chapter 17 Toxic Responses of the Ocular and Visual System

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Introduction to Ocular and Visual System Toxicology

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INTRODUCTION TO OCULAR AND VISUAL SYSTEM TOXICOLOGY

Environmental and occupational exposure to toxic chemicals, gases, and vapors, and side effects of systemic and ocular therapeutic drugs may result in structural and functional alterations to the eye and central visual system (Anger and Johnson, 1985; Grant and Schuman, 1993; Otto and Fox, 1993; Fox, 1998; Santaella and Fraunfelder, 2007; Bartlett and Jaanus, 2008). Almost half of all neurotoxic chemicals affect some aspect of sensory function (Crofton and Sheets, 1989). The most frequently reported sensory system alterations occur in the visual system (Anger and Johnson, 1985; Crofton and Sheets, 1989; Fox, 1998; Grant and Schuman, 1993). Approximately 3000 substances are toxic to the eye and visual system (Grant and Schuman, 1993). In many cases, alterations in retinal and visual function are the initial symptoms following chemical exposure (Hanninen et al., 1978; Damstra, 1978; Baker et al., 1984; Anger and Johnson, 1985; Mergler et al., 1987; Iregren et al., 2002). This suggests that sensory systems, and in particular the retina and central visual system, may be especially vulnerable to toxic insult. Alterations in the structure and/or function of the eye or central visual system are among the criteria utilized for setting permissible occupational or environmental exposure levels for many different chemicals in the United States (http://www.cdc.gov/niosh/npg/; http://www.epa.gov/iris/index.html). New or existing drugs may have visual side effects (Grant and Schuman, 1993; Novack, 2003; Brigell et al., 2005; Santaella and Fraunfelder, 2007). Subtle alterations in visual processing of information (eg, visual perceptual, visual motor) can have profound immediate, long-
term, and delayed effects on the mental, social, and physical health and performance of an individual. Among the elderly, reduced visual function is a major factor contributing to decreased ability to conduct routine activities of daily living, decreased ability to live independently, and increased risk of falls, car crashes, and other hazards. Ocular and visual system impairments can lead to increased occupational injuries, loss of productive work time, costs for providing medical and social services, lost productivity, and a distinct decrease in the overall quality of life.

The overall goals of this chapter are to provide essential information on ocular pharmacodynamics, pharmacokinetics, and drug metabolism; review the procedures for testing visual function; and evaluate and review the structural and functional alterations in the mammalian eye and central visual system produced by environmental and workplace chemicals, gases, and vapors, and major therapeutic drugs. Except where noted, all these compounds are referred to as chemicals and drugs. The adverse effects of these agents on the different compartments of the eye (ie, cornea, lens, retina, and retinal pigment epithelium [RPE]), central visual pathway (ie, optic nerve and optic tract), and the central processing areas (ie, lateral geniculate nucleus [LGN], visual cortex) are addressed (Table 17-1). To further understand the potential effects of these chemicals and drugs on the eye, the distribution of Phase I and Phase II drug metabolizing enzymes in ocular tissues are presented in Table 17-2. Table 17-3 provides examples of common signs, symptoms, and potential pathophysiological mechanisms of visual dysfunction associated with acute or chronic exposure to toxic compounds and selected drugs. Some of the symptoms are very nonspecific (eg, “blurred vision”) whereas others are more definitive.

