

Supplementary Materials: Retina-Targeted Delivery of 17 β -Estradiol by the Topically Applied DHED Prodrug

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Table S1. 2 \times 2 \times 3 factorial ANOVA of ex vivo steady-state transcorneal fluxes (J_{ss}) listed in Table 1.

Factor/Interaction	Type III Sum of Squares	Degree(s) of Freedom	F	<i>p</i>	η^2
Compound ¹	385,008	1	577	<0.001	0.472
Formulation ²	217,716	1	326	<0.001	0.267
Species ³	68,634	2	51.4	<0.001	0.084
Compound \times Formulation	56,421	1	84.6	<0.001	0.069
Compound \times Species	46,099	2	34.5	<0.001	0.056
Formulation \times Species	16,006	2	12.0	<0.001	0.020
Compound \times Formulation \times Species	10,226	2	7.66	0.003	0.013
Error	16,015	24			0.020

¹ E2 and DHED (2); ² Without and with HP β CD (2); ³ Rat, rabbit and pig (3).

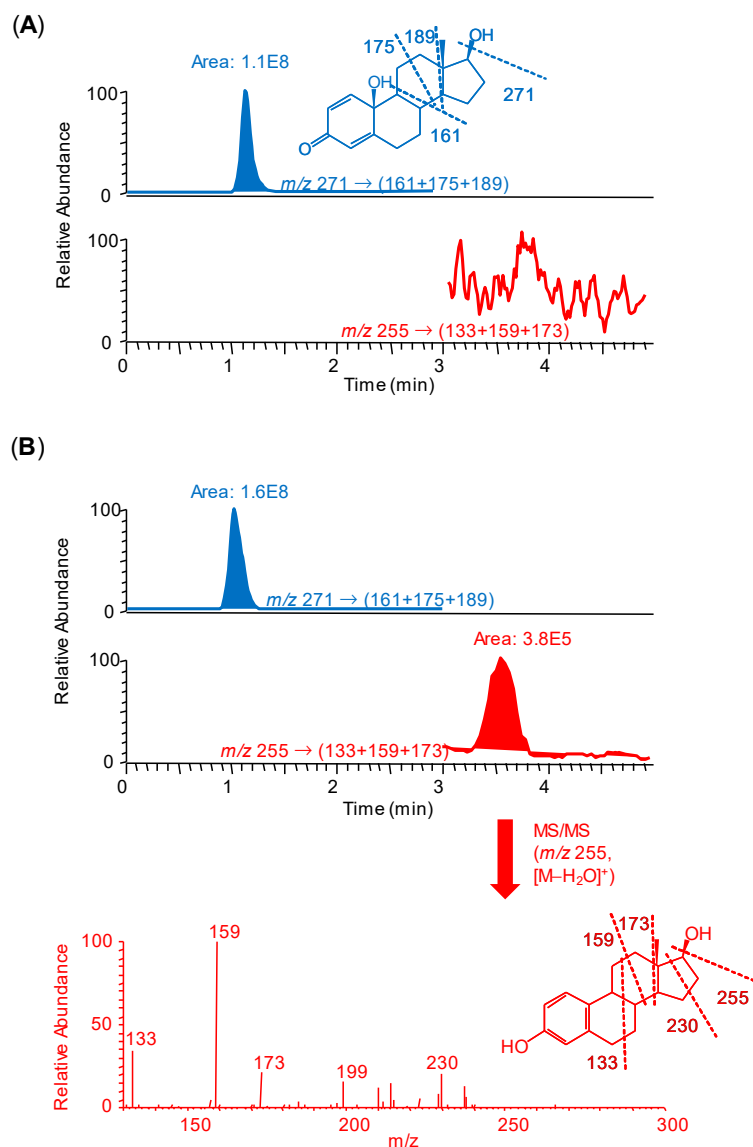


Figure S1. In vitro studies on DHED to E2 metabolism. **(A).** Evidence for no detectable DHED (blue) to E2 (red) conversion in the acceptor compartment of the diffusion chamber used as established by LC-APCI-MS/MS assay during a 2 h in vitro transport experiment of DHED (10 μ M) through pig cornea. Experiments in pig and rat cornea homogenates (20% *w/v*) after incubation of DHED (100 nmol/mL) at 37 °C yielded the same results as shown in chart **A**. **(B).** Conversion of DHED (blue) to E2 (red) occurred rapidly in the pig retina with 533 ± 158 pmol·min⁻¹ (mg protein)⁻¹ (average \pm SEM, $n = 3$) initial rate of formation.

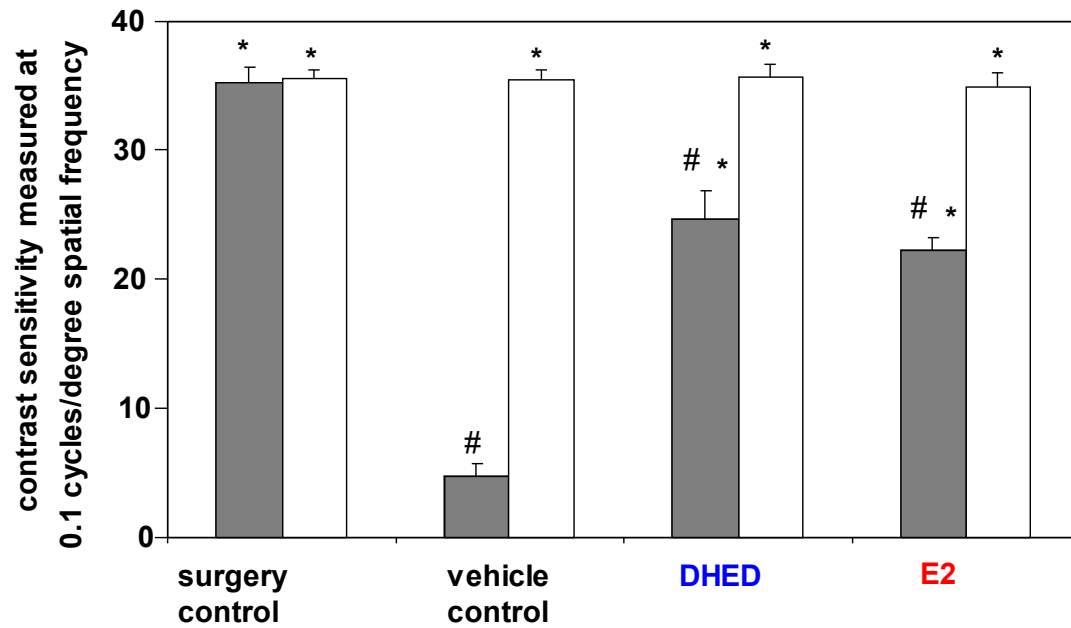


Figure S2. Neuroprotective effect of DHED-derived E2 on visual function measured as contrast sensitivity at a given spatial frequency in a rat model of glaucoma [17]. E2 eye drops (0.1% *w/v* in 20 % HPBC in saline vehicle) [3,19] served as positive control. DHED (0.1% *w/v* in 20 % *w/v* HPBC in saline vehicle) or 20 % *w/v* HPBC in saline vehicle was administered q.d. as eye drops for three weeks, analogously to that of E2 [3,19]. Contrast sensitivity was measured at a given spatial frequency for each treated eye individually in which IOP was increased (approximately by 25% from baseline [3]) and compared to the ipsilateral vehicle treated eye (white) (*; $p < 0.05$, $n = 3$ per group) or to the sham surgery control (#; $p < 0.05$; $n = 3$ per group) [35].