The Oral Iron Chelator Deferiprone Protects against Iron Overload–Induced Retinal Degeneration

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PURPOSE. Iron-induced oxidative stress may exacerbate age-related macular degeneration (AMD). Ceruloplasmin/Hephaestin double-knockout (DKO) mice with age-dependent retinal iron accumulation and some features of AMD were used to test retinal protection by the oral iron chelator deferoxamine (DFP).

METHODS. Cultured retinal pigment epithelial (ARPE-19) cells and mice were treated with DFP. Transferrin receptor mRNA (Tfrc), an indicator of iron levels, was quantified by qPCR. In mice, retinal oxidative stress was assessed by mass spectrometry, and degeneration by histology and electroretinography.

RESULTS. DFP at 60 μM decreased labile iron in ARPE-19 cells, increasing Tfrc and protecting 70% of cells against a lethal dose of H₂O₂. DFP 1 mg/mL in drinking water increased retinal Tfrc mRNA 2.7-fold after 11 days and also increased transferrin receptor protein. In DKOs, DFP over 8 months decreased retinal iron levels to 72% of untreated mice, diminished retinal oxidative stress by 70% of the untreated level, and markedly ameliorated retinal degeneration. DFP was not retina toxic in wild-type (WT) or DKO mice, as assessed by histology and electroretinography.

CONCLUSIONS. Oral DFP was not toxic to the mouse retina. It diminished retinal iron levels and oxidative stress and protected DKO mice against iron overload–induced retinal degeneration. Further testing of DFP for retinal disease involving oxidative stress is warranted. (Invest Ophthalmol Vis Sci. 2011;52:959–968) DOI:10.1167/iovs.10-6207

Iron is crucial for optimal cellular metabolism, but is also a potent generator of oxidative stress if present in excess, especially in the form of labile ferrous iron. Inability of the body to actively excrete excess iron leads to age-dependent iron accumulation in certain tissues, including the macula. Excess tissue iron generates reactive oxygen species (ROS) via the Fenton reaction, leading to oxidative damage. Free radicals and oxidative stress have been implicated in a growing number of conditions, from normal aging to cancer, diabetes, and neurodegenerative diseases, making iron overload or metabolic mishandling of iron an important target for therapeutic intervention.

Since iron catalyzes the production of the hydroxyl radical, the most damaging of the free radicals, it is likely to exacerbate oxidative damage in a tissue that is already prone to oxidative insult. Retinal pigment epithelial (RPE) cells and photoreceptors are especially vulnerable to oxidative damage due to high oxygen tension, ROS production by large numbers of mitochondria, and abundant, easily oxidized polyunsaturated fatty acids in photoreceptor membranes. Indeed, several neurodegenerative disorders with iron dysregulation feature retinal degeneration. These include the rare hereditary disorders aceruloplasminemia, Friedreich’s ataxia, and pantothenate kinase-associated neurodegeneration. Further, traumatic siderosis causes rapid retinal degeneration. Similarly, retinal degeneration in several mouse models is associated with retinal iron dysregulation.

Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss in the elderly worldwide. Although the pathogenesis of AMD is incompletely understood, growing evidence suggests that, in addition to inflammation, complement activation, and other hereditary and environmental influences, oxidative stress and iron may play important roles. We have demonstrated higher iron levels in AMD retinas than in age-matched controls, suggesting that iron-mediated oxidative stress may contribute to retinal degeneration in AMD. Supporting this hypothesis, patients lacking the ferroxidase ceruloplasmin (Cp) as a result of the autosomal recessive condition aceruloplasminemia, have retinal iron accumulation and early-onset macular degeneration. Similarly, Cp and hephaestin (Heph) double knockout (DKO) mice have age-dependent retinal iron accumulation, and, presumably as a result of the iron accumulation, have increased retinal oxidative stress, and retinal degeneration. This retinal degeneration shares some features of AMD, including photoreceptor and RPE death, RPE hypertrophy and autofluorescence, sub-RPE deposits including activated complement factor 3 (C3), and subretinal neovascularization. DKO mice also exhibit sparse macrophage infiltration between the RPE and outer segments, suggesting a chronic inflammatory component in their pathologic retinas.

