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Ophthalmic drug delivery considerations at the cellular level: drug-metabolising enzymes and transporters

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Ophthalmic drugs typically achieve < 10% ocular bioavailability. A drug applied to the surface of the eye may cross ocular–blood barriers where it may encounter metabolising enzymes and cellular transporters before it distributes to the site of action. Characterisation of ocular enzyme systems and cellular transporters and their respective substrate selectivity have provided new insight into the roles these proteins may play in ocular drug delivery and distribution. Altered metabolism and transport have been proposed to contribute to a number of ocular disease processes including inflammation, glaucoma, cataract, dry eye and neurodegeneration. As ocular enzyme and transport systems are better characterised, their properties become an integral consideration in drug design and development.

Keywords: eye, human, metabolism, ophthalmic drug, pharmacokinetics, transport

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1. Introduction

Eye disease can profoundly impact a patient's quality of life. As the population ages, the number of Americans with age-related eye disease is expected to double within the next 30 years [1]. Currently, it is estimated that blindness and visual impairment cost the US federal government > US$4 billion each year [1]. Thus, there is a need to develop more effective drug treatments to prevent the progression of ocular disease.

In recent years, there has been a shift in the treatment paradigm for many ocular disorders. Treatment of glaucoma, a neurodegenerative disorder associated with elevated intraocular pressure, has moved from the management of intraocular pressure to the prevention of neurodegeneration and maintenance of retinal function. Dry eye, which damages the ocular surface, is a common cause of patient visits to eye care specialists. Early dry eye treatment was palliative in the form of artificial tears until the market entry of Restasis® (cyclosporin A ophthalmic emulsion) in 2002, which targeted the immune component of the disease. Macular degeneration, a disease of the retina that can progress to vision loss, has been treated with systemic steroids in combination with retinal reattachment. New treatment strategies for macular degeneration involve intravitreal delivery of drugs that target choroidal vascularisation. Although ocular therapies have evolved, this area is still largely ignored compared with the much more established systemic therapeutics. However, many therapeutic principles that are applicable to systemic disorders are also applicable to ocular disorders.

The pharmacokinetic processes of absorption, distribution, metabolism and excretion determine the concentration of the drug delivered to the site of action. The activities of drug-metabolising enzymes and transporters play integral roles in these processes. In the past 15 years, many therapeutically important drugs for the treatment of systemic diseases were withdrawn from the market due to drug metabolism and drug–drug interaction issues. A few examples include the first non-sedating

antihistamine terfenadine (Seldane™), the non-steroidal anti-inflammatory bromfenac (Duract®), the antibiotic grepafloxacin (Raxar®) and the antidiabetic troglitazone (Rezulin®). These costly failures are now far less likely given the improved knowledge of drug-metabolising enzymes and better technologies in predicting and characterising drug metabolism pathways. A consideration of drug metabolism and drug–drug interaction issues has resulted in rational drug design and delivery, desirable pharmacokinetics/pharmacodynamics at the site of action and successful therapeutic outcome.

The need for information and understanding regarding metabolic pathways in ocular tissues has long been recognised [2,3]. There is a growing recognition of the role metabolic enzymes may play in governing the rate and extent of drug delivery to various ocular tissues. A recent review focused on the role of metabolism in ocular drug delivery and included a comprehensive overview of the distribution patterns of enzymes in different ocular tissues and their roles in prodrug delivery [4]. The present manuscript will review advances in the study of drug metabolism in the eye, focusing on the role these enzymes may play in ophthalmic drug metabolism and pathogenesis in ocular tissues.

Similar to ocular metabolism, the expression and function of membrane transporters at various layers of ocular epithelial and endothelial cells may significantly influence ocular drug efficacy by means of absorption, distribution and excretion. These transporters are as yet not studied as extensively or thoroughly as those expressed in other organs such as liver, intestine and kidney. The recent discoveries on ocular transporter systems and their impact, in association with ocular metabolising enzymes, on drug targeting will be discussed in this review.

2. Enzyme distribution in ocular tissues

Many enzyme systems that exist in systemic tissues have been identified in the various tissues of the eye (Table 1). Different enzyme systems and different enzyme isoforms are expressed in the different tissues. These differences probably arise from the unique functions served by each tissue. This manuscript will review the oxidative, hydrolytic and conjugating enzyme systems in the eye.

2.1 Cytochrome P450 monooxygenase system

Cytochrome P450 (CYP) enzymes constitute a superfamily of haem-containing proteins that are of paramount importance to commercial drug metabolism [5]. CYP enzymes are most highly expressed in the liver, but are also active in several other tissues, most notably, the small intestine, lung and kidney [6]. CYP enzymes catalyse many reactions, such as aromatic and side chain hydroxylation; *N*-, *O*- and *S*-dealkylation, *N*-oxidation, *N*-hydroxylation, sulfoxidation, deamination, dehalogenation and desulfuration [7]. In addition to xenobiotic metabolism, CYP enzymes play crucial roles in the metabolism, either biosynthesis or degradation, of endogenous substrates such as steroids, fatty acids, vitamins and other compounds [7].

Table 1. Drug-metabolising enzymes characterised in ocular tissues.

Enzyme	Substrates	Ref.
Oxidoreductase		
Aldehyde oxidase	Brimonidine	[26]
Ketone reductase	Levobunolol and ketanserin	[21,27,28]
Cyclooxygenase	Arachidonic acid	[114,115]
Monoamine oxidase	5-Hydroxytryptamine	[116]
Cytochrome P450	Broad array of commercial drugs and endogenous substrates	Table 2
Hydrolytic		
Aminopeptidase	L-leucine, L-alanine and L-arginine-4-methoxy-2-naphthylamide	[117,118]
Acetylcholinesterase	Naphthyl esters	[32]
Butyrylcholinestesase	Butyl esters	[32]
Carboxylesterase	Flestolol	[33]
Phosphatase	Phosphorylated amino acid residues	[31,119-121]
Aryl sulfatase	Sulfate esters	[31,122]
N-acetyl-β-glucosaminidase	Glucosamidated compounds	[31,123]
β-glucuronidase	Glucuronidated compounds	[123-125]
Conjugating		
Arylamine acetyltransferase	Aminozolamide and *p*-aminobenzoic acid	[38,39]
Glutathione *S*-transferase	Polyunsaturated fatty acids (i.e., 4-hydroxynonenal)	[40,41]

2.1.1 *Cytochrome P450 enzymes in the eye*

Shichi first identified the presence of a microsomal electron transfer system in the bovine retinal epithelium in 1969 [8]. With the development of more sensitive technologies, such as those employed in molecular biology and probe substrates for specific enzymes, researchers are now able to characterise enzyme systems at the gene, protein and functional activity levels in the eye. Following the first identification of the CYP monooxygenase system in ocular tissues, specific isoforms have been identified with specific ocular tissue distribution (Table 2).

The expression of CYP1A1 and CYP1A2, isoforms that are important to polyaromatic hydrocarbon metabolism, were detected in the mouse ciliary and iris epithelium using *in situ* hybridisation and immunohistochemistry [9,10]. The rat lens was found to express CYP2B1/2, which is phenobarbital inducible, and CYP2C11, which is an isoform specific to male rats, at the gene and protein levels [9]. The gene and

Table 2. CYP enzymes identified in ocular tissues.

Isozyme	Species	Ocular tissue	Ref.
CYP1A	Bovine, rabbit and mouse	Cornea, choroid retina and iris–ciliary body	[10,73,96]
CYP1B1	Human and mouse	Ciliary epithelium and trabecular meshwork	[17,16]
CYP2B	Rabbit and rat	Conjunctiva, cornea, ciliary epithelium and lens	[77,76]
CYP2C	Mouse	Cornea, ciliary body, lens and retina	[12]
CYP2J	Mouse	Specific tissue not reported	[11]
CYP3A	Rabbit, dog and human	Conjunctiva, cornea, choroid–retina, iris–ciliary body and lacrimal gland	[14,19,20]
CYP4B1	Human, bovine and rabbit	Conjunctiva, cornea, choroid–retina and iris–ciliary body	[103,102]
CYP39A1	Bovine	Ciliary epithelium	[126]
NADPH-reductase	Human and bovine	Corneal epithelium	[104]

CYP: Cytochrome P450.

protein expression of CYP2J, an enzyme involved in endogenous arachidonic acid and retinoic acid metabolism, and the expression of CYP2C, an enzyme involved in the metabolism of drugs such as diclofenac and propranolol, were reported in the mouse eye [11,12]. The authors recently detected mRNA transcript copies of CYP3A, an enzyme with broad substrate specificity and involved in the metabolism of > 50% of commercial drugs, in the rabbit lacrimal gland and conjunctiva [13]. The authors confirmed protein expression of CYP3A in the rabbit lacrimal gland and conjunctiva in addition to the iris–ciliary body [14]. Furthermore, the protein expression of CYP1A, CYP2D and NADPH reductase was detected in the rabbit lacrimal gland, conjunctiva, iris–ciliary body and choroid–retina (Figure 1) [14]. CYP4B1 mRNA and protein, an enzyme involved in arachidonic and retinoic acid metabolism, were detected in the rabbit corneal epithelium [15]. Finally, *CYP1B1* gene expression, an enzyme involved in retinoic acid biosynthesis and linked to primary congenital glaucoma [16], was detected in the human ciliary body, iris and non-pigmented ciliary epithelial cell line, and lower levels were detected in the cornea, retinal pigment epithelium and retina [17].

2.1.1.1 Tissue-specific distribution

The isoforms and levels of expression in different ocular tissues are specific to different tissues and different animal species and humans. To maintain optic path clarity, it is essential that the eye possesses defense mechanisms against blood circulation. Each ocular tissue is situated with strategic significance in order to serve its unique function. To explore these differences in enzyme distribution and to broaden our understanding of the roles served by various ocular tissues, the authors used testosterone metabolism as a probe for specific isoforms. The sites of testosterone hydroxylation can be monitored to probe specific CYP enzyme activity [18]. The hydroxylation of testosterone in various ocular tissues in rabbit, dog, monkey and human were measured and distinct activity patterns for each species in each tissue examined were found (Figure 2) [19,20]. Potentially, different patterns of enzyme expression may exist to enable the various ocular tissues to serve their unique functions. For example, Lee *et al.* [21] found that the corneal epithelium expressed the highest levels of NADPH-dependent ketone reductase activity compared with other ocular tissues such as the iris–ciliary epithelium, conjunctiva and the lens. The cornea functions as a physical, protective barrier to inner ocular tissues and the corneal epithelium is the outermost layer of cells in this tissue. It is logical that these cells would possess higher levels of detoxifying enzymes to serve as a defense function. Using this line of thought, the expression of specific CYP isoforms in different ocular tissues and the knowledge of the respective substrate specificity broadens our understanding of the roles that are served by these tissues.

2.2 Other oxidoreductase systems

2.2.1 Aldehyde oxidase

Aldehyde oxidase is a molybdenum-containing enzyme involved in the oxidative metabolism of nicotine [22] and retinoic acid synthesis [23]. Aldehyde oxidase gene expression was detected in rabbit ocular tissues [23] and its activity has been measured in bovine and rabbit ocular tissues including the ciliary body, retinal pigment epithelium–choroid, iris, retina and cornea but not the lens [24]. Brimonidine is a selective α_2-adrenoceptor agonist used to lower intraocular pressure. In liver extracts, briminodine undergoes aldehyde oxidase- mediated metabolism to its 2- and 3-oxobrimonidine derivatives and further metabolism to its 2,3-dioxobrimonidine derivative (Figure 3) [25]. Attar and colleagues studied the ocular disposition of brimonidine following a single topical dose to rabbits [26]. Using radiochemical detection following HPLC separation, aldehyde oxidase-mediated brimonidine metabolites in the rabbit conjunctiva, cornea and iris–ciliary body were detected. It was also found that the relative levels of enzyme activity in different ocular tissues varied in albino versus pigmented rabbits (Figure 4).

