Effect of Dietary Inducer Dimethylfumarate on Glutathione in Cultured Human Retinal Pigment Epithelial Cells

Kasey C. Nelson,1,2 Joanne L. Carlson,1,2 Melanie L. Newman,1 Paul Sternberg, Jr,2 Dean P. Jones,1 Terrance J. Kavanagh,5 Dolores Diaz,3 Jiyang Cai,1 and Mei Wu2

PURPOSE. To determine the effect of dimethylfumarate (DMF), an inducer of glutathione (GSH)-dependent detoxification, on intracellular GSH levels in cultured human retinal pigment epithelium (hRPE) cells, its mechanism of action, and its effect on hRPE cells subjected to oxidative injury.

METHODS. Established hRPE cell lines were treated with DMF and assayed by high-pressure liquid chromatography for intracellular and extracellular GSH levels. Quantification of γ-glutamylcysteine synthetase (GLCL) was determined through northern and western blot analyses, and activity was measured. Effects of pretreatment with DMF on GSH redox status of hRPE cells was determined. Sensitivity of hRPE cells to oxidative stress was determined using tert-butylhydroperoxide as the oxidative agent.

RESULTS. Dimethylfumarate caused a transient decrease followed by a significant increase in intracellular GSH. Glutathione increased maximally at 24 hours with 100 to 200 μM DMF. The initial decrease could be accounted for by the formation of a DMF-GSH conjugate. Dimethylfumarate treatment increased the steady state mRNA expression of the regulatory subunit of GLCL, but no increase was seen for the catalytic subunit. However, protein levels were increased for both, and the catalytic activity of GLCL was also increased. Whereas the initial decrease in GSH made hRPE cells more susceptible to oxidative damage, pretreatment with DMF under conditions that increased intracellular GSH protected hRPE cells against oxidative damage.

CONCLUSIONS. These results suggest a means by which the antioxidant capability of hRPE may be augmented without direct antioxidant supplementation. Specifically, a dietary compound that conjugates with GSH can induce GSH synthesis, increase GSH concentration, and improve protection by GSH-dependent detoxification pathways in hRPE. However, the early depletion of GSH before stimulated synthesis necessitates caution in prevention strategies using dietary inducers.


A ge-related macular degeneration (ARMD) is the leading cause of blindness in elderly Americans.1,2 Although a causal link between oxidative injury and ARMD has not been established, studies suggest that the primary source of dysfunction in ARMD may be linked to oxidative injury of the retinal pigment epithelium.3 In vitro studies have shown that the antioxidant glutathione (GSH) protects human retinal pigment epithelial (hRPE) cells from peroxide-induced injury.7 It has also been shown that consumption of a diet of antioxidant-rich fruits and vegetables is associated with higher levels of the reduced form of GSH.3 These findings suggest that nutritional or therapeutic means to increase GSH levels in the hRPE may provide approaches for reducing oxidative injury, thus affecting onset or progression of ARMD.

A broad class of naturally occurring and synthetic compounds is known to induce synthesis of GSH and/or GSH-dependent detoxification systems in mammalian cells. Dimethylfumarate (DMF) is a nonnutritive compound found in some fruits that has been shown to increase activity of GSH-dependent detoxification systems in vivo in various murine tissues6,7 and to increase GSH levels in human U1 monocyteid cells.9 It has relatively low toxicity, exemplified by its use as a food additive. This is further supported by chronic toxicologic studies of the parent compound, fumaric acid,9–11 and the treatment of psoriasis with its derivatives.12–14 Thus, we selected DMF as a model compound to determine whether dietary inducers increase GSH concentration in human retinal pigment epithelial cells and whether this increase can protect against oxidative injury.

