Investigating the Impact of Gut Dysbiosis on Liver-Targeted AAV Gene Therapy

Fateme Babaha [1], Erin Burnett [1], Maria Rita Peralta [2], Aomei Mo [1], Paul Batty [1,3], David Lillicrap [1]

1. Department of Pathology and Molecular Medicine, Queen's University, Kingston, Canada
2. Guy's and St Thomas' Hospital, NHS Foundation Trust, London, UK
3. Cancer Institute, University College London, London, UK

Bourses d'études supérieures du Canada Vanier Canada Graduate Scholarships

Introduction

- Gut-liver axis: Gut and liver have a bi-directional interaction which is vital for both organs. The liver secretes bile into the gut through the biliary tract and reciprocally, receives 75% of its blood supply through the portal vein. Also, enterally absorbed nutrients and microbial metabolites drain into the liver sinusoids that is important for hepatic immune function[1].
- Alterations in the gut bacterial composition, or dysbiosis, are recognized to alter the inflammatory state of the liver[2].
- How these changes influence liver-directed gene therapy is unknown.
- An altered liver microenvironment might affect vector transduction, levels of transgene expression, and the durability of expression.

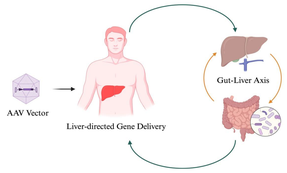


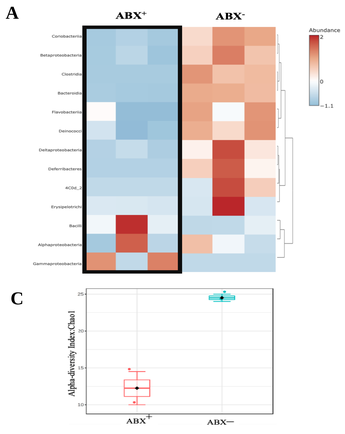
Figure 1: Schematic showing how AAV-gene delivery might be influenced by the gut-liver axis.

Aim

To evaluate the pattern of AAV-mediated transgene expression and immune response in mice following antibiotic-induced dysbiosis.

Results

Cohort 1 & 2: hA1AT transgene



Cohort 3 & 4 : FVIII transgene

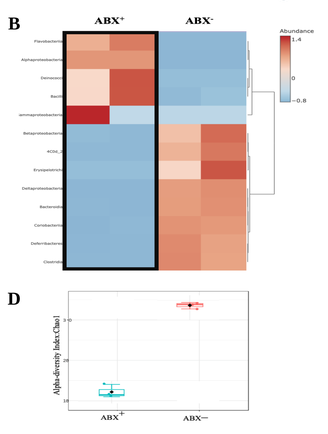


Figure 3: Microbiome analysis of cecal contents shows reduced taxonomic abundance and richness in ABX-treated compared to untreated mice

(A-B) Heatmaps showing relative abundance of bacterial class in ABX-treated and normal microbiome samples of cecal contents. Marked differences are seen in bacterial composition at class level between ABX-treated and untreated mice. Each column in the heatmap represents samples and rows the class of bacteria. (C-D) Alpha-diversity calculated by Chao1 a metric that measures within-sample diversity, shows a significant decrease in class richness in ABX-treated samples compared to untreated mice (t-test).

Conclusions

Liver-directed transgene expression and heterogeneity of responses were not influenced by gut microbiome manipulation. However, there is preliminary evidence of earlier loss of transgene expression in the ABX-treated mice that could be due to an anti-FVIII immune response.

Methods

- Manipulation of gut microbiota was achieved by oral administration of a combination of four antibiotics (ABX): Ampicillin, Metronidazole, Neomycin, and Vancomycin (10 mg/ml of each antibiotic per mouse per day for 10 days).
- DNA extracted from cecal contents was used for bacterial 16s ribosomal RNA(rRNA) sequencing.
- Two cohorts of ABX-treated and untreated wild type C57Bl/6 mice received human alpha 1 antitrypsin (A1AT) AAV5 vector.
- Two cohorts of C57bl/6 F8 knock-out (F8KO) with or without antibiotic treatment received AAV5-canine FVIII-SQ vector.

