TWO-STEP AAV8 GENE DELIVERY IN A CHILD WITH CRIGLER–NAJJAR SYNDROME TYPE I

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Crigler–Najjar syndrome type I, an orphan *UTG1A1* enzyme deficiency, manifests at birth with severe unconjugated hyperbilirubinemia. Here we describe sustained clinical response in a pediatric patient with Crigler–Najjar syndrome type I treated with two consecutive doses of AAV8 delivering the UTG1A1 coding sequence. Infusion I (6×10^{12} vg/kg) afforded sustained decrease in serum bilirubin, allowing substantial relaxation of the phototherapy from 12 h to 4 h daily. Infusion II at a double dose was made in 6 months; the decision was intended at complete elimination of phototherapy. However, the elimination led to a sharp increase in bilirubin levels necessitating resumption of phototherapy. The patient is currently stable on 4 h daily phototherapy for 80 weeks since the resumption and 107 weeks since the AAV8 therapy initiation. No toxic side effects were encountered. A slow incremental dynamics in serum bilirubin opens the issue of clinical advisability for subsequent infusions of the drug.

Keywords: Crigler–Najjar syndrome type I, AAV8, gene therapy

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Compliance with ethical standards: the therapy was approved by Ethics Committee at the Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology on Dec 01 2022, Protocol #12 for infusion I, and on Jul 20 2023, Protocol #7 for infusion II. The patient's legal representatives provided voluntary informed consents for the study and for each infusion of the drug.

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ДВУХЭТАПНАЯ AAV8-ГЕНОТЕРАПИЯ РЕБЕНКА С СИНДРОМОМ КРИГЛЕРА–НАЙЯРА 1-ГО ТИПА

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Синдром Криглера–Найяра 1-го типа (СКН1) является следствием дефицита фермента уридиндифосфатглюкуронозилтрансферы 1А1, кодируемого геном *UTG1A1* и проявляется при рождении тяжелой неконъюгированной гипербилирубинемией. В работе описан устойчивый клинический ответ у ребенка с СКН1, которого лечили двумя последовательными дозами AAV8-генотерапии, доставляющей кодирующую последовательность UTG1A1. Инфузия I (6×10^{12} вг/кг) обеспечила устойчивое снижение сывороточного билирубина, что позволило ослабить фототерапию с 12 ч до 4 ч в день. Инфузия II в двойной дозе была сделана через 6 месяцев; данное решение было принято с целью полной отмены фототерапии. Однако отмена привела к резкому повышению уровня билирубина, что потребовало возобновления фототерапии. В настоящее время состояние пациента стабильно на 4-часовой ежедневной фототерапии в течение 80 недель с момента возобновления и 107 недель с момента начала терапии генопрепаратом. Токсических побочных эффектов не наблюдалось. Плавный прирост уровня билирубина в сыворотке крови ставит вопрос о клинической целесообразности ежегодных инфузий препарата.

Ключевые слова: синдром Криглера–Найяра 1-го типа, AAV8, генная терапия

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Crigler-Najjar syndrome (CNs) is an orphan enzymopathy caused by loss-of-function variants in *UGT1A1* [1]. The lack of active uridine diphosphate glucuronosyltransferase 1A1, which leads to accumulation of bilirubin conjugates clinically represented by jaundice and severe neurological impairments, is potentially fatal in infancy. The diagnosis involves biochemical and molecular genetic tests for, respectively, unconjugated bilirubin and *UGT1A1* variants. No specific pathogenetic treatment for the condition has been proposed apart from transplantation of the liver in CNs type I, the severe form. The patients require constant observation and are on continual bilirubin clearance by plasmapheresis and (or) phototherapy, which is physiologically straining and profoundly affects the lifestyle options in young patients.

The potential of using adeno-associated virus serotype 8 for the treatment of Crigler-Najjar syndrome has been substantiated in preclinical studies *in vivo* [2, 3]. In 2023, D'Antiga et al. reported successful treatment of Crigler-Najjar syndrome (CNs) type I in adult patients using a gene drug with AAV-mediated delivery [4]. In pediatric patients, the efficiency could (speculatively) interfere with the high rates of growth and physiological renewal of liver cells. Here we describe a sustained clinical response (107 weeks by the time of writing) in a pediatric patient with CNs type I using *UGT1A1* coding sequence delivered by two consecutive doses of adeno-associated viral vector serotype 8 (AAV8). This is the first report on AAV therapy in a two-dose delivery mode.

Case description

Vector design and purification

cDNA from a healthy donor with wild-type *UGT1A1* sequence was used as a template. The coding sequence was cloned into pAAV-TBG plasmid. The pAAV-TBG-*UGT1A1* construct was packaged in HEK293 cells.