2.2.2 Ketone reductase

NADPH-dependent ketone reductase activity has been characterised in the corneal epithelium, iris–ciliary epithelium, conjunctiva and the lens [21]. Levobunolol and ketanserin both act to decrease the intraocular pressure and undergo ketone reductase-mediated metabolism in ocular tissues [27,28]. Ocular metabolism of levobunolol has been extensively studied in the authors' laboratory. Following topical administration to rabbits, levobunolol is rapidly absorbed and undergoes reductive metabolism to dihydrolevobunolol, an equipotent metabolite, in the corneal epithelium and iris–ciliary body [27]. In fact,

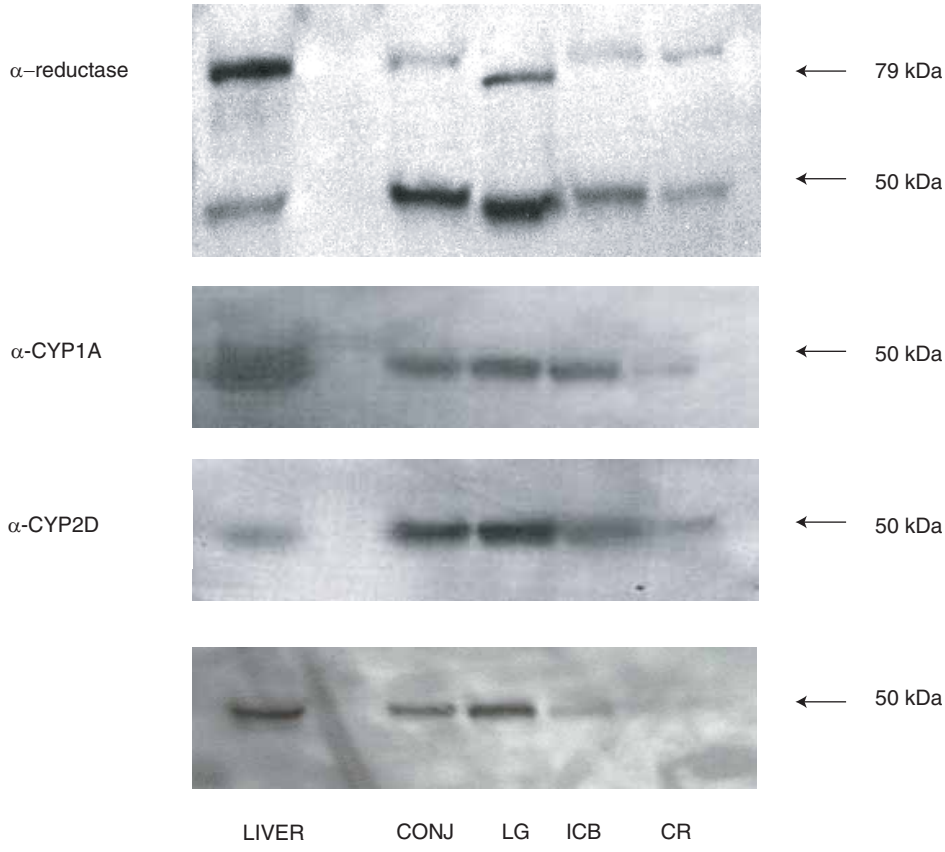

Figure 1. Western blot analysis of microsomal protein from rabbit liver and ocular tissues. The immunoblots were probed with rabbit polyclonal antibody raised against the indicated rat enzyme. The bands detected were between the 86 and 41.3 kDa molecular weight marker. Expected molecular weights: reductase protein ~ 79 kDa and CYP protein ~ 50 kDa. Each lane represents a loading of 10 µg microsomal protein. Reproduced from ATTAR M, LEE, VH, TANG-LIU DS *et al.*: Characterization of cytochrome P450 1A, 2D and 3A in the rabbit eye. *Presented at the sixth annual meeting of the Association of Ophthalmolgy and Pharmacology Therapeutics,* Kaui, Hawaii, USA (2003) [14].
CONJ: Conjunctiva; CR: Choroid–retina; CYP: Cytochrome P450; ICB: Iris–ciliary body; LG: lacrimal gland.

two-thirds of the ocular bioavailable dose was represented by dihydrolevobunolol. Examination of concentration–time profiles following topical levobunolol administration to rabbits revealed that the area under the curve was greater for dihydrolevobunolol versus levobunolol in the cornea, iris–ciliary body and aqueous humor (Figure 5) [27,29]. Furthermore, the terminal elimination half-life of dihydrolevobunolol was longer than the parent drug half-life. Together, these observations led the authors to conclude that the contribution of dihydrolevobunolol was important to the pharmacodynamic effect following levobunolol administration.

Ketone reductase activity in the cornea was found to be pH dependent [29]. The rate of dihydrolevobunolol formation increases with increasing pH and, thus, the buffering capacity of the ophthalmic formulation can impact drug therapy. A perceived drug–drug interaction in a combination dosage of levobunolol and pilocarpine was in fact related to the formulation pH of 5.5 and the strong buffering capacity of pilocarpine. At an acidic pH, levobunolol is > 99% ionised, which results in reduced corneal absorption and metabolism [30]. This study demonstrated the importance of metabolic considerations when developing combination formulations.

2.3 Hydrolytic enzymes

Several hydrolytic enzymes are active in the retina including acid phosphatase, aryl sulfatase, *N*-acetyl-β-glucosamidase and esterase [31]. Esterases are hydrolytic enzymes that are widely distributed throughout the body with broad substrate specificity. Various esterases, such as acetylcholinesterase, butyrylcholinesterase and carboxylesterase, have been identified to be ubiquitously expressed not only in the retina but in several ocular tissues [32,33]. There is an abundance of literature describing the expression and activity of various esterases in ocular tissues, in particular, relating to the activation of prodrugs. It has long been recognised that the distribution of esterases within different cell types can influence the rate and

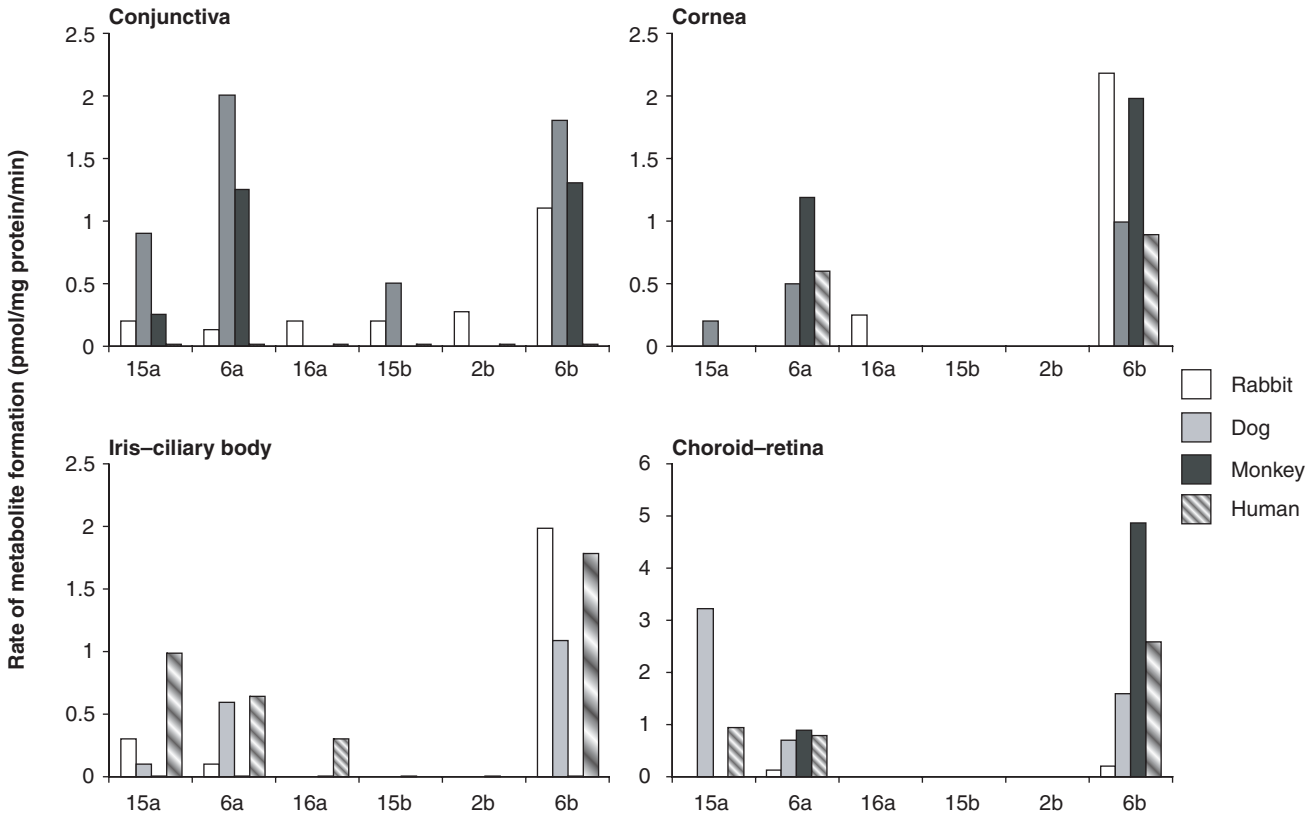

Figure 2. Rates of testosterone hydroxylation in microsomes prepared from ocular tissues from rabbit, dog, monkey and human. 15a- and 6a-testosterone hydroxylation are mediated by CYP2A, 16a-testosterone hydroxylation is mediated by CYP2B/2C and 15b-, 2b- and 6b-testosterone hydroxylation are mediated by CYP3A. This figure is produced from data originally presented by Cherukury *et al*. [19,20].
CYP: Cytochrome P450.

extent at which ester linkages would be hydrolysed [34]. For example, esterase activity towards naphthyl esters was greater in the iris–ciliary body tissue homogenates compared with corneal tissue homogenates for both bovine and rabbit eyes [34,35]. Furthermore, there was differential esterase expression within the different cell types of the rabbit cornea such that greater esterase activity was detected in the corneal epithelium as opposed to the stroma endothelium [35]. However, when the site of esterase-mediated hydrolytic metabolism of dipivefrin, an ophthalmic drug used to treat glaucoma, was studied, the major site of metabolism in rabbits was identified as the cornea [36]. Although higher rates of metabolism were detected in the iris–ciliary body, the fact that topically applied ophthalmic drug first encounters the cornea likely underlies this finding.

The potential of drug–drug interactions in man has been studied for combination therapy of dipivefrin and the cholinergic antiglaucoma agent, echothiophate iodide, which is an esterase inhibitor [37]. Coadministration of echothiophate iodide did not affect dipivefrin therapy. The authors of this study suggested that as echothiophate iodide is a cholinesterase inhibitor and dipivefrin is a phenol ester and is thus more likely to be metabolised by an arylesterase that is not subject to inhibition by echothiophate iodide. Similarly, prior administration of a cholinesterase inhibitor to rabbits did not affect the conversion of topically administered dipivefrin to adrenaline.

2.4 Conjugating enzyme systems

Arylamine acetyltransferase activity in the metabolism of *p*-aminobenzoic acid and aminozolamide, a drug developed to treat ocular hypertension that failed clinical trials, has been characterised in rabbit ocular tissues, namely the iris–ciliary body, corneal epithelium and stroma endothelium [38,39].

