Prophylactic corticosteroid treatment in mice improved AAV5 transgene expression through multiple mechanisms

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INTRODUCTION

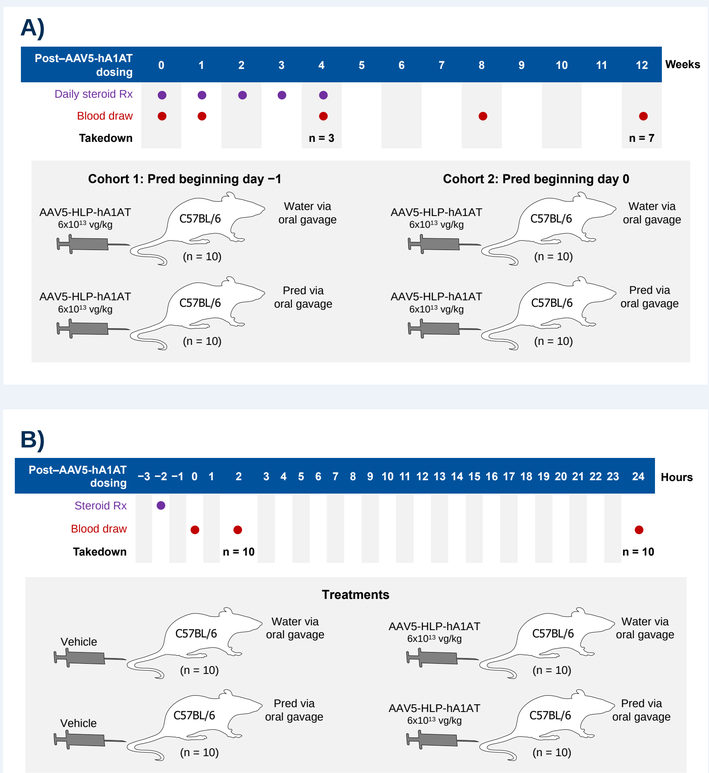
- Adeno-associated virus (AAV)-based gene therapy may stimulate immune responses that interfere with transduction. Corticosteroid treatment may reduce these and increase AAV-mediated gene expression[1–3]
- Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) is an AAV serotype 5 (AAV5) gene therapy vector that expresses a B-domain–deleted human factor VIII (hFVIII) 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 without potential activation of the clotting cascade and consumption of FVIII due to a tail-nick blood draw

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 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, RNA sequencing was performed on liver samples to identify potential molecular mechanisms. Targeted follow-up analyses were performed 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 hepatocytes were measured using droplet-digital PCR (ddPCR)
- RNAseq was performed on homogenised 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) and the AAV5 capsid protein, viral protein 3 (VP3), were assessed with immunohistochemistry (IHC)

In vitro methods

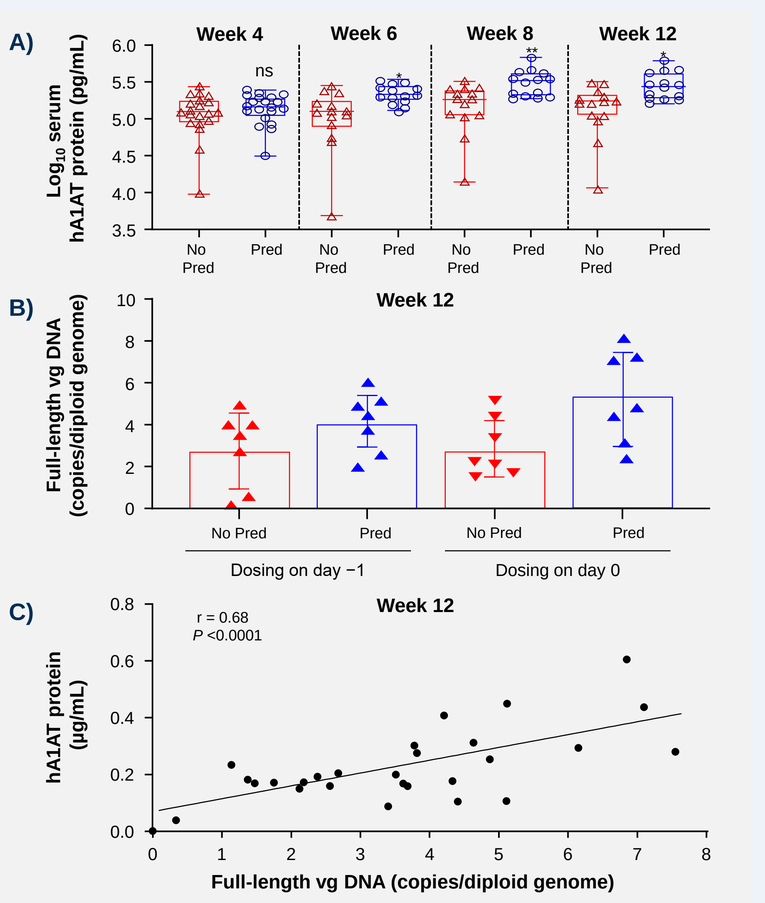
- Human and murine primary hepatocytes were treated with 2.5 µg/mL of prednisolone for 8 hours. RNA was extracted and PDGFRα was measured using ddPCR
- HepG2 cells were transduced with lentivirus containing a short-hairpin RNA (shRNA) targeting the PDGFRα mRNA. After 48 hours, cells were transduced with AAV5-hFVIII-SQ. DNA was harvested on day 4 after AAV transduction, and FVIII DNA was assessed with ddPCR