There are also differences in the pathologic features of the DKO versus AMD retinas: The width of the sub-RPE deposits in DKO is smaller than in AMD retinas, the number of hypertrophic RPE cells is greater in DKO, and the subretinal neovascularization in DKO more often originates from the retinal vasculature than from the choroid.
To determine whether iron dysregulation is the cause of retinal degeneration in DKO s and to develop a therapeutic model, we tested whether deferiprone can protect DKO retinas against iron accumulation and degeneration. Chelation therapy has, until recently, been used mainly for the treatment of acute iron toxicity and chronic transfusional iron overload in thalassemia and other conditions. Recently, iron-chelating drugs have been tested in additional categories of patients with normal body iron load, such as those with neurodegenerative, renal, and infectious diseases. Three widely used iron chelating drugs are deferoxamine, deferiprone (DFP), and deferasirox. Deferoxamine has been used for decades as the main iron chelating agent to treat transfusion-related hemochromatosis. It is administered via slow subcutaneous infusion over 8 to 12 hours or intravenously in some patients. Deferoxamine’s potential as a therapeutic agent is limited by the route of administration, as well as severe side effects at higher doses that include pigmentary retinopathy, bone dysplasia, and auditory toxicity. DFP is a low-molecular-weight iron chelator that can readily penetrate cells and is approved for use in Europe and Asia. The drug can decrease liver and cardiac iron levels in patients with transfusional iron overload. DFP can cross the blood-brain barrier and decrease brain iron levels in patients with Friedrich’s ataxia, which is associated with improved motor function in some patients. DFP is orally absorbed and binds iron in multiple subcellular and extracellular locations. Approximately 1% to 2% of patients with thalassemia given oral DFP develop reversible agranulocytosis, necessitating constant blood cell count monitoring. Deferasirox, another oral iron chelator, received FDA (U.S. Federal Drug Administration) approval in 2005 for treatment of transfusional iron overload, but there is no evidence to date that it can decrease brain or retinal iron levels.

Iron chelators may serve as protective agents against human retinal disorders in which iron accumulation or oxidative stress play an important role. Systemic chelation with deferoxamine protects the rodent retina from degeneration caused by light-induced damage and ischemia reperfusion injury and the rabbit retina from electroretinogram (ERG) changes induced by subretinal blood (Youssef TA, et al. IOVS 2002;43:ARVO E-Abstract 3000). Yet, deferoxamine’s clinical potential for retina protection is limited by route of administration and retinal toxicity, as discussed earlier. DFP seems to be an attractive candidate as it is orally absorbed, has been shown to cross the blood-brain barrier, and has shown efficacy and low toxicity in human clinical trials for diabetic nephropathy and primary glomerulonephritis. We tested whether oral DFP can chelate retinal iron in wild-type (WT) mice without adverse effects on retinal function. Further, we tested whether it can protect cultured retinal cells from oxidative stress and iron overloaded C57/BL6 mouse retinas from degeneration.

Materials and Methods

Cell Culture

The spontaneously immortalized human RPE cell line, ARPE-19 (ATCC, Manassas, VA) was cultured until confluent in 24-well plates ( Falcon; BD Biosciences, Bedford, MA) in 1:1 DMEM/F12, 20 μM l-glutamine (Invitrogen, Carlsbad, CA) supplemented with heat inactivated 10% FBS (HyClone, Logan, UT) and 1% penicillin-streptomycin. The cells were grown in 5% CO2 at 37°C. To evaluate the protective effect of DFP, confluent ARPE-19 cells were washed three times with MEM (Invitrogen, Carlsbad, CA) then pretreated for 30 minutes with various concentrations of DFP in MEM, followed by a 15 hour co-treatment with different concentrations of DFP and 200 μM H2O2 (Sigma-Aldrich, St. Louis, MO). Cell death was assayed after 15 hours (LDH Release Assay Kit; Roche, Basel, Switzerland). Cytotoxicity was expressed as a ratio between maximum LDH release in hydrogen peroxide-treated cells (200 μM) and minimum LDH release in cells incubated in MEM, and the percent viability was calculated. To visualize cell death, a cell viability assay was performed as suggested by the manufacturer (Live/Dead Assay; Invitrogen).

Animals

Female, 6-month-old C57BL/6 mice were obtained from The Jackson Laboratory. Female and male C57BL/6 WT mice, C57BL/6D mice with a targeted mutation in the Gp gene (Cp−/−), and mice with a naturally occurring mutation in the Hepb gene (Hepb−/−) were generated, as previously published. The Cp−/−Hepb−/− (or Cp−/−Hepb−/+) mice are referred to herein as DKO and Hepb−/− or Hepb−/+ animals.