The ophthalmological evaluation of the eye and the testing of visual function are discussed in this chapter, as the results from clinical, behavioral, and electrophysiological studies form the basis of diagnosis and understanding of adverse visual system effects. Many of the chemicals discussed below initially appear to have a single site and, by inference, mechanism of action, whereas others have several sites and corresponding mechanisms of action. However, a more in-depth examination reveals that, depending upon dose (concentration), many of these chemicals have multiple sites of action. A few examples illustrate the point. First, as described below in more detail, carbon disulfide produces optic nerve and optic tract degeneration and also adversely affects the neurons and vasculature of the retina, resulting in photoreceptor and retinal ganglion cell (RGC) structural and functional alterations (Raitta et al., 1974, 1981; Palacz et al., 1980; Seppalainen and Haltia, 1980; De Rouck et al., 1986; Eskin et al., 1988; Merigan et al., 1988; Fox, 1998). Second, gestational and postnatal exposure to inorganic lead clearly affects rod photoreceptors in developing and adult mammals, resulting in rod-mediated (scotopic) vision deficits; however, structural and functional deficits at the level of the RGCs, visual cortex, and oculomotor system also are observed (Fox and Sillman, 1979; Fox, 1984; Glickman et al., 1984; Otto and Fox, 1993; Lilienthal et al., 1988, 1994; Reuhl et al., 1989; Ruan et al., 1994; Fox et al., 1997, 2011; Rice, 1998; Rice and Hayward, 1999; He et al., 2000, 2003; Rothenberg et al., 2002; Nagpal and Brodie, 2009; Giddabasappa et al., 2011). Although both gestational and postnatal lead exposure produce scotopic electroretinographic (ERG) deficits, the amplitude changes are in
opposite directions and their underlying mechanisms are distinctly different. Finally, some environmental and occupational neurotoxicants (eg, acrylamide, lead) have been utilized for in vivo and in vitro animal models to examine the pathogenesis of selected retinal, neuronal, or axonal diseases; the basic functions of the retinocortical pathways; and/or the molecular mechanisms of apoptosis (Fox and Sillman, 1979; Vidyasagar, 1981; Lynch et al., 1992; He et al., 2000, 2003). The conceptual approach, format, and overall organization of this chapter on ocular and visual system toxicology for this edition of Casarett and Doull’s Toxicology: The Basic Science of Poisons were designed in anticipation that the main audience would be graduate and medical school students, ophthalmologists and occupational physicians, basic and applied science researchers interested in ocular and visual system toxicology, and those interested in having a basic reference source. To write this chapter, information was synthesized and condensed from several excellent resources on different aspects of ocular, retinal, and visual system anatomy, biochemistry, cell and molecular biology, histology, pharmacology, physiology, and toxicology (Dayhaw-Barker et al., 1986; Grant and Schuman, 1993; Albert et al., 2008; Bartlett and Jaanus, 2008; Wässle, 2004; Banh et al., 2005; Wu, 2010). The interested reader should consult these sources for more detail than is provided below. We gratefully acknowledge the use of the information in these sources as well as those cited in the text below.