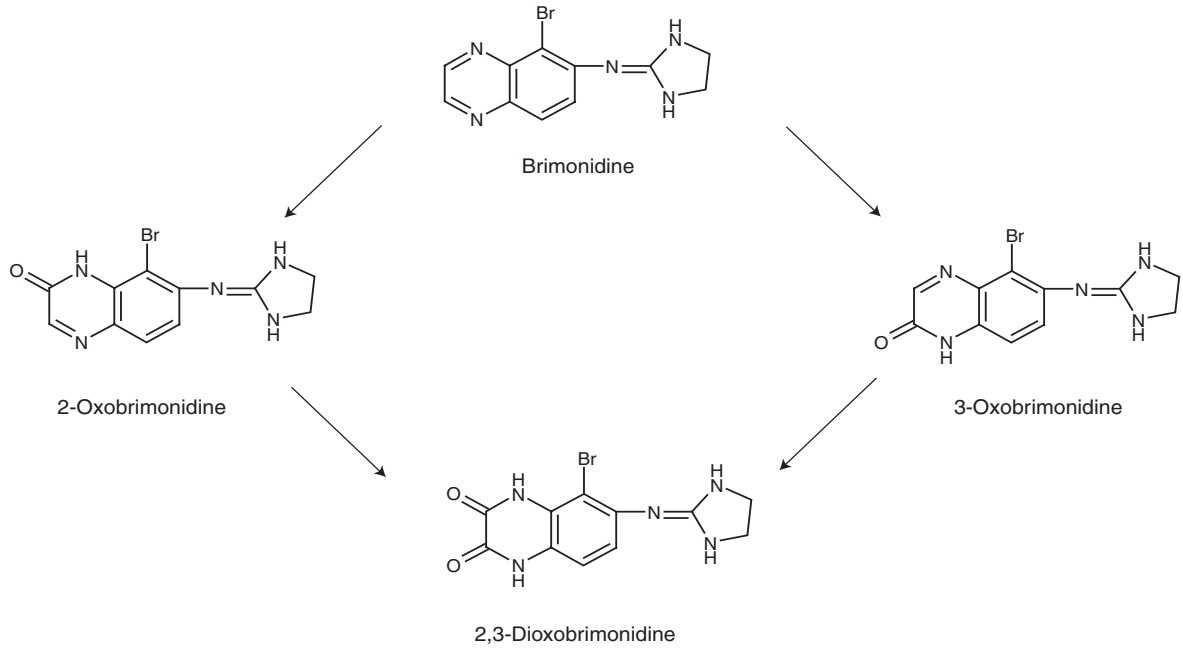

Figure 3. Metabolic pathway observed in rabbit ocular tissues following a single topical administration of 0.5% brimonidine to rabbits. This metabolic scheme was first presented by Acheampong *et al*. [26,25]**.**

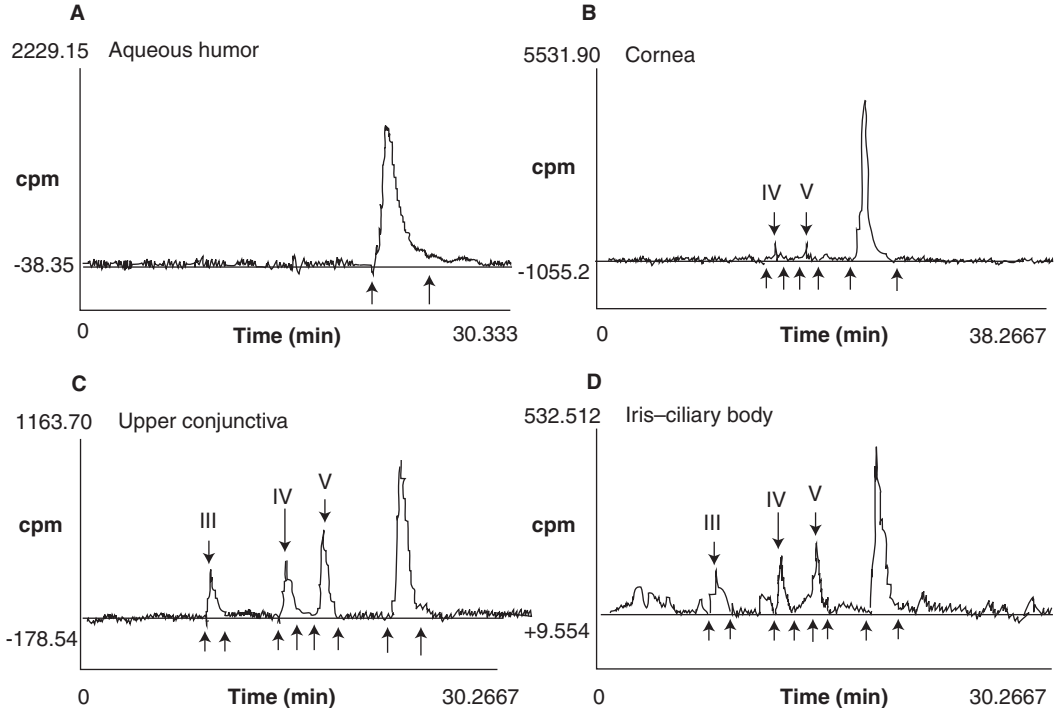

Figure 4. Typical HPLC radiochromatograms acquired following the analysis of A. aqueous humor, B. cornea, C. conjunctiva and D. iris–ciliary body extracts obtained following a single topical 0.5% [^{14}C]brimonidine administration of rabbits. Reprinted with permission from ACHEAMPONG AA, SHACKLETON M, TANG-LIU DD: Comparative ocular pharmacokinetics of brimonidine after a single dose application to the eyes of albino and pigmented rabbits. *Drug Metab. Dispos.* (1995) **23**:708-712 [26].
cpm: Counts per minute; HPLC: High performance liquid chromatography.

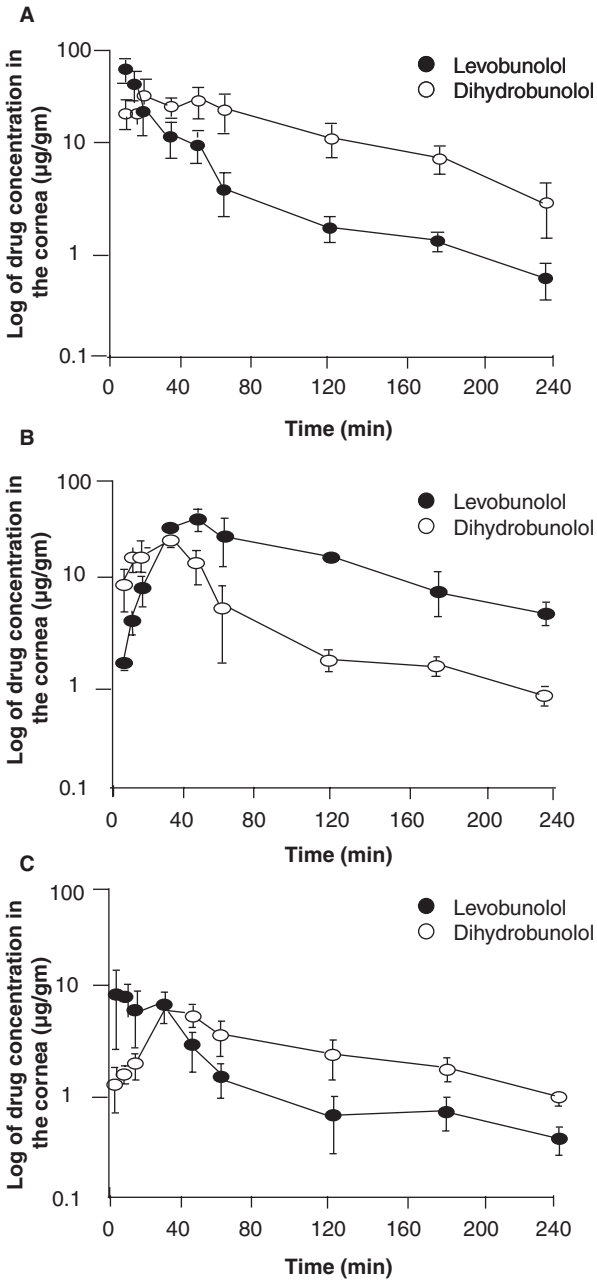

Figure 5. Concentration–time profiles of levobunolol and dihydrobunolol in the cornea (A), aqueous humor (B) and iris–ciliary body (C) following an opthalmic dose of levobunolol to rabbit eyes. Reprinted with permission from TANG-LIU DD, LIU S, NEFF J *et al.*: Disposition of levobunolol after an ophthalmic dose to rabbits. *J. Pharm. Sci.* (1987) **76**:780-783 [29].

Glutathione *S*-transferase has been identified in several ocular tissues including the cornea, retina, iris–ciliary body and sclera [40]. This enzyme system is an important adaptive response to oxidative stress, particularly in the retina [41].

3. Transporter expression in the eye

The treatment of many eye diseases, such as corneal keratitis, conjunctivitis, dry eye, eye allergies and glaucoma rely on topically applied medication. Some of these drugs exert their effect at the ocular surface whereas others may need to penetrate across the epithelial lining of the cornea and/or conjunctiva to reach their target sites in the eye. Expression and function of corneal and conjunctival transporters, in particular peptide and amino acid transporters, have been thoroughly reviewed in 2003 by Dey *et al.* [42]. Table 3 lists many transporter proteins identified to exist in the cornea and conjunctiva as well as other tissues in the eye.

Besides peptide and amino acid transporters, Ito *et al.* [43] reported the expression of the organic anion-transporting polypeptide-E (OATP-E) at both mRNA and protein levels in rat corneal epithelium. The OATP family proteins, as plasma membrane proteins, are responsible for the cellular uptake of various anionic and neutral molecules.

A functional study of organic cation transporters (OCTs) was carried out by Ueda *et al.* in rabbit conjunctiva epithelial tissue [44]. In this study, guanidine transport in the absorptive direction was significantly greater than in the secretive direction and was temperature and concentration dependent, suggesting an involvement of the transporter protein. This study also found that guanidine transport in the conjunctiva was significantly inhibited by dipivefrine, brimonidine and carbachol [44]. Therefore, several cationic amine type drugs may share this transport system to cross conjunctiva and gain access to the underlying ocular tissue.

Corneal expression of nucleoside/nucleobase transporters was studied by Majumdar *et al.* [45]. The antiviral nucleoside analogues acyclovir and ganciclovir appeared to permeate across the excised corneal tissues by simple passive diffusion without an involvement of the carrier proteins.

The blood–retinal barrier (BRB) consists of an outer barrier, the retinal pigmented epithelium, and an inner barrier, the retinal endothelium. The former prevents the free passage of molecules from the well-perfused choroid into the retina, whereas the latter prevents the free passage of molecules from the blood into the retina. The existence of these barriers pose special challenge to drug delivery in the posterior segment of the eye to treat retinal disorders such as age-related macular degeneration, diabetic retinopathy and proliferative vitreoretinopathy, which leads to blindness in severe cases. Conventional topical application of ocular drugs has not proven to be a useful route to treat these disorders. Expression of transporter proteins at the BRB, therefore, provides an opportunity for drug delivery to the retina and other tissues of the posterior eye.

Expression and function of BRB transporters, including amino acid, monocarboxylic acid and folate transporters, have been reviewed in 2003 by Duvvuri *et al.* [46]. These transporters were reported to usually exist in both the inner and outer BRB. However, Ocheltree *et al.* found that bovine or human retinal pigment epithelium (RPE) cell membrane did not

Table 3. Ocular distribution of known transporter proteins.

Transporters	Substrates	Species	Ocular expression site	Ref.
LAT1	Large neutral amino acids	Rabbit, human	Cornea	[127]
EAAC1	Glutamate	Human, pig	Pigment retinal epithelium	[128]
EAAT4	Glutamate	Human	Pigment retinal epithelium	[128]
PepT1	Dipeptides	Rabbit	Cornea, conjunctiva	[129,130]
PEPT2	Dipeptides	Rat	Retina	[131]
GLUT1	Glucose	Cow, human and rat	Cornea, conjunctiva, retina, iris–ciliary body	[132-135]
oatp-E	Organic anion	Rat	Cornea, retina, iris–ciliary body, pigment retinal epithelium	[43]
MCTs	Monocarboxylate	Human	Pigment retinal epithelium	[136]
NaDC3	Dicarboxylate	Mouse	Optic nerve, most layers of the retina, retinal pigment epithelium, ciliary body, iris and lens	[112]
Organic cation transporter 3	Organic cations	Rabbit, mouse and human	Conjunctiva, pigment retinal epithelium	[44,48]
P-glycoprotein	Large neutral or cationic compounds	Rabbit and human	Cornea, conjunctiva, retina, pigment retinal epithelium	[50-56]
MRP proteins	Large neutral or anionic compounds, glucuronide, glutathione, or sulfate conjugates	Human	Pigment retinal epithelium	[57]

EAAC: Excitatory amino acid carrier; EAAT: Excitatory amino acid transporter; GLUT: Glucose transporter; LAT: L-type amino acid transporter; MCT: Monocarboxylate transporter; MRP: Multidrug-resistance protein; NaDC: Na^+-coupled dicarboxylate transporter; oatp: Organic anion transporting polypeptide; PepT: Dipeptide transporter.

actively transport the model dipeptide substrate GlySar, suggesting against the expression of dipeptide transporter proteins in the RPE cells [47]. Expression and function of an OCT, OCT3, in the eye and in particular the RPE was reported by Rajan *et al.* [48]. The same study that identified the existence of organic anion transporter OATP-E in the cornea also reported expression in RPE [43]. As for the nucleoside/nucleobase transporters, Majumdar *et al.* [49] reported functional evidence for their expression in the retina.