DFP Treatment

The mice were treated per os with 1 mg/mL DFP (ApoPharma, Inc., Toronto, Ontario, Canada) in drinking water for various amounts of time, as indicated in the Results section.

Quantitative Real-Time PCR

Gene expression in the neurosensory retina and RPE/choroid samples obtained from DFP-treated and untreated WT and DKO mice was analyzed by quantitative (q)RT-PCR. The samples were acquired by removing the anterior segment and ciliary body and detecting the neurosensory retina from the underlying RPE/choroid tissue. RNA was isolated (RNeasy Mini Kit; Qiagen, Inc., Valencia, CA) according to the manufacturer’s protocol. The RNA was quantified with a spectrophotometer and stored at −80°C. cDNA was synthesized (TaqMan Reverse Transcription Reagents; Applied Biosystems, Inc. [ABI], Carlsbad, CA), according to the manufacturer’s protocol. Gene expression assays were obtained (Taqman; ABI) and used for qPCR analysis. Probes were used as transferrin receptor (Tfrc, Mm00441941_m1), vascular endothelial growth factor A (Vegfa, Mm00437304_m1), erythropoietin (Epo, Mm00433126_m1), CD68 antigen (Cd68, Mm03407435_m1), and complement component 3 (C3, Mm01232779_m1). Eukaryotic 18S rRNA (Hs99999901_s1) was used as an endogenous control. Real-time RT-PCR (Taqman; ABI) was performed on a sequence detection system (Prism model 7500; ABI) using the ΔΔCt method, which provides normalized expression values. The amount of target mRNA was compared among the groups of interest. All reactions were performed in biological (three mice or tissue culture wells) and technical (three qPCR replicates per biological sample) triplicates.

Immunofluorescence

The globes fixed in 4% PFA were rinsed in PBS, and the eye cups were generated by removing the anterior segment. The eye cups were cryoprotected in 30% sucrose overnight and embedded in optimal cutting temperature compound (OCT; Tissue-Tek, Sakura Finetek, Torrance, CA). Immunofluorescence was performed on 10-μm-thick sections as previously published. The primary antibody was rabbit anti-TR at 1:80 dilution (Santa Cruz Biotechnology, Santa Cruz, CA). Primary antibody was detected using fluorophore labeled secondary antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Control sections were treated identically but with omission of primary antibody (data not shown). The sections were analyzed by...
fluorescence microscopy with identical exposure parameters (model TE300 microscope; Nikon, Tokyo, Japan, with ImagePro software; Media Cybernetics, Bethesda, MD).

**Electroretinogram Recordings**

ERG recordings followed procedures described previously. In brief, mice were dark-adapted overnight and then anesthetized with a cocktail containing (in mg/kg body weight): 25 ketamine, 10 xylazine, and 1000 urethane. In each mouse, the pupils were dilated with 1% tropicamide saline solution (Mydriacil; Alconox, New York, NY) and the mouse was placed on a stage maintained at 37°C. Two miniature cups made of UV-transparent plastic with embedded platinum wires serving as recording electrodes were placed in electrical contact with the corneas. A platinum wire loop placed in the mouth served as the reference and ground electrode. ERGs were then recorded (Espion Electrophysiology System; Diagnosys LLC, Lowell, MA). The apparatus was modified by the manufacturer for experiments with mice by substituting LEDs with emission maximum at 365 nm for standard blue reference and ground electrode. ERGs were then recorded (Espion Electrophysiology System; Diagnosys LLC, Lowell, MA). The apparatus was modified by the manufacturer for experiments with mice by substituting LEDs with emission maximum at 365 nm for standard blue

