Enhancement of AAV Transduction in Muscle Cells In Vitro

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Introduction

- AAV9 is an efficient vector for gene delivery to muscle in vivo, however transduction of cells in culture is inefficient and necessitates use of high titers.
- Achieving adequate transduction in vitro is important for development of potency assays to support gene therapy clinical studies.
- Several agents are reported to increase transduction in HeLa or HEK293 cells, but few have been evaluated with AAV9, or in terminally differentiated muscle cells (myotubes).
- We sought to identify agents that enhance myotube transduction using AAV9 by screening multiple classes of agents with different mechanisms of action.
- A small panel of agents was screened in a dystrophin deficient murine muscle C2C12 cells using AAV9-CK8-luciferase vector and luciferase activity as readout.

Screening Panel

- Dystrophin-deficient C2C12 cells were differentiated, then treated with agent at 2 concentrations (low / high) for 2 h prior to transduction with AAV9-CK8-luciferase. Luminescence was then measured 96 h post-transduction.
- Agents that increased luciferase activity >2 fold versus DMSO vehicle control were considered top candidates.

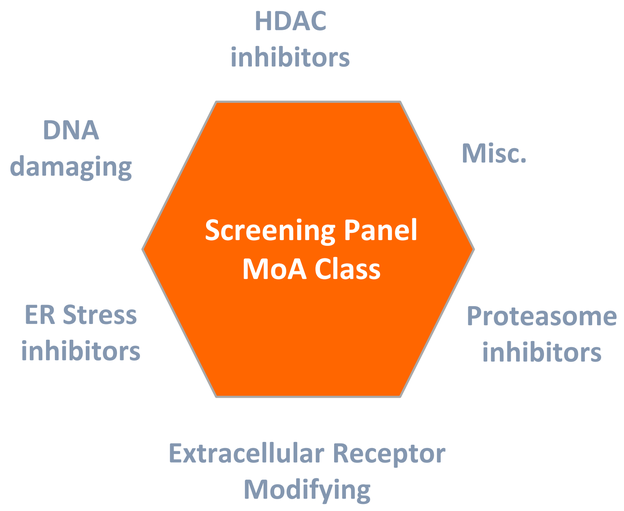

Figure 1. Classes of agents used in screening panel

Results

Top Agent Candidates that reproducibly enhance AAV9 transduction

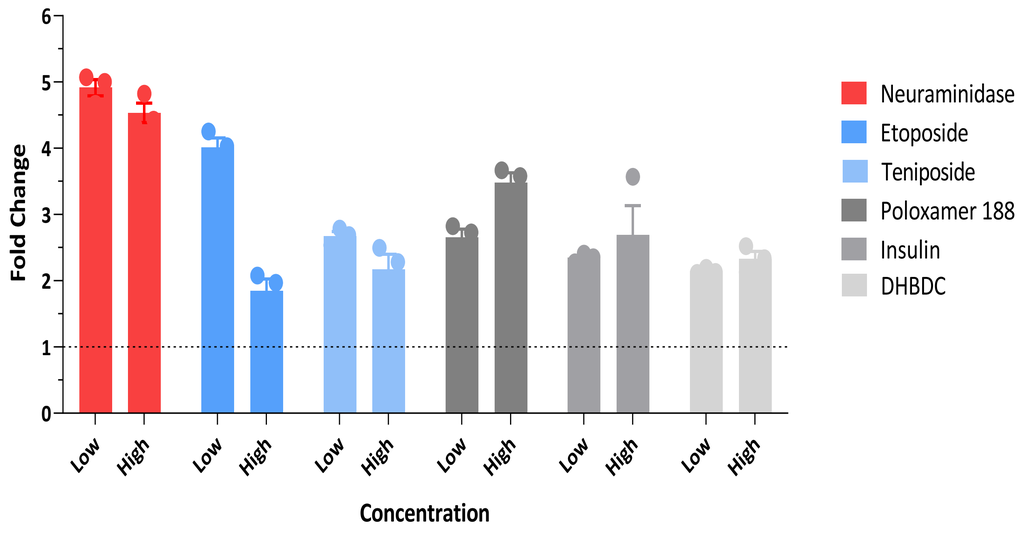

Figure 2. Identification of top candidates with >2-fold increase in luminescence

Dystrophin-deficient C2C12 cells transduced with AAV9-CK8-Luciferase 2 h post-treatment with agent at 2 concentrations (low and high). Luciferase assay was performed 96 h post-transduction. Values represented as fold change above vehicle (DMSO) + AAV9-CK8-luciferase (dashed line). Data are mean ± SD ($p < 0.05$).

Results (cont.)