METHODS

Methods of securing human tissues complied with the Emory University Human Investigation Review Board guidelines and the Declaration of Helsinki.
Cell Isolation and Culture Conditions

hRPE cell cultures were established from donor eyes obtained through the Georgia Eye Bank as previously described. Appropriate approval for their use was obtained from the Eye Bank and the Emory University Human Investigation Review Board. Primary cell cultures were established in 6-well tissue culture dishes with low-glucose (1 mg/ml) medium supplemented with fetal bovine serum (20%), amphotericin B (3 μg/ml), and gentamicin sulfate (50 μg/ml). Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum at 37°C under 95% air-5% CO2, and the medium was changed every 2 to 3 days. Cells were passaged in 75-cm² flasks every 7 to 14 days, and experiments were performed on hRPE cell cultures between the 3rd and 10th passages.

Incubations were performed in DMEM with 10% fetal bovine serum at 37°C under 95% air-5% CO2. The DMF (Sigma, St. Louis, MO) was dissolved in dimethyl sulfoxide (DMSO, Sigma) and then added to the medium, resulting in a final DMSO concentration of 0.2%. The controls were treated without DMSO only. Experiments were performed on cell lines at 75% to 90% confluence. Data from these replicate tissue culture dishes within the same experiment were averaged before the calculation of mean ± SEM.

GSH Analysis

For GSH studies, media were aspirated, and plates were washed with phosphate-buffered saline (PBS, pH 7.4) before addition of 1 ml per chloric acid (5% vol/vol) containing 0.2 M boric acid and 5 or 10 μM γ-glutamylglutamate as an internal standard. Contents were scraped and stored in 1.5-ml microcentrifuge tubes at −80°C before derivatization. Supernatant (300 μl) was mixed with 60 μl iodoacetic acid (7.4 mg/ml H2O) and adjusted to pH 9.0 ± 0.2 with a KOH/tetraborate solution (1 M KOH in saturated K2B4O7). After 20 minutes at room temperature, 300 μl of a dansyl chloride solution (20 mg/ml acetonitrile) was added. Samples were mixed and placed in the dark at room temperature for 24 hours. Five hundred microliters chloroform was then added to each sample and were stored in the presence of both the perchlorate precipitate and the chloroform layer at 0°C to 4°C until analysis through high-pressure liquid chromatography (HPLC).

Samples were centrifuged, and aliquots of the aqueous layer were collected for analysis. Separation was achieved on a 3-aminopropyl column (Custom LC, Houston, TX), with initial conditions of 80% solvent A (80% methanol) and 20% solvent B (4 M sodium acetate [pH 4.6] containing 64% methanol) run at 1 ml/min. After 10 minutes, a linear gradient of 20% solvent A and 80% solvent B was run during the next 20 minutes. From 30 to 46 minutes the conditions were maintained at 20% A and 80% B and returned to 80% A and 20% B in 2 minutes. Equilibration time for the next run was 12 minutes. Detection was obtained by fluorescence monitoring with a band-pass filter (305 to 395 nm excitation and 510 to 650 nm emission; Gilson Medical Electronics, Middleton, WI). Quantitation of glutathione was obtained by integration relative to the internal standard, γ-glutamylglutamate.

Isolation of Total RNA and Northern Blot Analysis

Total RNA was isolated by addition of TRIzol (Life Technologies, Gaithersburg, MD) to the plates. Cells were scraped, placed in 1.5-ml microcentrifuge tubes (Molecular Research Center Inc., Cincinnati, OH) and frozen in liquid nitrogen until resuspension in Formazol. Total RNA (20 μg/well) was separated by electrophoresis on a 3.7% formaldehyde-1.5% agarose gel. RNA was transferred to a positively charged nylon membrane (Schleicher and Schuell, Keene, NH) and cross-linked to the membrane by UV irradiation. The full length cDNAs for the mouse glutamate-cysteine ligase catalytic and regulatory subunits (GLCLc and GLCLR, respectively) were labeled with [α-32P]dATP (Dupont, Boston, MA) using a random prime labeling kit (Stratagene, La Jolla, CA), purified with NucTrap columns (Stratagene) and used at 8 × 105 cpm/ml of hybridization solution. Hybridizations were performed overnight at 60°C in a solution of 10% dextran sulfate, 2% sodium dodecyl sulfate (SDS), and 1 M NaCl, washed with 2 × SSC-0.1% SDS at 15 minutes for room temperature, then for 50 minutes at 60°C with 0.1 × SSC-0.1% SDS. The membranes were exposed to film with an intensifying screen (Biomax; Kodak, Rochester, NY) at −80°C for 24 hours. Membranes were stripped by boiling twice in 0.1% SDS for 15 minutes. Glyceraldehyde 3-phosphate dehydrogenase (G3PDH; Clonetech, Palo Alto, CA) was used as an internal standard. Autoradiographs were quantified by densitometric analysis and expressed as percentage of G3PDH mRNA expression levels.

