

Restoring visual function to the blind retina with a potent, safe and long-lasting photoswitch

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Supplementary Information

Supplementary Materials:

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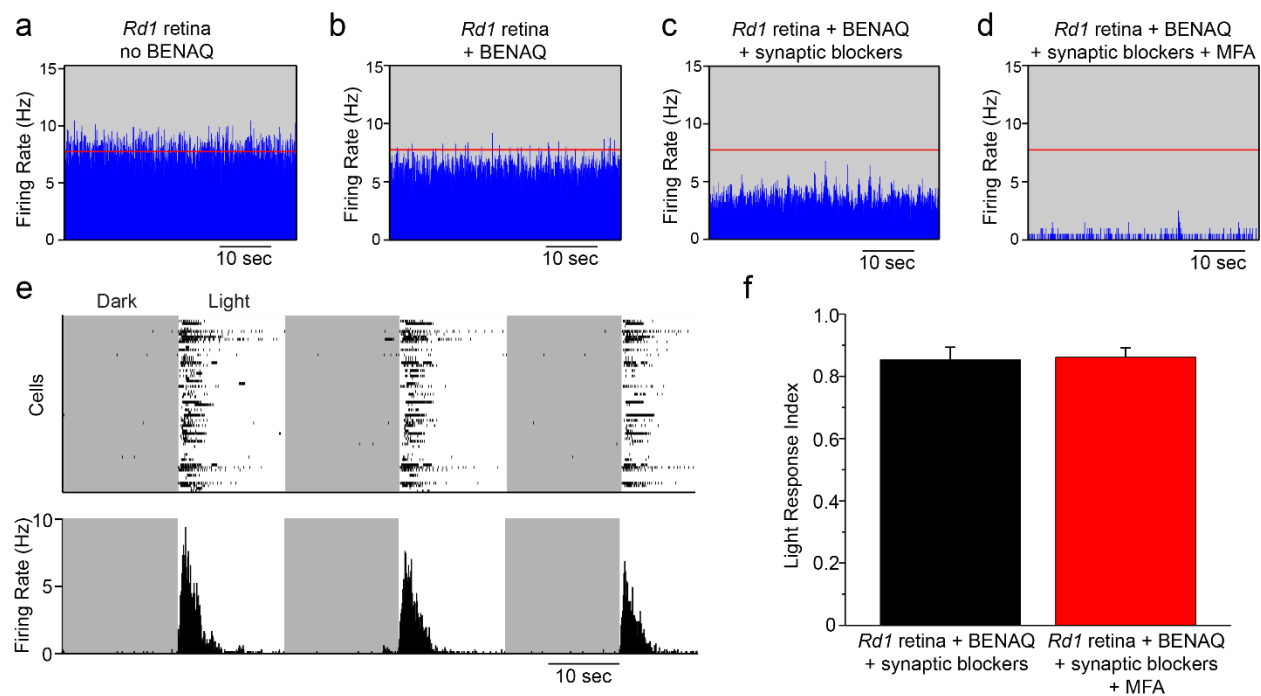


Figure S1. Effect of synaptic blockers and gap junction blockers on *rd1* RGC spontaneous activity and photosensitization.

a-d) MEA recording of average *rd1* RGC activity in the dark before BENAQ treatment (a), after BENAQ treatment (b), after application of synaptic blockers (c) and after addition of synaptic blockers + MFA (d). The average firing rate for the untreated retina is denoted by the red line.

BENAQ slightly reduces the spontaneous RGC activity, while block of synaptic transmission and gap junctions greatly reduces spontaneous RGC activity.

e) MEA recording of RGC activity from an *rd1* retina after treatment with BENAQ, synaptic blockers and MFA. Even in the absence of synaptic and electrical input, RGCs treated with BENAQ remain light sensitive.

f) Quantification of BENAQ-mediated light responses in synaptically isolated *rd1* RGCs prior to and after addition of MFA (n=4 retinas, p=0.88, t-test).