RESULTS

Prophylactic prednisolone increased transgene expression over 12 weeks

- Mice treated with prophylactic prednisolone on either day −1 or 0 before AAV5 dosing (**Figure 1A)** had higher transgene serum hA1AT protein levels beginning at week 6, possibly mediated by the reduced variation in expression levels in that group (**Figure 2A**)
- At week 12, mice treated with prophylactic prednisolone had higher levels of full-length vector genomes in hepatocytes than those who did not receive prednisolone (**Figure 2B, 2C**)

Figure 2. Effect of prophylactic prednisolone treatment on transgene expression and vector DNA. A) Serum hA1AT protein levels. 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; *P <0.05; **P <0.05 with a two-tailed student's t-test. Correlation was calculated with a Pearson's correlation. Liver samples were collected at takedown.
Symbols are individual data points. In panel A, data are median, Q1, Q3, minimum, and maximum. In panel B, data are mean ± standard deviation. "No Pred" includes mice who received control on day −1 and 0 before AAV; "Pred" includes mice who received prednisolone at on day −1 and 0 before AAV. In panel C, n = 28.
AAV, adeno-associated virus; hA1AT, human α1-antitrypsin; Pred, prednisolone; Q1, first quartile; Q3, third quartile; vg, vector genome; W, week.

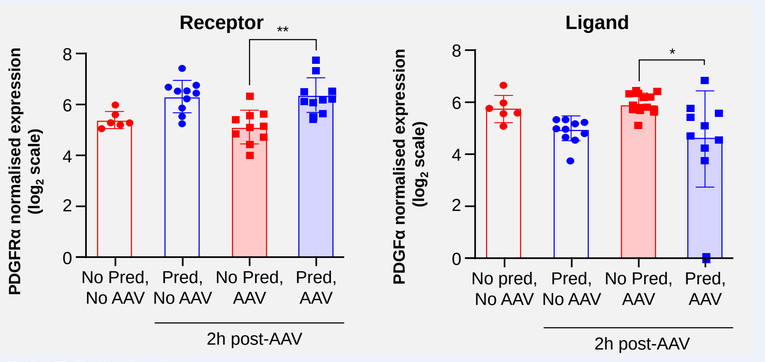
Prophylactic prednisolone suppresses innate immune responses within 24 hours of AAV5 dosing

- We performed RNAseq analyses of liver tissue to identify genes that were differentially expressed at 2 and 24 hours post-AAV5 dosing in response to both prophylactic steroid treatment and AAV5 dosing (**Figure 1B**)
- 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 (P <0.05) and significantly suppressed by prophylactic steroids at 24 hours post-AAV5 dosing *(P* <0.005; data not shown)

Prophylactic prednisolone treatment upregulates expression of AAV5 coreceptor PDGFRα

- We then used our RNAseq dataset to examine the effect of prophylactic corticosteroid treatment on genes involved in initial transduction efficiency
  - We assessed changes in expression of cell surface receptors known to facilitate uptake of AAV5 capsids, including PDGFRα
- At 2 hours post-AAV5 dosing, PDGFRα expression was significantly higher and expression of its ligand PDGFα was significantly lower in the livers of mice who received prednisolone before AAV5 compared with those who did not (**Figure 3**)