Isolation of GLCL Protein and Western Blot Analysis

Plates of hRPE cells were washed twice with PBS (pH 7.4) and PBS in frozen at −80°C. On thawing, samples were homogenized in the PBS plus peptidase inhibitors (100 μg/ml Pefabloc, 10 μg/ml TLCK, 1 μg/ml pepstatin A, 1 μg/ml aprotonin, and 1 μg/ml leupeptin [Boehringer Mannheim, Indianapolis, IN]) and centrifuged for 10 minutes at 16,000g at 4°C. Protein levels in the supernatants were assayed by the Bradford method (Bio-Rad, Hercules, CA), and a total of 70 μg protein from each homogenate per lane was separated by SDS-polyacrylamide gel electrophoresis. The proteins were transferred to polyvinylidene difluoride membrane (Millipore, Bedford, MA), and blots were incubated with blocking solution (3% bovine serum albumin, 3% nonfat dry milk, 1% ovalbumin, 1% normal goat serum, 0.1% Tween-20, and 0.1% NaN3). Blots were stained with rabbit polyclonal antisera raised against peptides from GLCLc and GLCLR. The secondary antibody was horseradish peroxidase-conjugated goat anti-rabbit IgG (Boehringer Mannheim) with binding detected by enhanced chemiluminescence (Amersham, Arlington Heights, IL). The autoradiographs were scanned and densitometry performed using the GEL-DOC system with commercial software (Molecular Analyst; Bio-Rad).

Reactive Oxygen Species Assay

The method used is based on that described by LeBel and Bondy. It involves the use of the nonfluorescent probe 2′,7′-dichlorofluorescein diacetate (DCFH-DA; Molecular Probes, Eugene, OR). DCFH-DA is a nonfluorescent compound that readily crosses cell membranes. Intracellularly, DCFH-DA is hydrolyzed to 2′,7′-dichlorofluorescein (DCFH), a nonfluorescent product that cannot cross cell membranes. If reactive oxygen species (ROS) are present, DCFH is oxidized by ROS to the highly fluorescent product 2′,7′-dichlorofluorescein (DCF).

The media were aspirated from cultured hRPE cells, and the cells were washed twice in DMEM. Cells were then incu-
bated in Hanks’ balanced salt solution (HBSS, Life Technologies) containing 40 \mu M DCFH-D Diaz for 30 minutes at 30°C in 5% CO₂. Cells were washed with DMEM twice and incubated in 100 \mu M DMF in DMEM for 1 and 3 hours at 37°C. After incubation, cells were washed with DMEM twice. Fluorescence measurements (FACScan; Becton Dickinson, MA) were then read, using an excitation wavelength of 488 nm and an emission wavelength of 588 nm. Controls consisted of hRPE cells incubated in DMEM without DMF and hRPE cells incubated in DMEM containing 300 \mu M H₂O₂ or 500 \mu M tert-butyldihydroperoxide (tBH).