EXPOSURE TO THE EYE AND VISUAL SYSTEM

Ocular Pharmacodynamics and Pharmacokinetics

Toxic chemicals and systemic drugs can affect all parts of the eye (Fig. 17-1; Table 17-1). Several factors determine whether a chemical can reach a particular ocular site of action, including the physiochemical properties of the chemical, concentration and duration of exposure/treatment, route of exposure, and the movement of the chemical into and across the different ocular compartments and barriers. The cornea and external adnexa of the eye, including the conjunctiva (the delicate membranes covering the inner surface of the eyelids and the exposed surface of the sclera) and eyelids are often exposed directly to chemicals (ie, acids, bases, solvents), gases, and particles, and drugs. The first site of action is the tear film: a three-layered structure with both hydrophobic and hydrophilic properties. The outermost tear film layer is a thin (0.1 μm) hydrophobic layer that is secreted by the meibomian (sebaceous) glands. This superficial lipid layer protects the underlying thicker (7 μm) aqueous layer that is produced by the lacrimal glands. The third layer, which has both hydrophobic and hydrophilic properties, is the very thin (0.02–0.05 μm) mucoid layer. It is secreted by the goblet cells of the conjunctiva and acts as an interface between the hydrophilic layer of the tears and the hydrophobic layer of the corneal epithelial cells. Thus, the aqueous layer is the largest portion of the tear film, and therefore water-soluble chemical compounds more readily mix with the tears and gain access to the cornea. However, a large proportion of the compounds that are splashed into the eyes is washed away by the tears and thus not absorbed.
The cornea, an avascular tissue, is considered the external barrier to the internal ocular structures. Once a chemical interacts with the tear film and subsequently contacts the cornea and conjunctiva, the majority of what is absorbed locally enters the anterior segment by passing across the cornea. In contrast, a greater systemic absorption and higher blood concentration occur through contact with the vascularized conjunctiva (Edelhauser, 2006; Fig. 17-2). The human cornea, which is approximately 500-μm thick, has several distinct layers, or barriers, through which a chemical must pass in order to reach the anterior chamber (see discussion on Cornea). The first is the corneal epithelium. It is a stratified squamous, nonkeratinized, and multicellular hydrophobic layer. These cells have a relatively low ionic conductance through apical cell membranes, and due to the tight junctions (desmosomes), they have a high-resistance paracellular pathway. The primary barrier to chemical penetration of the cornea is the set of tight junctions at the superficial layer of the corneal epithelial cells. Thus, the permeability of the corneal epithelium as a whole is low and only lipid-soluble chemicals readily pass through this layer. Bowman membrane separates the epithelium from the stroma. The corneal stroma makes up 90% of the corneal thickness and is composed of water, collagen, and glycosaminoglycans. It contains approximately 200 lamellae, each about 1.5- to 2.0-μm thick. Due to the composition and structure of the stroma, hydrophilic chemicals easily dissolve in this thick layer, which can also act as a reservoir for these chemicals. The inner edge of the corneal stroma is bounded by a thin, limiting basement membrane, called Descemet membrane, which is secreted by the corneal endothelium. The innermost layer of the cornea, the corneal endothelium, is composed of a single layer of large-diameter hexagonal cells connected by terminal bars and surrounded by lipid membranes. The endothelial cells have a relatively low ionic conductance through apical cell surface and a high-resistance paracellular pathway. Although, the permeability of the corneal endothelial cells to ionized chemicals is relatively low, it is still 100 to 200 times more permeable than the corneal epithelium. The Na⁺,K⁺-pump is located on the basolateral membrane while the energy-dependent Na⁺, (HCO₃⁻)-transporter is located on the apical membrane (Edelhauser, 2006).

There are two separate vascular systems in the eye (Flammer and Mozaffarieh, 2008; Nickla and Wallman, 2010): (1) the uveal blood vessels, which include the vascular beds of the iris, ciliary body, and choroid, and (2) the retinal vessels. In humans, the ocular vessels are derived from the ophthalmic artery, which is a branch of the internal carotid artery. The ophthalmic artery branches into (1) the central retinal artery, which enters the eye and then further branches into four major vessels serving each of the retinal quadrants; (2) two posterior ciliary arteries; and (3) several anterior arteries. In the anterior segment of the eye, there is a blood–aqueous barrier that has relatively tight junctions between the endothelial cells of the iris capillaries and nonpigmented cells of the ciliary epithelium. The major function of the ciliary epithelium is the production of aqueous humor from the plasma filtrate present in the stroma of the ciliary processes.

In humans and several widely used experimental animals (eg, monkeys, pigs, dogs, rats, mice), the retina has a dual circulatory supply: choroidal and retinal. The retinal blood vessels are distributed within the inner or proximal portion of the retina, which consists of the outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer
(IPL), and ganglion cell layer (GCL). The endothelial cells of capillaries of the retinal vessels have tight junctions similar to those that form the blood–brain barrier in the cerebral capillaries. These capillaries form the blood–retinal barrier, and under normal physiological conditions, they are largely impermeable to chemicals such as glucose and amino acids. However, at the level of the optic disc, the blood–retinal barrier lacks these tight-junction types of capillaries and thus hydrophilic molecules can enter the optic nerve head by diffusion from the extravascular space (Flammer and Mozaffarieh, 2008; Nickla and Wallman, 2010) and cause selective damage at this site of action. The outer or distal retina, which consists of the RPE, rod, and cone photoreceptor outer segments (ROS, COS) and inner segments (RIS, CIS), and the photoreceptor outer nuclear layer (ONL), are avascular. These areas of the retina are supplied by the choriocapillaris: a dense, one-layered network of fenestrated vessels formed by the short posterior ciliary arteries and located next to the RPE. Consistent with their known structure, these capillaries have loose endothelial junctions and abundant fenestrae; they are highly permeable to large proteins. Thus, the extravascular space contains a high concentration of albumin and \( \gamma \)-globulin (Sears, 1984).