Topo II inhibitors but not Topo I inhibitors enhanced AAV9 transduction

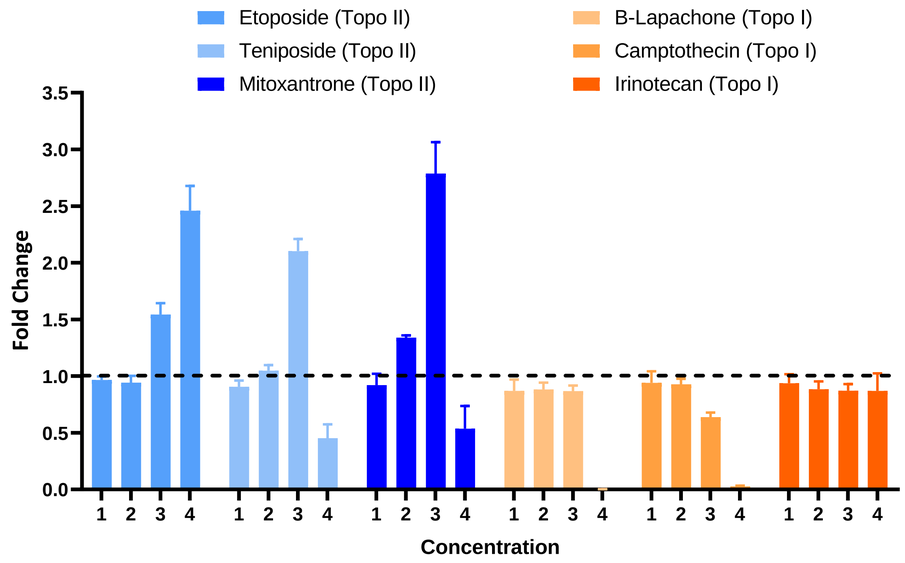

Figure 3. Fold change in luminescence signal from topoisomerase screen

Cells were treated with serial dilutions (1-high concentration to 4-low concentration) of Topo I or Topo II inhibitors for 2 h and then transduced with AAV9-CK8-Luciferase. Luciferase assay was performed at 96 h post-transduction. Values are represented as fold change above vehicle (DMSO) + AAV9-CK8-luciferase (dashed line). Data are mean ± SD ($p < 0.05$).

Dual treatment increased transduction demonstrating a combination effect

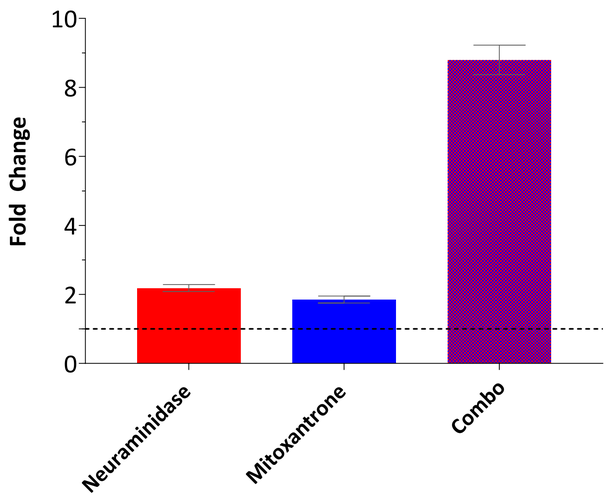

Figure 4. Fold change in luciferase activity following combination pre-treatment

Cells were pre-treated with neuraminidase, mitoxantrone, or a combination of both agents for 2 h and then transduced with AAV9-CK8-Luciferase. Luciferase assay was performed 96 h post-transduction. Values are represented as fold change above vehicle (DMSO) + AAV9-CK8-luciferase (dashed line). Data are mean ± SD ($p < 0.05$).

Mitoxantrone increased expression level and not kinetics of expression

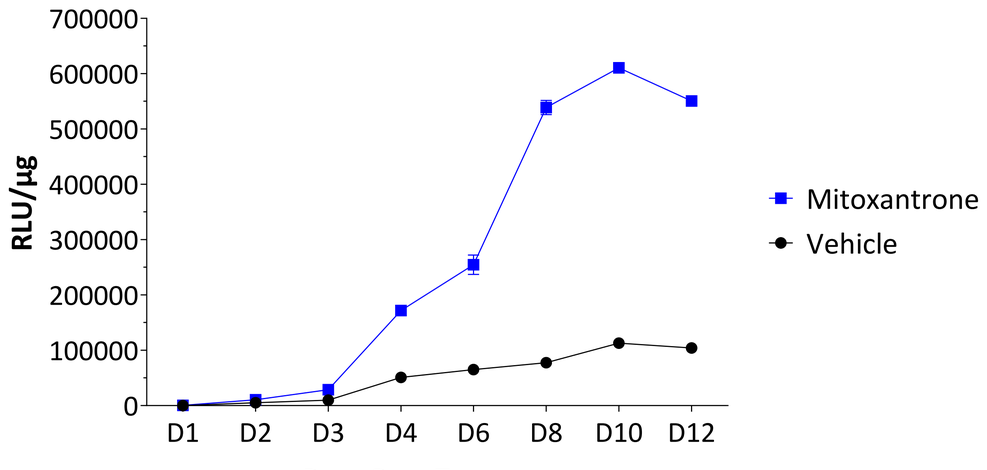

Figure 5. Kinetics of luciferase expression in mitoxantrone treated cells

Cells were pre-treated with either DMSO control or mitoxantrone and then transduced with AAV9-CK8-luciferase. Cells were harvested at multiple time points post-transduction and luminescence measured. Data are mean ± SD.

Conclusions

- Neuraminidase and Topo II inhibitors were among the top candidates in the screening panel and significantly increased AAV9 transduction in differentiated dystrophin-deficient C2C12 myotubes.
- All Topo II inhibitors tested enhanced AAV9 transduction while Topo I inhibitors tested exhibited no effect; data suggests that double strand DNA breaks induced by Topo II inhibitors may be important for their mechanism of action.
- Of the Topo II inhibitors tested, treatment with mitoxantrone had the largest fold change-increase in AAV9 transduction.
- Addition of mitoxantrone did not simply alter the kinetics of luciferase expression but rather increased the overall luciferase expression level.
- Agents from different classes with different MoAs e.g., neuraminidase and mitoxantrone may be combined to achieve a greater than additive effect on AVV9 transduction.
- Identification of these agents that significantly increased muscle cell transduction may advance the development of AAV9-based gene therapies including in vitro potency assays.