To date, only symptomatic treatment is available for these patients.

Causative therapeutic approaches to such disorders are expected to be most effective if applied already *in utero*, with the additional benefit that immune tolerance of a replacement protein may be induced, facilitating postnatal reapplication (Schneider *et al.*, 2002;

Waddington *et al.*, 2003). In the *Tabby* mouse, a well-characterized animal model of XLHED (Falconer, 1952), prenatal exposure to EDA1 via serial intravenous administrations to the dam corrected developmental abnormalities to a far greater extent compared with postnatal administration (Gaide and Schneider, 2003). This approach may, however, be suboptimal for achieving reproducible therapeutic drug concentrations in human fetuses, and would expose the mother to high serum levels of an exogenous molecule. We hypothesized that EDI200, an EDA1 replacement protein consisting of the receptor-binding domain of EDA1 and the Fc part of IgG1, may enter the fetal circulation also after injection into the amniotic fluid (AF), because the fetus swallows

AF regularly and the neonatal Fc receptor, which is present in rodent and human fetal intestine (Shah *et al.*, 2003), may facilitate intestinal absorption of Fc-containing proteins. AF could thus serve as a drug reservoir and provide for continuous drug uptake. Here, we report striking reversal of the XLHED phenotype of *Tabby* mice following a single intra-amniotic injection of EDI200.

Long-term stability of this recombinant protein in AF, i.e. the retention of binding to its cognate receptor, was confirmed *in vitro* under various conditions (Supplementary Figure S1 online). Pharmacokinetics following intra-amniotic injection of 35 μg EDI200 per amniotic sac (= 100 $\mu\text{g g}^{-1}$ of estimated fetal body weight) was studied in wild-

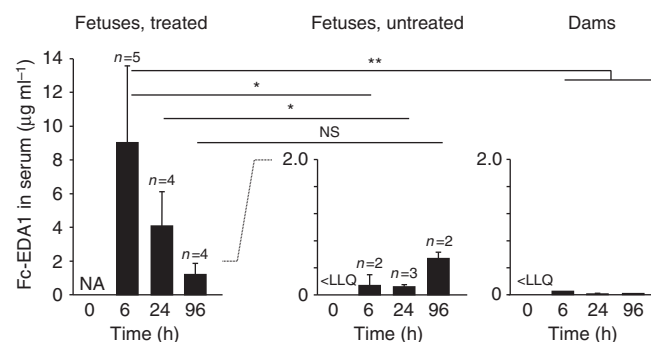


Figure 1. EDI200 pharmacokinetics after intra-amniotic administration to mice. EDI200 was injected into amniotic sacs of four pregnant wild-type mice carrying a total of 23 fetuses at day E15 (100 $\mu\text{g g}^{-1}$ of estimated fetal body weight). Some fetuses of each mother were left untreated. Another pregnant mouse and the fetuses of her served as negative controls. EDI200 serum concentrations were measured at different time points by receptor-binding ELISA. Highest serum levels were detected 6 hours after the injection. There was some leakage to untreated siblings and, to a lower extent, into the mother's circulation. NA, not applicable; * $P < 0.05$; ** $P < 0.01$.

Abbreviations: AF, amniotic fluid; EDA1, ectodysplasin A1; XLHED, X-linked hypohidrotic ectodermal dysplasia

