HEMOSTASIA E PAREDE VASCULAR: BIOLOGIA

AVALIAÇÃO DA VIABILIDADE E SEGURANÇA DA EXPRESSÃO A LONGO PRAZO DE HEMOPEXINA USANDO UM VETOR VIRAL (AAV8) EM UM MODELO ANIMAL DE ANEMIA FALCIFORME

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Objetivos: O heme extracelular medeia o dano tecidual e a ativação da coagulação na anemia falciforme (AF). A hemopexina (HPX) é uma proteína responsável por remover o excesso de heme da circulação. No entanto, estudos anteriores mostraram que os níveis de HPX estão esgotados na SCA devido à sobrecarga de heme livre. O uso de vetores virais baseados em vírus adeno-associado para expressar proteínas ausentes ou disfuncionais tem se tornado uma estratégia atraente para o tratamento de diversas doenças. Nosso objetivo foi explorar a viabilidade e segurança do uso de um vetor viral (AAV8) para obter a expressão de longo prazo de HPX em um modelo de camundongo de AF. **Material e métodos:** Foram utilizados dois vetores virais baseados em um vírus adeno-associado sorotipo 8 (AAV8) expressando HPX humano (hHPX) ou murino (mHPX). A expressão sustentada de hHPX usando o primeiro vetor foi relatada anteriormente. Camundongos C57Bl/6j ou Townes foram injetados por via intravenosa com um desses vetores ou com um controle (Empty). A expressão de HPX foi avaliada por qPCR, western blot ou Elisa. A segurança foi avaliada por parâmetros clínicos e hematológicos. **Resultados:** A expressão de hHPX foi confirmada em camundongos Townes em amostras de fígado após 28 semanas por PCR em tempo real e western blot transduzidos com 2 x 10^{13} vg/kg. No entanto, o hHPX circulante pode ser detectado em apenas 1/10 camundongos transduzidos (nível de HPX: 0,033 mg/mL). Para otimizar a expressão, mudamos para um vetor expressando mHPX, que aumentou os níveis plasmáticos de mHPX em até 3 vezes, de maneira dose-dependente, em camundongos C57Bl/6j. Camundongos Townes foram então tratados com a dose de vetor mais alta (1 x 10^{14} vg/kg) e observados por 28 semanas. Apesar de não serem observados diferenças nos níveis plasmáticos de mHPX entre os dois grupos (vetor e Empty), houve um aumento a nível transcricional na expressão de mHPX no grupo tratado com o vetor, sugerindo que o aumento que não vemos nos níveis plasmáticos pode indicar que essa HPX está sendo consumida. Os parâmetros hematológicos e bioquímicos não apresentaram diferenças entre os grupos. Como um dos parâmetros de segurança, que no caso do modelo Townes também pode ser considerado um parâmetro de eficácia, analisamos também o ganho de peso dos camundongos. Os animais foram acompanhados e pesados periodicamente. Na 8° e 18° semana observamos uma diferença significativa no ganho de peso nos animais tratados com o vetor ($p = 0.001$ e $p = 0.04$, respectivamente). **Discussão e conclusão:** Embora a transferência de genes de HPX a longo prazo seja viável em camundongos C57BL/6, a obtenção de níveis terapêuticos de HPX em um modelo animal de AF é desafiadora, possivelmente devido ao consumo de HPX. O padrão de ganho de peso observado em camundongos Townes que receberam o vetor rAAV8-mHPX sugere que a transferência gênica de HPX murina é um método capaz de modificar o fenótipo da AF observado nesta linhagem.

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HEMOSTASIA E PAREDE VASCULAR: DOENÇAS DA COAGULAÇÃO E FIBRINÓLISE

A SIMULATION STUDY TO PROVIDE GUIDANCE FOR INDIVIDUALS TRANSITIONING FROM EMICIZUMAB TO VALOCTOCOGENE ROXAPARVOVEC

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Aim: Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) is a gene therapy evaluated in the phase 3 GENEr8-1 trial that provides endogenous factor VIII (FVIII) production to prevent bleeding in people with severe hemophilia A and represents an alternative to emicizumab, a recombinant antibody mimicking the function of FVIII. Individuals receiving emicizumab were excluded from GENEr8-1 enrollment since emicizumab was then an investigational therapy. This study aimed to utilize pharmacokinetic simulations to provide guidance on maintaining hemostatic control while transitioning patients from emicizumab to valoctocogene roxaparvovec gene therapy. **Methods:** A published emicizumab pharmacokinetic model, based on data from the HAVEN clinical trials, was used to simulate in vivo emicizumab concentrations and merged with FVIII activity time-course data for 134 GENEr8-1 participants to estimate bleeding risk at weekly intervals post-infusion with valoctocogene roxaparvovec. The analysis examined 3 approved emicizumab dosing regimens for 2 transition scenarios; first, the last emicizumab dose given the

