Microdialysis and drug delivery to the eye

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Abstract

The eye presents unique challenges in both the development of tools for elucidating drug disposition as well as for the development of modes of drug delivery for treatment of ocular diseases. In this paper, we present a discussion of the anatomical and physiological characteristics and limitations present in the eye for microdialysis sampling of endogenous substrates and xenobiotics. To date, over twenty papers describing microdialysis approaches for assessment of ocular drug delivery and endogenous substrate characterization have been published. Although the majority of papers describe sampling of vitreous humor, recent efforts have been directed towards ocular anterior segment sampling using microdialysis. With this approach, an appreciable reduction in animal use has been realized. In addition, simultaneous examination of administered drug and endogenous substrates modulated by the drug is possible with this approach, facilitating construction of ocular pharmacokinetic/pharmacodynamic relationships through use of relevant surrogate markers. © 2000 Elsevier Science B.V.

Keywords: Ocular drug delivery; Pharmacokinetics; Aqueous humor; Vitreous humor; Blood–aqueous barrier; Pharmacodynamics

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1. Introduction

Microdialysis experimentation in ocular regions is a relatively recent event (late 1980s); implantation in brain and neck precede ocular sampling by over 20 years (1960s — dialysis sacs [1]). Requirements such as sight preservation, development of ways to circumvent surgically related artifacts, and needed improvements in probe design contributed to the relatively recent start for exploring the microdialysis approach for ocular sampling. In this paper, we examine the use of microdialysis for assessing the in vivo intraocular disposition of endogenous and exogenous substrates, intraocular drug delivery and pharmacokinetic/pharmacodynamic studies in the eye.

2. Eye as a sampling site

2.1. Anatomy and physiology

The eye is a unique organ system comprised of a number of important sites amenable to examination with microdialysis. In a simplistic sense, the eye can be viewed as a two compartment system (Fig. 1): the anterior segment (containing the cornea/conjunctiva/sclera externally and the anterior chamber, iris/pupil, posterior chamber and ciliary body internally) and the posterior segment (containing the lens, vitreous and rear ocular tissue layers [retina and choroid] internally, and the optic nerve and associated vasculature externally). The anterior segment has been described as a dynamic environment [2]. In contrast, the posterior segment is viewed as being somewhat static [3]. These descriptive terms have some validity, but can be misleading with respect to substrate flux through the system.

2.2. Anterior segment

2.2.1. Inflow dynamics

The physiology of aqueous humor formation and outflow within the anterior segment of the eye is complex. The anterior segment is involved primarily in intraocular pressure (IOP) regulation. The overall process of homeostatic IOP regulation is described as a balance between oncotic and hydrodynamic pressure and active secretory processes [2,4]. Aqueous humor, the fluid contained in the anterior and posterior chambers, is, in part, an ultrafiltrate of plasma. Aqueous humor contains <1% of the protein concentration of plasma, providing support for a theoretical “blood—aqueous barrier” for the eye [5]. The non-pigmented epithelial layer of the ciliary body is a component of this barrier. Zona occludens are present between non-pigmented epithelial cells of the ciliary body and contribute to barrier function [2]. Aqueous humor is produced by secretion and ultrafiltration processes at the ciliary body and flows into the posterior chamber. Aqueous humor proceeds via convective bulk flow from the posterior chamber through the pupil to the anterior chamber and out through the trabecular meshwork with eventual return to the systemic circulation (Fig. 1) [2,6–8].
The major portion of aqueous humor is secreted actively by the non-pigmented epithelial cells of the ciliary body. A number of potential active transport mechanisms have been described [2]. Endogenous substrates such as ascorbate and lactate are present at higher concentrations in aqueous humor than in plasma [5], consistent with active transport and metabolic processes in the ocular system.