**Assay of GLCL Activity**

The method was a modification of that of Fernandez–Checa and Kaplowitz, which we modified to perform using a 96-well plate. Monochlorobimane (mBCl) forms a fluorescent adduct with GSH more specifically than other bimanes and preferentially over other thiols present in solution. Cultured hRPE cells were treated with diethylmaleate in DMEM with 10% fetal bovine serum for 1 hour. Cells were then washed twice with PBS and scraped into a reaction buffer containing 100 mM Tris-HCl, 150 mM KCl, 20 mM MgCl₂, and 2 mM EDTA (pH 7.3). Cells were lysed by repeated aspiration through a 25-gauge needle, and 50 \mu l cytosol (0.05 mg protein) was added to 200 \mu l of the reaction buffer that contained 100 \mu M glutamate, 10 mM glycine, 3 mM adenosine triphosphate, 0.2 mM cysteine, 0.1 mM dithiothreitol, and 100 \mu M mBCl (from a stock of 100 mM in ethanol). The increase in fluorescence with time was used to quantify the enzymatic activity of GLCL. Fluorescence increase was converted to nanomoles GSH using standardized solutions of GSH and measuring the fluorescence increase after addition of 100 \mu M mBCl plus purified glutathione-S-transferase (GST).

**LDH Assay**

To measure viability of cells exposed to tBH, a lactate dehydrogenase (LDH) assay was used. PBS (0.9 ml) was added to a 100 \mu l sample of the reaction mixture, and the change in absorbance at 340 nm with time was immediately measured. Duplicates were measured for each plate. The cells were lysed by the addition of 100 \mu l 10% (vol/vol) Triton X-100 to the growth medium, and the rate of NADH oxidation was again measured. The ratios of the rates before and after lysing provide the percentage of LDH leakage and were used as a measure of cell viability, with 100% viability taken as the ratio for untreated control cells.

**RESULTS**

To determine whether DMF increased GSH in hRPE, cells were treated with increasing concentrations of DMF (0, 50, 100, 200, and 500 \mu M), and intracellular GSH concentrations were analyzed after 24 hours. The GSH content for hRPE cells under control conditions was 12.5 ± 1.5 nanomoles/mg protein. Glutathione concentration increased as a function of DMF concentration, with a maximal GSH level 2.5 times more than obtained in control samples at 200 \mu M DMF (Fig. 1). At 500 \mu M DMF, GSH was substantially decreased, and examination of cell viability with trypan blue showed 100% cell death after 24 hours (data not shown). Examination of cells cultured at 100 and 200 \mu M DMF did not reveal signs of toxicity (e.g., blebbing, lifting off the plates, or loss of trypan blue exclusion). Thus, the results show that treatment of hRPE cells with DMF caused an increase in intracellular GSH levels. The maximum increase was seen after treatment with 200 \mu M DMF, with higher DMF concentrations proving toxic.

To examine the time course of increase in GSH, measurements were performed at 1 to 24 hours after addition of 100 \mu M DMF to the media. Results showed that DMF caused a transient but substantial decrease in intracellular levels of GSH (Fig. 2). The lowest GSH levels were within 3 hours of addition.
indicating a rapid depletion of GSH as a consequence of adding DMF. Experiments with 200 μM DMF resulted in no detectable GSH at 1 and 3 hours (data not shown). However, in both cases, GSH had recovered substantially by 8 hours and had increased to levels considerably above those in control samples by 16 and 24 hours. Thus, the results show that DMF caused an immediate depletion of GSH that was followed by a recovery and overshoot of the initial GSH concentration. Examination of the cells treated with 100 μM and 200 μM DMF, using trypan blue exclusion, showed no evidence of loss of viability caused by diminished GSH (data not shown).

To determine whether the cause of the initial transient decrease in intracellular GSH levels in hRPE cells treated with DMF was caused by an efflux of GSH or glutathione disulfide (GSSG), the amounts of GSH, GSSG, and its most common oxidized derivative, cysteine-glutathione disulfide (CySSG), were measured in the media 1, 3, 8, 16, and 24 hours after DMF addition (Fig. 3). The amount of GSH in the medium of cells after DMF treatment was similar to the amount present in the medium of untreated cells. A GSSG concentration was not detectable in the medium. The amount of extracellular CySSG increased substantially by 24 hours with the DMF-treated cells but not in control cells. However, this increase was only 2 to 4 nanomoles/mg protein at 3 to 8 hours (compared with 12.5 nanomoles total cellular GSH). Thus, even though there was some increase in total GSH in the medium, this increase occurred much later than the time course of the cellular decrease and was not sufficient to account for the 11 nanomoles/mg protein loss in the cells.