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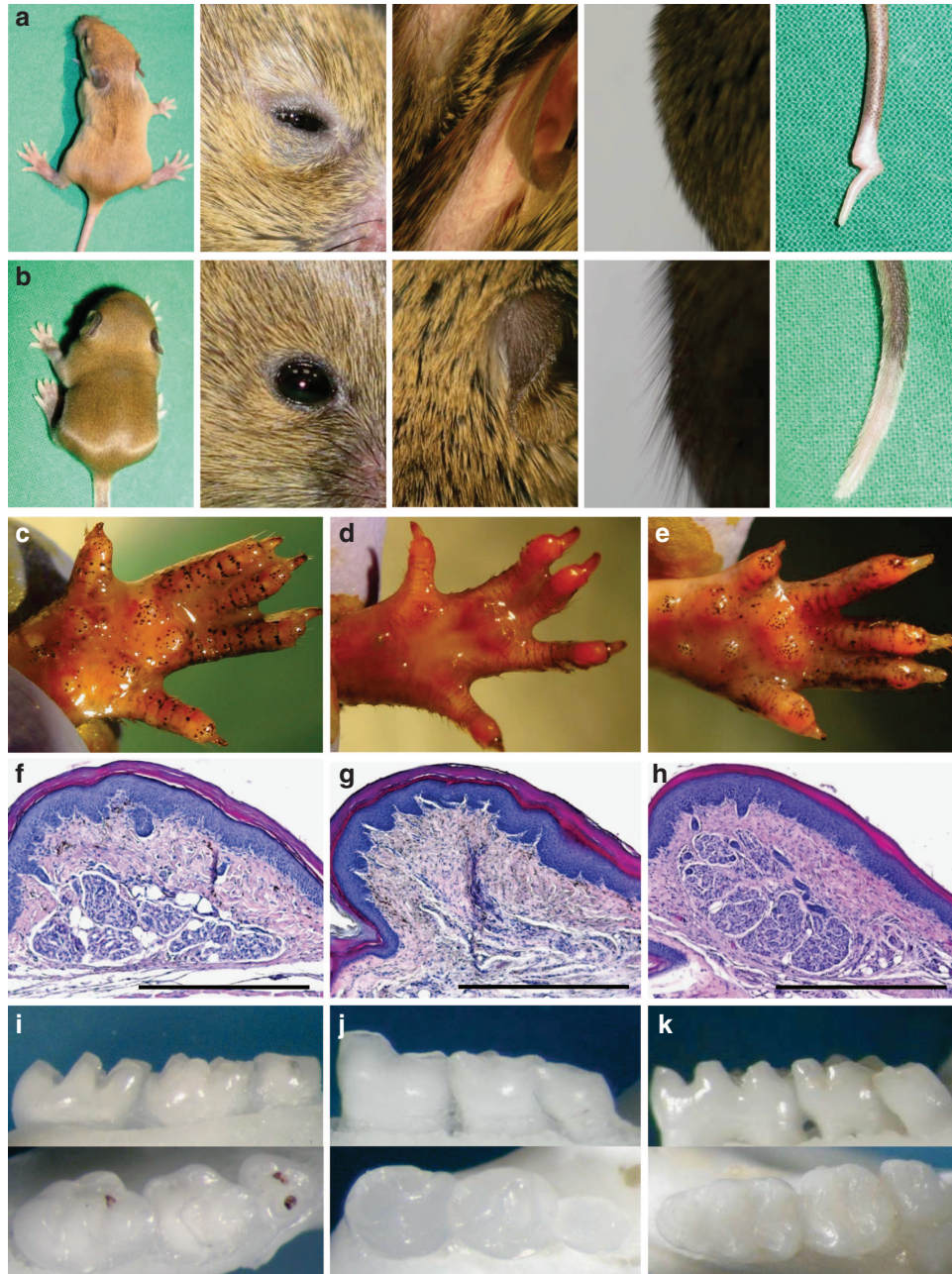


Figure 2. Phenotype after prenatal treatment with EDI200. *Tabby* mice were treated *in utero* (E15) by a single intra-amniotic injection of EDI200 (here $100 \mu\text{g g}^{-1}$) and investigated postnatally. Age-matched native *Tabby* mice (a) could be distinguished clearly from treated animals (b), which had a darker and denser coat, showed normal eye opening, retro-auricular and guard hair like wild-type mice, and a hairy, normally shaped tail. Starch-iodine tests of the paws revealed numerous dark spots in a characteristic pattern indicating functional sweat glands (c). Histological analysis of footpad sections (scale bar = 0.5 mm) confirmed the presence of fully developed eccrine sweat glands in the treated animals (f). Native *Tabby* mice (d, g) and wild-type mice (e, h) served as controls. Molars of treated *Tabby* mice (i) were altogether larger than those of native *Tabby* mice (j) and showed similar shape and cusps pattern as in wild-type animals (k).

