MINIREVIEW

Oxidative Stress Plays an Important Role in the Pathogenesis of Drug-Induced Retinopathy

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Several pharmaceutical agents have been associated with rare but serious retinopathies, some resulting in blindness. Little is known of the mechanism(s) that produce these injuries. Mechanisms proposed thus far have not been embraced by the medical and scientific communities. However, preclinical and clinical data indicate that oxidative stress may contribute substantially to iatrogenic retinal disease. Retinal oxidative stress may be precipitated by the interaction of putative retinal toxins with the ocular redox system. The retina, replete with cytochromes P450 and myeloperoxidase, may serve to activate xenobiotics to oxidants, resulting in ocular injury. These activated agents may directly form retinal adducts or may diminish ocular reduced glutathione concentrations. Data are reviewed that suggest that indomethacin, tamoxifen, thioridazine, and chloroquine all produce retinopathies via a common mechanism—they produce ocular oxidative stress. Exp Biol Med 229:607–615, 2004

Key words: oxidative; stress; drug; retinopathy; myeloperoxidase

Drug-induced retinopathy is an infrequent but serious complication associated with the use of a number of pharmacologically and structurally diverse compounds. Though a number of potential mechanisms have been proposed to explain these iatrogenic injuries, the etiology of drug-induced retinopathy is largely unknown. One theory contends that many retinal toxins are cationic amphiphilic drugs (CADs) that concentrate in lysosomes and produce phospholipidosis. Theoretically, these CADs could increase lysosomal pH, inhibiting enzymatic function within the organelle. Alternatively, such agents could disrupt the lysosomal membrane. Potentially, either mechanism could lead to the accumulation of phospholipids. Progressive phospholipidosis somehow impairs cellular function resulting in retinopathy. However, many pharmaceuticals commonly used today are CADs, and most do not produce retinopathy (Table 1; Ref. 1). In animal models, drug-induced retinal phospholipidosis has not been directly linked with diminished retinal function (1–4). In addition, only a few CADs have ever been shown to produce phospholipidosis in humans, and of those that do, there is little evidence that this produces significant clinical disease (1). Another proposed theory asserts that retinal toxicity is related to binding of toxic agents to ocular melanin. However, many drugs on the market today bind to melanin and produce no ocular toxicity (Table 2; Ref. 5). Furthermore, chloroquine and chlorpromazine, two compounds classically associated with retinopathies, produce similar retinal lesions in both pigmented and nonpigmented animals (5). Toxicologists have noted the lack of causation between melanin binding and ocular toxicity for decades (5, 6). It is possible that a number of mechanisms may be operative in the development of drug-induced retinopathy. Currently, there is no general mechanism accepted for most xenobiotic-induced retinal toxicities, making such injuries difficult to predict, avoid, and/or manage (6). Insight into one or more of the possible mechanisms by which drugs produce retinal damage may facilitate the development of future pharmaceuticals with reduced potential for precipitating injury.

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Table 1. Selected Amphiphilic Cationic Drugs Without Reported Retinal Toxicity

Agent	Pharmacological class
Amitriptyline	Tricyclic antidepressant
Chlorpheniramine	Antihistamine
Cyclobenzaprine	Centrally mediated muscle relaxant
Diphenhydramine	Antihistamine
Fenfluramine	Adrenergic agonist
Fluoxitene	Serotonin-specific reuptake inhibitor
Gentamicin	Aminoglycoside antibiotic
Imipramine	Tricyclic antidepressant
Lidocaine	Anesthetic
Methadone	Synthetic opioid
Propoxyphene	Synthetic opioid
Propranolol	β-blocker

