

Ocular Pharmacokinetics Of Pigment Epithelium-Derived Factor (PEDF) Following Adenovector-Based Gene Delivery Indicate That Low Levels Of PEDF Protein Are Therapeutic

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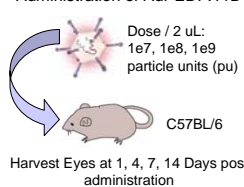
Purpose:

Pigment epithelium-derived factor (PEDF) is a secreted protein with proven anti-angiogenic and neuroprotective properties. PEDF delivery to the retina via adenovector-mediated gene transfer whether by intravitreal or periorcular delivery has been shown to inhibit choroidal neovascularization in animal studies. These experiments were designed to examine PEDF protein levels in ocular tissues following a single intraocular or periorcular injection of AdPEDF.11D (a second-generation replication-deficient adenovector)

Methods:

Adult female C57BL/6 mice were given a single intravitreal or periorcular injection of AdPEDF.11D (an E1-/partial E3-/E4-vector expressing human PEDF) into the right eye at doses of 1e9, 1e8, or 1e7 particle units (pu)/eye. Mice were sacrificed at 1, 4, 7, and 14 days post vector injection, and PEDF levels assessed in whole eyes and various ocular tissues (aqueous humor, cornea, iris/ciliary body, retina, RPE/Choroid, sclera and conjunctiva) using an enzyme-linked immunosorbant assay (ELISA).

Administration of AdPEDF.11D



Evaluate hPEDF by ELISA

Results:

Expression of PEDF following administration of AdPEDF.11D was evaluated at 1, 4, 7, and 14 days in murine whole eyes. The peak ocular concentration for all doses tested occurred within 24 hrs with PEDF expression declining thereafter. Both intravitreal and periorcular delivery resulted in dose-dependent PEDF protein expression. The highest dose (1e9 pu) delivered periorcularly gave comparable PEDF protein levels to that of the lowest dose (1e7 pu) delivered intravitreally at day one. This is particularly interesting, since the lowest dose of AdPEDF.11D (1e7 pu) administered intravitreally did not result in significant inhibition of choroidal neovascularization in the murine laser-induced rupture of Bruch's membrane model, yet 1e9 pu of AdPEDF.11D given periorcularly resulted in approximately 80% inhibition in this same model (Gehlbach *et al.*, 2003). This observation led us to investigate further the distribution of PEDF levels within various ocular fractions, especially the retina and the RPE/choroid. Preliminary data suggest that expression of only a small amount of PEDF protein at the right location is sufficient to inhibit aberrant choroidal neovascularization (Mori *et al.*, 2001 and 2002).

PEDF Levels in Whole Eyes

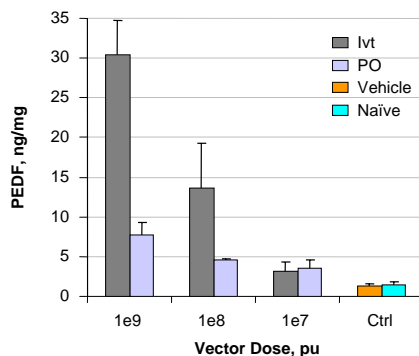


Figure 1. Dose dependant expression of hPEDF in whole eyes 1 day after administration of AdPEDF.11D. PEDF was measured by ELISA and total protein determined by the Bradford assay. Ivt = intravitreal; PO = periorcular; ctrl = controls.