**Quantitative Iron Detection**

The eyes from age-matched DFP-treated and untreated WT mice, as well as age-matched DFP-treated and untreated DKO mice were fixed in 4% PFA for several days. Eye cups were made by removing the anterior segment. The ciliary body was removed with a curved scalpel blade, and the neurosensory retina was detached from the underlying RPE/choroid tissue. Samples of the neurosensory retina and RPE/choroid (with sclera) were placed in separate tubes and allowed to dry at room temperature. Total nonheme iron was quantified using the bathophenanthroline-based spectrophotometric protocol described by Torrance and Bothwell. Briefly, preweighed tissues were snap frozen and kept at −80°C. Tissue digestion was performed overnight at 65°C in acid digest solution (0.1% trichloroacetic acid and 0.03 M HCl). After digestion, the tissue-acid mixture was vigorously vortexed, cooled to room temperature, and then centrifuged for 25 minutes at 3500 rpm in a centrifuge at room temperature (model 5415D; Eppendorf, Fremont, CA). The supernatant (20 µL) was added to 1 mL of chromogen reagent (2,2’5 M sodium acetate pretreated with Chelex 100 [Bio-Rad, Hercules, CA], 0.01% bathophenanthroline, and 0.1% thioglycolic acid). The absorbances were read at 535 nm. Iron level was assessed by comparing absorbances of tissue-chromogen samples with serial dilutions of iron standard (Sigma-Aldrich, Inc.).

**Assessment of Oxidative Stress**

Mass spectrometry was used for biochemical analysis of isoprostane F2α-VI levels, a specific marker of oxidative stress. Immediately after euthanatization, the retinas were isolated and snap frozen on dry ice. The retinas were homogenized, and total lipids were extracted as previously published.

**Statistical Analysis**

The mean ± SE were calculated for each comparison pair. The means between each pair were compared by t-test. P < 0.05 was considered statistically significant (GraphPad software; San Diego CA).

**RESULTS**

**DFP Chelated Labile Iron and Protected against H2O2-Induced Cell Death in Cultured Retinal Cells**

To determine whether DFP can protect retinal cells against iron-catalyzed oxidative stress, we used confluent human retinal pigment epithelial cells (ARPE-19 cell line). First, we assessed DFP’s ability to chelate labile iron within these cells. Since Tfrc mRNA stability is regulated by labile iron levels, with decreased labile iron resulting in increased Tfrc mRNA, we quantified Tfrc mRNA levels after exposing the cells to various concentrations of DFP. At DFP concentrations of 60 µM and higher, Tfrc mRNA levels were increased (Fig. 1A). Next, the cells were treated with 200 µM H2O2, which caused death of 100% of the cells, as measured by the LDH release assay and the fluorescent cell viability assay (Figs. 1B, 1C). This cytotoxicity was markedly diminished when DFP was applied 30 minutes before and during H2O2 exposure. The lowest concentration that chelated enough labile iron to cause a measurable increase in Tfrc mRNA (60 µM) was also the lowest concentration able to protect more than 50% of the cells. DFP itself was not cytotoxic up to the highest concentration tested, 10 mM (data not shown).

**Oral DFP in WT Mice Decreased Labile and Total Retinal Iron**

Wild-type mice, 6 months of age, that were given DFP in drinking water for 24 hours and killed the following morning had decreased retinal labile iron levels, as indicated by increased Tfrc mRNA levels in the neural retina (Fig. 2A). RPE/choroid Tfrc mRNA levels did not change (Fig. 2B). Longer DFP treatment, for 11 days, resulted in a larger increase in Tfrc mRNA (almost threefold) in the neural retina (Fig. 2C). The RPE/choroid showed a similar trend, but the increase was not statistically significant (Fig. 2D). To test whether transferrin receptor protein (TIR) levels change with the treatment and to localize TIR, retinas from mice treated for 11 days with DFP were compared with untreated controls using immunofluorescence with an anti-TIR antibody. The DFP-treated mice had increased TIR immunoreactivity in all retinal cell layers (Figs. 2E1, 2E2). In 9-month-old WT mice after 3 months of treatment, DFP decreased total nonheme iron levels in the neural retina (Fig. 2F), but not in the RPE/choroid (Fig. 2G).

**Oral DFP Exhibited No Retinal Toxicity as Assessed by ERG and Histology**

After treatment with DFP, no retinal toxicity was detected by ERG. Wild-type mice treated for 11 days or 3 months with DFP manifested no changes in ERG relative to age- and genotype-matched untreated controls (Table 1). The absence of retinal toxicity in 9-month-old WT mice was also confirmed by the normal retinal morphology after 3 months of treatment (Fig. 3). After 6 months of treatment with DFP, 8-month-old DKO mice had an increase in all ERG wave amplitudes (though not statistically significant at this age; see the Discussion section) relative to untreated age-matched DKO controls (Table 2).