To further test whether the initial decrease in intracellular GSH levels in hRPE cells could have been caused by oxidation induced by DMF, we analyzed GSSG in cells treated with DMF. The results showed that GSSG decreased during this period (Fig. 4). Although the ratio of GSH to GSSG decreased during this period, the decrease in the ratio was caused by the decrease in intracellular GSH rather than an increase in GSSG. To further test whether DMF created oxidative stress in hRPE cells, we used DCFH-DA hydrolysis and oxidation to DCF as a measure of ROS generation. For positive controls, cells were treated with concentrations of two compounds known to produce intracellular ROS, 300 μM H2O2 and 500 μM tBHP.19–21 Cells treated with 100 μM DMF for 3 hours had no detectable increase in fluorescence compared with controls, whereas positive controls showed a greater fluorescence than respective untreated cells (Fig. 5). Thus, the data indicate that DMF did not induce oxidative stress, when measured by this fluorescent indicator. Together with the GSH and GSSG data, the results indicate that neither oxidative stress nor GSH efflux can account for the initial transient decrease of GSH in cells exposed to DMF.

Previous studies have shown that inducers of GSH-dependent detoxification systems share a common feature of being Michael acceptors,22–25 compounds that react with GSH to form a conjugate. Dimethylfumarate has features of a Michael acceptor and therefore is expected to form a conjugate with GSH. To determine whether a DMF-GSH conjugate is formed and could account for the early loss of GSH that was observed, we examined extracts of DMF-treated cells by HPLC. After treatment with dansyl chloride, a peak eluting approximately 1.4 to 1.8 minutes before GSH that was not present in untreated cells was consistently observed (Fig. 6). This peak was also present in chromatograms of extracts from incubation of 50 μM DMF and 50 μM GSH in HBSS (pH 7.4) that had been similarly derivatized. This peak was not seen in 50 μM GSH without DMF, nor in mixtures of S-carboxymethyl GSH plus DMF. The peak was eliminated by treating cell extracts with γ-glutamyl-transpeptidase, an enzyme that degrades GSH conjugates, before derivatization and HPLC. Thus, the results indi-
Glutathione in Dimethylfumarate-Treated hRPE Cells

To determine whether increased intracellular GSH caused by a 3-hour pretreatment with DMF altered the sensitivity of the hRPE cells to oxidative stress, three different cell lines were incubated with tBH, a model oxidant that has been used extensively with cultured hRPE cells.4,19 Because the cell lines differ in their sensitivity to tBH, we initially exposed the cell lines to increasing concentrations of tBH for 24 hours to establish the highest tBH concentration at which the cells exhibited a 0% to 10% loss of cell viability, measured by trypan blue exclusion (Fig. 9A, concentration identified as b). These tBH concentrations ranged from 250 to 300 μM. Each cell line was then incubated with 100 μM DMF for 3 hours followed by the aforementioned concentration of tBH for 24 hours. After 24 hours, viability was measured by LDH release into the media. Although this release is traditionally used to measure necrosis, our recent studies of oxidant-induced cell death in hRPE cells indicate that LDH release probably represents necrosis that occurs in vitro secondary to apoptosis.19 When compared with untreated control cells, cells pretreated with DMF showed a 98% reduction in viability at a tBH concentration that caused a 0% to 2% reduction in viability of cells treated with DMSO only (Fig. 9B). Thus, cells were more sensitive to oxidative stress during the initial decrease of GSH after DMF exposure. It is likely this increased sensitivity was caused by lower GSH levels during oxidative stress, because low levels of GSH have been associated with oxidative cell death in previous studies.28,29