The efflux pump transporter P-glycoprotein (P-gp), well known and well characterised in systemic tissues, has been reported to exist in both the cornea and conjunctiva at functional and molecular levels [50-54]. In both tissues, P-gp was localised to the mucosal membrane, facilitating the efflux of substrates into the tear side of the eye. This expression is beneficial to remove harmful toxins from the ocular epithelial linings, but, at the same time, could restrict topical absorption and lower ocular bioavailability of lipophilic drugs such as cyclosporin A and erythromycin. Coadministration of P-gp inhibitors at nontoxic dose levels could facilitate drug absorption across the cornea. In BRB, P-gp serves as a biological barrier for gate keeping in addition to the physical barrier of the tight junction in RPE and retinal endothelium that restricts paracellular diffusion [55]. In human RPE cells, P-gp is expressed at both apical and basolateral cell surfaces, possibly serving a protective function for the neural retina to clear unwanted substances from the subretinal space [56]. In addition to P-gp, the multi-drug resistance-associated protein (MRP) was also found in both cell line and primary culture of human RPE cells [57]. MRPs export organic anions and glucuronide, glutathione or sulfate conjugated compounds.

It is essential to realise that, in many cases, transporter studies in the eye have been limited to confirming their expression at transcriptional and translational levels, whereas further detailed studies in various ocular tissues (e.g., transporter expression level and localisation, substrate selectivity, transporter activity kinetics, species differences and so on) will be necessary to effectively use this route to allow site-specific carrier-mediated drug delivery.

4. Properties of drug metabolising enzymes: polymorphism, inhibition and induction

When considering drug metabolism, there are three issues of particular importance to drug pharmacokinetics; namely, polymorphism, inhibition and induction. As more is learnt about the expression and activity of drug-metabolising enzymes and transporters in ocular tissues, this information can be incorporated into the improved design of ophthalmic drug treatment.

4.1 Polymorphism

There is considerable interindividual variability in CYP expression [5,58]. Evolutionary and environmental pressures have resulted in considerable genetic variability built into different populations [59]. Variability in CYP activity is a critical issue in drug therapy as it will affect the concentration of the drug that will reach the intended target [58]. Phillips *et al.* reported that drugs frequently cited in adverse drug reaction studies were more likely to be metabolised by at least one enzyme with a variant allele known to cause poor metabolism than any randomly selected drug [60]. Historically, the major polymorphisms that have clinical implications to the oxidation of drugs are those of CYP2D6 and CYP2C19 [5,58,61].

Timolol undergoes CYP2D6-mediated hepatic metabolism following oral administration. In ophthalmology, timolol is used to treat ocular hypertension. Adverse events, specifically excessive β-blockade, are associated with ophthalmic timolol therapy and relate to the patient's CYP2D6 genotype [62,63]. The mechanism proposed to underlie this event involves the systemic absorption of topically applied timolol such that exposure levels exceed a critical point. These high exposure levels may result from a poor metaboliser patient phenotype and/or competitive inhibition of CYP2D6-mediated metabolism by oral drugs. However, it has been argued that due to the theoretical route of ophthalmic timolol into the systemic circulation via the nasolacrimal duct, with absorption through the nasal mucosa and venous delivery to the heart, the impact of a first-pass hepatic effect should be minimal [64]. Thus, an alternative explanation may be related to the impact of ocular tissue CYP2D6-mediated metabolism. In the past, it has been assumed that timolol is unlikely to undergo ocular metabolism to achieve clinical significance in the eye or body. This assumption is likely to be based on early metabolism studies conducted in rabbits that demonstrated minimal metabolism in ocular tissues [65]. However, these studies were conducted 20 years ago and significant advances have been made in the methodology and technology used to study extrahepatic metabolism. In fact, CYP2D expression and activity in rabbit ocular tissues has been observed [14]. Potentially, polymorphic CYP2D expressed in ocular tissues may affect ophthalmic timolol therapy. Recently, a frameshift mutation was identified in human brain CYP2D7 and thought to impact on local codeine to morphine metabolism [66]. Similar polymorphism in ocular CYP2D enzymes may alter the exposure levels of timolol and other substrate drugs.

The effect of a hepatic polymorphic enzyme phenotype has been investigated for arylamine acetylation in the rabbit eye. Aminozolamide undergoes arylamine acetyltransferase metabolism in the corneal epithelium and iris–ciliary body of rabbits [38]. However, the *in vitro* and *in vivo* ocular acetylation activities do not correlate with a hepatic acetylation phenotype [39]. Flestolol metabolism by carboxylesterase has been studied in rabbit eyes and appears to be polymorphic. The results from this study demonstrated that carboxylesterase slow and fast metaboliser phenotypes measured in the blood correlated with the metaboliser phenotype measured in cornea, but not other ocular tissues [33]. Together, these findings suggest further study is required to better understand the impact of polymorphism in ocular metabolism. In particular, does polymorphism impact ophthalmic drug bioavailability and why are there differences between ocular and systemic expression? Perhaps this differential expression in polymorphism arises from particular local requirements to serve ocular needs. For example, polymorphism in *CYP1B1*, which is expressed in human ocular tissues, is linked to primary congenital glaucoma [16]. In addition, polymorphism in glutathione *S*-transferase has been linked as a genetic risk factor in the development of cataracts in Japanese and Estonian patients [67,68].

Although it is clear that much like other tissues, polymorphism exists in ocular enzymes, the data does not support a link between ocular and systemic phenotypes. This fact presents a compelling reason as to why ocular metabolism should be studied as the presence or absence of systemic polymorphism does not predict of ocular enzyme polymorphism.

4.2 Inhibition

Inhibition of drug-metabolising enzymes is an important issue in drug pharmacokinetics. Identifying potential inhibitors can aid in predicting drug–drug interactions and/or unfavourable pharmacokinetic profiles produced following drug coadministration. Competitive inhibition of drug metabolism may alter drug or metabolite concentrations to cause either therapeutically and toxicologically significant consequences. It has recently been observed that CYP3A activity in the lacrimal gland can be inhibited by ketoconazole [13], which is given orally for the treatment of ocular fungal infections such as fungal keratitis [69-71]. An interesting question is raised as to what effect these azole antifungals may exert on CYP3A-mediated metabolism in ocular tissues. Systemic drug interactions with ketoconazole have been extensive and the clinical consequences are severe. Therefore, azoles will most definitely affect the pharmacokinetics of drugs metabolised by CYP3A in the eye. Although, to the authors' knowledge there has been no clinical report of this type of drug–drug interaction, it is still important to consider this possibility in ocular drug therapy. Furthermore, could the inhibition of CYP-mediated metabolism in ocular tissues be targeted in drug design strategies? The importance of CYP- and myeloperoxidase-mediated generation of reactive oxygen species in the retina to the development of drug-induced retinopathy is recognised [72]. Understanding these metabolic pathways and eventually being able to target them for therapeutic intervention may offer new opportunities for drug design.

4.3 Induction

Induction of enzyme gene expression is a protective physiological response against chemical exposure and represents an important class of drug–drug interaction [52,58]. The

Table 4. Examples of prodrugs and soft drugs used in ophthalmology.

Prodrugs
Dipivalyl adrenaline (Propine®)
Fluoromethalone-17-acetate (Flarex®)
Prednisolone acetate (Pred Forte®)
Latanoprost (Xalatan®)
Travoprost (Travatan®)
Fluoromethalone-17-acetate (Flarex®)
Unoprostone (Resecula®)
Soft drugs
Fluoromethalone (FML®)
Loteprednol etabonate (Alrex® and Lotemax®)

induction of CYP enzymes can underlie the lack of efficacy observed in some patients following chronic drug treatment or the increased generation of active metabolites.

Zhao and Shichi were the first to demonstrate specific CYP isoform induction in a specific ocular tissue [10], which confirmed their previous observation that CYP1A activity in the liver and eye were under the same genetic control [73]. C75BL/6 mice received a single intraperitoneal injection of β-naphthoflavone, a CYP1A1/1A2 inducer, 48 h prior to sacrifice. Following β-naphthoflavone treatment, the ciliary nonpigmented epithelium possessed the greatest immunoreactivity toward anti-CYP1A1/1A2 antibodies. Furthermore, the maximal level of immunoreactivity in the ciliary non-pigmented epithelium was ~ 25% of that observed in liver tissue. Some level of CYP1A1/1A2 induction was also observed in other ocular tissues such as the choroid, retinal pigmented epithelium, cornea epithelium and iris epithelium. The authors proposed that as the ciliary epithelium is involved in aqueous humor formation by acting as a 'metabolic ultrafiltration' system to the blood plasma, then drug-metabolising enzymes in these tissues may play a critical role in metabolic detoxification of plasma prior to its secretion as aqueous humor.

Dexamethasone induces the expression and activity of CYP3A and other enzymes in the liver, following oral administration to humans and animals or *in vitro* treatment of hepatocytes. The effect of dexamethasone on CYP activities in ocular tissues has been investigated in the author's laboratory following both systemic and topical administration. CYP3A is expressed in various ocular tissues collected from rabbit, dog and human eyes (Table 2) [14,19,20]. When rabbits received intraperitoneal injections of dexamethasone 75 mg/kg/day for 4 days, CYP3A corneal activity increased fourfold, as measured by testosterone 6β-hydroxylation [74]. Furthermore, 4 – 30-fold increases in CYP2A, CYP2B and CYP2C activities were also detected in corneal, conjunctival and retinal tissues. It was also found that topical treatment with dexamethasone every hour for 4 h for 5 days increased CYP3A activity, two/fivefold, in the lacrimal gland and conjunctiva of rabbits as measured by benzyloxyquinoline dealkylation and testosterone hydroxylation [13].

Phenobarbital is also a CYP enzyme inducer with a different induction profile than dexamethasone. Treatment *in vitro* with phenobarbital increases CYP2A, CYP2B, CYP2C, CYP2E and CYP3A enzyme activity in human hepatocytes [75]. The induction of CYP in the cornea, conjunctiva and ciliary epithelium of rabbits following daily intraperitoneal injections of phenobarbital 80 mg/kg/day for 4 days was demonstrated as measured by immunohistochemical analysis [76]. Furthermore, studies in the author's laboratory revealed that rabbits receiving daily intraperitoneal injections of phenobarbital 80 mg/kg/day for 4 days had increased CYP2B activity of ≤ 15-fold in the cornea, increased CYP2B and CYP2C activity of ≤ 10-fold in the iris–ciliary body and increased CYP2A and CYP3A activity of ≤ 4-fold in the choroid retina, as measured by testosterone hydroxylation [74]. Phenobarbital was also found to increase the expression levels of CYP2B1/2 and CYP2C11 in the rat lens [77]. Other enzyme systems can be induced in ocular tissues. For example, the induction of glutathione *S*-transferase has been observed in retinal pigment epithelial cell lines in response to the chemopreventative agent oltipraz and in resopnse to oxidative stress [41,78].

5. Metabolism and transporter structure–activity relationship-guided drug discovery

Based on the knowledge of metabolic pathways, many interactions between drug-metabolising enzymes and drug substrates are predictable. These considerations have been incorporated in drug design and delivery. A molecule can be synthesised for good ocular penetration and metabolised to the active species following penetration or arrival at the site of action. The synthetic strategy can also involve making inactive derivatives of a known active metabolite as the molecule of delivery. A third approach can be the effective deactivation of the drug at the site of adverse effects. Table 4 lists examples of prodrugs and soft drugs used in ophthalmology.

5.1 Prodrug

A prodrug is a pharmacologically inactive compound that is usually designed to enhance absorption of the drug into the cell where ubiquitously expressed enzymes metabolise and release the active drug. For example, the activity of esterases, amidases, oxidoreductases, ketoreductases and acetyltransferases in the eye have been targeted to release active ophthalmic drugs that are delivered as prodrugs.