2.2.2. Outflow dynamics

Aqueous humor outflow is driven primarily by pressure-dependent flow of aqueous humor through the trabecular meshwork, and secondarily by pressure-independent flow of aqueous humor via the uveoscleral outflow pathway. The pressure-dependent pathway is driven by the higher IOP relative the episcleral venous pressure (\( \sim 16-20 \) mm Hg versus 7–9 mm Hg; Fig. 2) [4]. Aqueous flows through progressively smaller pores of the trabecular meshwork, into the canal of Schlemm, and ultimately is collected into the episcleral venous system.

2.3. Posterior segment

Vitreous humor, a viscoelastic connective tissue [3], which is \( \sim 99\% \) water, contains substrates that confer a gelatinous consistency: chondroitin sulfates, hyaluronates, glycosaminoglycans, and collagen [4]. In contrast to aqueous humor, which is continuously regenerated in adults, no new vitreous is produced [9]. Vitreous can be viewed, from a drug distribution standpoint, as a static unstirred fluid; small drug or solute transport occurs primarily via diffusion [9,10]. The retina, contiguous to the outer margins of the vitreous, is nourished primarily by retinal arteries. Active transport of endogenous and exogenous substrates from the retinal vasculature has been reported [11]. Flux of these substrates through vitreous still occurs via diffusion [12,13].

The lens (or crystalline lens) is a relatively dense organ that is sensitive to external stimulation, which can result in cataract generating cascades [14]. Sampling of this tissue can be performed only with loss of the tissue or subsequent nonreversible decrease in visual performance due to cataract formation. The lens contains 35% protein, the highest of any organ in the body [4], and 65% water.

3. Challenges in assessing drug disposition in the ocular system

The anatomical size of the eye presents a number of difficulties for appropriate examination of both normal and pathologic conditions. Inherent in these difficulties is the sensitivity of the eye to immunoprotective cascades following manipulation [14,15]. Protection of visual function is also a major concern for any procedures proposed for observation of ocular pathophysiology or ocular pharmacokinetic/pharmacodynamic experimentation. The anterio-posterior diameter of the normal adult globe is \( \sim 24.5 \) mm [16]. The anterior chamber aqueous humor volume is \( \sim 200 \) µl; the posterior contains \( \sim 55 \) µl, similar to the rabbit [17]. The depth is \( \sim 3.5 \) mm. The vitreous chamber contains \( \sim 2 \) ml of vitreous humor [4]. The chamber depth is much larger than that in the aqueous chambers. Differences in the dynamics of aqueous versus vitreous humor turnover provide clues to the challenges for targeted drug delivery to these sites. In general, absorption through corneal tissue increases with increased lipophilicity of the administered substrate [18]. Many eye diseases of the anterior segment can be effectively treated via the topical route [19]. However, the mean residence time of topically administered drug at the cornea versus cul-de-sac may be important in efficacy [10]. Much effort has been directed towards formulation development for increasing the mean residence time of pharmacologic agents in the cul-de-sac of the eye for enhanced intraocular exposure [20]. Limited evaluation of the ocular pharmacokinetics of ophthalmics in humans has been conducted, however, due to the intrusive procedures...
that are required. As a consequence, no relationships between drug intraocular concentrations and pharmacologic responses have been developed [21,22]. In contrast to accessibility of drug to the anterior segment, the posterior locations in the eye (e.g., retina and optic nerve) provide challenges to effective pharmacotherapy. Not only is the location more distant, but intraocular barriers such as the lens and static reservoirs such as the vitreous humor limit distribution of drugs to the retina following topical administration [3]. Treatment approaches for target tissues in these remote regions include systemic administration of drugs, peribulbar or retrobulbar injections, intravitreal injection [21,23], and surgical insertion of drug releasing devices to the vitreal spaces [24,25]. The nature of vitreous distributional characteristics may cause localized peaks in drug concentrations following intravitreal injections approaching toxic levels [12,22].

