

Evaluation of 4-Methylpyrazole as a Potential Therapeutic Dark Adaptation Inhibitor

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ABSTRACT *Purpose:* To investigate whether 4-methylpyrazole (4-MP; fomepizole; Antizol), an alcohol dehydrogenase inhibitor that delays dark adaptation in laboratory animals, is a possible pharmaceutical agent for the treatment of Stargardt disease. *Methods:* Healthy adults were given intravenous infusions of either 4-MP or placebo during six weekly visits to assess effects on dark adaptation. *Results:* Each participant exhibited a linear, rod- and cone-mediated, log-based response during the initial phase of dark adaptation during both placebo and 4-MP sessions. There were no statistically significant differences between the linear slopes of the 4-MP and placebo testing sessions ($\alpha = 0.05$). *Conclusions:* 4-MP does not appear to be a sufficient inhibitor of the human visual cycle to be considered further as a clinical treatment for Stargardt disease or similar ocular disorders at this time; however, additional testing of 4-MP inhibition of production of lipofuscin's A2E fluorophore in mouse models of Stargardt disease is still warranted.

KEYWORDS A2E; dark adaptation inhibition; lipofuscin; 4-methylpyrazole; Stargardt disease

INTRODUCTION

Stargardt disease (STGD1) is an autosomal recessive macular dystrophy that progressively impairs central vision, has an onset during adolescence or early adulthood, and has a prevalence of nearly 1/10,000.¹ The visually impairing pathologic features of STGD1 include accumulation of fluorescent lipofuscin pigments such as A2E² in the cells of the retinal pigment epithelial (RPE) layer, leading to RPE dysfunction and secondary degeneration of the photoreceptor cells.³ Inhibition of visual cycle function, either pharmacologically or through severe light restriction, has been proposed as a way to reduce the accumulation of these retinotoxic lipofuscins.^{4–6}

The visual cycle involves a series of biochemical reactions responsible for supplying 11-*cis*-retinal to the opsin proteins of the rods and cones.⁷ With light absorption, the 11-*cis*-retinal chromophore is isomerized to the all-*trans* configuration⁸ and eventually released from the opsin protein into the photoreceptor disk membranes where it can form Schiff base adducts with phosphatidylethanolamine (PE). These *N*-retinyl-PE Schiff bases are removed from

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the disk membranes in an ATP-dependent process by an integral membrane protein, ABCR (ABCA4), and delivered to an all-*trans*-retinol specific dehydrogenase, which reduces the aldehyde to all-*trans*-retinol (vitamin A) and is then transported to the RPE where it is enzymatically processed back to an 11-*cis* configuration suitable for regeneration of rod and cone photopigments.⁷ The majority of the all-*trans*-retinal goes through the cyclical enzymatic reactions leading back to 11-*cis*-retinal. However, a small portion of the highly reactive all-*trans*-retinal molecules can undergo further reactions with *N*-retinyl-PE to form the fluorophores that constitute the lipofuscin of the RPE.⁹ The major fluorescent component of lipofuscin is the bis retinoid *N*-retinylidene-*N*-retinylethanolamine (A2E),² and many studies have documented the toxicity of A2E to RPE.^{10,11} Excessive accumulation of these toxic compounds in the RPE is believed to be the main contributor to the loss of central vision in STGD1, which is caused by mutations in the human *ABCR* (*ABCA4*) gene.¹²

Several different methods have been proposed to limit the amount of all-*trans*-retinal available to form A2E in Stargardt-related pathologies. Travis and colleagues reported that visual cycle inhibition, through a presumed retinal alcohol dehydrogenase inhibitor such as 13-*cis*-retinoic acid (Accutane, Roche Pharmaceuticals, Nutley, NJ, USA), can limit the amount of A2E formed.^{4,11} Another proposed intervention is inhibition of RPE65, a protein critical for the formation of 11-*cis*-retinoids in the RPE, by synthetic antagonistic molecules specific to membrane-bound mRPE65.⁵ In yet another approach, *N*-(4-hydroxyphenyl)-retinamide (HPR) was shown to produce a reversible, dose-related reduction in serum retinol and retinol binding protein (RBP) in an animal model with advanced lipofuscin accumulation (*Abca4*^{-/-} mutant).⁶ Although these drugs hold promise for the future, they are either too toxic for long-term administration in humans or they are many years away from clinical use as they must undergo rigorous testing prior to FDA approval. In the meantime, it is useful to examine whether or not there are other currently FDA-approved pharmaceuticals that inhibit dark adaptation that could be brought into clinical practice much more rapidly. One such drug is 4-methylpyrazole (4-MP; fomepizole; Antizol, Orphan Medical, Minneapolis, MN, USA; Fig. 1), an FDA-approved intravenous antidote for methanol and ethylene glycol poisoning, which inhibits a wide variety