Retinal Anatomy

The retina is a highly compact, metabolically active, neural structure that is approximately 100- to 500-μm thick and occupies the innermost layer of the eyeball (Fig. 1; Ref. 6). Composed of distinct layers, the retina receives nourishment from the vascular choroid that lies just above the retinal pigmented epithelium (RPE). The RPE is composed of a monolayer of cells, rich in melanin, and serves a number of crucial metabolic functions including the phagocytosis and removal of shed photoreceptor cell outer segments (7). Directly below the RPE reside the photoreceptor cells whose outer segments are enveloped by pseudopod-like extensions from the RPE (8). The photoreceptor cells, composed of rod and cone cells, contain the photosensitive pigment rhodopsin. Activated by light, conformational changes in rhodopsin initiate the neuronal impulses associated with sight. The terminal folds of the rods and cones are continually shed as new disks replace them. To prevent accumulation, the RPE phagocytizes the shed disks. After phagocytosis by the RPE, shed photoreceptor cell outer segments are digested by powerful oxidants produced by myeloperoxidase. The rods and cones contain a number of obligatory cysteines that function to maintain the disk-like invaginations of the photoreceptor cells, and these cysteines are vulnerable to oxidation from the proximal RPE (9). To protect the photoreceptor cells from oxidative and structural injury, Müller cells, rich in glutathione, traverse the entire thickness of the retina (6). Immediately below the photoreceptor cells reside the bipolar cells. The bipolar cells synapse proximally with the photoreceptor cells and distally with cells composing the final layer of the neuroretina—the ganglion cells. Bipolar cells also communicate laterally with horizontal cells and amacrine cells. The bipolar cells process signals produced from photoreceptor cells, and the ganglion cells translate these signals into action potentials.

Table 2. Selected Drugs That Bind to Melanin Without Reported Retinal Toxicity[a]

Agent	Pharmacological class
Atropine	Antimuscarinic
Bupivicaine	Anesthetic
Clindamycin	Lincomycin antibiotic
Codeine	Opioid
Haloperidol	Neuroleptic
Imipramine	Antidepressant
Lidocaine	Anesthetic
Labetalol	β-blocker
Methadone	Synthetic opioid
Noradrenaline	Adrenergic agonist
Propranolol	β-blocker
Rifampicin	Rifamycin antibiotic
Salbutamol	β-agonist
Sumatriptan	5-HT_1 agonist
Timolol	β-blocker

[a] Adapted from Ref. 5.

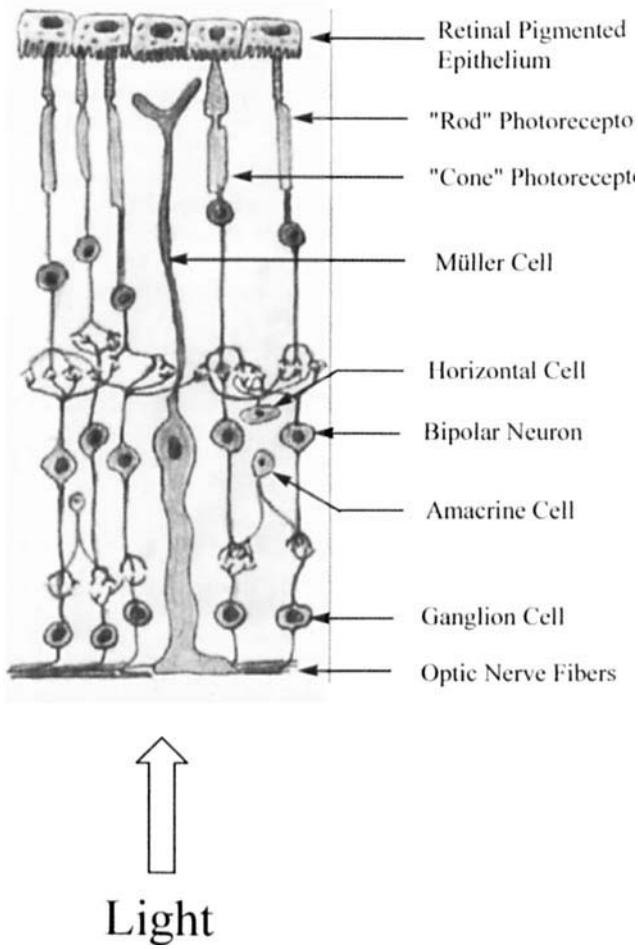

Figure 1. Retinal anatomy. (Adapted from Darnell J, Lodish H, and Baltimore D. Nerve cells, and the electric properties of cell membranes. In: Molecular Cell Biology. New York: W.H. Freeman and Company, pp762–814, 1990.)