3.1. Intraocular disposition of drugs following systemic versus topical administration

In order to optimize therapy for localized illness or disease, a delicate balance between enhanced efficacy and minimization of side effects must be maintained. This is particularly true of treatment approaches for a number of important eye diseases [22]. Many agents used to treat a broad spectrum of eye diseases also may illicit systemic side effects. The unique physiology of the eye provides a pathway for drug delivery to the systemic circulation with bypass of first pass metabolism effects [26,27]. Thus, systemic exposure to topically administered drugs is possible [26]. Studies have been conducted in order to assess these effects. In fact, some investigators have proposed the exploitation of the ocular route of administration for treatment of systemic diseases [28,29]. Pharmacokinetic studies have been conducted in order to address this issue. Mosstetter and colleagues [30] evaluated the ocular and systemic disposition of cyclosporin following topical administration in the rabbit. They found that subtherapeutic concentrations of cyclosporin A were obtained following administration of 2% eye drops in both aqueous humor and plasma; undetectable concentrations were observed in plasma. Ocular tissues evaluated included cornea and aqueous humor. This study required 30 rabbits, as a single rabbit was used for each time point and \( n = 6 \) per time point were evaluated. Lee [18] examined the systemic disposition of a series of beta-adrenergic antagonists following topical administration to the pigmented rabbit. In this study, trends with regard to physicochemical drug properties and absorption pathways were assessed. Examination of the efficiency of nasolacrimal punctum occlusion for minimization of systemic exposure also was examined. Several studies of this type have developed compartmental pharmacokinetic models to describe intraocular absorption and disposition [31,32]. In general, these approaches are very animal-resource intensive [33]. Microdialysis provides a unique opportunity to answer these relevant questions with the use of much fewer animals.

3.2. Standard approaches to assessment of ocular pharmacokinetics and pharmacodynamics

In general, the intrusive nature of experimentation for assessment of ocular pharmacokinetics of ophthalmics has limited these studies to animal models [10,33]. Rabbits are the species of choice for most ocular pharmacokinetic experiments, although work in the cat, dog and primate has been reported [12,34]. The rabbit is an ideal subject in some regards: it has anatomical similarities to the human eye in size and aqueous humor volume, and is a relatively inexpensive species to use for these types of studies, which are extremely animal-resource intensive in nature. The rabbit eye has a number of appreciable differences from the human eye: thinner corneal thickness (0.35 mm versus 0.52 mm in humans [10]), slower blink reflex [10], a nictitating membrane (absent in humans [35]), and virtually no uveoscleral outflow pathway [10]. These differences must be taken into account in the interpretation of results from a pharmacokinetic and pharmacodynamic point of view. Of course, the pharmacodynamic effects may be species specific. A typical rabbit ocular pharmacokinetic study involves the use of 30–48 rabbits; \( n = 6 \) per time point. A single ~100 \( \mu l \) sample of aqueous humor is aspirated via a syringe (paracentesis sampling) following local or systemic anesthesia of the rabbit. As a usual course, this procedure is terminal; the rabbit is sacrificed prior to or immediately following the sampling.
procedure. The procedure is repeated for the desired number of time points. Inter-animal and inter-eye variability in aqueous humor drug concentrations can be appreciable [36,37]. Miller and colleagues [38] conducted an ocular pharmacokinetic study with repeated aqueous humor and vitreous humor sampling in rabbits; this approach is the exception rather than the rule. The volume of aqueous humor or vitreous humor extracted was extremely small relative to the sample volume required for most analytical techniques (~ 7 µl). Local anesthesia was also a requirement for this approach; this may provide confounding factors in evaluating the absorption profiles for the drugs under examination [15,39]. Pharmacodynamic studies may involve a non-intrusive measure such as IOP determination or the employment of sophisticated equipment such as fluorophotometry to assess aqueous humor flow [4,6,7,40]; no correlation to ocular drug disposition is possible using this approach. Other pharmacodynamic studies involve initiation of a disease state such as inflammation, scoring of the extent and following the course of healing or quantitation of ocular tissue accumulation or inhibition of endogenous substrates following a topical or systemic dosing regimen of the drug under examination [41].