same day as infusion, and second, given 4 weeks after valoctocogene roxaparvovec. **Results:** Discontinuation of emicizumab the day of valoctocogene roxaparvovec infusion compared with continued dosing for 4 weeks offered similar levels of hemostatic control. The time course for bleeding risk was comparable across the emicizumab dosing regimens for both scenarios. An algorithm to provide guidance for discontinuing emicizumab was developed based on these results. To guide how treatment decisions for emicizumab discontinuation may vary among individuals, theoretical case examples were developed based on participants of GENEr8-1. **Conclusions:** Regardless of the emicizumab dose or dosing regimen, pharmacokinetic simulations showed no meaningful difference in the risk of bleeding related to FVIII and FVIII equivalent activity determined by the dynamic balance of decaying emicizumab levels and increasing gene therapy –derived endogenous FVIII. These original data suggest individuals on emicizumab prophylaxis can safely transition to valoctocogene roxaparvovec. COIs for presenting author. Suresh Agarwal is an employee and stockholder of BioMarin Pharmaceutical Inc.

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QUANTITATIVE PHARMACOKINETIC MODEL TO CHARACTERIZE AND EXTRAPOLATE LONG-TERM FVIII ACTIVITY LEVELS IN PATIENTS WITH SEVERE HEMOPHILIA A TREATED WITH VALACTOCOGENE ROXAPARVOVEC

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Aim: Valoctocogene roxaparvovec delivers a B-domain deleted factor VIII (FVIII) coding sequence with an adeno-associated virus vector to reduce bleeding and FVIII concentrate use in people with severe hemophilia A. This study aimed to characterize the long-term trajectory of transgene-derived FVIII activity using a linear mixed effects (LME) model to estimate mean and median FVIII activity levels 5 years post-infusion. **Methods:** In GENEr8-1, an open-label, single-arm, multicenter phase 3 trial, 134 participants with severe hemophilia A received a single 6 x 10^{13} vg/kg dose of valoctocogene roxaparvovec. FVIII activity was assessed using the chromogenic substrate assay and one-stage assay. A previously published quantitative pharmacokinetic (PK) modelwas updated to extrapolate FVIII activity levels to 5 years post-infusion. Ln-transformed FVIII activity values from week 76 to 104 were fit to the LME model with random effects for participants on slope and intercept using a restricted maximum likelihood method with the lmer package in the R statistical computing software (R Foundation for Statistical Computing, Vienna, Austria). The precision of parameter estimates and model diagnostics were evaluated to confirm goodness-of-fit. The model and extrapolation approach was further qualified by comparing to observed FVIII activity at week 156. **Results:** The final LME model dataset included 928 observations from 120 participants. The long-term trajectory of FVIII activity was consistent with first-order elimination kinetics starting at week 76. Model parameter estimates were consistent with the previously published model; diagnostic plots showed no major deficiencies. FVIII activity was extrapolated to 5 years post-gene transfer. Mean and median FVIII activity extrapolations at week 156 were consistent with observed values, confirming adequacy of the model. **Conclusions:** Pharmacokinetic modeling indicates valoctocogene roxaparvovec-derived FVIII activity levels will remain in the mild hemophilia range for ≥5 years post-gene transfer for the majority of patients treated. **COIs for presenting author: Suresh Agarwal** is a paid employee of BioMarin Pharmaceutical Inc. and holds stock ownership with BioMarin Pharmaceutical Inc.

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BLEEDING, FVIII ACTIVITY, AND SAFETY 3 YEARS AFTER GENE TRANSFER WITH VALOCTOCOGENE ROXAPARVOVEC: RESULTS FROM GENER8-1

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