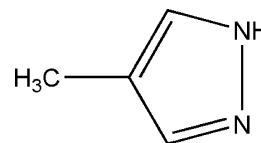


FIGURE 1 Chemical structure of 4-methylpyrazole (4-MP).

of alcohol dehydrogenases¹³ and is known to be an excellent inhibitor of the visual cycle in rodents.^{14,15} In the living rat eye, it specifically targets the alcohol dehydrogenase that oxidizes 11-*cis*-retinol to 11-*cis*-retinal in the RPE (known as RDH5),¹⁶ while the alcohol dehydrogenase activity in the rod outer segments responsible for the reduction of all-*trans*-retinal to all-*trans*-retinol is unaffected (Fig. 2).^{14,15,17} After infusion, 4-MP rapidly distributes to total body water and is eliminated by dose-dependent, nonlinear zero-order kinetics at a rate of 4–15 $\mu\text{mol/L}^{-1}/\text{hr}^{-1}$ ⁽¹⁸⁾ and it is recommended in the package insert that 4-MP be redosed at 12-hr intervals to maintain therapeutic blood levels. At clinically approved doses, 4-MP has little acute toxicity, and long-term chronic oral administration in monkeys and rats has shown no toxicity.^{19,20} Visual cycle effects of 4-MP in humans have never been examined, however. In this study, we test whether or not 4-MP administered at clinically approved doses can significantly inhibit dark adaptation in normal subjects. If a positive result is obtained, further examination of its utility in Stargardt patients would be definitely warranted.

MATERIALS AND METHODS

Participants were recruited from the University of Utah campus. All nonpregnant, nonlactating adults without significant ocular pathologies were eligible to participate in the study. The protocol was approved by the University of Utah Institutional Review Board (IRB), and informed consents were obtained. Each participant received a basic eye examination in which ocular histories were obtained. Those with previous pathology were excluded, and a urine-based pregnancy test was performed on all female participants. A total of nine volunteers were eligible.

The study was conducted in a double-blind fashion with each subject serving as his or her own control. Each participant received either placebo (0.9% saline) or a 15 mg/kg dose of 4-MP (Orphan Medical, Minneapolis, MN, USA) dissolved in 0.9% saline as a 30-min intravenous (i.v.) infusion. Subjects returned a total of six times to receive three sessions of placebo and

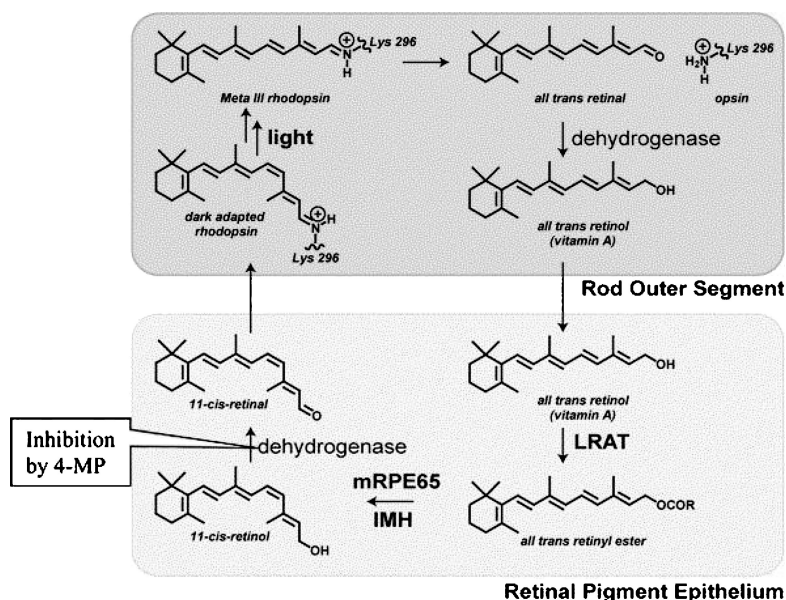


FIGURE 2 Mammalian visual cycle showing the site of enzymatic inhibition of RDH5 by 4-MP. Modified figure reprinted from Xue et al. with permission from the publisher.¹⁷