Early Models of Ocular Injury

It was noted as early as the 1920s that iodate (IO_3^-), a stable oxidizing agent, produced retinal injuries following systemic administration (6). Iodate was first administered

during the preantibiotic period as an antimicrobial agent under the trade name of Septojod (6, 10). Shortly after its initial use, several cases of blindness ensued (10). In animal models, iodate primarily injures the retinal pigmented epithelium, producing a pigmented retinopathy with degeneration of the rod outer segments (ROSs; Ref. 6). When administered to rabbits, iodate produces an initial acute decline in soluble sulfhydryl concentrations, followed 16 hrs post-dose by a dramatic increase in total and free sulfhydryl concentrations, suggesting significant denaturation of retinal proteins (11). Interestingly, co-administration of cysteine blocks the retinotoxic effects of iodate (12).

Another simple iodinated compound, iodoacetate ($C_2H_2IO_2^-$), also produces retinal injuries in animal models (13). Iodoacetate is an inhibitor of glyceraldehyde-3-phosphate dehydrogenase (G-3-PD) and therefore is an inhibitor of glycolysis (6). Because photoreceptor cells depend extensively on glycolysis for energy, it was initially thought that iodoacetate produced retinal injury by inhibition of G-3-PD with subsequent loss of cellular energetics and photoreceptor cell death. However, following the administration of iodoacetate, morphologic changes in the photoreceptor cells precede changes in enzymology; electroretinogram (ERG) changes are evident within minutes of injection (14). Furthermore, administration of iodoacetate alters the free sulfhydryl content on photoreceptor cells (15). It has been suggested that rapid alteration of photoreceptor cell sulfhydryls produces the retinal toxicity rather than inhibition of glycolysis (6). Iodoacetate produces a pigmented retinopathy with retinal lesions similar to those observed in patients with retinitis pigmentosa (14, 16). In addition to inhibiting G-3-PD, iodoacetate is a powerful electrophilic alkylating agent and readily forms covalent adducts with sulfhydryls, including those of cysteine and glutathione (14, 17). As noted with iodate injections, co-administration of cysteine blocks the retinotoxic effects of iodoacetate (12). Therefore, it is likely that the depletion of photoreceptor cell sulfhydryls is the result of electrophilic substitution of iodoacetate on crucial ROS cysteines. Bubis *et al.* have shown that iodoacetate alkylates a number of transducin cysteine residues, including Cys347, from isolated bovine retinas, yielding a dysfunctional protein (18). In a similar manner, iodoacetate likely diminishes ocular reduced glutathione and perturbs the redox balance within the retina.

Retinal toxicity following the administration of both iodate and iodoacetate is enhanced by preadministration of the oxidizing agent potassium permanganate (19).