Dipivalyl adrenaline (Propine®) was one of the first prodrugs used in ophthalmology to treat glaucoma. The dosage is 0.1% dipivalyl ester of adrenaline. The increased lipophilicity of the prodrug allows for > 10-fold drug absorption into rabbit ocular tissues compared with the absorption of adrenaline [79]. The greatest amount of drug was detected in the cornea, which is also the major site of hydrolysis [36]. This approach reduces the ocular surface hyperemia typically associated with a high

ocular dose of adrenaline, range of 0.5 – 2% ophthalmic solution. The same strategy has been used for latanoprost (Xalatan®) and travoprost (Travatan®). Both latanoprost and travoprost are used for the treatment of glaucoma as isopropyl ester prodrugs designed to enhance absorption into the cornea of the active drug. Following topical latanoprost treatment in patients, it was rapidly and completely hydrolysed subsequent to ocular absorption, with the major detectable species being latanoprost acid in the aqueous humor [80].

5.2 Soft drug

Soft drug design avoids oxidative metabolism and instead relies on hydrolytic enzymes to achieve predictable and controllable drug metabolism [61]. This approach has been applied in the design of loteprednol etabonate (Alrex®; Lotemax®), a soft steroid. Ophthalmic steroids are associated with undesirable side effects, both local and systemic. The soft drug approach allows for this corticosteroid to act locally at the eye and then undergo local metabolism to inactive components and thus avoid the undesirable effects. Following topical administration to rabbits, loteprednol etabonate was well absorbed and rapidly deactivated, primarily in the cornea [81]. This approach is thought to produce a drug that is locally active but has a low propensity to cause unwanted side effects at other tissues. In corticosteroid responder patients, fluorometholone (FML®) was less likely to increase intraocular pressure compared with dexamethasone [82]. This is likely to be due to the high susceptibility of fluorometholone to undergo metabolism to inactive metabolites in the cornea [82].

Transporter proteins naturally shuttling nutrients inward and toxins outward of cells could be 'tricked' into transporting drug molecules sharing a similar structure with their natural substrates. Therefore, opportunities exist to explore the boundaries of transporter substrate selectivity by means of molecular engineering such as mutational studies or the construction of chimeric proteins. Some transporters expressed in the eye have well-defined structure–activity relationship, such as amino acid and peptide transporters. Other ocular transporters, such as organic cation, organic anion transporters and efflux pumps (P-gp and MRP), seem to accept a wider spectrum of substrates.

Because of the wide substrate selectivity of the OATP and organic cation transporters, the finding of oatp-E and OCT3 expression in the corneal epithelium and retina has important implication for drug design. The exact substrate specificity of the OATP isoforms is not completely known. It has been reported that their substrates (mostly amphipathic organic anions) include not only endogenous compounds such as bile salts, steroid conjugates, thyroid hormones and anionic oligopeptides, but also drugs, toxins and other xenobiotics [83]. Examples of drugs transported by OATP transporters include many statins (such as pravastatin and rosuvastatin), flunixin, fexofenadine and digoxin.

Physiologically, OCT3 may participate in the clearance of dopamine and histamine from the subretinal space, whereas pharmacologically it could be involved in the disposition of the retinal neurotoxin $MPP(^{+})$, a typical substrate for the OCT proteins. OCT3 is expressed in the pigment retinal epithelial cells as well as several cell types of the neural retina, including photoreceptor, ganglion, amacrine and horizontal cells [48]. Therefore, OCT3 may contribute to organic amine drug distribution in the retina by serving as a gate for these drugs across the outer BRB, following which allow access into neural retinal cells for potential intracellular targeting. For example, brimonidine, a neuroprotectant for the retina [84], has been shown to competitively inhibit transport of other substrates of the OCT [44].

6. Disease

Mutations occurring in transporter proteins may be the cause of certain ocular disease. Pseudoxanthoma elasticum (PXE) is an autosomal inherited disease characterised by progressive dystropic calcification of the elastic structures in the skin, eyes and cardiovascular system. The ocular abnormalities include retinitis pigmentosa, comet-like streaks, pinpoint white lesions of the choroid and angioid streaks. PXE is caused by mutations in *MRP6*, encoding a MRP, as a member of the ATP-binding cassette transporter gene family [85,86].

Altered metabolism may also underlie the pathogenesis of ocular disease. The production of reactive metabolites or changes in the metabolism rates of endogenous gene regulators such as steroids and retinoids may elicit undesirable effects.

6.1 Cataract

When ocular metabolism at a specific site becomes saturated, reactive and/or toxic metabolites could be formed locally and cause tissue damage. This pathophysiological mechanism may explain the observation of cataract formation following an overdose of acetaminophen in mice [87]. When mice received an overdose of acetaminophen in the presence of the CYP1A1/1A2 inhibitor, diallyl sulfide, cataract development was prevented [88]. The protective effect of diallyl sulfide presumably results from the inhibition of biotransformation of acetaminophen to the reactive metabolite *N*-acetyl-*p*-benzoquinone (NAPQI) by CYP1A1/1A2. Mice intracamerally injected with NAPQI developed cataract [89].

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, commonly referred to as statins, are widely prescribed as oral drugs to lower plasma low-density lipoprotein cholesterol levels. A strong relationship between systemic exposure levels and the cataractogenic potential of this class of compounds has been observed in dogs [90]. Lovastatin induced opacity in organ-cultured rat lens, and adversely affected cell structure and proliferation of cultured epithelial cells from human and rabbit lenses [91]. The mechanism underlying these observations is not yet clear; however, it has been suggested that the exposure of the outer cortical region of the lens to statins is somehow associated with cataractogenesis [90]. The exposure levels achieved of statins at clinically therapeutic doses have

not been associated with the development of cataract in man [92-94]. However, the concomitant administration of a drug that inhibits CYP metabolism may be significant if this results in increased exposure levels of statins and associated adverse effects [92,95]. The role of ocular CYP metabolism in chronic statin treatment and association with cataract formation still needs to be confirmed.

6.2 Neurodegeneration

CYP1A1 expression and activity were examined in bovine retinal pigment epithelial cells following exposure to benzo(a)pyrene (BaP) [96]. BaP is an important constituent of cigarette smoke. This compound was found to induce CYP1A1 mRNA levels and protein content in retinal epithelial cells. CYP1A1 is known to activate polyaromatic hydrocarbon compounds to toxic metabolites, including the metabolism of BaP to benzo(a)pyrene-7,8-diol-9,10-epoxide (BPDE), which can then form DNA adducts [97]. Indeed, covalent adducts between the mutagenic metabolite BPDE and DNA were detected in bovine retinal epithelial cells by immunocytochemical staining following BaP treatment as well as other indications of DNA damage. This study data suggests a mechanism by which exposure to cigarette smoke may underlie degenerative disorders of the retina.

6.3 Ocular lens dislocation

Molybdenum deficiency is associated with ocular lens dislocation. Aldehyde oxidase activity requires molybdenum as a necessary cofactor. Molybdenum deficiency is associated with prolonged total parenteral nutrition in patients [98]. Alternatively, molybdenum cofactor deficiency can arise from an autosomal recessive disorder characterised by the lack of aldehyde oxidase activity [99]. Altered retinoic acid synthesis resulting from the absence of aldehyde oxidase activity may underlie this developmental disorder.

6.4 Primary congenital glaucoma

Mutation in the *CYP1B1* gene is associated with primary congenital glaucoma (PCG) [16]. *CYP1B1* is expressed in the human ciliary body, and iris and in human cells of nonpigmented ciliary epithelium and trabecular meshwork [16,17]. Many studies have been conducted to understand the underlying molecular mechanisms that link PCG to a mutation in the *CYP1B1* gene. Libby *et al.* reported that *CYP1B1* knockout mice presented histological abnormalities resembling humans with PCG in terms of abnormal ocular drainage structures [100]. Interestingly, it was found that the phenotypic effect of the *CYP1B1* mutation could be modified by tyrosinase activity. This group proposed that tyrosinase deficiency affects angle development through its effects on L-dihydroxyphenylalanine (L-dopa) levels. In fact, the administration of L-dopa-enriched water to the mothers of mice deficient in both *CYP1B1* and tyrosinase throughout their ocular development was found to prevent severe angle dysgenesis present in mice whose mothers were left untreated. The authors hypothesised that altered *CYP1B1* metabolism of all-*trans* retinoic acid affected cell proliferation in neural cells and may ultimately result in a metabolic disturbance effecting L-dopa levels that contributes to abnormal anterior segment development. Through understanding how this mutation may lead to the development of PCG, drug therapy may be developed such as some form of prenatal treatment for the mothers of children with *CYP1B1* mutations to limit the severity of their disease [101].

6.5 Inflammation

Schwartzman and colleagues were the first to describe a novel metabolic pathway for arachidonic acid (AA) that was CYP dependent in the corneal epithelium of human, bovine and rabbit eyes [102,103]. AA is an important precursor to a number of cell mediators and the specialised location in the corneal epithelium suggested a physiological function in activating this endogenous compound. Using functional assays and western blotting this group also described the presence of haem oxygenase and NADPH reductase in the human corneal epithelium, and compared the levels with bovine corneal epithelium and human liver microsomes [104]. NADPH reductase is an essential partner to CYP haem protein. In addition, haem oxygenase partners with NADPH reductase to mediate the oxidative degradation of haem. Thus, haem oxygenase may have an important physiological role in determining levels of CYP enzyme activity.

Schwartzman's group demonstrated that 12*(R)*-hydroxyeicosatetraenoic acid, a CYP-dependent metabolite of AA formed in bovine corneal epithelial microsomes, inhibits the Na^+,K^+-ATPase in the cornea [105]. This pump functions to maintain an electrochemical gradient across the corneal epithelium. Another active metabolite is formed, namely 12*(R)*-hydroxyeicosatrienoic acid, which was found to be a potent vasodilator, to stimulate protein influx into the aqueous humor of the eye and to be a potent angiogenic factor [106]. As the effects of 12*(R)*-hydroxyeicosatrienoic acid on the rabbit eye mimic the response to an inflammatory stimulus, the authors hypothesised that inflammation is in some part mediated by 12*(R)*-hydroxyeicosatrienoic acid following injury to the cornea. When human tear film was collected from subjects with inflamed eyes and compared with that from normal eyes, increased levels of 12*(R)*-hydroxyeicosatrienoic acid were detected [107]. Furthermore, it was found that the levels of these endogenous metabolites increased following contact lens-induced hypoxic stress in rabbits [108], thus suggesting these metabolites may be potential mediators of contact lens complications. In rabbit corneal epithelial cells, hypoxia stimulated the production of these CYP-derived inflammatory eicosanoids relative to cells subjected to normoxic conditions [109]. Using this *in vitro* cell culture system, the group identified CYP4B1 as being involved in the production of these hypoxia-induced eicosanoids [15]. CYP4B1 mRNA levels were found to be increased in hypoxia-treated corneal epithelial cells compared with control cells.

Recently, retinoic acids, namely 9-*cis* and all-*trans* retinoic acids, were found to increase the expression of CYP4B1 and enhance the production of these inflammatory eicosanoids in rabbit corneal epithelial cells [110]. Retinoids are known mediators of the wound healing process in many tissues and are thus integral components of the inflammatory response. This work provided a link between wound healing and inflammation in the ocular surface.

7. Conclusion

In the coming years the knowledge of metabolic and transport systems in ocular tissues will broaden. The understanding of how these proteins can affect pharmacokinetics and pharmacodynamics or how they may underlie disease processes will be strengthened. Together, this information will result in the development of more effective drug therapy to treat and prevent the progression of eye disease.

8. Expert opinion

Metabolising enzymes and transporters are endogenously expressed in the eye and serve as a defensive mechanism to regulate the entry and exposure of drugs and endogenous substrates. It is not fully elucidated as to whether these systems coexist in ocular tissues to work in concert or are individually expressed to serve unique functions.

The metabolism and transport of ophthalmic drugs should be considered in drug design. Metabolism may limit ophthalmic drug bioavailability or generate active species at the ocular site of action. Drug therapy may modulate drug-metabolising enzymes and/or transporters. Mechanisms underlying therapeutic failures, which can include lack of efficacy or safety, may be explained by considering the potential of drugs to interact with proteins that mediate the processes of absorption, distribution, metabolism and elimination.