type mice at day 15 of gestation (E15). All maternal animals and 93% of the treated fetuses survived the procedure. EDI200 serum levels were measured at different time points after injection both in treated and untreated fetuses as well as in the dams. Intra-amniotic injection

of EDI200 resulted in mean fetal serum levels of 9.0 and $1.2 \mu\text{g ml}^{-1}$ at 6 and 96 hours, respectively. After 6 hours, this corresponds to 180 ng of EDI200 per fetus or 0.5% of the injected protein, assuming a total serum volume of approximately $20 \mu\text{l}$ in an E15 mouse

fetus. Interestingly, there was a low level of EDI200 transfer into the circulation of untreated siblings and that of the pregnant dam (Figure 1). The drug was partially and slowly redistributed from treated fetuses, in which EDI200 concentration diminished over time, to

untreated siblings that witnessed a parallel increase in the serum levels (up to $0.57 \mu\text{g ml}^{-1}$). Maternal EDI200 serum levels remained $<0.1 \mu\text{g ml}^{-1}$ at the time points investigated. Thus, intra-amniotic administration of EDI200 at E15 resulted in substantial fetal uptake with minimal maternal exposure.

This approach was then evaluated in pregnant *Tabby* mice with doses of 100, 10, and $1 \mu\text{g g}^{-1}$ of estimated fetal body weight. The surgical procedure was conducted under isofluran anesthesia plus perioperative analgesia with metamizole and was approved by the local government authorities. All treated *Tabby* mouse fetuses of the high and intermediate dose cohorts survived the E15 intra-amniotic injection and were born without complications. They were easily distinguishable from native *Tabby* mice already in the second week after birth. Later, normal eye opening, retro-auricular and guard hair as in wild-type mice, and a normally shaped tail tip (Figure 2a and b) were evident. Starch-iodine tests revealed regular sweat production at the paws (Figure 2c–e). Normal eccrine sweat glands (Figure 2f–h) were detected in footpads of these animals. In addition, size and shape of the molars resembled those of wild-type mice (Figure 2i–k).

Thus, in contrast to a single maternal intravenous injection of $400 \mu\text{g}$ of EDI200 in pregnant *Tabby* mice at E15, which corrected the XLHED phenotype in the offspring only partially (unpublished own data), a single intra-amniotic dose of $3.5 \mu\text{g}$ or above resulted in complete phenotypic correction. No adverse effects were observed. All treated *Tabby* mice survived to adulthood and showed normal behavior and fertility. The lower dose of $1 \mu\text{g g}^{-1}$ body weight yielded only a partial restoration of normal ectoderm development, with less guard and/or tail hair and fewer sweat glands present (Supplementary Table S1 online). Interestingly, but less relevant to human singleton gestations, a dose-dependent correc-

tion was also observed for untreated littermates (Supplementary Table S1 online)—explained by partial leakage of the drug to neighboring fetuses. As expected from previous studies (Gaide and Schneider, 2003), the maternal *Tabby* phenotype was not visibly altered by EDI200 administration, regardless of the dose. All dams remained fertile and no adverse effects of the treatment could be detected during an observation period of 6–9 months.

The EDA1 signaling pathway is well conserved among vertebrates (Pantalacci *et al.*, 2008) and findings in animal models should therefore be transferable to human XLHED patients. Early corrective treatment would increase their life expectancy, would have a high impact on the quality of life, and substantially reduce medical expenses. Intra-amniotic drug delivery might be most beneficial if attempted during mid-gestation, when sweat gland development is not yet completed (Ersch and Stallmach, 1999). This approach is likely to have a good benefit/risk ratio, supported by the broad experience with amniocentesis, and may represent a novel paradigm for treatment of disorders in early human development.

CONFLICT OF INTEREST

Kenneth Huttner is an employee of Edimer Pharmaceuticals Inc.; Pascal Schneider holds shares in this company. Holm Schneider is a member of the clinical advisory board of Edimer Pharmaceuticals.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/jid>

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