Ocular Oxidations: Cytochrome P450 and Myeloperoxidase

The eye has metabolic capabilities to oxidatively activate xenobiotics. Although not extensively studied to date, cytochrome P450 enzymes appear to be expressed in both the anterior and posterior portions of the eye (20). These enzymes, including CYP1A1/1A2 and CYP4A, are constitutively expressed and are capable of being induced and inhibited (21, 22). Of interest, rats with hereditary retinal degeneration appear to express higher concentrations of P450 relative to controls (23). The retina also appears to have meaningful concentrations of both monoamine oxidase (MAO) A and B as well as a retinal specific amine oxidase (RAO) and xanthine oxidase (24–27). In addition, the retina contains large amounts of myeloperoxidase, an enzyme with great oxidizing potential (28). Myeloperoxidase is released from activated phagocytic cells, primarily neutrophils and monocytes (29). However, the retinal pigmented epithelium is also capable of phagocytosis, a function necessary for the maintenance of vision (28, 30). Retinal pigmented epithelial cells phagocytize the shed disks of the photoreceptor ROSs. Significant defects in this phagocytotic process may result in retinal degeneration (31). The RPE uses myeloperoxidase to catalyze the formation of the potent oxidizer hypochlorous acid (HOCl) from hydrogen peroxide (H_2O_2) and chloride ions (28). Within the phagosomes of the RPE, the shed photoreceptor cell outer segments are oxidatively destroyed. Importantly, hypochlorite ion (^-OCL) is an excellent oxidizer and is noted for the oxidation of aromatic amines, sulfhydryls, and phenols (29). The presence of myeloperoxidase within the RPE may be relevant to drug-induced retinopathy, as this enzyme is known to activate arylamines and phenols to cytotoxic intermediates (32). Substrates oxidized by myeloperoxidase have strongly been associated with idiosyncratic drug-induced toxicity, especially neutropenia and hepatotoxicity (29, 32, 33).

Indomethacin, Tamoxifen, and Thioridazine

Indomethacin has been associated with retinal injuries, yet it is not a CAD nor is it noted for extensive binding to melanin (34, 35). Indomethacin was introduced in 1963 as an anti-inflammatory agent for the treatment of rheumatoid arthritis. It inhibits both cyclooxygenase I and II and has analgesic properties that are distinct from its anti-inflammatory effects (36). Indomethacin's retinal toxicity is unique among the nonsteroidal anti-inflammatory agents, suggesting that the retinal toxicity associated with this agent is unrelated to inhibition of cyclooxygenase. Morphologically, retinal injuries following the use of indomethacin have been described as a pigmented retinopathy with atrophy in the macula and a waxy disk (34, 35, 37). These morphological changes have been accompanied by depressed ERGs and constricted visual fields (34, 35). In addition to retinal toxicity, indomethacin has been associated with neutropenia and hepatotoxicity (38). Metabolically, indomethacin is sequentially O-dealkylated to produce *O*-des-methylindomethacin (DMI) and then N-deacylated by microsomal systems to yield desmethyldeschlorobenzoylindomethacin (DMBI), a metabolite that can easily form a quinone imine following further oxidation (Fig. 2; Ref. 39). Unconjugated DMBI is a major circulating

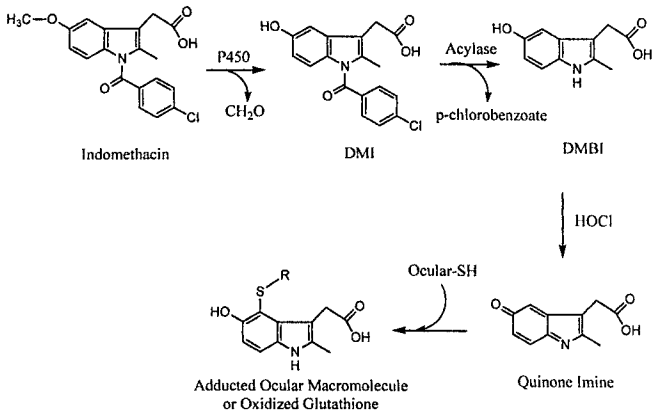

Figure 2. Oxidation of indomethacin to a reactive quinone imine with subsequent adduction to an ocular/retinal macromolecule or ocular glutathione. (Adapted from Ref. 39.)

metabolite of indomethacin (39). Ju *et al.* have demonstrated that a quinone imine can be formed from DMBI under oxidizing conditions and can be trapped by either glutathione or *N*-acetylcysteine (39). Ju *et al.* propose that this electrophilic quinone produces neutropenia by alkylation of vital intracellular macromolecules with resultant leukocyte death (39). Because the eye also contains microsomal enzymes and myeloperoxidase, it is possible that indomethacin is metabolized in ocular tissues in the same manner that it is metabolized in neutrophils. DMBI, a known substrate for myeloperoxidase, may be oxidized in the RPE to a stable quinone imine. This electrophilic quinone imine could form adducts with vital ocular macromolecules and/or diminish reduced glutathione concentrations, leading to retinopathy (Fig. 2).