Assessing the contribution and impact of metabolism and transport in ophthalmic tissues will pose novel challenges. For example, the vectorial nature of drug traffic in relation to the location of enzymes and transporters must be considered. For instance, the contribution of CYP3A metabolism and P-gp interplay will affect drug exposure in the conjunctiva, depending on whether the substrate is delivered systemically versus topically. The same applies to drug delivery to the posterior retina. Figure 6 is adapted from a model first proposed and then reviewed by Benet *et al.* describing metabolic and transport considerations at the small intestine versus the liver [111]. It illustrates the vectorial nature of the conjunctival epithelium and depicts the situations where the drug may be delivered topically or systemically. Drugs delivered topically will encounter efflux prior to metabolism versus drugs or endogenous substrates delivered systemically will encounter metabolism prior to efflux. The interplay of CYP3A and P-gp in the conjunctiva will generate scenarios where drug absorption could be increased or decreased, or

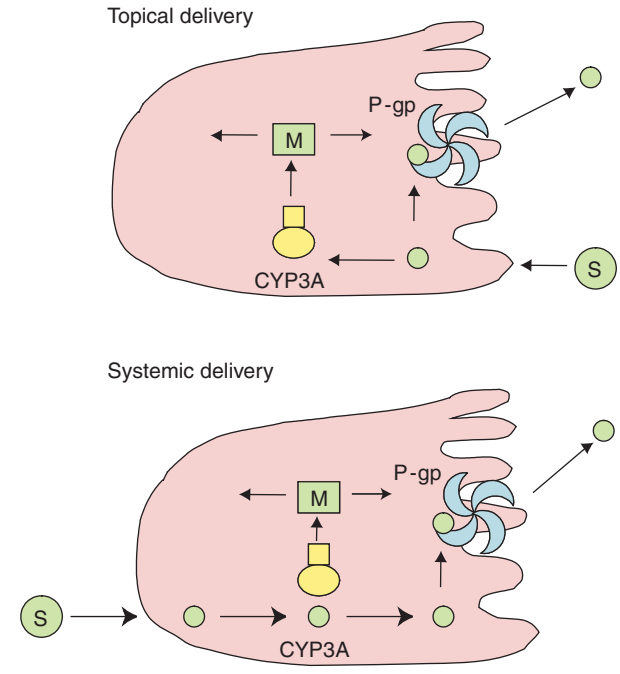

Figure 6. Illustration demonstrating the vectorial considerations that impact topical drug bioavailability versus systemic drug or endogenous substrate bioavailability.
CYP: Cytochrome P450; M: Metabolite; P-gp: Polyglycoprotein; S: Substrate.

alternatively a drug may undergo recycling. These effects would be based on the vectorial nature of the drug traffic and overlapping or lack of substrate specificity. Together, these effects may influence what chemical stimulants are present in the cell to elicit an effect. In fact, alternations in enzyme and transporter functions, working in concert, have been proposed to underlie ocular disease processes such as Canavan's disease [112].

Another area that warrants more attention is the variability and polymorphism associated with drug-metabolising enzymes and transporters. Studies in the polymorphic *CYP1B1* area elucidate the role of genetic expression of proteins in pathogenesis. It is not clear whether the variability of ocular enzymes correspond to the same extent as those expressed in the liver and whether this variability may translate to the large differences in clinical efficacy of ocular drugs such as responders versus nonresponders.

In the past decades, significant advances in ocular therapeutics through novel and potent pharmacological agents have been witnessed. The chronological progression has been tremendous: for the treatment of glaucoma, the move from 2 – 4% pilocarpine four-times daily, to 0.5% timolol (β-blocker) two-times daily, to 0.2% brimonidine three-times daily (α-agonist), to 0.03% latanoprost (prostaglandin) daily. With each progression to a new pharmacologically based technology, there was a significant improvement

in drug potency and duration of action. These advances result in more effective treatment options and better patient compliance. Although mostly delivered as ophthalmic solutions, the high potency supports low active concentrations and the long drug action allows for twice- or once-daily dosing, which results in much improved patient compliance and, therefore, therapeutic success. The development of newer compounds, such as brimonidine and latanoprost, have benefited from the availability of modern laboratory tools and, therefore, their ocular metabolic fate has been fully characterised as an integral part of drug development.

Much emphasis has been placed on drug delivery to the posterior segment of the eye in recent years so that effective therapies for diseases such as macular degeneration and proliferative vitreoretinopathy can be developed. Potential therapeutic agents include 5-fluorouracil, triamcinolone, retinoic acid, daunorubicin and macugen. Some of these agents, such as daunorubicin, are known substrates of transporters [113]. Although the knowledge base for transporters in the back of the eye is still developing, it is important to keep in mind the locations and roles of these transporters that may be a key factor in the drug kinetics and distribution profile.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Vision problems in the US: prevalence of adult vision impairment and age-related eye disease in America. *Prevent Blindness America* (2002).
2. ZIMMERMAN TJ, LEADER B, KAUFMAN HE: Advances in ocular pharmacology. *Ann. Rev. Pharmacol. Toxicol.* (1980) 20:415-428.
3. LEE VH: Precorneal, corneal and postcorneal factors. In: *Ophthalmic Drug Delivery Systems*. AK Mitra (Ed.), Marcel Dekker, Inc., New York, NY, USA (1993):59-81.
4. DUVVURI S, MAJUMDAR S, MITRA AK: Role of metabolism in ocular drug delivery. *Curr. Drug Metab.* (2004) 5:507-515.
5. PARKINSON A: An overview of current cytochrome P450 technology for assessing the safety and efficacy of new materials. *Toxicol. Pathol.* (1996) 24:48-57.
6. PARKINSON A: Biotransformation of xenobiotics. In: *Casarett & Doull's Toxicology: the basic science of poisons.* CD Klaassen (Ed.), McGraw-Hill, New York, NY, USA (1996):113-186.
7. WILKINSON GR: Pharmacokinetics. In: *Goodman and Gilman's: the pharmacological basis of therapeutics*. AJ Hardman, LE Limbird, A Goodman Gilman (Eds), McGraw-Hill, New York, NY, USA (2001):3-30.
8. SHICHI H: Microsomal electron transfer system of bovine retinal pigment epithelium. *Exp. Eye Res.* (1969) 8:60-68.
   • The first published report of a microsomal electron transfer system in any ocular tissue.
9. MCAVOY M, SINGH AK, SHICHI H: *In situ* hybridization of Cyp1a1, Cyp1a2 and Ah receptor mRNAs expressed in murine ocular tissues. *Exp. Eye Res.* (1996) 62:449-452.
10. ZHAO C, SHICHI H: Immunocytochemical study of cytochrome P450 (1A1/1A2) induction in murine ocular tissues. *Exp. Eye Res.* (1995) 60:143-152.
11. XIE Q, ZHANG QY, ZHANG Y *et al.*: Induction of mouse CYP2J by pyrazole in the eye, kidney, liver, lung, olfactory mucosa, and small intestine but not in the heart. *Drug Metab. Dispos.* (2000) 28:1311-1316.
12. TSAO CC, COULTER SJ, CHIEN A *et al.*: Identification and localization of five CYP2Cs in murine extrahepatic tissues and their metabolism of arachidonic acid to regio- and stereoselective products. *J. Pharmacol. Exp. Ther.* (2001) 299:39-47.
13. ATTAR M, LING KH, TANG-LIU DD *et al.*: Cytochrome P450 3A expression and activity in the rabbit lacrimal gland: glucocorticoid modulation and the impact on androgen metabolism. *Invest. Ophthalmol. Vis. Sci.* (2005) (In press).
   • The first published report of CYP3A expression and activity, an enzyme responsible for steroid deactivation, in the lacrimal gland, a steroid sensitive tissue.
14. ATTAR M, LEE, VH, TANG-LIU DS *et al.*: Characterization of cytochrome P450 1A, 2D and 3A in the rabbit eye. *Presented at the sixth annual meeting of the Association of Ophthalmolgy and Pharmacology Therapeutics*, Kaui, HI, USA (2003).
15. MASTYUGIN V, AVERSA E, BONAZZI A *et al.*: Hypoxia-induced production of 12-hydroxyeicosanoids in the corneal epithelium: involvement of a cytochrome P4504B1 isoform. *J. Pharmacol. Exp. Ther.* (1999) 289:1611-1619.
16. STOILOV I, AKARSU AN, SARFARAZI M: Identification of three different truncating mutations in cytochrome P4501B1 (CYP1B1) as the principal cause of primary congenital glaucoma (Buphthalmos) in families linked to the GLC3A locus on chromosome 2p21. *Hum. Mol. Genet.* (1997) 6:641-647.
17. STOILOV I, AKARSU AN, ALOZIE I *et al.*: Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1. *Am. J. Hum. Genet.* (1998) 62:573-584.
18. WAXMAN DJ, ATTISANO C, GUENGERICH FP *et al.*: Human liver microsomal steroid metabolism: identification of the major microsomal steroid hormone 6 beta-hydroxylase cytochrome P450 enzyme. *Arch. Biochem. Biophys.* (1988) 263:424-436.
19. MADHU C, DINH V, BABUSIS D *et al.*: Tissue specific distribution of cytochrome P450 isozymes in the monkey eye. *Annual meeting of the Association for Research in Vision and Ophthalmology Proceedings*, Fort Lauderdale, Florida, USA (1999).
20. MADHU C, DINH V, BABUSIS D *et al.*: Expression of cytochrome P450 isoenzymes in dog and human eye. *Fourteenth annual meeting of the International Society for the Study of Xenobiotics Proceedings* (1999).

21. LEE VH, CHIEN DS, SASAKI H: Ocular ketone reductase distribution and its role in the metabolism of ocularly applied levobunolol in the pigmented rabbit. *J. Pharmacol. Exp. Ther.* (1988) 246:871-878.

22. BERKMAN CE, PARK SB, WRIGHTON SA *et al.*: *In vitro–in vivo* correlations of human *(S)*-nicotine metabolism. *Biochem. Pharmacol.* (1995) 50:565-570.

23. HUANG DY, FURUKAWA A, ICHIKAWA Y: Molecular cloning of retinal oxidase/aldehyde oxidase cDNAs from rabbit and mouse livers and functional expression of recombinant mouse retinal oxidase cDNA in Escherichia coli. *Arch. Biochem. Biophys.* (1999) 364:264-272.

24. SHIMADA S, MISHIMA H, KITAMURA S *et al.*: Nicotinamide *N*-oxide reductase activity in bovine and rabbit eyes. *Invest. Ophthalmol. Vis. Sci.* (1987) 28:1204-1206.

25. ACHEAMPONG AA, CHIEN DS, LAM S *et al.*: Characterization of brimonidine metabolism with rat, rabbit, dog, monkey and human liver fractions and rabbit liver aldehyde oxidase. *Xenobiotica* (1996) 26:1035-1055.

26. ACHEAMPONG AA, SHACKLETON M, TANG-LIU DD: Comparative ocular pharmacokinetics of brimonidine after a single dose application to the eyes of albino and pigmented rabbits. *Drug Metab. Dispos.* (1995) 23:708-712.

27. TANG-LIU DD, LIU S, NEFF J *et al.*: Disposition of levobunolol after an ophthalmic dose to rabbits. *J. Pharm. Sci.* (1987) 76:780-783.

28. SCHOENWALD RD, ZHU J: The ocular pharmacokinetics of ketanserin and its metabolite, ketanserinol, in albino rabbits. *J. Ocul. Pharmacol. Ther.* (2000) 16:481-495.

29. TANG-LIU DD, SHACKLETON M, RICHMAN JB: Ocular metabolism of levobunolol. *J. Ocul. Pharmacol.* (1988) 4:269-278.

30. TANG-LIU DD, RICHMAN JB: The effect of pilocarpine on ocular levobunolol absorption from ophthalmic solutions. *J. Ocul. Pharmacol.* (1994) 10:605-615.

31. ESSNER E, GORRIN GM, GRIEWSKI RA: Localization of lysosomal enzymes in retinal pigment epithelium of rats with inherited retinal dystrophy. *Invest. Ophthalmol. Vis. Sci.* (1978) 17:278-288.

32. LEE VH, CHANG SC, OSHIRO CM *et al.*: Ocular esterase composition in albino and pigmented rabbits: possible implications in ocular prodrug design and evaluation. *Curr. Eye Res.* (1985) 4:1117-1125.

33. STAMPFLI HF, QUON CY: Polymorphic metabolism of flestolol and other ester containing compounds by a carboxylesterase in New Zealand white rabbit blood and cornea. *Res. Commun. Mol. Pathol. Pharmacol.* (1995) 88:87-97.