Distance (μm)	Median LRI	95% confidence interval
Target	.60	.41 to .78
200-400	.01	0.00 to 0.03
400-600	-.01	-0.02 to 0.00
600-800	0.00	-0.01 to 0.01
800-1200	0.00	-0.02 to 0.02

Table S1. Responses of BENAQ-treated *rd1* RGCs to targeted small spot illumination.

BENAQ pharmacokinetic parameters	rabbit plasma	BENAQ vitreous humor	BENAQ retina	BENAQ choroid
T _{max} (hours)	ND	24	24	168
C _{max} (ng/g or ng/mL)	ND	520	89100	20200
t _{1/2} elimination (hours)	ND	130	571	ND
AUC _{0-last} (ng*h/g or ng*h/mL)	ND	66200	2.09x10 ⁷	5.74x10 ⁶

AUC _{0-inf} (ng*h/g or ng*h/mL)	ND	76600	6.08x10 ⁷	ND
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Table S2. Pharmacokinetic parameters of BENAQ after intraocular injection in rabbits.

T_{max}: time point at which maximal concentration was detected. C_{max}: maximal concentration (units: retina - ng/g, choroid – ng/g, vitreous humor – ng/mL). t_{1/2}: half-life. AUC: Area Under the Curve. ND: Not Determined. BENAQ not detected in plasma.

Treatment	Vehicle		30µM BENAQ		100µM BENAQ	
	1	2	1	2	1	2
Animal number						
Organ/finding						
Left Eye	Y	Y	Y	Y	Y	Y
Mononuclear cell infiltrates, bulbar conjunctiva, at limbus	1	0	0	0	0	1
Mononuclear cell infiltrates, vitreous	1	2	1	1	2	2
Mononuclear cell infiltrates, optic disc	0	1	0	0	1	1
Mononuclear cell infiltrates, optic nerve	0	2	0	0	0	0
Material, fibrillar/precipitates, vitreous	P	P	P	P	P	P
Lenticular degeneration, posterior lens, subcapsular	0	0	0	0	0	0
Retinal degeneration	0	0	0	0	0	0
Retinal detachment	0	0	0	0	0	0

Right Eye	Y	Y	Y	Y	Y	Y
Mononuclear cell infiltrates, bulbar conjunctiva, at limbus	1	0	0	0	0	1
Mononuclear cell infiltrates, vitreous	1	2	1	1	2	2
Mononuclear cell infiltrates, optic disc	0	1	0	0	1	1
Material, fibrillar/precipitates, vitreous	P	P	P	P	P	P
Lenticular degeneration, posterior lens, subcapsular	0	0	0	0	0	0
Retinal degeneration	0	0	0	0	0	0
Retinal detachment	0	0	0	0	0	0

Table S3. Tabulated rabbit ocular histopathologic data after intravitreal injection of vehicle or BENAQ.

Labels: Y=tissue analyzed, P=present, 0=absent, 1=minimal amount/severity, 2=mild amount/severity. Ocular tissues were evaluated histologically at 15 days after a single injection of 50 μ L vehicle (Lucentis formulation saline) (n=4 eyes), 600 μ M BENAQ (n=4 eyes) or 2 mM BENAQ (n=4 eyes), for a predicted final vitreal concentration of 30 μ M or 100 μ M BENAQ, assuming a 20x dilution in rabbit vitreous (1mL volume). No signs of retinal toxicity were observed in any of the vehicle or BENAQ treated retinas. The presence of eosinophilic material and minimal to mild mononuclear cell infiltrates (lymphocytes and macrophages) was detected in the vitreous in all vehicle-injected and BENAQ-injected eyes, most likely a result of the injection procedure itself. 50% of the eyes injected with saline or 2 mM BENAQ displayed minimal

mononuclear cell infiltrates in the bulbar conjunctiva at the limbus. This finding when present at this limited intensity is usually a spontaneous observation in Dutch Belted rabbits used in laboratory studies.