Tamoxifen is a selective estrogen receptor modulator (SERM), primarily indicated for breast cancer prevention and treatment, that has been shown to produce retinal toxicity (40). Interestingly, like indomethacin, tamoxifen has also been associated with neutropenia and hepatotoxicity (41–43). Retinal toxicity associated with the use of tamoxifen is marked by pigmented retinopathy, a decrease in visual acuity, bilateral macular edema, and yellow/white spots in the paramacula (40). Most ocular toxicities have been associated with extended use (greater than 1 year) and may occur in up to 6% of treated subjects (40). The metabolism of tamoxifen has rigorously been studied. After oral administration, tamoxifen is extensively metabolized to *N*-desmethyl tamoxifen, 4-hydroxy-tamoxifen, a side-chain primary alcohol, and a variety of polar conjugates (43). Tamoxifen is excreted mainly as polar conjugates (43). 4-Hydroxytamoxifen, a substrate for myeloperoxidase, may further be oxidized to a relatively stable quinone methide (Fig. 3; Ref. 44). Alternatively, 4-hydroxytamoxifen may further be metabolized to 3,4-dihydroxytamoxifen by either P450 or tyrosinase (45). The activity of tyrosinase, an enzyme crucial for the production of melanin, is particularly high in the retinal pigmented epithelium (46). 3,4-

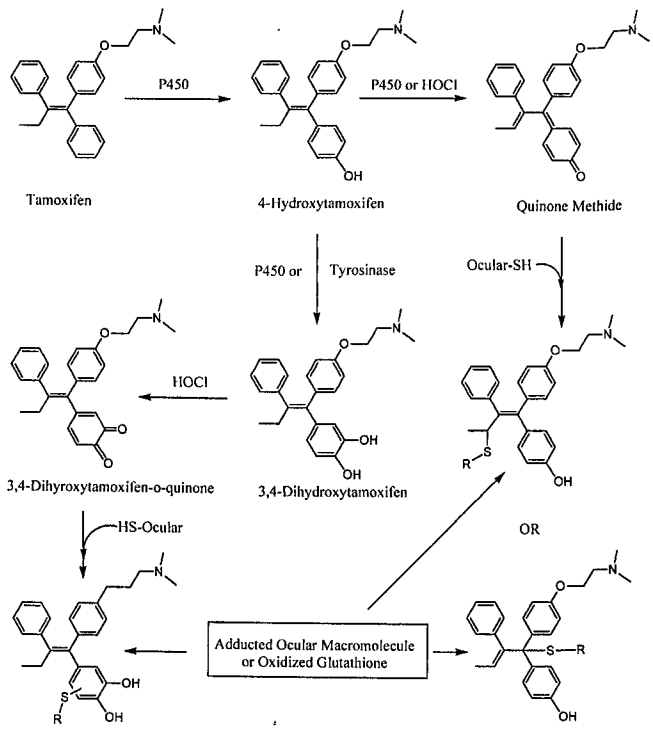

Figure 3. Oxidation of tamoxifen to reactive quinones with subsequent adduction to an ocular/rentinal macromolecule or ocular glutathione. (Adapted from Refs. 44 and 45.)

Dihydroxytamoxifen may further be oxidized to a reactive ortho-quinone (Fig. 3; Ref. 45). These reactive quinones are capable of forming covalent adducts with cellular DNA, proteins, and glutathione (47–49). Because the retina expresses high activities of both myeloperoxidase and tyrosinase, it may be particularly efficient at forming reactive quinones from tamoxifen. Alkylation of crucial retinal macromolecules or oxidation of free glutathione may produce oxidative cellular stress and photoreceptor cell death (Fig. 3).