The particles were purified in an iodixanol stepwise density gradient (OptiPrep, StemCell Technologies, USA) at 350,000 g, +18 °C for 1.5 hours (Optima-XPN ultracentrifuge; Beckman, USA). The 60% fraction and a half of the 40% iodixanol fraction were collected, purified by dialysis (Spectrum™, Fisher Scientific #0867140; pore size 100 kDa) against (1x) PBS/350 mM NaCl/0.001% Pluronic F-68 buffer at 4°C for 14–18 h, concentrated with Amicon® centrifugal filters, pore size 100 kDa (Millipore Sigma, USA) and additionally sterilized by 0.22 µm syringe filtration. The product, alphaglucuronosyltransferasegene unoparvovec, was quantitated by real-time PCR with plasmid DNA as a reference. All purity indicators were found to be within the reference limits for clinical use; the product contained endotoxin <0.64 EU/ml (Endosafe® LAL; Charles River Endosafe, USA), BSA < 159 ng/ml (BSA ELISA Kit; Wuhan Fine Biotech, PRC), HEK293 residual proteins <2 ng/ml (HEK293 HCP ELISA Kit; Cygnus Technologies, USA) and was *Mycoplasma*-negative.

Preclinical study

In vitro tests were carried out on HeLa cells (ATCC, USA).

Animal study was authorized by the Institutional Animal Care and Use Committee at the Pirogov University. *In vivo* tests on C57BL6 mice were performed in three rounds: dose-response assessment, overdose toxicity assessment and fade-out assessment in adolescence. The control group received identical volume of dilution buffer instead of the drug in all experiments.

Dose-response assessment was performed on 8-month-old mice in groups $n(c)=11$, $n(L)=11$, $n(M)=11$, $n(H)=11$. Toxicology and biodistribution studies involved monitoring of leukocyte counts and biochemical tests at 3, 4, and 5 weeks post-infusion, and post-mortem assessment of blood parameters and biodistribution of the drug to muscle and viscera on week 6. Immunohistochemistry revealed selective biodistribution of the human *UGT1A1* coding sequence to the liver. The hUGT1A1 signal increased incrementally with the dose by both the intensity and the spread into the liver parenchyma from veins of the lobules (Fig. 1).

Overdose toxicity assessment of the maximum of potential therapeutic dose was performed by administering a 1.2×10^{14} vg/kg dose to 8-month-old mice in control and experimental groups ($n = 6$ each) with the same check points and controlled parameters as above. No significant toxicity effects were detected.

Fade-out assessment in adolescence was assessed on 5-week-old mice ($n = 24$) received tail vein 1.2×10^{13} vg/kg drug injections. At 2 weeks post-infusion and then every 3 weeks until the end of experiment (at the age of 19 weeks), groups of mice ($n = 6, 4, 4, 4, 6$) were euthanized with livers taken for qPCR analysis of viral genome copies and quantitative protein detection by immunohistochemistry (liver sections stained with hUGT1A1-specific antibody). Quantitative PCR showed a 2-fold decrease in the vector DNA content every 6 weeks (4-fold over the experiment). Quantitative protein analysis showed that hUGT1A1 levels increased up to 5 weeks post-infusion and remained stable until the end of the experiment.

Patient

A girl with CNs type I, confirmed by *UGT1A1* gene sequencing and manifested as severe unconjugated hyperbilirubinemia, under continuous observation at the Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology, was maintained on 12 h daily phototherapy, which afforded a neat confinement of indirect bilirubin levels within the toxicity limit of 300–350 µmol/l.

The AAV therapy was approved by Ethics Committee at the Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology on Dec 01 2022, Protocol #12 for infusion I, and on Jul 20 2023, Protocol #7 for infusion II. Application for clinical trial phase I–II No. 13571. The patient's legal representatives provided voluntary informed consents for the study and for each infusion of the drug.

Treatment and outcome

The gene therapy was initiated at an age of 7 years 5 months. The patient received two intravenous infusions of alphaglucu ronosyltransferasegene unoparvovec: 6×10^{12} viral genomes per kg body weight (vg/kg) on week 0 and 1.2×10^{13} vg/kg on week 27 (Fig. 2). The safety was monitored by transaminase levels and patient-reported well-being. The efficacy was assessed by reduction in serum bilirubin levels. A chart of indirect bilirubin levels over 3.5 years, including an extensive time length preceding the treatment, is given in Fig. 2. The phototherapy regimen was continuously adjusted in accordance with the indirect bilirubin levels. Both infusions were accompanied by prednisolone support to mitigate immune reactions: the patient received prednisolone daily, in oral doses reduced stepwise from 1 mg/kg to 0 in the course of 8 and 2 weeks since the infusion, respectively (Fig. 2). In addition, on day of infusion II,