three sessions of 4-MP in random order. Sessions were 1 week apart to allow for complete elimination of the drug, which has a half life of approximately 12 hr. Five minutes after each i.v. session, a small amount of blood was drawn to measure 4-MP plasma concentrations. Pupils were dilated with 1% tropicamide, and then participants were placed in a brightly lit environment (≈ 4200 cd/m²) for 5 min to ensure photoreceptor bleaching and to mimic the light intensity experienced on a bright, sunny day. A black eye patch was placed over the nondominant eye, and dark adaptation measurements were taken using a Goldmann-Weekers Adaptometer (Haag-Streit, Switzerland). Measurements were recorded until there were no decreases in threshold light intensity (typically 25–45 min from start of test).

Plasma samples were analyzed following published HPLC procedures²¹ to ensure therapeutic 4-MP levels using a Bondapak C18 column (3.9 × 300 mm) (Waters, Milford, MA, USA). Samples were eluted isocratically with a mixture of 7.5% acetonitrile in 0.5 mM potassium phosphate buffer (pH 6) at a flow rate of 1.1 ml/min on a Waters HPLC system with single wavelength detection at 220 nm. The 4-MP peak was quantified by external standardization against synthetic 4-MP to ensure therapeutic dosing levels (8.2–24.6 mg/L) were achieved in each 4-MP session.

All participants demonstrated a log-based, linear decline in threshold for roughly 7 to 10 min after photoreceptor bleaching. The slope of these linear responses was

used to determine if 4-MP inhibited dark adaptation. Dark adaptation results were quantified and transferred to statistical software (Origin 7.0, Northampton, MA, USA). Slope averages of each placebo and 4-MP dark adaptation test were compared, and independent *t*-tests ($\alpha = 0.05$) were performed to measure the significance of the results.

RESULTS

Seven participants completed all six visits of three infusions of placebo and three infusions of 4-MP. In all cases, the subjects achieved the desired therapeutic range of 4-MP ranging from 14.9 to 22.7 mg/L, and as the recommended therapeutic dosing interval is 12 hr, we are confident that therapeutic levels were maintained throughout dark adaptation testing. One participant withdrew from the study after one placebo and one 4-MP infusion, and another participant was withdrawn from the study after three infusion sessions based on minor skin irritation at the i.v. site and moderate dizziness after each 4-MP infusion. Other participants reported side effects consistent with the package insert including lingering metallic taste and mild dizziness. No other complications were noted or reported.

Figure 3 demonstrates typical dark adaptation results from one of the participants. Table 1 summarizes the average log-based linear slopes of participants' dark adaptation results during placebo and 4-MP test sessions. Independent *t*-tests run at the $\alpha = 0.05$

