

# Blind Dogs That Can See

## Pharmacological Treatment of Leber Congenital Amaurosis Caused by a Defective Visual Cycle

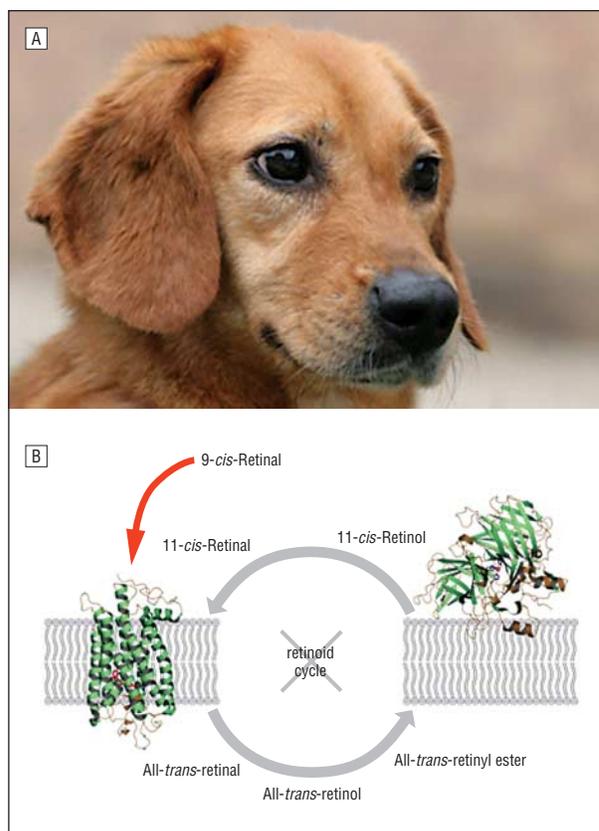
**P**ETERSEN-JONES AND COLLEAGUES PERFORMED an important proof-of-principle study titled "Improvement of Visual Performance With Intravitreal Administration of 9-*cis*-Retinal in *Rpe65*-Mutant Dogs,"<sup>1</sup> published in this issue of the *Archives*. As a component of comprehensive preclinical studies, its primary goal was to determine the efficacy of 9-*cis*-retinal in restoring visual function assessed by both electroretinography (ERG) and functional vision testing in *Rpe65*-mutant dogs. Because

See also page 1442

dogs generally have high levels of circulating retinoids,<sup>2</sup> the researchers injected 9-*cis*-retinal directly into 1 eye of 7 Briard *Rpe65*<sup>-/-</sup>-mutant dogs (Figure, A).<sup>3,4</sup> The results were striking. In 5 of 7 dogs, 9-*cis*-retinal injection resulted in increased rod ERG responses and improved functional vision. Moreover, 3 injected dogs exhibited increased 33-Hz flicker amplitudes characteristic of cone-mediated responses. These positive effects lasted for about 10 weeks. More important, a second injection of 9-*cis*-retinal at 20 or 29 weeks after the first injection in 2 (dogs 5 and 6) of the 7 dogs partially restored vision again, providing a potential dosing strategy for humans. These encouraging results provide impetus for the development of intravitreal devices that promote sustained delivery of 9-*cis*-retinal as a therapy for conditions resulting from a genetic blockade of the retinoid (visual) cycle (Figure, B).

### PHOTOTRANSDUCTION AND THE RETINOID (VISUAL) CYCLE

Human vision depends on a photochemical photoisomerization reaction that converts vitamin A–derived 11-*cis*-retinal, bound either to rod opsin (rhodopsin) or cone opsins, to all-*trans*-retinal.<sup>5</sup> To sustain vision, this chromophore needs to be continuously regenerated through a chain of reactions called *the retinoid cycle*, which occur in photoreceptor cells and the retinal pigment epithelium<sup>3</sup> (Figure, B). Lack of 11-*cis*-retinal regeneration results in a severe form of blindness called *Leber congenital amaurosis* (LCA) (reviewed in Travis et al<sup>4</sup>). In particular, 2 extensively studied enzymes are implicated in this disease: lecithin to retinol acyltransferase (LRAT), which produces the substrate for the isomerase, and the retinoid isomerase (or *Rpe65*) itself.