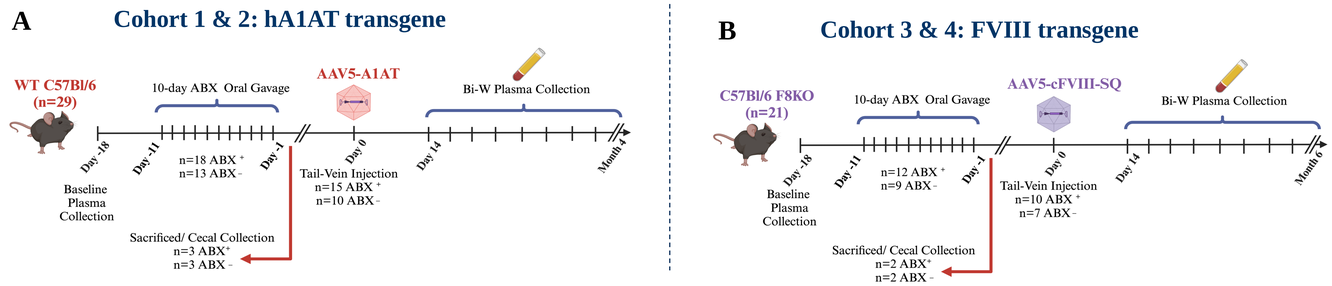


Figure 2: AAV-gene delivery of two transgenes in mice with or without microbiome manipulation.

Mice with normal and manipulated microbiome received (A) hA1AT or (B) FVIII transgene and were followed for 4 and 5 months. Plasma samples were collected. Levels of serum A1AT and plasma FVIII activity levels were assessed using ELISA and Chromogenic assay, respectively. DNA from cecal content was analysed for microbiome alterations in ABX-treated and untreated mice. A1AT: Alpha 1 antitrypsin.

Results

Cohort 1 & 2: hA1AT transgene

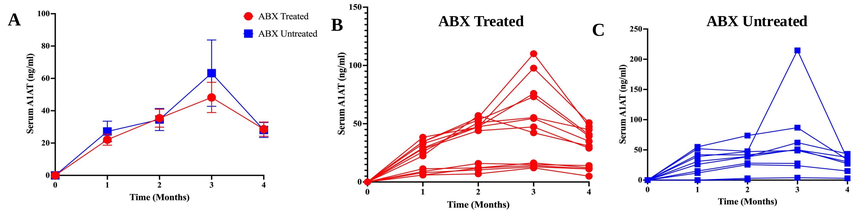


Figure 4: Levels of serum hA1AT measured by human A1AT Elisa: In normal mice, starting from 2 weeks post-delivery, hA1AT expression was seen that peaked at 3 months (48.2- 63.2 ng/ml) followed by a decline at 4 months (Figure 2A). A1AT expression pattern in individual samples are presented in B and C. No significant difference is observed in serum A1AT levels between AXB-treated and untreated samples. Data represented as mean ±SEM

Cohort 3 & 4 : FVIII transgene

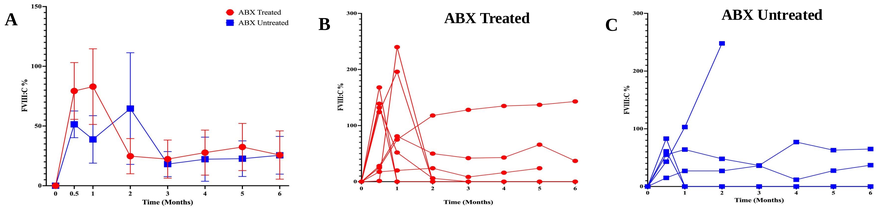


Figure 5: Levels of canine FVIII activity (FVIII:C) measured by Chromogenic assay. (A) In F8KO/ABX-treated mice, peak FVIII:C expression was seen 2-weeks post-delivery (FVIII:C 63%), with a fall (mean FVIII:C = 20%) at 2 months, which remained stable for the next 4 months. In non-ABX treated mice, FVIII:C expression started 2-weeks post-delivery, peaked at 2 months (FVIII:C 46%), then dropped and stabilized (mean FVIII:C = 10%) for the remainder of the study. FVIII expression pattern in individual samples are presented in B and C. No significant difference was seen in levels of transgenic FVIII:C in ABX-treated and untreated mice. Data represented as mean ±SEM

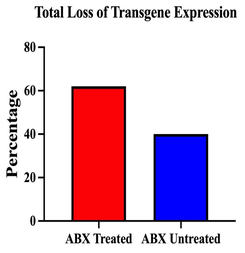


Figure 6: Total loss of expression in F8KO mice could be due to FVIII inhibitor formation.

In mice that received cF8, a drop in mean FVIII:C occurred due to anti-FVIII Ab formation, with total loss of expression in 62% ABX-treated and 40% untreated mice.

Acknowledgments

CBR THE CENTRE FOR BLOOD RESEARCH · BIOMARIN · CIHR IRSC

References

1) Hsu CL, Schnabl B. The gut–liver axis and gut microbiota in health and liver disease. Nature Reviews Microbiology. 2023

2) Doré J, Simrén M, Buttle L, Guarner F. Hot topics in gut microbiota. United European Gastroenterology Journal. 2013;1(5):311-8.