Time Course

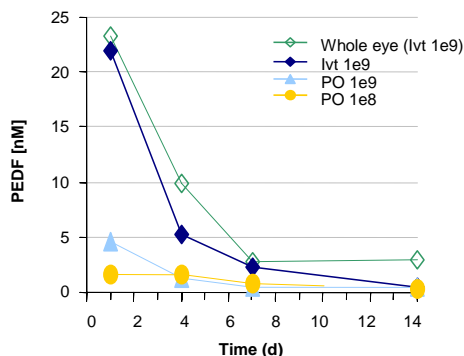


Figure 2. Time course of hPEDF expression. The highest level of hPEDF is detected within 24 hrs after administration of AdPEDF.11D. Regardless of the delivery method PEDF levels decline steadily with a $t_{1/2}$ of approximately 40 hrs for intravitreal delivery and 50 hrs for periorcular delivery. Note the higher level of expression following intravitreal delivery. Open diamonds = whole eye data for intravitreal administration; solid diamonds = sum of PEDF found in ocular fractions after intravitreal delivery; triangles = sum of PEDF found in ocular fractions after periorcular delivery of 1e9 pu; solid circles = sum of PEDF found in ocular fractions after periorcular delivery of 1e8 pu. The amount of PEDF in the whole eye after periorcular delivery is similar to the sum of the parts (data not shown)

Distribution

time (days)	Cornea	Iris/Ciliary Body	Retina	RPE / Choroid	Sclera	Conjunctiva
Naive 0	1.1	0.5	0.4	0.7	9.7	0.7
Ivt 1e9 1	193.9	50.1	12.3	49.4	123.6	44.6
Ivt 1e9 4	31.2	12.8	8.4	0.9	32.4	19.0
Ivt 1e9 7	6.6	2.9	0.4	0.7	28.2	14.4
Ivt 1e9 14	0.5	0.3	0.4	0.3	7.2	0.4
PO 1e9 1	13.0	2.3	0.8	7.9	73.5	7.1
PO 1e9 4	0.4	0.8	0.2	0.5	17.0	8.8
PO 1e9 7	1.3	0.3	0.3	0.6	1.5	2.0
PO 1e9 14	0.7	0.4	0.2	0.5	0.4	5.8
PO 1e8 1	13.1	0.7	0.4	2.5	17.3	1.4
PO 1e8 4	0.8	2.6	0.3	0.4	0.4	14.7
PO 1e8 7	0.7	3.0	0.2	0.7	0.7	8.4
PO 1e8 14	0.5	0.5	0.2	0.3	0.5	0.9

Figure 3. Ocular distribution of hPEDF, in ng/mg of total protein, after a single injection of AdPEDF.11D. At day 1 intravitreal delivery results in PEDF distributed largely towards the front of the eye (40% in the cornea vs. 12 % for periorcular), and periorcular delivery shows greater distribution towards the rear of the eye (70% in the sclera vs. 25% for intravitreal). Ivt = intravitreal; PO = periorcular

Efficacy

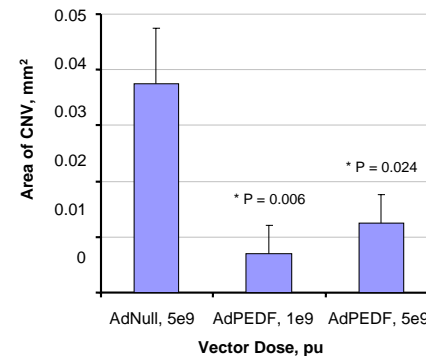


Figure 4. Efficacy of hPEDF in a mouse model of wet AMD. Gehlbach *et al.*, 2003, has demonstrated that an adenoviral vector expressing PEDF significantly suppresses choroidal neovascularization following periorcular administration, when compared to AdNull delivery. The vector used for that experiment was the same one used in this study (see fig. 2). * = Compared to AdNull

Conclusions:

Small amounts of PEDF protein at the right location are sufficient for inhibition of ocular neovascularization. Periorcular delivery of 1e9 particles of AdPEDF.11D, which results in lower overall level of PEDF expression when compared to the same dose via intravitreal administration (fig. 2), account for nearly 80% inhibition of choroidal neovascularization in a CNV model (fig. 4). These data suggest that low levels of PEDF protein may also be adequate to provide therapeutic benefit in humans, and warrant the use of adenovector-based gene delivery for the treatment of ocular neovascular diseases.