**Figure.** Treatment of Briard *Rpe65*<sup>-/-</sup>-mutant dogs with the synthetic *cis*-retinoids. A, View of a treated Briard *Rpe65*<sup>-/-</sup>-mutant dog. B, Chemistry of vision: phototransduction and the retinoid (visual) cycle (reviewed in von Lintig et al<sup>3</sup>). Mutations in *Rpe65* are associated with Leber congenital amaurosis<sup>4</sup> because they are required for 11-*cis*-retinal production. 9-*cis*-Retinoids can be used to overcome this blockage and regenerate functional visual pigments, called *isorhodopsin*, in rods that have characteristics similar to rhodopsin. The depicted protein structures are of rhodopsin (left) and *Rpe65* (right).

### LEBER CONGENITAL AMAUROSIS

Leber congenital amaurosis is a recessively inherited, degenerative retinal disease characterized by nystagmus and a dramatic decrease in sensitivity to light, resulting in severe vision loss at birth as assessed by ERG despite an initially normal-appearing retina. The prevalence of LCA is 1 of every 90 000 live births. Mutations in *Rpe65* and *LRAT* account for about 10% of all LCA cases.<sup>6</sup> Partial vision was restored in humans with LCA due to *Rpe65* mutations by using

recombinant adeno-associated virus-mediated gene transfer of wild-type *Rpe65* complementary DNA. These studies on animal models and humans, expertly summarized in a recent review,<sup>7</sup> raised hopes that LCA can be treated in the near future.

### RAPID RESTORATION OF VISION IN ANIMAL MODELS WITH LCA AND IN PATIENTS WITH LCA

#### Mice

Proof-of-principle studies showing the efficacy of exogenous retinoids as chromophore replacements were introduced in 2000.<sup>8</sup> Since then, steady progress has been made with retinoids in drug development and as experimental tools to study the retinoid cycle (summarized in Palczewski<sup>9</sup>).

#### Dogs

Gearhart et al<sup>1</sup> provide a further advance by showing the efficacy of artificial retinoid treatment in a large animal model, the Briard breed of dog,<sup>10</sup> that has a naturally occurring mutation in the gene encoding *Rpe65*. This work follows in the footsteps of gene transfer experiments in these same animals, which were performed approximately a decade ago.<sup>11</sup> Rescue by a single injection can last several weeks, with rods faring better than cones, perhaps because rods degenerate more slowly.

#### Humans

QLT Inc (<http://www.qltinc.com/newsCenter/2010/100420.htm>) recently announced a phase 1b clinical proof-of-principle study of QLT091001 for the treatment of LCA (<http://clinicaltrials.gov/ct2/show/NCT01014052?term=qlt&rank=1>). This phase 1b trial is a short-term, open-label, single-center study designed to evaluate the safety profile and effects on retinal function in 8 pediatric subjects (aged 5-14 years) diagnosed as having LCA due to inherited deficiency of *Rpe65* or LRAT.

### DURATION OF TREATMENT: HOW MUCH CHROMOPHORE DO WE NEED?

Each human eye contains about 5 nmol of visual pigments.<sup>12</sup> So, if steady-state light bleaches 0.01% to 0.1% (100 lux)<sup>13</sup> and we assume 1 second for signaling and regeneration of visual pigment, our consumption of chromophore during a 12-hour active day would be approximately 22 to 220 nmol/d, or approximately 6 to 60 µg/d of 11-*cis*-retinal for full regeneration. While these assumptions are imprecise and will require revision, this calculation still highlights the fact that such small amounts of chromophore can easily be provided. Clinically effective chromophore dosing could be even lower, and full regeneration may not be required. The recommended daily consumption of vitamin A for adults is 900 µg. Thus, the calculated estimate is only about 5% of the retinoids required to maintain health.