34. LEE VH, IIMOTO DS, TAKEMOTO KA: Subcellular distribution of esterases in the bovine eye. *Curr. Eye Res.* (1982) 2:869-876.

35. LEE VH, MORIMOTO KW, STRATFORD RE Jr: Esterase distribution in the rabbit cornea and its implications in ocular drug bioavailability. *Biopharm. Drug Dispos.* (1982) 3:291-300.

36. ANDERSON JA, DAVIS WL, WEI CP: Site of ocular hydrolysis of a prodrug, dipivefrin, and a comparison of its ocular metabolism with that of the parent compound, adrenaline. *Invest. Ophthalmol. Vis. Sci.* (1980) 19:817-823.

37. MINDEL JS, YABLONSKI ME, TAVITIAN HO *et al.*: Dipivefrin and echothiophate. Efficacy of combined use in human beings. *Arch. Ophthalmol.* (1981) 99:1583-1586.

38. PUTNAM ML, SCHOENWALD RD, DUFFEL MW *et al.*: Ocular disposition of aminozolamide in the rabbit eye. *Invest. Ophthalmol. Vis. Sci.* (1987) 28:1373-1382.

39. CAMPBELL DA, SCHOENWALD RD, DUFFEL MW *et al.*: Characterization of arylamine acetyltransferase in the rabbit eye. *Invest. Ophthalmol. Vis. Sci.* (1991) 32:2190-2200.

40. SRIVASTAVA SK, SINGHAL SS, BAJPAI KK *et al.*: A group of novel glutathione *S*-transferase isozymes showing high activity towards 4-hydroxy-2-nonenal are present in bovine ocular tissues. *Exp. Eye Res.* (1994) 59:151-159.

41. SINGHAL SS, GODLEY BF, CHANDRA A *et al.*: Induction of glutathione *S*-transferase hGST 5.8 is an early response to oxidative stress in RPE cells. *Invest. Ophthalmol. Vis. Sci.* (1999) 40:2652-2659.

42. DEY S, ANAND BS, PATEL J *et al.*: Transporters/receptors in the anterior chamber: pathways to explore ocular drug delivery strategies. *Expert Opin. Biol. Ther.* (2003) 3:23-44.

43. ITO A, YAMAGUCHI K, TOMITA H *et al.*: Distribution of rat organic anion transporting polypeptide-E (oatp-E) in the rat eye. *Invest. Ophthalmol. Vis. Sci.* (2003) 44:4877-4884.

44. UEDA H, HORIBE Y, KIM KJ *et al.*: Functional characterization of organic cation drug transport in the pigmented rabbit conjunctiva. *Invest. Ophthalmol. Vis. Sci.* (2000) 41:870-876.

45. MAJUMDAR S, TIRUCHERAI GS, PAL D *et al.*: Functional differences in nucleoside and nucleobase transporters expressed on the rabbit corneal epithelial cell line (SIRC) and isolated rabbit cornea. *AAPS PharmSci.* (2003) 5:E15.

46. DUVVURI S, MAJUMDAR S, MITRA AK: Drug delivery to the retina: challenges and opportunities. *Expert Opin. Biol. Ther.* (2003) 3:45-56.

47. OCHELTREE SM, KEEP RF, SHEN H *et al.*: Preliminary investigation into the expression of proton-coupled oligopeptide transporters in neural retina and retinal pigment epithelium (RPE): lack of functional activity in RPE plasma membranes. *Pharm. Res.* (2003) 20:1364-1372.

48. RAJAN PD, KEKUDA R, CHANCY CD *et al.*: Expression of the extraneuronal monoamine transporter in RPE and neural retina. *Curr. Eye Res.* (2000) 20:195-204.

49. MAJUMDAR S, MACHA S, PAL D *et al.*: Mechanism of ganciclovir uptake by rabbit retina and human retinal pigmented epithelium cell line ARPE-19. *Curr. Eye Res.* (2004) 29:127-136.

50. DEY S, GUNDA S, MITRA AK: Pharmacokinetics of erythromycin in rabbit corneas after single-dose infusion: role of P-glycoprotein as a barrier to *in vivo* ocular drug absorption. *J. Pharmacol. Exp. Ther.* (2004) 311:246-255.

51. DEY S, PATEL J, ANAND BS *et al.*: Molecular evidence and functional expression of P-glycoprotein (MDR1) in human and rabbit cornea and corneal epithelial cell lines. *Invest. Ophthalmol. Vis. Sci.* (2003) 44:2909-2918.

52. KAWAZU K, YAMADA K, NAKAMURA M *et al.*: Characterization of cyclosporin A transport in cultured rabbit corneal epithelial cells: P-glycoprotein transport activity and binding to

cyclophilin. *Invest. Ophthalmol. Vis. Sci.* (1999) 40:1738-1744.

53. YANG JJ, KIM KJ, LEE VH: Role of P-glycoprotein in restricting propranolol transport in cultured rabbit conjunctival epithelial cell layers. *Pharm. Res.* (2000) 17:533-538.

54. SAHA P, YANG JJ, LEE VH: Existence of a P-glycoprotein drug efflux pump in cultured rabbit conjunctival epithelial cells. *Invest. Ophthalmol. Vis. Sci.* (1998) 39:1221-1226.

- The first published report of P-gp expression and activity in the conjunctival epithelium.

55. GREENWOOD J: Characterization of a rat retinal endothelial cell culture and the expression of P-glycoprotein in brain and retinal endothelium *in vitro*. *J. Neuroimmunol.* (1992) 39:123-132.

56. KENNEDY BG, MANGINI NJ: P-glycoprotein expression in human retinal pigment epithelium. *Mol Vis.* (2002) 8:422-430.

57. AUKUNURU JV, SUNKARA G, BANDI N *et al.*: Expression of multi-drug resistance-associated protein (MRP) in human retinal pigment epithelial cells and its interaction with BAPSG, a novel aldose reductase inhibitor. *Pharm. Res.* (2001) 18:565-572.

58. LIN JH, LU AY: Inhibition and induction of cytochrome P450 and the clinical implications. *Clin. Pharmacokinet.* (1998) 35:361-390.

59. SACHSE C, BROCKMOLLER J, BAUER S *et al.*: Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am. J. Hum. Genet.* (1997) 60:284-295.

60. PHILLIPS KA, VEENSTRA DL, OREN E *et al.*: Potential role of pharmacogenomics in reducing adverse drug reactions: a systematic review. *JAMA* (2001) 286:2270-2279.

61. LIN JH, LU AY: Role of pharmacokinetics and metabolism in drug discovery and development. *Pharmacol. Rev.* (1997) 49:403-449.

62. EDEKI TI, HE H, WOOD AJ: Pharmacogenetic explanation for excessive beta-blockade following timolol eye drops. Potential for oral-ophthalmic drug interaction. *JAMA* (1995) 274:1611-1613.

63. ISHII Y, NAKAMURA K, TSUTSUMI K *et al.*: Drug interaction between cimetidine and timolol ophthalmic solution: effect on heart rate and intraocular pressure in healthy Japanese volunteers. *J. Clin. Pharmacol.* (2000) 40:193-199.

64. NOVACK GD: Excessive B-blockade with timolol eye drops. *JAMA* (1996) 275:985.

65. PUTTERMAN GJ, DAVIDSON J, ALBERT J: Lack of metabolism of timolol by ocular tissues. *J. Ocul. Pharmacol.* (1985) 1:287-296.

66. PAI HV, KOMMADDI RP, CHINTA SJ *et al.*: A frameshift mutation and alternate splicing in human brain generate a functional form of the pseudogene cytochrome P4502D7 that demethylates codeine to morphine. *J. Biol. Chem.* (2004) 279:27383-27389.

67. SEKINE Y, HOMMURA S, HARADA S: Frequency of glutathione-*S*-transferase 1 gene deletion and its possible correlation with cataract formation. *Exp. Eye Res.* (1995) 60:159-163.

68. JURONEN E, TASA G, VEROMANN S *et al.*: Polymorphic glutathione *S*-transferases as genetic risk factors for senile cortical cataract in Estonians. *Invest. Ophthalmol. Vis. Sci.* (2000) 41:2262-2267.

69. SCOTT IU, FLYNN HW Jr, MILLER D *et al.*: Exogenous endophthalmitis caused by amphotericin B-resistant Paecilomyces lilacinus: treatment options and visual outcomes. *Arch. Ophthalmol.* (2001) 119:916-919.

70. THOMAS PA: Fungal infections of the cornea. *Eye* (2003) 17:852-862.

71. ARTHUR RR, DREW RH, PERFECT JR: Novel modes of antifungal drug administration. *Expert Opin. Investig. Drugs* (2004) 13:903-932.

72. TOLER SM: Oxidative stress plays an important role in the pathogenesis of drug-induced retinopathy. *Exp. Biol. Med.(Maywood.)* (2004) 229:607-615.

73. SHICHI H, ATLAS SA, NEBERT DW: Genetically regulated aryl hydrocarbon hydroxylase induction in the eye: possible significance of the drug-metabolizing enzyme system for the retinal pigmented epithelium-choroid. *Exp. Eye Res.* (1975) 21:557-567.

74. MADHU C, DINH V, BABUSIS D *et al.*: Cytochrome P450 isozyme activities in rabbit ocular tissues. *Thirteenth annual meeting of the International Society for the Study of Xenobiotics Proceedings*, Cairns, Australia (1998).

75. MADAN A, GRAHAM RA, CARROLL KM *et al.*: Effects of prototypical microsomal enzyme inducers on cytochrome P450 expression in cultured human hepatocytes. *Drug Metab. Dispos.* (2003) 31:421-431.

76. MATSUMOTO K, KISHIDA K, MANABE R *et al.*: Induction of cytochrome P450 in the rabbit eye by phenobarbital, as detected immunohistochemically. *Curr. Eye Res.* (1987) 6:847-854.

77. TANAKA H, HIRAYAMA I, TAKEHANA M *et al.*: Cytochrome P450 expression in rat ocular tissues and its induction by phenobarbital. *J. Health Sci.* (2002) 48:346-349.

78. NELSON KC, ARMSTRONG JS, MORIARTY S *et al.*: Protection of retinal pigment epithelial cells from oxidative damage by oltipraz, a cancer chemopreventive agent. *Invest. Ophthalmol. Vis. Sci.* (2002) 43:3550-3554.

79. WEI CP, ANDERSON JA, LEOPOLD I: Ocular absorption and metabolism of topically applied adrenaline and a dipivalyl ester of adrenaline. *Invest. Ophthalmol. Vis. Sci.* (1978) 17:315-321.

80. SJOQUIST B, STJERNSCHANTZ J: Ocular and systemic pharmacokinetics of latanoprost in humans. *Surv. Ophthalmol.* (2002) 47 (Suppl. 1):S6-S12.

81. DRUZGALA P, WU WM, BODOR N: Ocular absorption and distribution of loteprednol etabonate, a soft steroid, in rabbit eyes. *Curr. Eye Res.* (1991) 10:933-937.

82. KASS M, CHEETHAM J, DUZMAN E *et al.*: The ocular hypertensive effect of 0.25% fluorometholone in corticosteroid responders. *Am. J. Ophthalmol.* (1986) 102:159-163.

83. HAGENBUCH B, MEIER PJ: The superfamily of organic anion transporting polypeptides. *Biochim. Biophys. Acta* (2003) 1609:1-18.

84. WOLDEMUSSIE E, RUIZ G, WIJONO M *et al.*: Neuroprotection of retinal ganglion cells by brimonidine in rats with laser-induced chronic ocular hypertension. *Invest. Ophthalmol. Vis. Sci.* (2001) 42:2849-2855.

85. YOSHIDA S, HONDA M, YOSHIDA A *et al.*: Novel mutation in ABCC6 gene in a Japanese pedigree with pseudoxanthoma elasticum and retinitis pigmentosa. *Eye* (2005) 19:215-217.

86. RINGPFEIL F, LEBWOHL MG, CHRISTIANO AM *et al.*:

Pseudoxanthoma elasticum: mutations in the MRP6 gene encoding a transmembrane ATP-binding cassette (ABC) transporter. *Proc. Natl. Acad. Sci.USA* (2000) 97:6001-6006.

87. SHICHI H, GAASTERLAND DE, JENSEN NM *et al.*: Ah locus: genetic differences in susceptibility to cataracts induced by acetaminophen. *Science* (1978) 200:539-541.