Following systemic exposure to drugs and chemicals by the oral, inhalation, dermal, or parenteral route, these compounds are distributed to all parts of the eye by the blood in the uveal blood vessels and retinal vessels (Fig. 17-3). Most of these drugs and chemicals rapidly equilibrate with the extravascular space of the choroid where they are separated from the retina and vitreous body by the RPE and endothelial cells of the retinal capillaries, respectively. Hydrophilic molecules with molecular weights less than 200 to 300 Da can cross the ciliary epithelium and iris capillaries and enter the aqueous humor (Sears, 1984). Thus, the corneal endothelium, the cells responsible for maintaining normal hydration and transparency of the corneal stroma, could be exposed to chemical compounds by the aqueous humor and limbal capillaries. Similarly, the anterior surface of the lens can also be exposed as a result of its contact with the aqueous humor. The most likely retinal target sites following systemic drug and chemical exposure appear to be the RPE and photoreceptors in the distal retina because the endothelial cells of the choriocapillaris are permeable to proteins smaller than 50 to 70 kDa. However, the cells of the RPE are joined on their basolateral surface by tight junctions, zonula occludens, that limit the passive penetration of large molecules into the neural retina (Strauss, 2005; Mecklenburg and Schraemeyer, 2007).

The presence of intraocular melanin plays a special role in ocular toxicology. First, it is found in several different locations in the eye: pigmented cells of the iris, ciliary body, RPE, and uveal tract. Second, it has a high binding affinity for polycyclic aromatic hydrocarbons, electrophiles, calcium, and toxic heavy metals such as aluminum, iron, lead, and mercury (Meier-Ruge, 1972; Potts and Au, 1976; Dräger, 1985; Ulshafer et al., 1990; Eichenbaum and Zheng, 2000). Although this initially may play a protective role, it also results in the excessive accumulation, long-term storage, and slow release of numerous drugs and chemicals from melanin. For example, atropine binds more avidly to pigmented irides and thus its duration of action is prolonged (Bartlett and Jaanus, 2008). In addition, the accumulation of chloroquine in the RPE produces an 80-fold higher concentration of chloroquine in the retina relative to liver (Meier-Ruge, 1972). Similarly,
lead accumulates in the human retina such that its concentration is 5 to 750 times that in other ocular tissues (Eichenbaum and Zheng, 2000).

**Nanoparticles and Ocular Drug Delivery**

Ocular drug delivery and targeting create significant challenges and obstacles for most drugs that must enter the eye and reach their site of action. The main ocular target sites of importance for disease treatment and neuroprotection are the anterior segment and posterior retina. As noted above, there are numerous superficial barriers, blood–retina barriers, transporters, depot sites, and the like that restrict bioavailability, decrease therapeutic efficacy, and increase side effects. One new approach involves development of nanoscale preparations for drug delivery, which can substantially enhance penetration from the cornea, deliver a wide variety of drugs and molecules, and increase the concentration and contact time of drugs with these tissues (Diebold and Calonge, 2010). A wide variety of nanoformulations have been considered including matrix-embedded materials and membrane-bound reservoirs (nanoencapsulation). Formulations being developed are solid lipid nanoparticles containing lipids, phospholipids, and/or metals; liposomes; nanosuspensions, and emulsions; and the use of biocompatible coatings such as chitosan (Diebold and Calonge, 2010; Nagpal et al., 2010; Seyfoddin et al., 2010). Metallic particles that enable remote magnetic targeting of drug delivery also are under development. The preparations being developed as pharmaceutical vehicles for ocular drug delivery should have low toxicity to ocular tissues (Prow, 2010). For a wide variety of nonocular purposes, many engineered nanomaterials are being developed. Among the thousands of materials being developed and incorporated into hundreds of consumer products, to date almost no attention has focused on their potential for ocular toxicity other than in vitro assessments of potential phototoxicity (discussed elsewhere).