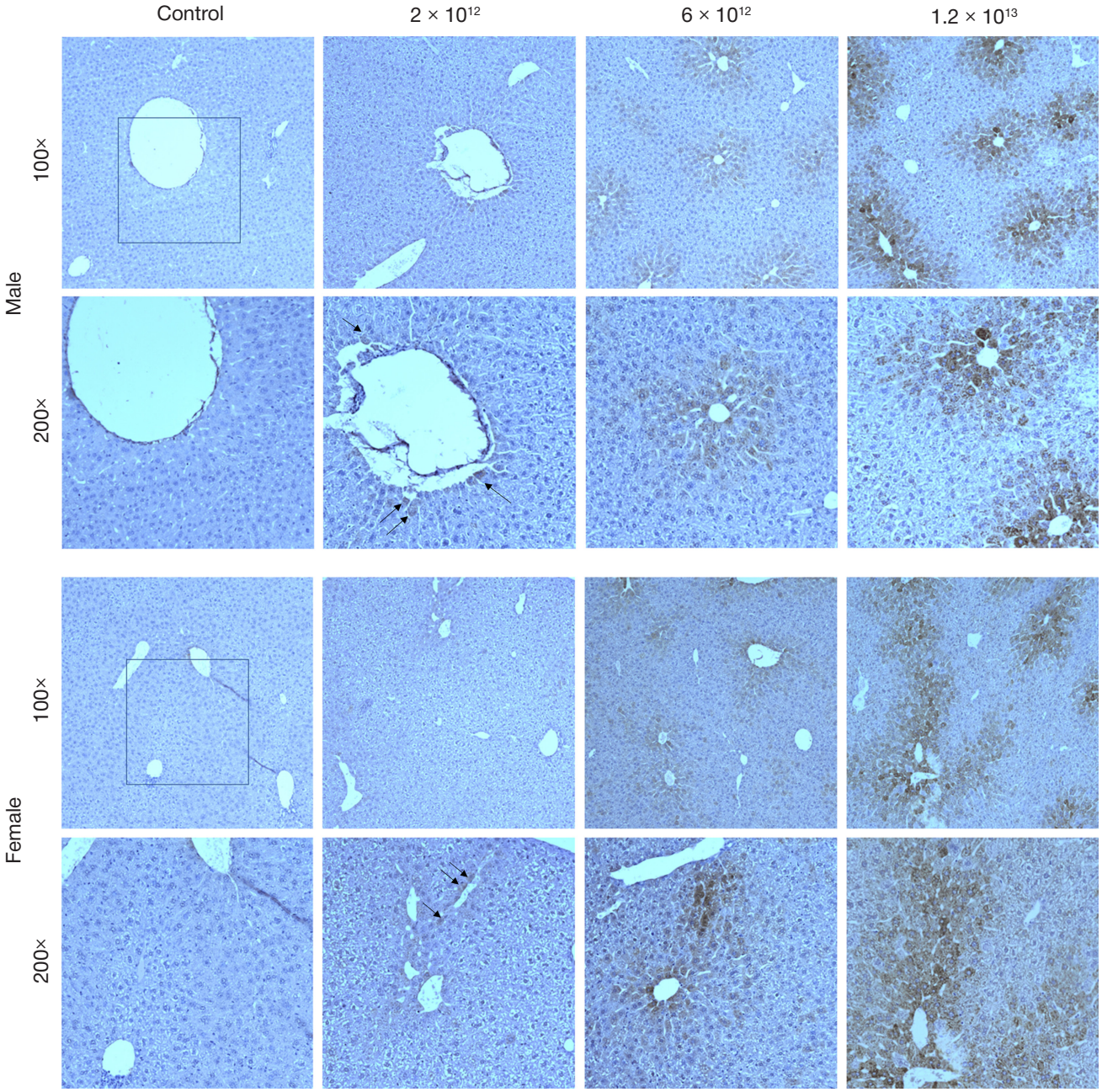

Fig. 1. Immunohistochemistry for human UDP-glucuronosyltransferase 1A1 in mouse liver, post-infusion week 6. The sections counterstained with hematoxylin were assessed using antibodies to human UGT1A1 topped with HRP-conjugated second antibody. Vector doses, vg/kg, are indicated. *Black arrows* in the low-dose images indicate perivascular localization of the signal

the patient received 250 mg (10 mg/kg) methylprednisolone intravenously.

No neutralizing antibodies to AAV8 were detectable before infusion I. The positive immunological reaction with AAV8 epitopes persisted post-infusion.