**DFP Treatment Decreased Total Iron Pools within the Neural Retina and RPE/Choroid of DKO Mice**

Similar to its effects on WT mice, in 9-month-old DKOs, DFP significantly reduced the total iron pool within the neural
Green fluorescence. Photomicrographs of ARPE-19 cells labeled in a fluorescent cell viability assay.

Isoprostane F2\textsubscript{alpha} VI is a prostaglandin-like compound formed by the free radical-catalyzed peroxidation of arachidonic acid. It serves as a quantitative, specific marker of oxidative stress in the retina\textsuperscript{46} and the brain.\textsuperscript{51} DFP treatment for 5 months significantly reduced isoprostane F2\textsubscript{alpha}-VI levels in the neural retinas of treated DKO mice (age 9 months) relative to untreated age-matched control DKOs (Fig. 6A). Erythropoietin (Epo), a cytokine regulating hematopoiesis, can be considered a marker of oxidative stress because Epo mRNA levels can be upregulated by oxidative and nitrosative stress.\textsuperscript{52} Consistent with a decrease in oxidative stress, DFP treatment for 6 months significantly lowered retinal Epo mRNA levels (Fig. 6B), whereas mRNA levels of another HIF regulated proangiogenic gene, Vegfa, remained unchanged in both neurosensory retina (Fig. 6C) and RPE/choroid (Fig. 6D). DFP treatment significantly reduced neurosensory retina mRNA levels of the monocye/macrophage specific glycoprotein Cd68 (Fig. 6E) and was associated with a trend toward decreased C3 mRNA levels in the RPE/choroid (Fig. 6F).

**DISCUSSION**

In this study, we tested whether chelation with a low-molecular-weight, cell-permeant, central nervous system-permeant iron chelator, such as DFP, is a safe and effective therapy for iron-exacerbated retinal degeneration. Our data showed that in mice, oral DFP was not retina toxic, caused a decrease in retinal labile iron after short-term administration, and decreased retinal iron stores after long-term administration. Further, it diminished oxidative stress in DKO mouse retinas and provided marked protection against retinal degeneration.

Since transferrin receptor (Tfrc) mRNA is stabilized by a decrease in labile iron, we tested chelation of labile iron in WT mice through quantification of Tfrc mRNA. After only 1 day of oral DFP treatment, qPCR demonstrated a significant increase in retinal Tfrc mRNA levels (Fig. 2). Eleven days of treatment was sufficient to upregulate TfR protein enough to detect increased anti-TfR immunofluorescence throughout retinas of treated mice compared with untreated controls. Unlike chelation of free, labile iron, longer DFP treatment was required to produce a measurable reduction in total retinal iron

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**FIGURE 1.** DFP treatment decreased labile iron and protected ARPE19 cells from H\textsubscript{2}O\textsubscript{2}-induced cell death. (A) Relative Tfrc mRNA levels as determined by qPCR in ARPE-19 cells after 15 hours of treatment with different concentrations of DFP, as indicated. (B) Percentage of cell viability as determined by LDH assay. DFP protected the ARPE-19 cells against H\textsubscript{2}O\textsubscript{2}-induced cell death. (C) Fluorescence photomicrographs of ARPE-19 cells labeled in a fluorescent cell viability assay. **Green:** live cells as detected by calcein fluorescence. Error bars ± SEM.
levels. Three months of treatment in WT mice or 6 months of treatment in DKO mice significantly reduced total neural retina iron stores.

Unlike deferoxamine, which is a large, positively charged molecule, DFP is only 139 Da in mass and is neutral in the circulation, whether free or bound to iron, and readily pene-

**FIGURE 2.** DFP treatment in WT mice chelated labile iron, increasing retinal Tfrc mRNA and protein levels. Wild-type mice had significantly increased Tfrc mRNA levels, as measured by qPCR in the neural retina after DFP treatment for 1 day (A; n = 3 mice per group, 6 months old) and 11 days (C; n = 4 mice per group, 6 months old). The difference in Tfrc expression is not statistically significant in the RPE/choroid in both groups (B, D). Representative fluorescence retinal photomicrographs from 11-day untreated control (E1) and DFP-treated (E2) WT mice. The DFP-treated mice showed increased anti-TfR immunoreactivity throughout the retina. Scale bar, 25 μm. Immunoreactivity was quantified by measuring the mean pixel intensity within the RPE and neural retinas (n = 3 mice per group, 6 months old) and is shown as the mean (E3). Three months of treatment with DFP significantly reduced total iron in the neural retina (F), but not in the RPE/choroid (G). *Significant difference (P < 0.05). Error bars ± SEM.