To determine whether increased intracellular GSH pretreatment with DMF for 24 hours protects cells from cell death associated with oxidative injury, three different cell lines were used for study. Because the cell lines differed in their sensitivity to tBH, we initially performed experiments with various concentrations of tBH to establish the lowest concentration that resulted in approximately 90% cell death (Fig. 9A, concentration identified as c). These tBH concentrations ranged from 500 to 500 μM. Dishes of cells from these cell lines were then pretreated with 100 μM DMF for 24 hours, followed by the appropriate concentration of tBH for 24 hours. Cells with increased GSH caused by treatment with DMF exhibited a 12% loss of viability, whereas cells treated with DMSO only showed...
an 85% decrease in viability (Fig. 9C). Together with the above data, the results show that the increased sensitivity of cells to oxidative stress after DMF treatment was transient; the eventual increase in GSH protected the cells against oxidative damage.

** DISCUSSION **

Epidemiologic data and animal studies have provided evidence that oxidative mechanisms may contribute to the progression of ARMD,\(^3\) and a large-scale interventional study is currently under way to assess whether supplementation with antioxidants and/or zinc protects against onset or progression of ARMD.\(^4\) Our present study was devised to gain information on an alternate strategy, namely, whether a nonnutritive compound that increases GSH-dependent detoxification enzymes can be used to enhance cellular defenses against oxidative injury.

Our experiments were performed with established normal cell lines of human RPE. Histologic studies show that RPE cells are affected early in the progression of macular disease,\(^5\) and animal studies show that these cells are vulnerable to light-induced injury. In vivo, the RPE cells are exposed to high partial pressures of oxygen, and the cells in the macula are exposed to focused light. Thus, although the evidence remains circumstantial, it seems possible that oxida-

** FIGURE 6. **HPLC chromatograms identifying a possible DMF-GSH conjugate. (A) Chromatogram of lysate of hRPE cells treated with DMSO only for 1 hour. The peak at approximately 35 minutes represents GSH. (B) Chromatogram of lysate of hRPE cells treated with DMF for 1 hour. Note the reduction of the GSH peak at approximately 35 minutes, and the appearance of a novel peak, labeled X, at approximately 33 minutes. The decreased area of the GSH peak in solutions containing DMF or in DMF-treated cells was consistent, as was the appearance of a novel peak approximately 2 minutes before GSH.

** FIGURE 7. **Northern blot analysis of the effect of 100 \(\mu\)M DMF on the catalytic subunit (GLCLc) and the regulatory subunit (GLCLR) of \(\gamma\)-glutamylcysteine ligase after 1-, 3-, and 6-hour treatments. G3PDH was used as a control for loading. Data are composed of three experiments using established cell lines from three different donor eyes.
TABLE 1. Expression of the Catalytic Subunit (GLCLc) and the Regulatory Subunit (GLCLR) of γ-Glutamylcysteine Ligase after Treatment with 100 μM DMF.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (h)</th>
<th>n</th>
<th>GLCLc Expression</th>
<th>GLCLR Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>1</td>
<td>2</td>
<td>83 (72–93)</td>
<td>123 (87–158)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>64 (47–91)</td>
<td>127 (94–135)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2</td>
<td>121 (72–169)</td>
<td>203 (42–265)</td>
</tr>
<tr>
<td>DMSO + DMF</td>
<td>1</td>
<td>3</td>
<td>63 (48–77)</td>
<td>122 (100–192)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>76 (67–90)</td>
<td>221 (132–371)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3</td>
<td>99 (74–117)</td>
<td>465 (233–666)</td>
</tr>
</tbody>
</table>

Integrated optical densities were corrected for loading relative to G3PDH expression. Values represent mean optical densities (range) expressed as a percentage of the control.

tive injury contributes to macular disease development and/or progression. Although RPE cell cultures used in the present study retain morphology characteristic of RPE, they differ from in vivo RPE because they have a decreased content of pigment granules and have acquired the capacity to proliferate. But these cultured cells remain sensitive to light-induced toxicity and chemical-induced oxidative injury. Thus, these cell cultures provide a useful in vitro model for study of potential mechanisms to protect the RPE from oxidative damage.