Infusion I reduced the serum bilirubin 2-fold, the minimum achieved throughout weeks 2–9. As the daily phototherapy doses were gradually reduced to 4 h by week 8, serum bilirubin concentrations rebound to ~250 μmol/l starting from week 10. The clinical decision on infusion II was intended at complete elimination of phototherapy. Infusion II on week 27 produced no toxic effects and afforded a further ~20% reduction in serum bilirubin concentrations by week 29. Considering the positive dynamics, the phototherapy support was discontinued, but resumed 2 weeks later at the same limited dose of 4 h, as serum bilirubin concentrations rapidly increased to ~400 μmol/l. The re-initiated phototherapy provided rapid correction of serum bilirubin concentrations to ~250 μmol/l. The liver enzyme profiles remained normal throughout the observation period. At the time of writing (week 97), the patient is stable on phototherapy administered 4 h daily, without systemic complaints.

Case discussion

The treatment produced no toxic side effects. Although complete discontinuation of the phototherapy proved unfeasible at this stage, the treatment afforded a lasting 3-fold reduction in daily exposure compared with initial values.

CONCLUSION

The use of liver-specific gene therapy in pediatric patients is associated with accelerated loss of the restored function,

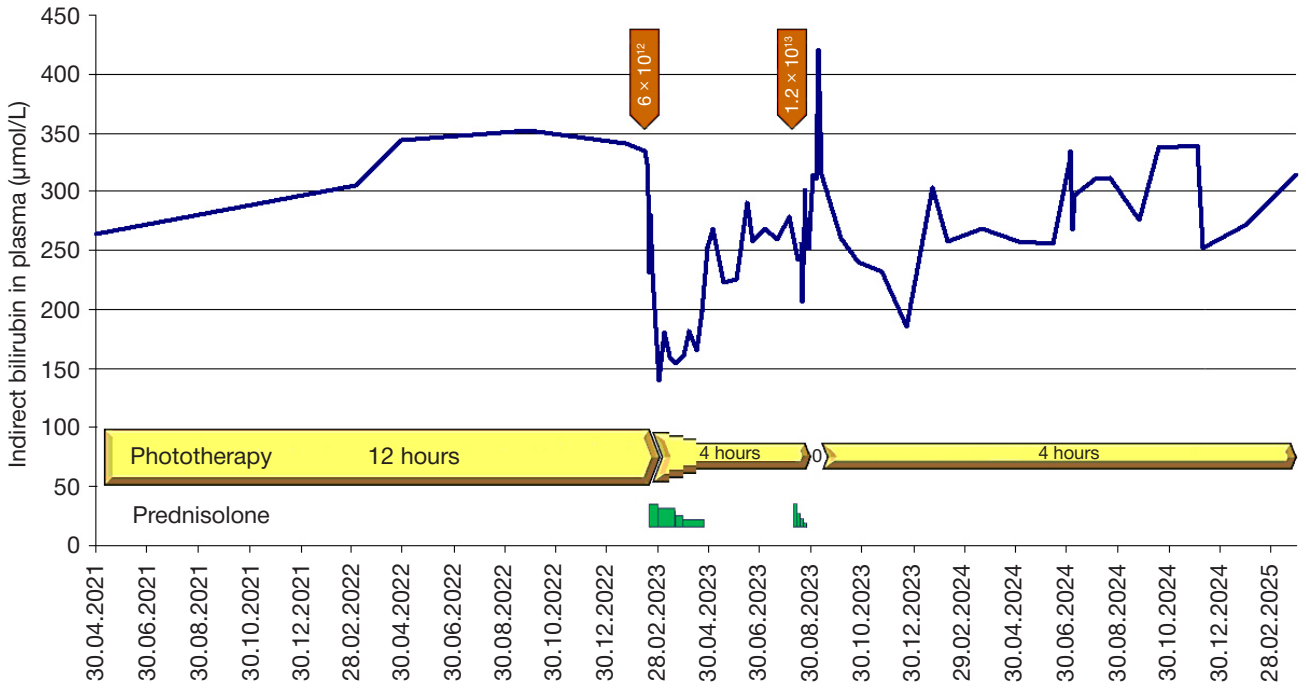

Fig. 2. Dynamics of indirect bilirubin levels with regard to alphaglucuronosyltransferasegene unoparvovec infusions I and II, adjusted phototherapy regimens and prednisolone support (oral doses, starting from 1 mg/kg daily)

apparently due to the high physiological regeneration capacity and liver growth. The opportunity of safe repeated administration of AAV8 vector allows satisfactory control of the critical biochemical indicator.

The Gene Therapy for Crigler-Najjar Syndrome Type I is currently in a Phase 1/2 trial (https://clinicaltrials.gov/study/NCT06641154) and could be applicable to other patients aged 3 months to 10 years.

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