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A. Amplitudes with or without 11 Days’ Treatment with DFP

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<tr>
<td>Control</td>
<td>372 ± 25.7</td>
<td>394 ± 30.56</td>
<td>188 ± 15.4</td>
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<tr>
<td>DFP treated</td>
<td>361 ± 31.3</td>
<td>398 ± 36.9</td>
<td>183 ± 7.8</td>
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<td><em>P</em></td>
<td>0.789</td>
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B. Amplitudes with or without 3 Months’ Treatment with DFP

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<tr>
<td>Control</td>
<td>216 ± 31.8</td>
<td>286 ± 52.5</td>
<td>154 ± 33.1</td>
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<tr>
<td>DFP treated</td>
<td>209 ± 37</td>
<td>261 ± 23.9</td>
<td>145 ± 16.5</td>
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<tr>
<td><em>P</em></td>
<td>0.906</td>
<td>0.660</td>
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*Mice (n = 3 per group) had access to drinking water, with or without DFP, starting at 2 months of age for 6 months and were killed at 8 months of age. The a- and b-wave amplitudes are shown for saturating light stimuli. Data are the mean ± SEM amplitudes at the 6-month treatment time point.

*Calculated for DKO-untreated and DKO DFP-treated mice.*
Thus, oral DFP does not appear to upregulate retinal Vegf. The DFP-induced reduction in Epo in DKOs may result from diminished retinal oxidative stress (which has been shown to upregulate Epo) or from improved retinal oxygenation. The former explanation is more likely, as anemic 3-month-old HepbKO mice, which do not have retinal iron overload, do not have retinal Epo mRNA upregulation compared with age-matched WT mice (not shown).

While testing for retinal protection, we observed other systemic effects of DFP.

Most DKO mice manifest progressive ataxia and die by 6 to 9 months, because of brain iron accumulation within specific brain regions, especially the substantia nigra and striatum, with loss of dopaminergic neurons (Harris ZL et al., manuscript in preparation). DFP treatment decreased nonheme iron in DKO brains (not shown). This was associated with prevention of ataxia in these mice and a markedly increased lifespan (not shown).

Our study suggests that DFP may serve as a protective agent against retinal diseases in which iron-induced oxidative stress...
has been implicated, including AMD. The dose used in this study, 1 mg/mL in drinking water, results in an approximate dose in mice of 150 mg/kg/d. This dose is higher than that typically used in thalassemic patients (75–100 mg/kg/d), but provides proof-of-principle that DFP can be therapeutic in the retina. Experiments are in progress to assess ocular pharmacokinetics in mice and larger animals. In humans, a single 50-mg/kg dose can result in blood levels as high as 300 μM, which is more than five times the dose needed to provide significant protection to ARPE-19 cells exposed to a lethal dose of hydrogen peroxide.56

Although DFP is approved for use for iron overload disorders in Europe and Asia, approximately 1% of thalassemic patients taking oral DFP develop reversible agranulocytosis, requiring frequent blood cell count monitoring.53,57 DFP binds iron with higher affinity than it does copper or zinc, but may diminish levels of these latter metals. Thus levels of iron, copper, and zinc should be monitored in any future clinical trial of DFP for AMD, especially given the evidence that zinc supplementation is protective against AMD58 and that the elderly may have reduced zinc intake. Unlike intracellular zinc chelation, which could be detrimental, it is possible that copper chelation would be helpful, as copper can produce reactive oxygen species. Extracellular zinc chelation could also be helpful, as zinc deposits in Bruch’s membrane and may promote drusen formation.59 Since DFP is currently approved only for treatment of patients with iron overload, any consideration of its potential use in patients with localized iron dysregulation, as opposed to iron overload, must incorporate careful assessment of dose, as novel adverse effects and/or adverse effects at lower doses could be anticipated.

While further safety and pharmacokinetic studies are needed before initiating a clinical trial for retinal disease, this study suggests that DFP holds promise for prevention or treatment of retinopathies involving iron overload or oxidative stress, including AMD, diabetic retinopathy, and aceruloplasminemia.

**Acknowledgments**

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