Our previous studies showed that cultured hRPE cells have the capacity to synthesize the antioxidant GSH. Moreover, the sensitivity of these cells to oxidative injury was decreased by supplying amino acid precursors of GSH, and this sensitivity was increased by inhibiting GSH synthesis. Thus, hRPE cells are sensitive to oxidative stress, and enhanced synthesis of GSH protects against oxidative stress in these cells. The present studies show that cellular GSH also can be increased by a nonnutritive dietary compound and that pretreatment with this compound also protects against oxidative injury. Thus, the results support the principle that antioxidant defenses can be enhanced in the hRPE by nutritionally or therapeutically increasing GSH synthesis.

Although DMF ultimately caused a large increase in intracellular GSH concentrations, it immediately depletes the cells of GSH. Our evidence suggests that this depletion was not caused by increased efflux of GSH from the cells or by an oxidative insult. The results suggest that DMF formed a conjugate with GSH that was exported and/or metabolized by the cell. A significant increase in extracellular GSH and CySSG concentration at 16 to 24 hours after DMF addition (Fig. 3) provides evidence that the efflux of GSH increased only after intracellular concentrations were increased. Therefore, our evidence supports the conclusion that a DMF-GSH conjugate, or a decrease in GSH per se, acts to induce the synthesis of glutathione. If induction is stimulated by a GSH conjugate, then it may be possible to stimulate synthesis with a more potent conjugate under conditions that do not deplete GSH.

The increase in GSH after treatment with DMF is consistent with previous studies showing transcriptional regulation of GLCL. However, in contrast to results showing increased expression of catalytic subunit only, DMF appeared to induce activity in hRPE cells in a previously unrecognized manner, by increasing the expression of the mRNA and the resultant protein for the regulatory subunit. An increase in the amount of regulatory subunit may result in an increased half-life of the catalytic subunit or alter its kinetic properties to account for the twofold increase in activity of GLCL.

Despite these very promising observations concerning increased GSH synthesis, it is unclear whether the initial GSH decrease would pose a health risk, whether GSH increases in the RPE can be achieved in vivo, whether such increases can be sustained over long periods, and whether such increases can protect against oxidative processes that may contribute to...
ARMD. The initial decrease in GSH concentration with DMF suggests that intermittent therapy may pose a risk. However, if long-term exposure allows a sustained elevation of GSH, the use of DMF as a therapeutic agent for the prevention and treatment of ARMD may nonetheless be feasible. Alternatively, other inducers that increase intracellular GSH levels without causing a transient decrease, such as oltipraz,43 may be effective and more useful therapeutically.

The present finding of an increase of GSH in cultured hRPE cells caused by DMF treatment suggests a possible mechanism that could contribute to epidemiologic findings of an association of fruit and vegetable consumption and reduced risk of ARMD.34,45 Plants contain a variety of compounds that induce detoxication enzymes, including the rate-limiting enzyme of GSH synthesis, GLCL. People who consume diets high in fruits and vegetables may have higher GSH concentration in their RPE cells as a consequence of this induction and therefore may have enhanced antioxidant defenses. In support of this hypothesis, a previous demographic study showed increased plasma GSH in subjects who reported higher fruit and vegetable consumption on a food frequency questionnaire.5

In conclusion, our results show that a nonnutritive dietary compound results in increased intracellular GSH in hRPE cells and consequent protection against oxidative injury. These results suggest that consumption of diets high in such compounds, or therapeutic administration of such compounds, could enhance antioxidant defenses and protect against ARMD. However, early depletion of GSH by DMF also suggests that experimental use of such a strategy must be undertaken cautiously to determine whether the transient decrease in GSH could contribute to increased susceptibility to oxidative injury.

References