4. Evolution of microdialysis for in-the-eye sampling

Microdialysis, in its present form, evolved from early experimental attempts to evaluate extracellular fluid compartments rather than blood as a sampling site for analyzing endogenous compounds present in various organs and tissues [42]. In the 1960s, attempts were made to implant "dialysis sacs" containing 6% dextran in saline into the subcutaneous tissue of the neck and into the parenchyma of the cerebral hemispheres of dogs. Ten weeks later, the dialysis sacs were removed and analyzed for amino acid content [1]. Many of the earlier experiments targeted brain tissue sampling sites. By 1991, over 600 articles were published using microdialysis, primarily in the neurosciences [43]. There has been substantial growth in the number of reports of various applications of this tool since that time. Other sampling sites have been targeted in recent years. Blood and peripheral organs and tissues such as adipose tissue, adrenal, heart, liver, muscle, ovary, uterus, and eye have been sampled via microdialysis [43,44].

4.1. Vitreous humor microdialysis sampling

Earlier studies in the eye with microdialysis sampling were conducted in a rabbit [5] or cat [12] model. Adachi and coworkers [46,47] used a rather unusual species, the pigeon. In all of the above-mentioned studies, vitreous humor was the site of examination. For many of these studies, microdialysis probes were positioned in close proximity to the retina in the vitreous humor in order to determine the time course of exogenously administered solutes and to characterize the disposition of endogenous substrates.

Gunnarson and colleagues [45] reported the earliest use of microdialysis in the eye. These authors were examining the rate of appearance of radio-labeled water versus mannitol in vitreous following intra-carotid injection in albino rabbits (1.5–2 kg); the probe was positioned next to the retina. They also examined endogenous concentrations of amino acids and the effect of KCl perfusion media on endogenous substrate concentrations of taurine and phosphoethanolamine. The probe design consisted of a 3-mm loop of cuprofan B4 membrane matrix of 3000 molecular weight cut off. Probe recovery was determined by the in vitro water recovery method (ranged from 7 to 10%). Ben–Nun and coworkers [12,48] designed a microdialysis probe for placement into the vitreous space of adult cats (2.2–5 kg) for the examination of beta-aminoglycosides (gentamicin). It has been noted that access to posterior eye spaces for drug therapy is difficult [21]. Using microdialysis, these authors examined the disposition of gentamicin following an intravitreal bolus dose. Because flow is limited in vitreous [3], this study was conducted to examine whether the drug elimination kinetics differed between diseased versus control eyes. This procedure facilitated a detailed examination of intravitreal distribution pharmacokinetics; such examination would not be possible without animal resource intensive studies. These investigators also examined the utility of microdialysis for regional drug delivery of gentamicin to vitreous;
with this approach, it was possible to effectively deliver therapeutically relevant concentrations to vitreous with avoidance of dramatic spikes in localized drug concentrations — a possible consequence of administration by intravitreal injection [22].

Waga and colleagues [14,49,50,68] provided a crucial examination of possible long-term placement of microdialysis probes in vitreous chambers of rabbits. These authors were the first to develop a viable surgical procedure and to characterize carefully the pathophysiology of probe placement in the vitreous for up to 161 days. They also assessed probe function (average functional span of 21 days). In their earlier work they used a tube probe design with a polycarbonate-polyether copolymer membrane with a 20,000 molecular weight cut-off; probe recovery was determined by the in vitro recovery method using tritiated water-spiked saline (8–40%; membrane length not specified). Their work also constituted the first examination of intravitreal disposition of endogenous substrates in conscious animals using microdialysis. A concentric probe design was used for their later experiments. Nerve growth factor (NGF) was delivered to rabbit vitreous via a polyether sulphone (PES) microdialysis membrane with a 100,000 dalton cutoff; NGF intravitreal concentrations of up to $10^{-11}$ M were observed [68].

