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The effect of prophylactic corticosteroid treatment on adeno-associated virus mediated gene therapy and potential mechanisms of action

Harris S, Handyside B, Zhang L, Yates B, Xie L, Sihn C-R, Murphy R, Bouwman T, Baridon B, Su C, Bullens S, Ismail AM, Bunting S, Fong S

BioMarin Pharmaceutical Inc., San Rafael, CA

Introduction

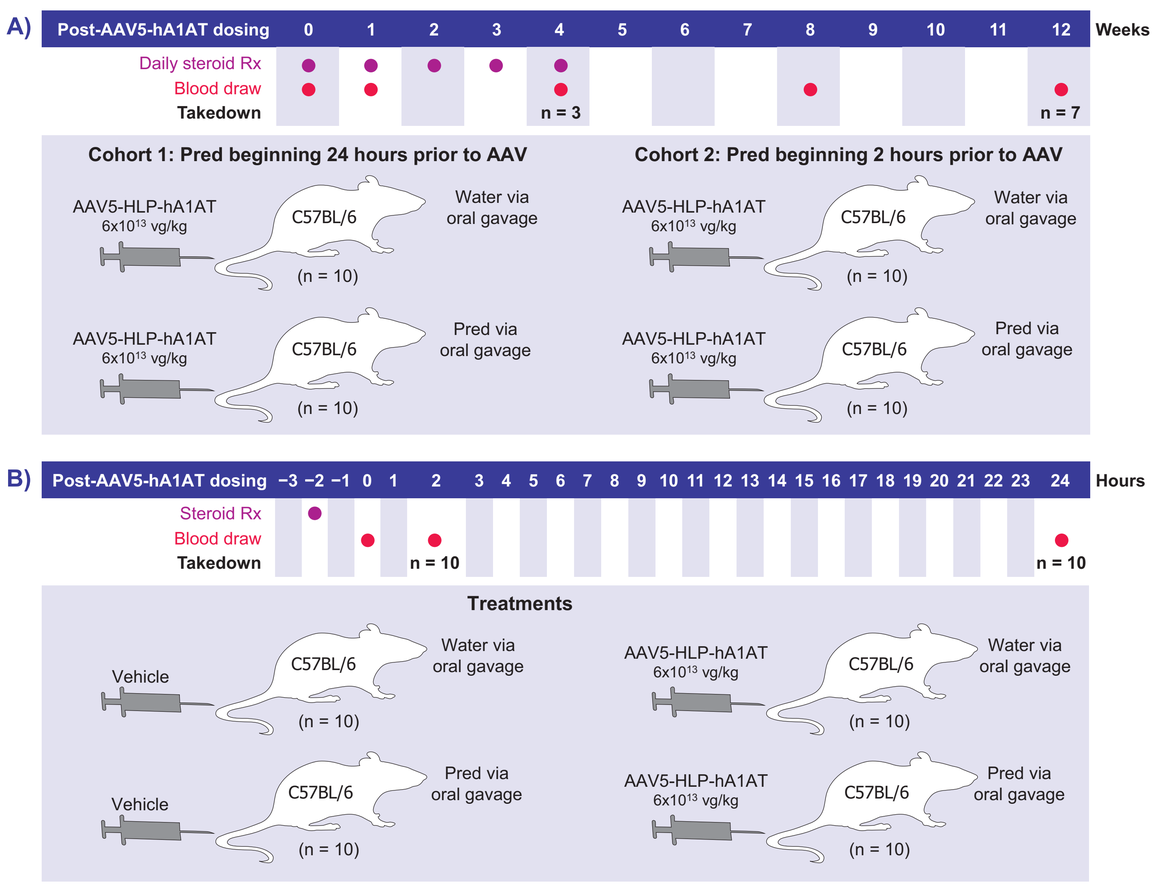
- Adeno-associated virus (AAV)-based gene therapy may stimulate immune responses that interfere with transduction; corticosteroid treatment may reduce these and increase AAV-mediated gene expression[1–3]
- Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) is an AAV serotype 5 (AAV5) gene therapy vector that expresses a B-domain–deleted human factor VIII (FVIII) from a hepatocyte-specific promoter[3-6]
  - In mice, treatment with prednisolone starting 1 week after AAV5-hFVIII-SQ dosing did not affect FVIII expression[7]
- Here, we examined the effect of prophylactic prednisolone treatment prior to AAV5-mediated gene therapy on transgene expression in mice and investigated early mechanisms of action

