TITLE PAGE

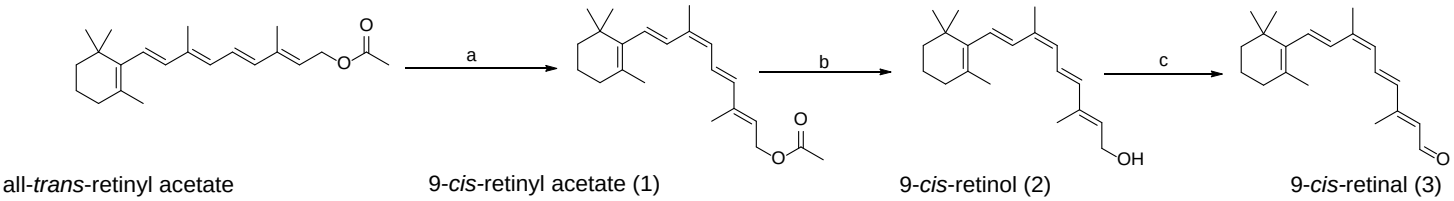
Retinal-chitosan conjugates effectively deliver active chromophores to retinal photoreceptor cells in blind mice and dogs

Songqi Gao, Shirin Kahremany Jianye, Zhang, Beata Jastrzebska, Janice Querubin, Simon M Petersen-Jones, and Krzysztof Palczewski

Department of Pharmacology and Cleveland Center for Membrane and Structural Biology, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA (S. G., S. K., J. Z., B. J. K. P.)

Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, 736 Wilson Road, D-208., East Lansing, MI 48864, USA (J. Q., S. M. P-J.)

SUPPLEMENTARY FIGURES



all-*trans*-retinyl acetate 9-*cis*-retinyl acetate (1) 9-*cis*-retinol (2) 9-*cis*-retinal (3)

Figure 1. Synthesis pathway of 9-*cis*-retinal (3). a) $(benzonitril)_2PdCl_2$, trimethylamine, hexane at 65 ^oC, 20 h; b) NaOH, ethanol at 40 ^oC, 30 min; c) MnO_2, methylene chloride 24 h.

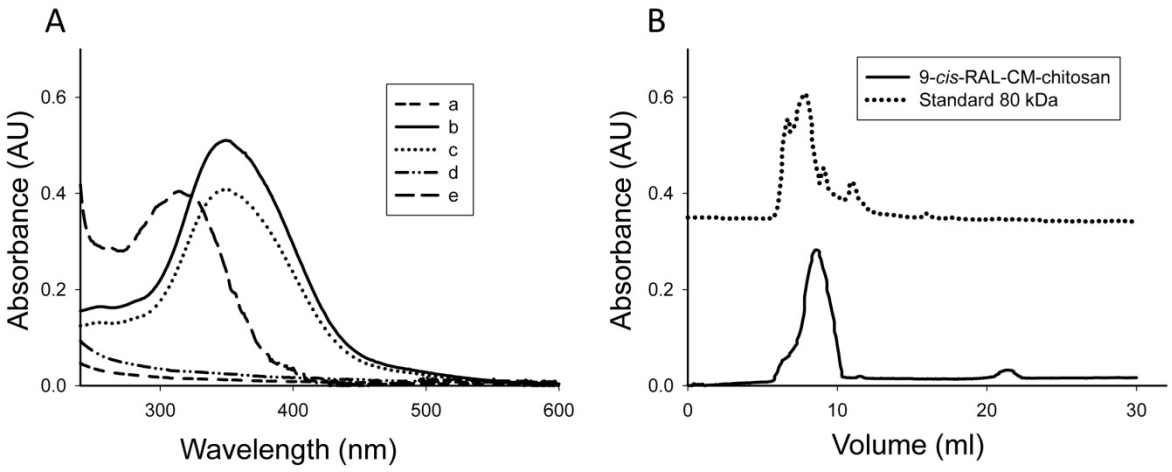


Figure 2. UV-visible absorbance spectra and MW estimation of CM-chitosan conjugates. (**A**) UV-visible absorbance spectra: a, CM-chitosan; b, 9-*cis*-RAL-CM-chitosan conjugate; c, 9-*cis*-RAL oxime; d, CM-chitosan after hydroxylamine treatment; e, product after reduction of Schiff-base in 9-*cis*-RAL-CM-chitosan conjugate. (**B**) MW estimation; gel filtration chromatography reveals that the MW of the CM-chitosan conjugate is about 80 kDa.