Louzada-Junior et al. [51] and Stempels et al. [52] examined the effect of ischaemia on the health of the retina using microdialysis sampling of vitreous humor; probes were placed adjacent to the retina for sampling of endogenous amino acid markers such as glutamate and O-phosphoserine in vitreous humor. Anesthetized rabbits were used for this animal model. Hughes and colleagues [53] examined the pharmacokinetics of acyclovir and ganciclovir following intravitreal injection with microdialysis in anesthetized New Zealand white rabbits. For this work, they used a concentric probe design (CMA-12, CMA/AB Microdialysis, Stockholm, Sweden). Adachi [54] studied glutamate and nitric oxide neurotoxicity induced in cat retinal injury in vivo using microdialysis.

In an elegant study of pigeon diurnal fluctuation in pineal gland versus vitreal concentrations of melatonin under light–dark cycles [46,47] the phasic nature of dopamine versus melatonin concentrations in vitreous was demonstrated as a consequence of exposure to light–dark versus continuous-dim-light cycles (Fig. 3). Studies such as these demonstrate the power of the microdialysis approach for mechanistic investigations in the eye as well as other sites in the body. Summaries of all published studies using microdialysis sampling of the vitreous humor/retina are presented in Table 1.

4.2. Aqueous humor microdialysis sampling

Unique challenges are presented for the employment of microdialysis sampling in aqueous humor. The dynamics of homeostatic regulation of IOP or aqueous humor formation rate, for example, directly

![Fig. 3. Vitreal melatonin (solid squares) and dopamine (open circles) concentrations of pigeons under dark–light cycles measured using microdialysis; panel A: mean values ($n = 3$); panel B: a representative pigeon. From S. Ebihara et al., Biol Signals. 1997; 6:233–240. Used by permission of Elsevier Science, Inc.](image-url)
Table 1
Ocular studies using microdialysis: vitreous humor/retina