Methods

Study design

- Two studies of prophylactic corticosteroid use before AAV5 dosing were performed to evaluate effects over 12 weeks **(Figure 1A)** and investigate mechanisms within 24 hours of AAV5 administration **(Figure 1B)**
  - The reporter vector AAV5-HLP-hA1AT expressing the serum protein human α1-antitrypsin (hA1AT) from a hepatocyte-specific promoter was used instead of AAV5-hFVIII-SQ to allow serial blood sampling via tail-nick without potential activation of the clotting cascade and consumption of FVIII

Figure 1. Design of the A) 12-week study and B) 2- and 24-hour study of prophylactic corticosteroid use before AAV5 treatment in mice



AAV5-HLP-hA1AT, adeno associated virus vector serotype 5 hybrid liver promoter human α1 antitrypsin; Pred, prednisolone; Rx, treatment.

- In the 12-week study, serum hA1AT expression and vector DNA levels in hepatocytes were assessed
- In the 24-hour study, RNAseq was performed on liver samples to identify potential molecular mechanisms, with targeted follow-up analyses focusing specifically on mechanisms of increased AAV transduction and immune suppression

Analytical methods

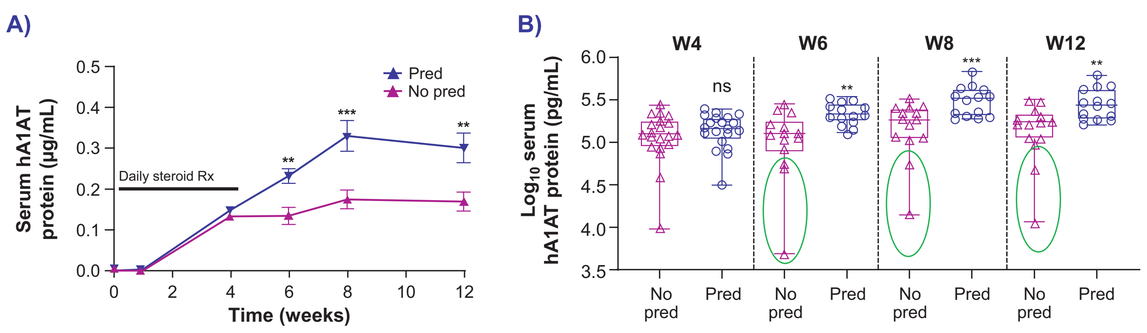
- Expression of serum protein hA1AT was measured with an enzyme-linked immunosorbent assay
- Levels of vector DNA and full-length vector genomes in the liver were measured using droplet-digital PCR
- Hepatic distribution of vector genomes was assessed with in situ hybridization
- RNAseq was performed on homogenized liver samples; differential expression was determined using edgeR software in R (R Foundation for Statistical Computing, Vienna, Austria). Pathway enrichment analyses were performed using the MSigDB hallmark gene set
- Hepatic expression and distribution of the protein platelet-derived growth factor receptor-α (PDGFRα) was assessed with immunohistochemistry

Results

Prophylactic prednisolone increased transgene expression and decreased variability over 12 weeks

- Mice treated with prophylactic prednisolone before AAV5 dosing had higher transgene expression beginning at week 6 **(Figure 2A)**
- This result was potentially mediated by decreasing the number of mice with lower responses; low responders who did not receive prednisolone are circled in green in **Figure 2B**

Figure 2. Effect of prophylactic prednisolone treatment on transgene expression. A) Serum hA1AT protein levels. B) Variation in serum hA1AT protein levels

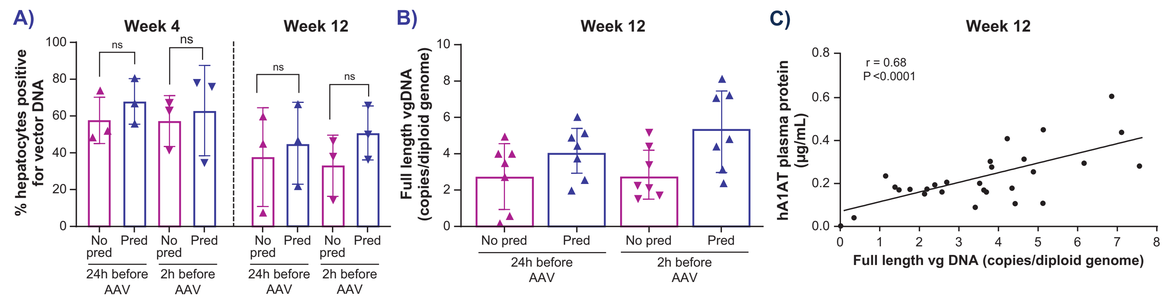


ns, not significant; ** P <0.05; *** P <0.005 with a two-tailed Student's t-test. In panel A, data are mean ± SEM. In panel B, data are median, Q1, Q3, minimum, and maximum. In panel B, circles and triangles are individual data points. "No pred" includes mice who received control 2 or 24 h before AAV; "pred" includes mice who received prednisolone at 2 or 24 h before AAV. hA1AT, human α1-antitrypsin; pred, prednisolone; Q1, first quartile; Q3, third quartile; Rx, dosing; SEM, standard error of the mean; W, week.