Several phenothiazines, used in the treatment of schizophrenia, have been noted to produce retinal injury (37). During the initial development of NP-207, a piperidylchlorophenothiazine, retinal injuries were observed after administration of this agent to clinical trial subjects (50). Decreases in visual acuity, constriction of visual fields, abnormal ERGs, and "salt and pepper" retinopathy were noted with doses of 400 to 800 mg per day (51). Ocular changes and lesions were observed within 2 to 3 months of dosing. Due largely to the retinal damage, further clinical studies with this agent were halted (52). Other phenothiazines have been noted to produce retinopathies similar to those reported from the use of NP-207. Agents producing retinal changes appear to be primarily limited to the aminopropyl and piperidine phenothiazines, possibly because these agents require larger doses than the more potent piperazine derivatives (6). Both chlorpromazine and thioridazine have been implicated in the development of pigmented retinopathies with associated declines in visual

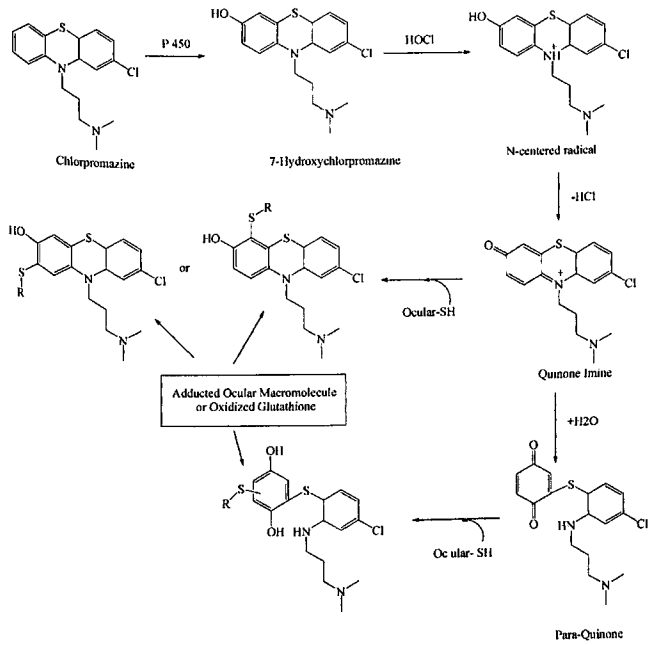

Figure 4. Oxidation of chlorpromazine to reactive quinones with subsequent adduction to an ocular/retinal macromolecule or ocular glutathione. (Adapted from Neptune and McCreery, 1978; Ref. 62)

acuity (53, 54). Retinal toxicities have been reported to a greater degree with thioridazine than with chlorpromazine. However, chlorpromazine may yield more pigmentary changes associated with the lens and cornea (6). Like indomethacin and tamoxifen, thioridazine and chlorpromazine have been reported to produce neutropenia (agranulocytosis) and hepatotoxicity in addition to retinopathies (55). Elevated hepatic transaminases have been reported in up to 5% of subjects receiving thioridazine, and fatalities have been reported as a result of thioridazine-associated agranulocytosis (56, 57). Approximately 1% of subjects treated with chlorpromazine develop jaundice (57). Both chlorpromazine and thioridazine are hydroxylated at positions 3 and 7 of the phenothiazine ring system (58, 59). In addition, the phenothiazines, including chlorpromazine and thioridazine, are substrates of myeloperoxidase (60, 61). Furthermore, 3-hydroxychlorpromazine, 7-hydroxychlorpromazine, and 3,7-dihydroxychlorpromazine produce quinone and quinone imine metabolites following oxidations *in vitro* (59, 62). Such quinone imines would likely form under the strong oxidant conditions (hypochlorous acid) associated with retinal myeloperoxidase. These quinones and quinone imines would likely adduct crucial retinal macromolecules or oxidize free glutathione, yielding ocular oxidative stress and cellular injury (Fig. 4).