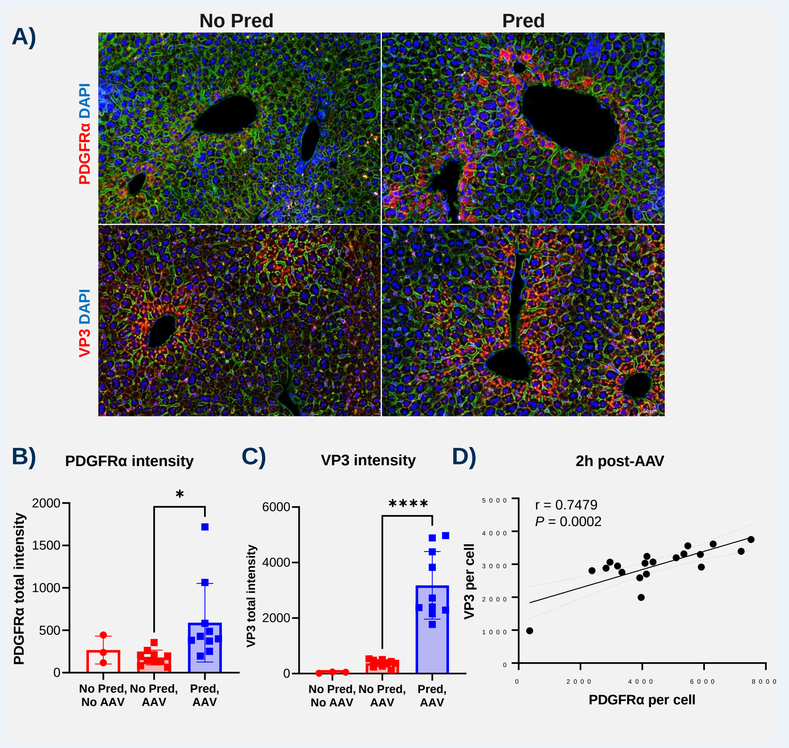
Figure 3. Normalised PDGFRα and ligand PDGFα RNA expression in liver tissue



**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; PDGFα, platelet-derived growth factor alpha; PDGFRα, PDGF receptor alpha; Pred, prednisolone; SD, standard deviation.

- PDGFRα protein assessed with IHC was also significantly higher in the hepatocytes of mice dosed with prophylactic prednisolone before AAV5 treatment than in those who were not (**Figure 4A, 4B**)
  - Prophylactic prednisolone treatment increased capsid uptake in hepatocytes, as assessed with IHC of capsid protein VP3, compared with those that did not receive prednisolone (**Figure 4C**)
  - In addition, PDGFRα expression was positively correlated with VP3 on a per-cell basis (**Figure 4D**)

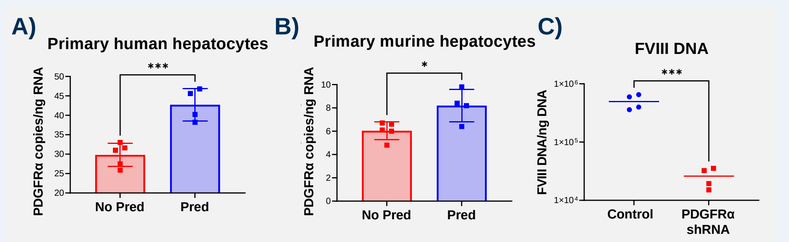
Figure 4. Prednisolone increased PDGFRα expression in hepatocytes and AAV5 capsid uptake 2 hours post-AAV5 dosing. A) Localisation of PDGFRα and VP3 protein in liver. Intensity of B) PDGFRα and C) VP3 staining in the liver. D) Correlation of PDGFRα and VP3 staining



****P <0.0001; *P <0.05 with a one-way ANOVA followed by a Tukey's multiple comparison 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; ANOVA, analysis of variance; DAPI, 4′,6-diamidino-2-phenylindole; h, hour; PDGFRα, platelet-derived growth factor receptor alpha; Pred, prednisolone; SD, standard deviation; VP3, viral protein 3.

- We used additional experiments to confirm these results indicating that prednisolone upregulates PDGFRα, in turn increasing AAV5 capsid uptake
  - In both human and murine primary hepatocytes treated with prophylactic prednisolone, PDGRFα transcript expression was upregulated (**Figure 5A, 5B**)
  - Knockdown of PDGFRα in HepG2 cells with shRNA significantly decreased AAV5 transduction, as indicated by reduced FVIII DNA levels (**Figure 5C**)

Figure 5. Prednisolone treatment upregulates PDGFRα expression in A) primary human and B) murine hepatocytes. C) Knockdown of PDGFRα decreases AAV5 transduction



***P < 0.001; *P < 0.05 with an 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; FVIII, factor VIII; PDGFRα, platelet-derived growth factor receptor alpha; Pred, prednisolone; SD, standard deviation; shRNA, short hairpin RNA.

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 innate immune response
  - Upregulation of the AAV5 coreceptor PDGFRα on hepatocytes and downregulation of its competitive ligand PDGFα
  - 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
- Translatability to humans is supported by the in vitro human hepatocyte model and is currently being assessed in a clinical trial (NCT04323098)

References

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Disclosures

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