### PRESERVATION OF CONES IN LCA

In LCA, the expression of cone-specific genes is down-regulated and accompanied by cone degeneration at an early age.<sup>14</sup> Chromophore is required during cone opsin synthesis for successful opsin trafficking such that without 11-*cis*-retinal, cones may degenerate because of opsin mislocalization.<sup>15</sup> Early cone photoreceptor losses in *Rpe65*-LCA suggest that robust *Rpe65*-based visual chromophore production is important for cones, and the residual retained cone structure and function support the speculation that alternative pathways are critical for cone photoreceptor survival.<sup>16</sup> Mice treated long term with retinylamine, a very potent visual cycle inhibitor highly selective for *Rpe65*, also showed a decline in the number of cones that was ameliorated by 9-*cis*-retinoids. These results suggest that long-term lack of chromophore leads to progressive loss of cones in mice and humans. Therapy for patients with LCA should be geared toward early adequate delivery of chromophore to cone photoreceptors.<sup>17</sup>

### WHAT IS THE FATE OF SPENT CHROMOPHORE?

A fundamental question remains: what happens with spent *cis*-chromophore? Are its all-*trans*-retinoid products toxic to the retina? These issues have yet to be fully resolved experimentally, but there are already sufficient data to make an educated prediction. Both 11-*cis*-retinal and 9-*cis*-retinal are converted to all-*trans*-retinal by light. This *trans*-aldehyde is reduced, esterified, and stored in the eye in specific structures called *retinosomes*.<sup>18</sup> *Rpe65*<sup>-/-</sup> mutant mice store large amounts of long-chain fatty acid all-*trans*-retinyl esters that increase with aging, and their retinas slowly degenerate. Moreover, it was experimentally demonstrated that this retinal degeneration is caused by the constitutive activity of opsin.<sup>19</sup> *Rpe65*<sup>-/-</sup>*Gnat1*<sup>-/-</sup> double-knockout mice that do not express the G protein transducin and thus lack continuous activation of phototransduction by daily light exposure do not develop rod photoreceptor degeneration. This finding suggests that retinyl ester oil droplets, which are also found in liver stellate (Ito) cells, do not directly cause retinal degeneration. Van Hooser et al<sup>20</sup> showed that when opsin is charged with 9-*cis*-retinal, the demand for retinol by the *Rpe* decreases significantly (Figure 1C, in Van Hooser et al). These results suggest that retention-release of retinoid in the eye returns to normal after treatment with *cis*-retinoid. Finally, one should consider that retinoids enter the eye via both passive and active transport, and they are eliminated from the eye either via metabolism, facilitated transport, or diffusion back into circulation. If liver stores of vitamin A are depleted, then vitamin A deficiency and blindness ensue, a major health issue in developing countries. If vitamin A were stable in the eye for the life of an individual, this problem would not occur, as just a slight daily retention of retinoid would lead to significant accumulation in the eye. Under laboratory conditions, it would be impossible to produce vitamin A deprivation in the eye, but that is not what is experimentally observed. Thus, *Lrat*<sup>-/-</sup> mutant mice cannot accumulate

significant amounts of retinyl esters.<sup>21</sup> Only simple retinoids are provided by the diet, so slow retinal degeneration ensues as a result of constitutive opsin activity. In those patients with *Lrat*<sup>-/-</sup> treated with 9-*cis*-retinoid, spent retinal does not seem yet to produce a significant identifiable toxic effect.

## LOOKING TOWARD THE FUTURE

Retinoid replacement has become a possible therapeutic strategy for the treatment of specific eye diseases.<sup>9</sup> The exquisite native system for retinoid delivery from digestion to blood circulation can also be hijacked to deliver artificial retinoids to the eye. The current study by Gearhart et al<sup>1</sup> constitutes an important initial step on the road to further develop 9-*cis*-retinoids for treatment of retinal disease. As with every drug and alternative therapy, adverse effects of these interventions must be carefully weighed against their potential benefits.

Krzysztof Palczewski, PhD

**Author Affiliation:** Department of Pharmacology, Case Western Reserve University, Cleveland, Ohio.

**Correspondence:** Dr Palczewski, Department of Pharmacology, Case Western Reserve University, 10900 Euclid Ave, Cleveland, OH 44106 (kxp65@case.edu).

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