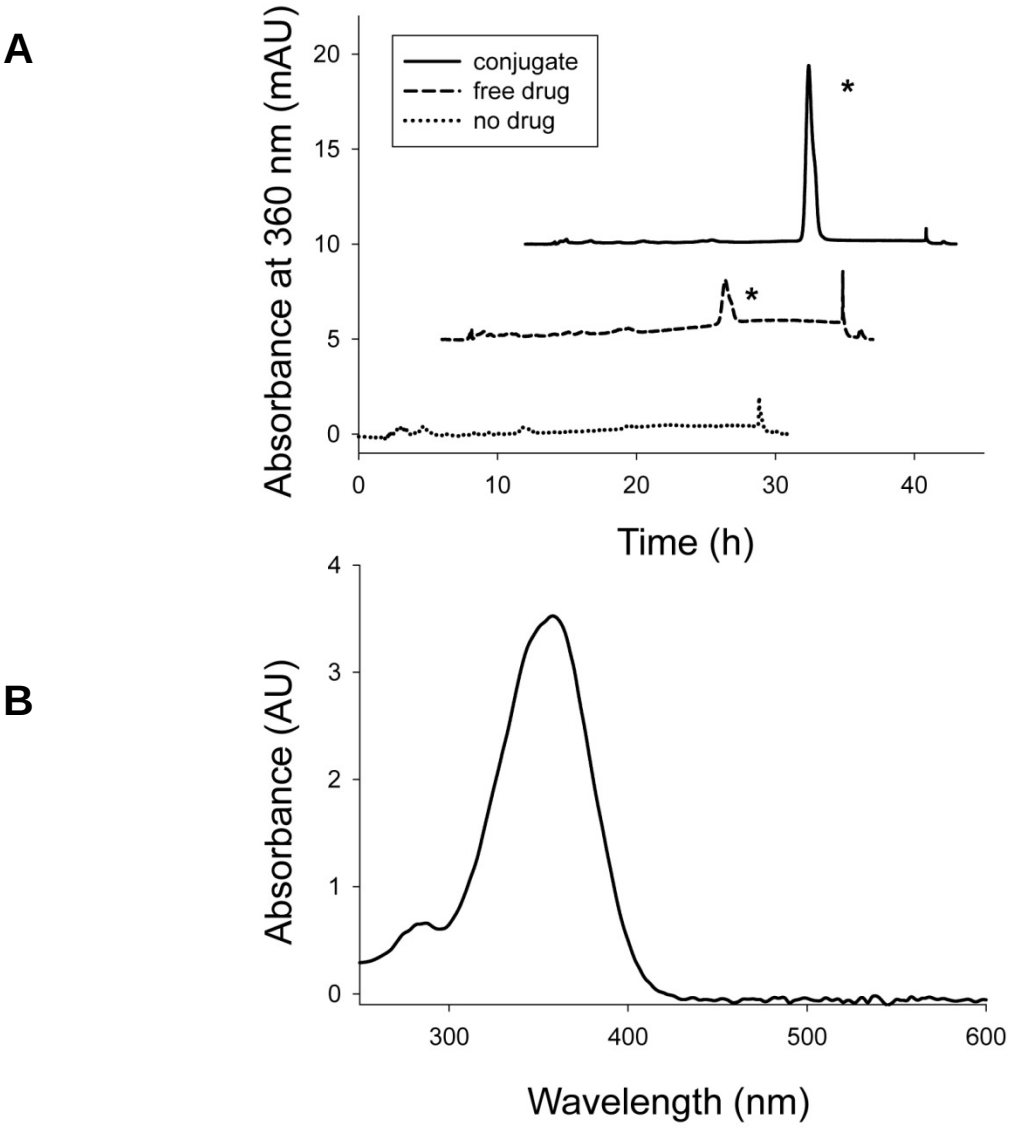


Figure 3. HPLC analysis of 9-*cis*-retinoid in eye samples obtained from *Lrat*$^{-/-}$ mice. (**A**) The 9-*cis*-retinoid peaks (indicated by asterisks) were detected in eye samples 24 h after oral administration of either free 9-*cis*-RAL or the 9-*cis*-RAL-CM-chitosan conjugate at a dose of 45 mg/kg of 9-*cis*-RAL or 9-*cis*-RAL equivalent. The chromatogram reveals that more 9-*cis*-retinoid accumulated in eyes of *Lrat*$^{-/-}$ mice that received the conjugate. (**B**) UV-visible absorbance spectrum of 9-*cis*-retinoid.