88. ZHAO C, SHICHI H: Prevention of acetaminophen-induced cataract by a combination of diallyl disulfide and *N*-acetylcysteine. *J. Ocul. Pharmacol. Ther.* (1998) 14:345-355.

89. QIAN W, AMIN RH, SHICHI H: Cytotoxic metabolite of acetaminophen, *N*-acetyl-*p*-benzoquinone imine, produces cataract in DBA2 mice. *J. Ocul. Pharmacol. Ther.* (1999) 15:537-545.

90. GERSON RJ, MACDONALD JS, ALBERTS AW *et al.*: On the aetiology of subcapsular lenticular opacities produced in dogs receiving HMG-CoA reductase inhibitors. *Exp. Eye Res.* (1990) 50:65-78.

91. RAO PV, ROBISON WG Jr, BETTELHEIM F *et al.*: Role of small GTP-binding proteins in lovastatin-induced cataracts. *Invest. Ophthalmol. Vis. Sci.* (1997) 38:2313-2321.

92. SMEETH L, HUBBARD R, FLETCHER AE: Cataract and the use of statins: a case-control study. *QJM* (2003) 96:337-343.

93. BOCCUZZI SJ, BOCANEGRA TS, WALKER JF *et al.*: Long-term safety and efficacy profile of simvastatin. *Am. J. Cardiol.* (1991) 68:1127-1131.

94. HARRIS ML, BRON AJ, BROWN NA *et al.*: Absence of effect of simvastatin on the progression of lens opacities in a randomised placebo-controlled study. Oxford Cholesterol Study Group. *Br. J. Ophthalmol.* (1995) 79:996-1002.

95. EINARSON TR, METGE CJ, ISKEDJIAN M *et al.*: An examination of the effect of cytochrome P450 drug interactions of hydroxymethylglutaryl-coenzyme A reductase inhibitors on healthcare utilization: a Canadian population-based study. *Clin. Ther.* (2002) 24:2126-2136.

96. PATTON WP, ROUTLEDGE MN, JONES GD *et al.*: Retinal pigment epithelial cell DNA is damaged by exposure to benzo[a]pyrene, a constituent of cigarette smoke. *Exp. Eye Res.* (2002) 74:513-522.

97. ZHANG Y, WU X, GUO D *et al.*: Two-step error-prone bypass of the (+)- and (-)-trans-anti-BPDE-N2-dG adducts by human DNA polymerases eta and kappa. *Mutat. Res.* (2002) 510:23-35.

98. SARDESAI VM: Molybdenum: an essential trace element. *Nutr. Clin. Pract.* (1993) 8:277-281.

99. PARINI R, BRISCIOLI V, CARUSO U *et al.*: Spherophakia associated with molybdenum cofactor deficiency. *Am. J. Med. Genet.* (1997) 73:272-275.

100. LIBBY RT, SMITH RS, SAVINOVA OV *et al.*: Modification of ocular defects in mouse developmental glaucoma models by tyrosinase. *Science* (2003) 299:1578-1581.

•• This paper elucidates the underlying mechanism that links mutation in CYP1B1 and the development of primary congenital glaucoma. Through understanding the roles played by metabolic enzymes in ocular tissues, more effective drug therapy may be developed.

101. ALWARD WL: Biomedicine. A new angle on ocular development. *Science* (2003) 299:1527-1528.

102. SCHWARTZMAN ML, MASFERRER J, DUNN MW *et al.*: Cytochrome P450, drug metabolizing enzymes and arachidonic acid metabolism in bovine ocular tissues. *Curr. Eye Res.* (1987) 6:623-630.

103. SCHWARTZMAN ML, DAVIS KL, NISHIMURA M *et al.*: The cytochrome P450 metabolic pathway of arachidonic acid in the cornea. *Adv. Prostaglandin Thromboxane Leukot. Res.* (1991) 21A:185-192.

104. ABRAHAM NG, LIN JH, DUNN MW *et al.*: Presence of haem oxygenase and NADPH cytochrome P450 (c) reductase in human corneal epithelium. *Invest. Ophthalmol. Vis. Sci.* (1987) 28:1464-1472.

105. SCHWARTZMAN ML, BALAZY M, MASFERRER J *et al.*: 12*(R)*-hydroxyicosatetraenoic acid: a cytochrome-P450-dependent arachidonate metabolite that inhibits Na^+,K^+-ATPase in the cornea. *Proc. Natl. Acad. Sci.USA* (1987) 84:8125-8129.

106. MURPHY RC, FALCK JR, LUMIN S *et al.*: 12*(R)*-hydroxyeicosatrienoic acid: a vasodilator cytochrome P450-dependent arachidonate metabolite from the bovine corneal epithelium. *J. Biol. Chem.* (1988) 263:17197-17202.

107. MIEYAL PA, DUNN MW, SCHWARTZMAN ML: Detection of endogenous 12-hydroxyeicosatrienoic acid in human tear film. *Invest. Ophthalmol. Vis. Sci.* (2001) 42:328-332.

• Metabolic products from local ocular metabolism can be measured in tears, which may serve as important biomarkers to monitor disease progression and treatment.

108. DAVIS KL, CONNERS MS, DUNN MW *et al.*: Induction of corneal epithelial cytochrome P450 arachidonate metabolism by contact lens wear. *Invest. Ophthalmol. Vis. Sci.* (1992) 33:291-297.

109. VAFEAS C, MIEYAL PA, URBANO F *et al.*: Hypoxia stimulates the synthesis of cytochrome P450-derived inflammatory eicosanoids in rabbit corneal epithelium. *J. Pharmacol. Exp. Ther.* (1998) 287:903-910.

110. ASHKAR S, MESENTSEV A, ZHANG WX *et al.*: Retinoic acid induces corneal epithelial CYP4B1 gene expression and stimulates the synthesis of inflammatory 12-hydroxyeicosanoids. *J. Ocul. Pharmacol. Ther.* (2004) 20:65-74.

111. BENET LZ, CUMMINS CL, WU CY: Unmasking the dynamic interplay between efflux transporters and metabolic enzymes. *Int. J. Pharm.* (2004) 277:3-9.

112. GEORGE RL, HUANG W, NAGGAR HA *et al.*: Transport of *N*-acetylaspartate via murine sodium/dicarboxylate cotransporter NaDC3 and expression of this transporter and aspartoacylase II in ocular tissues in mouse. *Biochim. Biophys. Acta* (2004) 1690:63-69.

113. WANG EJ, CASCIANO CN, CLEMENT RP *et al.*: Cooperativity in the inhibition of P-glycoprotein-mediated daunorubicin transport: evidence for half-of-the-sites reactivity. *Arch. Biochem. Biophys.* (2000) 383:91-98.

114. DAMM J, RAU T, MAIHOFNER C *et al.*: Constitutive expression and localization of COX-1 and COX-2 in rabbit iris and ciliary body. *Exp. Eye Res.* (2001) 72:611-621.

115. KULKARNI PS, SRINIVASAN BD: Cyclooxygenase and lipoxygenase pathways in anterior uvea and conjunctiva. *Prog. Clin. Biol. Res.* (1989) 312:39-52.

116. WALTMAN A, SEARS M: Catechol-*O*-methyl transferase and monoamine oxidase activity in the ocular tissues of albino rabbits. *Invest. Ophthalmol.* (1964) 3:601.

117. STRATFORD RE Jr, LEE VH: Ocular aminopeptidase activity and distribution in

the albino rabbit. *Curr. Eye Res.* (1985) 4:995-999.

118. STRATFORD RE Jr, LEE VH: Aminopeptidase activity in albino rabbit extraocular tissues relative to the small intestine. *J. Pharm. Sci.* (1985) 74:731-734.
119. AZZAROLO AM, BJERRUM K, MAVES CA *et al.*: Hypophysectomy-induced regression of female rat lacrimal glands: partial restoration and maintenance by dihydrotestosterone and prolactin. *Invest. Ophthalmol. Vis. Sci.* (1995) 36:216-226.
120. MIRCHEFF AK, MILLER SS, FARBER DB *et al.*: Isolation and provisional identification of plasma membrane populations from cultured human retinal pigment epithelium. *Invest. Ophthalmol. Vis. Sci.* (1990) 31:863-878.
121. LI DW-C, XIANG H, FASS U *et al.*: Analysis of expression patterns of protein phosphatase-1 and phosphatase-2A in rat and bovine lenses. *Invest. Ophthalmol. Vis. Sci.* (2001) 42:2603-2609.
122. FEENEY L: Lipofuscin and melanin of human retinal pigment epithelium. Fluorescence, enzyme cytochemical, and ultrastructural studies. *Invest. Ophthalmol. Vis. Sci.* (1978) 17:583-600.
123. CABRAL L, UNGER W, BOULTON M *et al.*: Regional distribution of lysosomal enzymes in the canine retinal pigment epithelium. *Invest. Ophthalmol. Vis. Sci.* (1990) 31:670-676.
124. HAYASAKA S, SEARS ML: Distribution of acid phosphatase, beta-glucuronidase, and lysosomal hyaluronidase in the anterior segment of the rabbit eye. *Invest. Ophthalmol. Vis. Sci.* (1978) 17:982-987.
125. ROBERTSON MJ, ERWIG LP, LIVERSIDGE J *et al.*: Retinal microenvironment controls resident and infiltrating macrophage function during uveoretinitis. *Invest. Ophthalmol. Vis. Sci.* (2002) 43:2250-2257.
126. IKEDA H, UEDA M, IKEDA M *et al.*: Oxysterol 7alpha-hydroxylase (CYP39A1) in the ciliary nonpigmented epithelium of bovine eye. *Lab. Invest.* (2003) 83:349-355.
127. JAIN-VAKKALAGADDA B, DEY S, PAL D *et al.*: Identification and functional characterization of a Na^+-independent large neutral amino acid transporter, LAT1, in human and rabbit cornea. *Invest. Ophthalmol. Vis. Sci.* (2003) 44:2919-2927.
128. MAENPAA H, GEGELASHVILI G, TAHTI H: Expression of glutamate transporter subtypes in cultured retinal pigment epithelial and retinoblastoma cells. *Curr. Eye Res.* (2004) 28:159-165.
129. ANAND BS, MITRA AK: Mechanism of corneal permeation of l-valyl ester of acyclovir: targeting the oligopeptide transporter on the rabbit cornea. *Pharm. Res.* (2002) 19:1194-1202.
130. BASU SK, HAWORTH IS, BOLGER MB *et al.*: Proton-driven dipeptide uptake in primary cultured rabbit conjunctival epithelial cells. *Invest. Ophthalmol. Vis. Sci.* (1998) 39:2365-2373.
131. BERGER UV, HEDIGER MA: Distribution of peptide transporter PEPT2 mRNA in the rat nervous system. *Anat. Embryol. (Berlin)* (1999) 199:439-449.
132. BILDIN VN, ISEROVICH P, FISCHBARG J *et al.*: Differential expression of Na:K:2Cl cotransporter, glucose transporter 1, and aquaporin 1 in freshly isolated and cultured bovine corneal tissues. *Exp. Biol. Med.* (2001) 226:919-926.
133. GHERZI R, MELIOLI G, DE LUCA M *et al.*: High expression levels of the 'erythroid/brain' type glucose transporter (GLUT1) in the basal cells of human eye conjunctiva and oral mucosa reconstituted in culture. *Exp. Cell Res.* (1991) 195:230-236.
134. TAKATA K, KASAHARA T, KASAHARA M *et al.*: Ultracytochemical localization of the erythrocyte/HepG2-type glucose transporter (GLUT1) in cells of the blood-retinal barrier in the rat. *Invest. Ophthalmol. Vis. Sci.* (1992) 33:377-383.
135. TAKATA K, KASAHARA T, KASAHARA M *et al.*: Ultracytochemical localization of the erythrocyte/HepG2-type glucose transporter (GLUT1) in the ciliary body and iris of the rat eye. *Invest. Ophthalmol. Vis. Sci.* (1991) 32:1659-1666.
136. PHILP NJ, WANG D, YOON H *et al.*: Polarized expression of monocarboxylate transporters in human retinal pigment epithelium and ARPE-19 cells. *Invest. Ophthalmol. Vis. Sci.* (2003) 44:1716-1721.

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