

All Trans-Retinoic Acid Regulation of Transgene Expression

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INTRODUCTION:

Adenovectors are a useful protein expression system for the treatment of ocular diseases, such as age-related macular degeneration (AMD).¹ They efficiently transduce a variety of ocular cells following intravitreal (IVT) administration.² Typically, *in vivo* transgene expression is transient and is known to decrease from initial levels.³ An important therapeutic benefit of adenovirus gene transfer for some treatment purposes would be persistence of transgene expression. All trans-retinoic acid (ATRA) treatment has been shown to induce transgene expression *in vitro*.⁴ In the current studies, we investigate two routes (systemic and topical) of ATRA administration in stimulating expression of a marker gene, as well as a therapeutic transgene, pigment epithelium-derived factor (PEDF) after a single, IVT injection of adenovector, and the feasibility of repeated re-expression in the same animal.

PURPOSE:

To determine the parameters for transgene re-expression in the eye using systemic and topically administered ATRA following a single intravitreal (IVT) injection of an adenovector protein expression system.

METHODS:

To assess the feasibility and determine parameters for the use of ATRA for transgene re-expression, C57BL/6 mice received a single IVT injection of 1e9 particles (pu) of adenovector (AdL.11D or AdPEDF.11D) at Day 0. Animals then received a single, intramuscular (IM) or topical dose of ATRA (1 or 2 mM) on day 28, 59 or 89 post vector injection. Animals were sacrificed 24 hours post ATRA injections, eyes harvested and assayed for luciferase activity or PEDF levels. To determine whether transgene re-expression could be repeated within the same animal, animals received a single, IVT injection of AdL.11D (1e9 pu) at Day 0. Animals then received multiple intramuscular ATRA (1 mM) injections on days 28, 59 and 89. Animals were sacrificed 24 hours post ATRA injection, eyes harvested and assayed for luciferase activity.

Transgene Expression Initially is High and Transient Following a Single, Intravitreal Injection of Adenovector

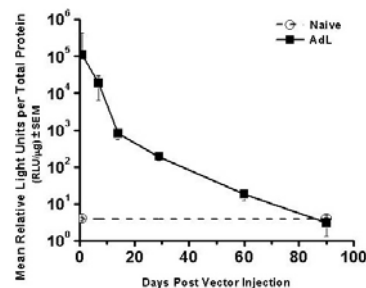


Figure 1. Luciferase Expression Profile Following a Single, IVT Injection of Adenovector.

Luciferase activity in whole mouse eyes following a single, IVT injection of AdL.11D (1e9 pu). On 1, 7, 14, 29, 60 and 90 days post vector injection, eyes were harvested and luciferase activity quantitated per manufacturer's instructions (Promega, Madison, WI). Data are expressed as the mean ± SEM (error bars) of 5 separate experiments (n= ~5 animals/treatment group/time point).

Result: Luciferase expression increased up to approximately 4-logs on day 1 post injection and then declined to background levels by day 90.

Systemically Administered ATRA Stimulates Transgene Re-Expression

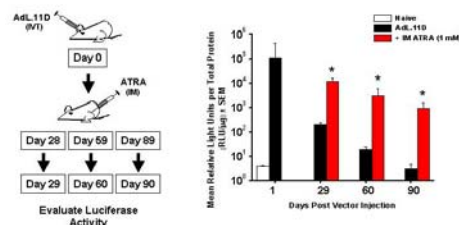


Figure 2. Systemically Administered ATRA Can Stimulate Luciferase Re-Expression at days 29, 60 and 90.

C57BL/6 mice received a single, IVT injection of AdL.11D (1e9 pu) on Day 0. Animals then received a single, IM dose of ATRA (1 mM) on day 28, 59 or 89 post vector injection. Approximately 24 hours post ATRA injection, eyes were harvested and luciferase activity quantitated per manufacturer's instructions (Promega, Madison, WI). Data are expressed as the mean ± SEM (error bars) of 4 separate experiments (n= ~5 animals/treatment group/time point).

Result: Following a single, IM injection of ATRA on day 28 or 59 or 89, luciferase activity increased approximately 2-logs above vector alone on day 29, 60 and 90, respectively. There is a statistically significant difference between luciferase activity in the ATRA treated versus vector only treated groups at all time points (P < 0.05, two-tailed Student's t-test).

Transgene Re-Expression by ATRA can be Repeated

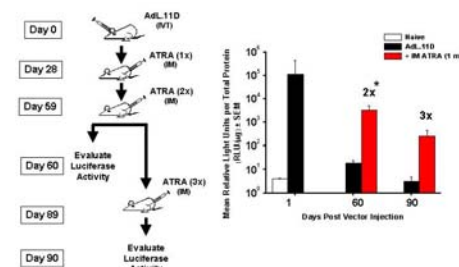


Figure 3. Multiple Systemic Injections of ATRA Stimulates Luciferase Re-Expression 2 and 3 Times.