Figure 4

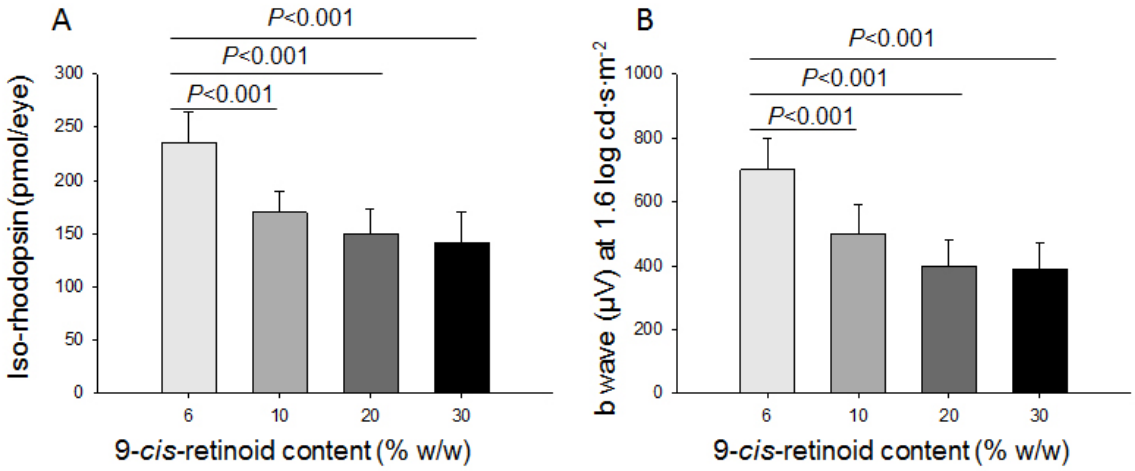


Figure 4. Percentage of 9-*cis*-retinoid contained in 9-*cis*-RAL-CM-chitosan conjugates affect iso-Rho levels and ERG responses. Iso-Rho levels and ERG responses in *Lrat*$^{-/-}$ mice were determined 2 days after oral gavage of the conjugates at a dose of 90 mg/kg of 9-*cis*-RAL equivalent. A water-soluble conjugate containing 6% 9-*cis*-retinoid leads to a significantly higher ocular level of isoRho and ERG response, as compared to three water-insoluble conjugates containing 10%, 20% and 30% 9-*cis*-retinoid, (P < 0.001). (**A**) Iso-Rho levels versus weight percentages of 9-*cis*-retinoid in the conjugate. (**B**) ERG b-wave amplitudes versus weight percentages of 9-*cis*-retinoid in the conjugate. Data are presented as the mean ± SD, n=5 for each group.