Chloroquine

Chloroquine was developed in the 1940s in hopes of producing an effective antimalarial agent with reduced systemic toxicity (37). The effort was largely successful. Except for infrequent cases of retinopathy, chloroquine is a generally well-tolerated medication. Retinopathy associated with the use of chloroquine is qualitatively similar (pigmented retinopathy) to those of the other classic retinal toxins (37). However, chloroquine does not produce hepatotoxicity or neutropenia, unlike tamoxifen, indomethacin, and thioridazine. Such a difference in the toxicity profile of chloroquine suggests a different mechanism for the associated retinopathy. Although theoretically possible following hydroxylation in position 6, studies to date indicate that chloroquine does not form a quinone metabolite to any significant degree (63). 6-Hydroxychloroquine has not been identified as a metabolite of chloroquine (64). When incubated with myeloperoxidase, chloroquine does not produce a reactive metabolite nor does it produce a chloroquine-glutathione adduct (63). However, when administered to male albino rats, chloroquine produces dose-dependent decreases in both retinal glucose-6-phosphate dehydrogenase (G-6-PD) activity and reduced retinal glutathione (GSH) concentrations (65). Reduced GSH concentrations are dependent on available reducing equivalents from nicotinamide adenine dinucleotide phosphate (NADPH) and the pentose phosphate pathway. Therefore, inhibition of retinal G-6-PD activity would diminish concentrations of retinal reduced NADPH and subsequently retinal GSH concentrations (65, 66). In addition to declines in reduced glutathione concentrations, retinal lipid peroxidation was enhanced in this rodent model after chloroquine treatment (65). Activities of glutathione-*S*-transferase and superoxide dismutase were unaffected (65). Pretreatment of animals with bacterial lipopolysaccharide, an activator of myeloperoxidase, further diminished retinal GSH concentrations and increased retinal lipid peroxidation (65). These toxicological findings are consistent with the proposed plasmocidal activity of chloroquine. Chloroquine has been shown to inhibit the pentose phosphate pathway in *Plasmodium* and thus diminish reduced glutathione concentrations (67). Diminished reduced GSH concentrations inhibit the detoxification of ingested ferriprotoporphyrin IX, leading to plasmodial membrane lipid peroxidation and subsequent parasite death (68). Of interest, subjects deficient in erythrocyte G-6-PD appear to be more resistant to malarial infections (69–71). Furthermore, co-administration of glutathione depleters with chloroquine enhances the plasmocidal activity (72, 73). The plasmodial parasite and the mammalian retina are both exquisitely sensitive to oxidative damage; chloroquine appears to produce oxidative injury to both by limiting the availability of reduced glutathione (65, 74).

Discussion

The retina, containing the RPE, is a metabolically active organ with tremendous oxidative capabilities similar to an activated neutrophil or macrophage (75). Following phagocytosis of shed photoreceptor disks, retinally produced oxidants destroy the discarded outer segments and

prevent their continued accumulation. Evolutionary pressures have selected for mechanisms that protect the fragile retina from oxidative injury. Müller cells actively excrete reduced glutathione throughout the neuroretina (76–81). Melanin, found within the RPE and choroid, possesses nucleophilic properties and thus serves as a protective structure, defending the neuroretina against oxidatively produced reactants (5, 82–84). However, the photoreceptor cells, rich in structural sulfhydryls, are particularly susceptible to oxidants produced in close proximity (9, 65, 85, 86).