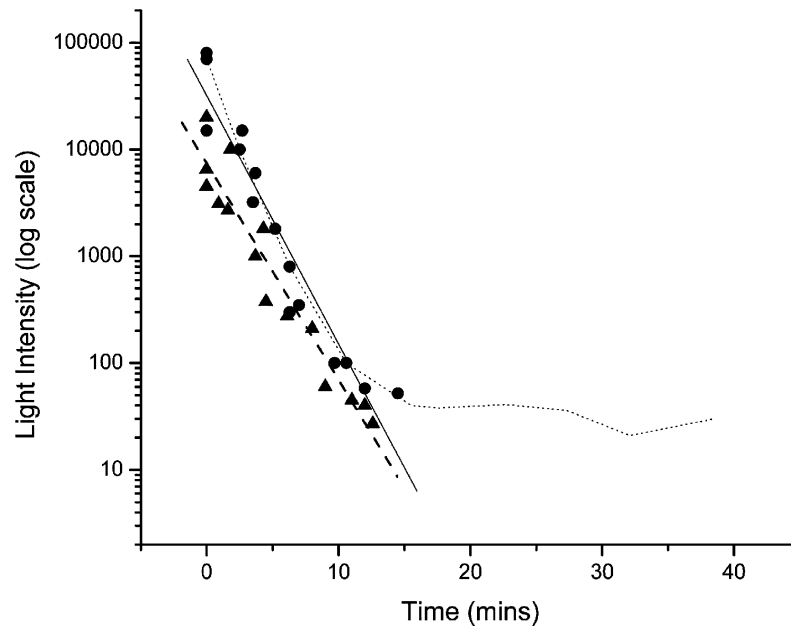


FIGURE 3 Dark adaptation results for subject 7. Solid circles are light intensity thresholds recorded during the subject's three placebo infusions, and solid triangles are light intensity thresholds recorded during the subject's three 4-MP infusions. Best-fit regression lines for the placebo and 4-MP infusions are given by the solid and dashed lines, respectively. The dotted curve is the complete dark adaptation curve from a single placebo session demonstrating achievement of stable full dark adaptation.

significance level showed no statistically significant difference between the average log-based linear slopes of dark adaptation measurements during placebo or 4-MP sessions on any individual subject. Final minimum thresholds of the placebo and 4-MP sessions were also recorded and compared. These results likewise showed no statistical difference.

TABLE 1 Summary of Log Based Linear Slopes Of Dark Adaptation

Subject	Average placebo slope	SD	Average 4-MP slope	SD	p value ^a
1	-0.10	0.03	-0.13	0.02	0.19
2	-0.17	0.14	-0.20	0.05	0.81
3	-0.19	0.08	-0.25	0.03	0.25
4	-0.07	0.03	-0.10	0.05	0.43
5 ^b	-0.15	N/A	-0.18	0.17	0.90
6	-0.24	0.03	-0.23	0.02	0.53
7	-0.30	0.08	-0.24	0.07	0.42
8	-0.25	0.06	-0.23	0.04	0.76
9 ^c	-0.28	N/A	-0.22	N/A	N/A
Average	-0.194	0.064	-0.197	0.056	0.82 ^d

N/A, not applicable; SD, standard deviation.

^aIndependent *t*-tests with $\alpha = 0.05$.

^bPatient discontinued after three measurements due to an adverse drug reaction.

^cPatient withdrew after two measurements.

^dPaired *t*-test ($\alpha = 0.05$) of average placebo and 4-MP slope populations.

DISCUSSION

Stargardt disease (STGD1) is an appealing target for pharmacological intervention because its genetic basis is well defined, and its mechanism of degeneration offers rational therapeutic approaches, namely the inhibition of A2E formation by blockade of enzymatic reactions of the visual cycle. Proof of principle has been shown in animal models, but for a variety of reasons widespread use of these pharmaceuticals is still a long way off. In this study, we have examined the potential use of 4-MP because it is well-known to inhibit dark adaptation in laboratory animals by inhibiting the oxidation of 11-*cis*-retinol to 11-*cis*-retinal, and it is already FDA approved for other medical conditions which opens the possibility of off-label uses by American physicians. In our pilot study, normal volunteers received weekly placebo or therapeutic doses of 4-MP intravenously in random order over a 6-week period. STGD1 patients were not used in this early-phase study because they would be expected to have higher variability of dark adaptation rates depending on their stage of the disorder, which would make data interpretation more challenging.²²

Although our study was powered to detect a 10% inhibition of dark adaptation, none was noted. We therefore conclude that, based solely on dark adaptation results, 4-MP is not likely to be a successful

intervention against Stargardt disease at currently approved dosages. Dose escalation could be considered as rats tolerate acute dosages up to 30 times higher, but we are concerned that some of our subjects were already experiencing mild but potentially significant side effects such as dizziness and a metallic aftertaste. Other outcome measures in long-term human clinical studies with an oral version of 4-MP could be considered such as inhibition of lipofuscin formation, but current quantitative noninvasive autofluorescence measurement methods are probably not sensitive enough to make this approach feasible. Recent work with similar visual cycle inhibitors, namely fenretinide, have shown reduced levels of A2E production with minimal delays in dark adaptation.⁶ These results indicate that further testing, such as an efficacy study designed to measure A2E levels in mouse models of Stargardt disease treated with 4-MP, is still necessary to explore the possibilities of the use of 4-MP in treatment of Stargardt disease. In our opinion, despite our disappointing results with 4-MP, pharmacological inhibition of the visual cycle with newly developed drugs still has great potential for the treatment of Stargardt disease and related clinical disorders, and we look forward to their future clinical trials.

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