**Ocular Drug Metabolism**

Metabolism of xenobiotics occurs in all compartments of the eye by well-known Phase I and II xenobiotic-biotransforming enzymes. Drug metabolizing enzymes such as acetylcholinesterase, carboxylesterase (also known as pseudocholinesterase: see Chap. 6), alcohol and aldehyde dehydrogenase, aldehyde and aldose reductase, catalase, monoamine oxidase A and/or B, and Cu²⁺/Zn²⁺ superoxide dismutase as well as several types of proteases are present in the tears, iris–ciliary body, choroid, and retina of many different species (Shanthaverrappa and Bourne, 1964; Waltman and Sears, 1964; Bausher, 1976; Atalla et al., 1998; Crouch et al., 1991; Watkins et al., 1991; Gondhowiardjo and van Haeringen, 1993; Downes and Holmes, 1995; Gaudet et al., 1995; Behndig et al., 1998; King et al., 1999; Nakamura et al., 2005; Choudhary et al., 2006; Fox et al., 2011; Table 17-2).

Cytochrome P450 (CYP) is a superfamily of hemoproteins involved with xenobiotic and endogenous metabolism. There are age-, gender-, and region-specific differences (ie, cornea, ciliary and lens epithelium, and retina) in CYP expression in the developing and adult human and rodent eye (Nakamura et al., 2005; Choudhary et al., 2006; Doshi et al., 2006; Lee et al., 2006). Mutations or alterations in CYP expression (eg, CYP1B1) during
development can produce ocular diseases and defects such as primary congenital glaucoma and corneal opacity (Choudhary et al., 2006). Numerous other CYP family members are located in the eye and respond to different drugs and toxicants. For example, CYP1A1 and CYP1A2 are found in all bovine and mouse ocular tissues except the lens and can be induced by 3-methylcholanthrene and β-naphthoflavone (Shichi and Nebert, 1982; Zhao and Shichi, 1995). Moreover, CYP4A1 in the mouse and CYP4B1 in the rabbit are present in corneal epithelium and can be induced by phenobarbital and the peroxisome proliferator clofibrate (Shichi, 1996; Zhao et al., 1996; Mastyugin et al., 1999). This corneal epithelial CYP monooxygenase metabolizes arachidonic acid to two of its major metabolites: 12(R)-HETE [12(R)-hydroxy-5,8,10,14-eicosatrienoic acid] and 12(R)-HETRe [12(R)-hydroxy-5,8,14-eicosatrienoic acid] (Schwartzman, 1997; Asakura et al., 1994; Mastyugin et al., 1999). In the corneal epithelium, 12(R)-HETE is a potent inhibitor of Na+,K+-ATPase, whereas 12(R)-HETRe is a potent angiogenic and chemotactic factor (Schwartzman, 1997).

The Phase II conjugating enzymes found in bovine, rabbit, and rat ocular tissues include UDP glucuronosyltransferase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, and N-acetyltransferase (Awasthi et al., 1980; Shichi and Nebert, 1982; Penn et al., 1987; Watkins et al., 1991; Srivastava et al., 1996; Singh and Shichi, 1998; Nakamura et al., 2005; see Table 17-2). Although the activity of these enzymes varies between species and ocular tissues, the whole lens appears to have low biotransformational activity. Metabolically, the lens is a heterogeneous tissue, with glutathione-S-transferase activity found in the lens epithelium and not in the lens cortex or nucleus (Srivastava et al., 1996). Overall, these findings suggest that ocular tissues that contact the external environment have a blood supply possessing both CYPs and Phase II conjugating enzymes, especially those enzymes related to glutathione conjugation. The presence and need for a competent glutathione conjugation system is clearly understandable in ocular tissues that directly interact with UV radiation, light, and xenobiotics. Further work is still needed to determine the presence and activity of other CYP family members in ocular tissue, the various factors (ie, age, gender, tissue-specific, xenobiotics, etc) that regulate their expression, and their endogenous and exogenous substrates.