Prophylactic prednisolone increased vector DNA in the liver

- At week 12, mice treated with prophylactic prednisolone had more hepatocytes that stained positive for vector DNA, as well as higher levels of full-length vector genomes than those who did not receive prednisolone **(Figure 3)**

Figure 3. Effect of prophylactic corticosteroids on levels of vector DNA over 12 weeks. A) Percent of hepatocytes staining positive for vector DNA. B) Levels of full-length vector genomes in the liver. C) Correlation between levels of full-length vector DNA and hA1AT protein in the liver

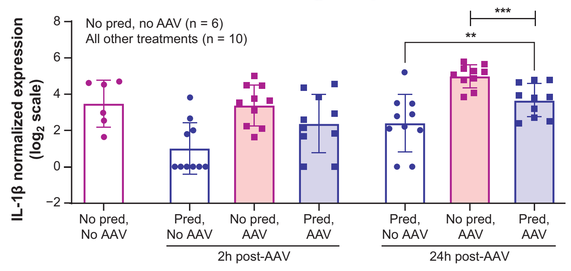


ns, not significant with a two-tailed Student's t-test. Correlation was calculated with a Pearson's correlation. Liver samples were collected at takedown. Bar graphs show mean ± SD. Circles are individual data points. In panel A, each bar represents average of 3 data points. In panel C, n = 28. AAV denotes AAV5-HLP-hA1AT dosing. AAV, adeno-associated virus; AAV5-HLP-hA1AT, AAV serotype 5 hybrid liver promoter hA1AT; h, hour; hA1AT, human α1-antitrypsin; pred, prednisolone; SD, standard deviation; vg, vector genome.

Prophylactic prednisolone suppresses innate immune responses within 24 hours of AAV5 dosing

- We performed RNAseq analyses of liver tissue to identify genes that were differentially expressed in response to both prophylactic steroid treatment and AAV5 dosing
- Pathway enrichment analyses of differentially expressed genes suggested that AAV5 transduction activates innate immune responses and prophylactic corticosteroid treatment modulates them (data not shown)
  - Expression of interleukin-1β, a marker of the inflammasome, was induced by AAV5 and suppressed by prophylactic steroids at 24 hours post-AAV5 dosing **(Figure 4)**

Figure 4. Normalized IL-1β RNA expression levels across treatment groups

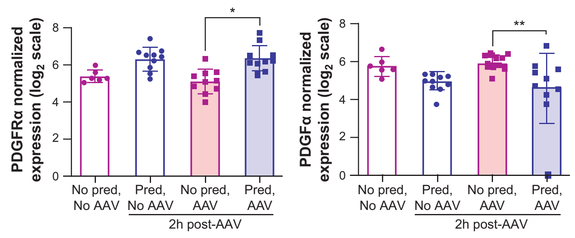


** P < 0.05; *** P < 0.005 with a Welch's unpaired t-test. Data are mean ± SD; circles and squares are individual data points. AAV denotes AAV5-HLP-hA1AT dosing. AAV, adeno-associated virus; AAV5-HLP-hA1AT, AAV serotype 5 hybrid liver promoter human α1-antitrypsin; h, hour; IL-1β, interleukin-1β; pred, prednisolone; SD, standard deviation.

Prophylactic prednisolone treatment upregulates expression of AAV5 co-receptor PDGFRα

- We hypothesized that prophylactic corticosteroid treatment might also increase the initial transduction efficacy
  - We used our RNAseq dataset to investigate changes in expression of cell surface receptors known to facilitate uptake of AAV5 capsids, including PDGFRα
- At 2 hours post-AAV dose, PDGFRα expression was significantly higher in the livers of mice who received prednisolone before AAV5 compared with those who did not, and expression of its ligand PDGFα was significantly lower **(Figure 5)**