Peripherin/rds, a tetra-spanning membrane protein, is responsible for the morphogenesis and stabilization of photoreceptor outer segment disk membranes in vertebrates (9, 85). This protein contains 13 cysteine residues, of which 7 are highly conserved in vertebrates (9). At least six of the seven conserved cysteines are crucial for correct pheripherin/rds conformation and thus proper morphology of the photoreceptor outer segments (9). It appears that some or all of the conserved cysteine residues contribute to intramolecular disulfide bonds. Furthermore, an identified mutant variant of the conserved cysteine residues, denoted C214S, has been linked to autosomal dominant retinitis pigmentosa (9). Deletions in nonconserved cysteines, 118 and 119, have also been linked to autosomal dominant retinitis pigmentosa (9). Other retinal degenerative diseases including macular dystrophy and retinitis punctata albescens have been associated with mutations located within a 150-residue intradiskal loop containing the 7 conserved cysteines (9). Therefore, it is apparent that alterations of peripherin/rds cysteines may yield structural aberrations in photoreceptor cells that are associated with significant retinal pathology. Xenobiotic-induced oxidation of crucial cysteine residues of the integral membrane photoreceptor cell protein peripherin/rds may lead to the disorganization and deterioration of outer disk segments and ultimately receptor cell death. A number of other photoreceptor proteins, including arrestin, opsin, and transducin, contain crucial cysteine amino acids that upon alteration may lead to retinopathy (18, 87, 88).

Polymorphisms associated with drug oxidations and conjugations have been linked to xenobiotic-induced toxicities (89). Myeloperoxidase, an enzyme that produces the potent oxidant perchlorous acid, is polymorphically expressed (90, 91). Located primarily in neutrophils and the retina, myeloperoxidase has been associated with oxidations yielding idiosyncratic drug-induced toxicities (29, 32, 33). In addition to myeloperoxidase, glutamate cysteine ligase (GCL) and glutathione-*S*-transferase (GST), enzymes regulating the production and use of glutathione, are polymorphically expressed as well (89, 92). Variations resulting in reduced activities of both GCL and GST have been linked to xenobiotic toxicities and retinal lipid peroxidation. Therefore, it is possible that variants in the expression and regulation of retinal myeloperoxidase, GST, and GCL may predispose specific individuals to drug-induced retinal injury.

Ingestion of antioxidants may protect against the development of retinal diseases (94–96). Several studies, including ARED and LAST, indicate that dietary intake of antioxidants may be beneficial for those at risk of developing retinal disease (94, 97). Much of the interest in the use of antioxidants for retinal diseases emerges from growing evidence that supports an oxidative etiology for age-related macular degeneration (86, 98). Data suggest that supplementation with vitamins A, C, and E, zinc, and lutein may prove beneficial in some subjects at risk for the development of macular degeneration (99–102). Vitamins C and E have been shown to help maintain concentrations of reduced glutathione in ocular tissues and prevent oxidative damage (101). Drug-induced retinal injury and age-related macular degeneration both appear to be related to oxidative injury (103). Macular degeneration may be precipitated in part by lifelong exposure to oxidative injury from exposure to environmental oxidants in susceptible subjects (86). Similarly, when exposed acutely to drugs that alter the redox balance in the retina, susceptible individuals may develop retinal injuries.

Summary

The eye contains meaningful concentrations of cytochromes P450, monoamine oxidase, xanthine oxidase, and myeloperoxidase. Like other organs with high oxidative potential, the retina is subject to injury from reactive metabolites generated from proximal enzymes. Simple oxidants like iodate and iodoacetate produce retinal injuries strikingly similar to those observed following the administration of more complex molecules, including indomethacin, tamoxifen, thioridazine, and chloroquine. Drugs that lead to retinal toxicity are pharmacologically and structurally diverse, yet most have one characteristic in common: they all produce oxidative stress in at least one other cell type. In general, animal models indicate that the co-administration of pro-oxidants exacerbate retinal injuries, whereas antioxidants afford some protection against injury. Clinicians have been suspicious for years that oxidative stress contributes significantly to progressive retinal disease, and some now advocate the judicious use of antioxidants for macular degeneration. Clearly, more research is needed, but compelling preclinical and clinical data suggest that iatrogenic retinal injury results from oxidative stress induced by these agents.

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