C57BL/6 mice received a single, IVT injection of AdL.11D (1e9 pu) on Day 0. Animals then received multiple IM doses of ATRA (1 mM) on day 28, 59, and 89 post vector injection. Approximately 24 hours post final ATRA injection (day 60 or 90), eyes were harvested and luciferase activity quantitated per manufacturer's instructions (Promega, Madison, WI). Data are expressed as the mean ± SEM (error bars) of 3 separate experiments (n= ~5 animals/treatment group/time point).

Result: Pulsatile expression of a transgene is possible. Following 2 or 3 IM injections of ATRA, luciferase activity was increased approximately 2-logs above vector alone. There is a statistically significant difference between luciferase activity in the ATRA treated versus vector only treated group at day 60 post vector injection (P < 0.05, two-tailed Student's t-test).

Topically Administered ATRA Stimulates Transgene Re-Expression

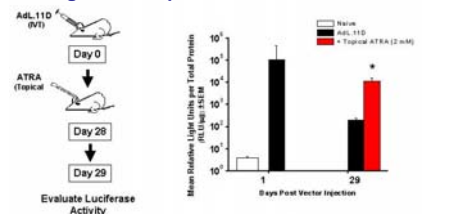


Figure 4. Topically Administered ATRA Stimulates Luciferase Re-Expression.

C57BL/6 mice received a single, IVT injection of AdL.11D (1e9 pu) on Day 0. Animals then received a single, topical dose of ATRA (2 mM) on day 28 post vector injection. Approximately 24 hours post ATRA injection, eyes were harvested and luciferase activity quantitated per manufacturer's instructions (Promega, Madison, WI). Data are expressed as the mean ± SEM (error bars) of 3 separate experiments (n= ~5 animals/treatment group/time point).

Result: Following a single, topical dose of ATRA, luciferase activity increased approximately 2-logs above vector alone. There is a statistically significant difference between luciferase activity in the ATRA treated versus vector only treated group (P < 0.05, two-tailed Student's t-test).

Topically Administered ATRA Stimulates Therapeutic Transgene Re-Expression

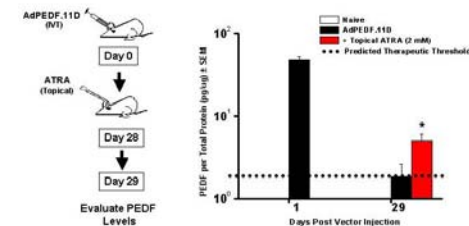


Figure 5. Topically Administered ATRA Stimulates PEDF Re-Expression.

C57BL/6 mice received a single, IVT injection of AdPEDF.11D (1e9 pu) on Day 0. Animals then received a single, topical dose of ATRA (2 mM) on day 28 post vector injection. Approximately 24 hours post ATRA injection, eyes were harvested and PEDF levels quantitated by ELISA (GenVec, Inc). The predicted therapeutic threshold is indicated by the dashed line.⁵ Data are expressed as the mean ± SEM (error bars) of 3 separate experiments (n= ~5 animals/treatment group/time point).

Result: Following a single, topical dose of ATRA, PEDF levels were statistically significant, and increased approximately 2-fold above vector alone (P < 0.05, two-tailed Student's t-test). This is within the range predicted to be therapeutically relevant.

CONCLUSIONS:

- Re-expression of transgene following a single intravitreal administration of adenovector was achieved using the clinically relevant compound, ATRA.
- Transgene re-expression can be induced multiple times from a single, intravitreal administration of adenovector in a single animal.
- Topical administration of ATRA appears to be a feasible route of inducing transgene re-expression.
- Pulsatile regulation of a therapeutic transgene, PEDF, in the eye was demonstrated and led to PEDF levels above the predicted therapeutic threshold level.

REFERENCES:

1. D'Amato RJ et al. Angiogenesis inhibition in age-related macular degeneration. *Ophthalmology*. 1995; 102:1261-62.
2. Mori K et al. Intraocular adenoviral vector mediated gene transfer in proliferative retinopathies. *Invest Ophthalmol Vis Sci*. 2002; 43: 1610-15.
3. Yang Y et al. Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. *Proc Natl Acad Sci USA*. 1994; 91:4407-11.
4. Mountford JC et al. All trans-retinoic acid increases transgene expression in MSCV-transduced cells, via a mechanism that is retinoid receptor dependent but independent of cellular differentiation. *Human Gene Ther*. 2005; 16: 132-38.
5. Mori K. et al. AAV-Mediated Gene Transfer of Pigment Epithelium-Derived Factor Inhibits Choroidal Neovascularization. *IOVS*. 2002; 43:1994-2000.