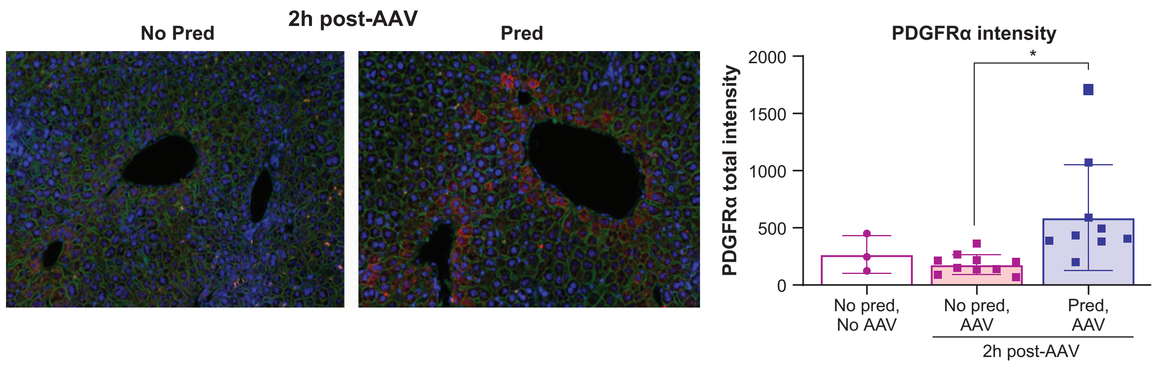
Figure 5. Normalized PDGFRα and ligand PDGFα RNA expression in liver tissue



* P < 0.01; ** P < 0.05 with a Welch's unpaired t-test. AAV denotes AAV5-HLP-hA1AT dosing. Data are mean ± SD; circles or squares are individual data points. AAV, adeno-associated virus; AAV5-HLP-hA1AT, AAV serotype 5 hybrid liver promoter human α1-antitrypsin; h, hour; ns, not significant; PDGFα, platelet-derived growth factor alpha; PDGFRα, PDGF receptor alpha; pred, prednisolone.

- PDGFRα protein levels as assed by immunohistochemistry were also significantly higher in the hepatocytes of mice dosed with prophylactic prednisolone before AAV5 treatment compared to those who were not **(Figure 6)**

Figure 6. PDGFRα protein levels in hepatocytes following AAV5 administration



* P < 0.01 with a Welch's unpaired t-test. AAV denotes AAV5-HLP-hA1AT dosing. Bar graphs show mean ± SD; circles or squares are individual data points. Images are at 20x magnification and DAPI staining indicates viable hepatocytes and T-lectin stains the sinusoids within the liver tissues. It delineates the outer boundaries of the hepatocytes and enables the image analysis quantification of the signal by allowing visualization of the hepatocyte cell. AAV, adeno-associated virus; AAV5-HLP-hA1AT, AAV serotype 5 hybrid liver promoter human α1-antitrypsin; DAPI, 4′,6-diamidino-2-phenylindole fluorescent stain; h, hour; ns, not significant; PDGFα, platelet-derived growth factor alpha; PDGFRα, PDGF receptor alpha; pred, prednisolone.

Conclusions

- Prophylactic corticosteroid treatment before AAV5 administration improved transgene expression through multiple mechanisms that increased the uptake of vectors by hepatocytes
  - Suppression of the acute immune response
  - Upregulation of the AAV5 co receptor PDGFRα and downregulation of its competitive ligand PDGFα on hepatocytes
- Events that occur within 24 hours of AAV5 dosing may affect transgene expression weeks later
- Prophylactic corticosteroids may be an actionable strategy for improving AAV5 mediated-transgene expression

References

1. Nathwani, et al. *NEJM* . 2011.365:2357 65; **2.** George, et al. *NEJM.* 2017;377:2215 27; **3**. Nathwani, et al. *NEJM.* 2014; 317(21):1994 2004; **4.** Rangarajan, et al. *NEJM.* 2017;377:2519 30; **5.** Pasi, et al. *NEJM.* 2020;382:29 40; **6.** Bunting, et al. *Mol Ther.* 2018;26:496 509; **7.** Zhang, et al. *Mol Ther.* 2020;17:13 20.

Conflict of interest

Britta Handyside, Lening Zhang, Bridget Yates, Lin Xie, Choong-Ryoul Sihn, Ryan Murphy, Taren Bouwman, Brian Baridon, Cheng Su, Sherry Bullens, Ashrafali M. Ismail, Stuart Bunting, Sylvia Fong are employees and stockholders of BioMarin Pharmaceutical Inc.

Acknowledgements

Funding for this study was provided by BioMarin Pharmaceutical Inc. Medical writing support was provided by Kathleen Pieper, PhD, of AlphaBioCom, LLC, and funded by BioMarin Pharmaceutical Inc.

Presented at the Congrès Français d'Hémostase: May 10–12, 2023, Saint Malo, France