Central Visual System Pharmacokinetics

The penetration of potentially toxic compounds into visual areas of the central nervous system (CNS) is governed, like other parts of the CNS, by the blood–brain barrier (Fig. 17-3). The blood–brain barrier is formed through a combination of tight junctions in brain capillary endothelial cells and foot processes of astrocytic glial cells that surround the brain capillaries. Together these structures serve to limit the penetration of blood-borne compounds into the brain and in some cases actively exclude compounds from brain tissue. The concept of an absolute barrier is not correct, however, because the blood–brain barrier is differentially permeable to compounds depending on their size, charge, and lipophilicity. Compounds that are large, highly charged, or otherwise not very lipid soluble tend to be excluded from the brain, whereas smaller, uncharged, and lipid-soluble compounds more readily penetrate into the brain tissue. In addition to entering the CNS
through this nonspecific semipermeable diffusion barrier, some specific nutrients including ions, amino acids, and glucose enter the CNS through selective transport mechanisms. In some cases, toxic compounds may be actively transported into the brain by mimicking the natural substrates of active transport systems. A few areas of the brain lack a blood–brain barrier; consequently, blood-borne compounds readily penetrate into the brain tissue in these regions.

**Light and Phototoxicity**

The most important oxidizing agents are visible light and UV radiation, particularly UV-A (320–400 nm) and UV-B (290–320 nm), and other forms of electromagnetic radiation. Light- and UV-induced photooxidation leads to generation of reactive oxygen species, and oxidative damage that can accumulate over time. Higher-energy UV-C (100–290 nm) is even more damaging. Fortunately, at sea level the atmosphere filters out virtually all UV-C and all but a small fraction of UV-B derived from solar radiance (AMA Report, 1989). The cornea absorbs about 45% of light with wavelengths below 280 nm, but only about 12% between 320 and 400 nm. The lens absorbs much of the light between 300 and 400 nm and transmits 400 nm and above to the retina (Banh et al., 2005). Absorption of light energy in the lens triggers a variety of photoreactions, including the generation of fluorophores and pigments that lead to the yellow–brown coloration of the lens. Sufficient exposure to infrared radiation, as occurs to glassblowers, or microwave radiation will also produce cataracts through direct heating of the ocular tissues.

Drugs and other chemicals can serve as mediators of photo-induced toxicity in the cornea, lens, or retina (Dayhaw-Barker et al., 1986; Roberts, 2001, 2002; Glickman, 2002). This occurs when the chemical structure allows absorption of light energy in the UV or visible spectrum and the subsequent generation of activated intermediates, free radicals and reactive oxygen species. Chemical structures likely to participate in such phototoxic mechanisms include those with tricyclic, heterocyclic, or porphyrin ring structures because, with light, they produce stable triplet reactive molecules leading to free radicals and reactive oxygen species. The propensity of chemicals to cause phototoxic reactions can be predicted using photophysical and in vitro procedures (Roberts, 2001; Glickman, 2002).

The phototoxic properties of chemicals are being exploited for photodynamic therapies where photoactive chemicals are delivered to pathological tissues such as cancerous tumors or inappropriate angiogenic vessels in age-related macular degeneration. Wavelength-specific light is introduced to the tissue causing the photoactive chemical to activate thereby initiating a free-radical cascade that kills the pathological tissues. These agents also are being developed to utilize long wavelengths near the red/infrared end of the spectrum where the irradiation penetrates deeper into the tissue. A high-throughput screening assay has been developed to rapidly screen candidate chemicals for phototoxicity (Butler et al., 2010). However, photoactive chemicals that are not intentionally introduced for photodynamic therapy also can generate phototoxic reactions. Two examples, which exhibit in vitro ocular phototoxicity, are fullerene (Roberts et al., 2008) and titanium dioxide nanomaterials (Sanders et al., 2012).