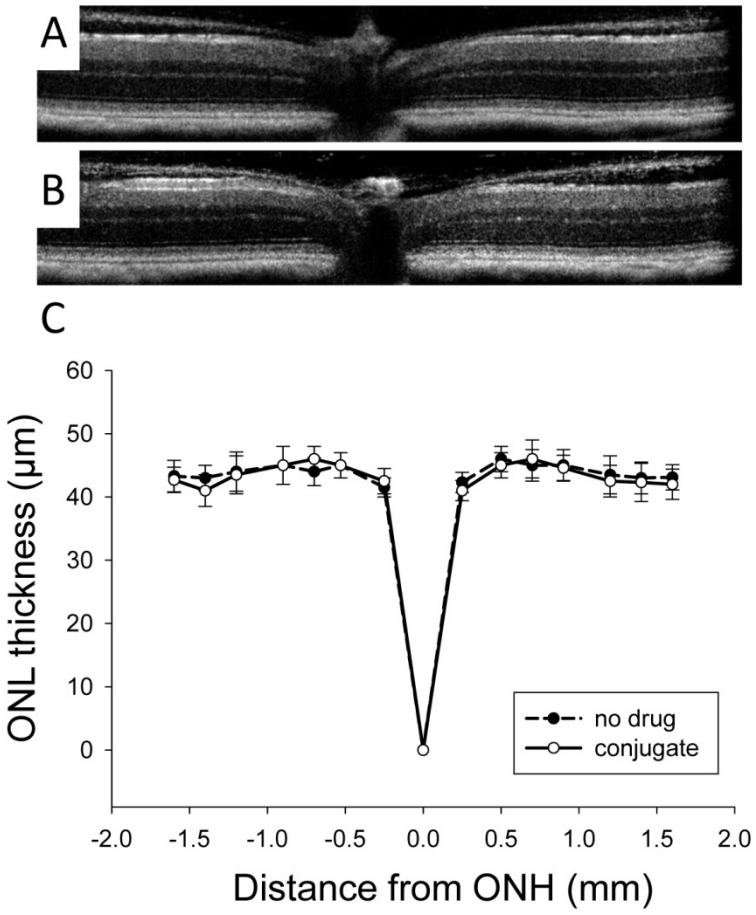


Figure 5. ONL morphology of *Lrat*$^{-/-}$ mice treated with 9-*cis*-RAL-CM-chitosan conjugate is normal. (**A** and **B**) SD-OCT images of the retina of 8-week-old *Lrat*$^{-/-}$ mice treated with (**A**) or without (**B**) the conjugate display no obvious differences; (**C**) Quantification of ONL thickness in *Lrat*$^{-/-}$ mice; there was no significant difference between conjugate-treated and untreated control groups. Data are presented as the mean ± SD, n=9 for each group.

SUPPLEMENTARY TABLES

Table 1.

Table1. Scotopic ERG response amplitudes from dogs treated with 100% 9-*cis*-RAL-CM-chitosan.

	3 cd·s·m^{-2}				10 cd·s·m^{-2}			
	a-wave		b-wave		a-wave		b-wave	
Time (days)	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Pre	0.5	0	1	0	1	0	3.5	0
3	12.5	1	48.5	7	18	4	59	11
10	21	4	47.5	9.5	35	9.5	60.5	17.5
32	4	2.5	15	5.5	12	4	16	4.5
38	2.5	0	12.5	4.5	7.5	2	11.5	6
54	0	0	2	6	1	3	3	5

Table 2. Scotopic ERG response amplitudes from dogs treated with 50% 9-*cis*-RAL-CM-chitosan.

	3 cd·s·m^{-2}				10 cd·s·m^{-2}			
	a-wave		b-wave		a-wave		b-wave	
Time (days)	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Pre	0	0	0	0	0	0	2	0
8	0	8	5.5	19	6.5	13.5	12.5	21.5
21	2	6	7.5	10	8.5	15	8.5	13

Table 3. Scotopic ERG response amplitudes from dogs treated with 25% 9-*cis*-RAL-CM-chitosan.

	3 cd·s·m^{-2}	10 cd·s·m^{-2}

	a-wave		b-wave		a-wave		b-wave	
Time (days)	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Pre	0	0	0	0	0	0	1.5	0
8	3.5	0	19.5	8	9	2	22.5	11
21	0.5	1	6	4	2.5	3.5	5.5	4.5

Table 4. Photopic ERG response amplitudes from dogs treated with 100% 9-*cis*-RAL-CM-chitosan.

	3 $cd \cdot s \cdot m^{-2}$				10 $cd \cdot s \cdot m^{-2}$			
	a-wave		b-wave		a-wave		b-wave	
Time (days)	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Pre	0	0	0	0	1.3	0	3	0
3	3	0	4.5	0	3	2	7.5	2
10	3.3	1.5	3.5	2	7.5	2	7.2	4.2
32	1	1	2	1	2.5	1	2.4	1.2
38	0.5	0	0.5	0	1	1	1	1
54	0	0	0	0	2	0	2	0

Table 5. Photopic ERG response amplitudes from dogs treated with 50% 9-*cis*-RAL-CM-chitosan.

	3 $cd \cdot s \cdot m^{-2}$				10 $cd \cdot s \cdot m^{-2}$			
	a-wave		b-wave		a-wave		b-wave	
Time (days)	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Pre	0	0	0	0	0.5	0	1.5	0
8	0	0	2	0.5	1	0	2.5	1
21	0	0	0	0.5	0	1	0	1.5

Table 6. Scotopic ERG response amplitudes from dogs treated with 25% 9-*cis*-RAL-CM-chitosan

	3 cd·s·m^{-2}				10 cd·s·m^{-2}			
	a-wave		b-wave		a-wave		b-wave	
Time (days)	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2
Pre	0	0	0	0	0	0	0	0
8	0.5	0.5	3.5	2.5	1	1	6	3
21	0	0	0	0	0	0	0	0