<table>
<thead>
<tr>
<th>Author</th>
<th>Probe description</th>
<th>Animal used/placement</th>
<th>Anesthetized/Conscious</th>
<th>Problem examined</th>
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<tr>
<td>Gunnarson et al.</td>
<td>3-mm loop: membrane- cuprophan B4 3000 mwco, 0.23 mm o.d.; 60 mm long (2×4 cm cannula); recovery 7–10% in vitro.</td>
<td>Albino rabbits 1.5–2 kg; probe inserted through guide channel penetrating sclera through small incision at upper nasal part of eye 2–2.5 mm from limbus; probe penetrated vitreous with tubing loop less than 0.3 mm from retinal surface nasally.</td>
<td>Anesthetized: i.m. flunisoneum/ Fentanyl.</td>
<td>Vitreal ocular disposition of a carotid-artery-administered mixture of ¹⁴C mannitol and triitated water, and endogenous amino acids near the retinal surface.</td>
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<tr>
<td>Ben-Nunn et al.</td>
<td>10-mm concentric design–membrane regenerated cellulose (cuprammonium rayon), 35000 mwco, 200 µm o.d.; tungsten rod 150 µm o.d.; modified concentric design; recovery 5% in vitro.</td>
<td>Adult domestic cats (2.5–5 kg), lateral canthotomy followed by suture of upper eyelid to expose upper temporal eyelid. Limbal suture to R eye at 9 o’clock position; open tenon’s capsule over pars plane; creation of a 8–10 mm diameter window; 20-ga needle to create hole, insertion of probe just behind the posterior pole of the lens; perfusate was normal saline; perfusion 3.5 µl/min.</td>
<td>Anesthetized–i.v. alfaxalone/alfadalone acetate, ventilated followed by gallamine triethiodide (flaxedil) for paralysis and alfaxalone/alfadalone maintenance i.v. infusion.</td>
<td>Gentamicin sulfate 10 mg/0.25 ml injected subconjunctivally or intravitreally; samples up to 8 h; examined pharmacokinetics of dosing placement: dosing near probe tip versus away; huge differences observed (7–9 h); second study of efficacy following intravitreal injection of Staph aureus; 48 h later, intravitreal injection of gentamicin; observed injected eyes had lower gentamicin than non (aqueous humor conc.-cleared 2× higher); 3rd examined delivery using MD; more controlled delivery observed; no localized highs.</td>
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<td>Waga et al., 1991</td>
<td>Protecting tube o.d. 0.6 mm; 0.4 mm i.d. for probe membrane, polycarbonate-polyether copolymer. 0.4 µm; i.d. 400 µm; 20,000 mwco; long opening dimensions not specified; recovery 8–40% (average 21%). In vitro using H saline solution. Last paper: high permeability membrane of a new type (polyether sulphone, PES, cutoff at 10000 daltons)</td>
<td>Rabbits (not specified what type or weight); opened conjunctiva at limbus; 20-ga needle inserted on nasal side of eye ~4–6 mm behind limbus and brought through to other side: tube-per-se design with protective sleeve over line except an exposed surface of unspecified area.</td>
<td>Anesthetized For surgery: flunisoneum/Fentanyl. Perfused with BSS 4–6 times per week at 2 ul/min or 4 ul/min.</td>
<td>Examined rabbits with probes in place from 7 to 161 days; carefully examined pathology of probe placement and made observations of eye health and probe function; average probe function ~21 days; also examined endogenous amino acids; Examined the disposition of ceftazidime in blood and vitreous following systemic and intravitreal administration of ceftazidime. Differences noted in disposition of inflated versus intact eyes. (2000) 125I labelled NGF (nerve growth factor) delivered to rabbit vitreous using microdialysis.</td>
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<tr>
<td>Louzaada-Junior et al.</td>
<td>Mono-probe 0.5 cm in length with mwco 3000.</td>
<td>Male rabbits (2.0–2.5 kg); into vitreous through the nonvascular pars plana region of the sclerotic coat after puncture with a surgical needle; probe positioned 1 mm from retinal surface; perfusion with kreb’s-bicarbonate saline 4–9 µl/min.</td>
<td>Anesthetized with thiopentanol i.v.</td>
<td>Ischemia introduced: increase IOP by venous puncture needle introduced into anterior chamber connected to a saline reservoir of heparin sulfate. 120 mmHG applied to saline solution through a sphynanometer. Mechanically transmitted pressure to vitreous chamber which provoked collapse of arterioles to retina resulting in total ischemia; maintained 45 min followed by collection of microdialysis samples for 5 min; amino acids were measured; examine pre and post ischemia; (reperfusion).</td>
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Table 1. Continued

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<tr>
<td>Stempels et al.</td>
<td>CMA probe 0.6 o.d. mm shaft, a 0.52 mm o.d. probe of 3 mm and 20 kD mwco; in vitro recovery was 17–24% for catecholamines and acidic metabolites.</td>
<td>Adult male Dutch pigmented rabbits; placed removable scleral entry port with removable closing plug; probe inserted here mid-vitreous; ringsers solution with Ca(^{2+}) 0.175 mM, 2 (\mu)l/min</td>
<td>Anesthetized with diazepam and ketamine during surgery and during probe implantation and sample collection (kept under halothane).</td>
<td>Observed rabbits up to 6 months after scleral entry port surgery; probes removed after each experiment and reinserted for each new experiment.</td>
</tr>
<tr>
<td>Hughes et al. [53].</td>
<td>CMA-12, 4 mm, polycarbonate, 20 kD mwco; in vitro recovery 15%; isotonic phosphate buffered saline pH 7.4 at 2 (\mu)l/min.</td>
<td>New Zealand white rabbits (2–4 kg); probe guide inserted 3–4 mm below corneal scleral limbus through the pars plana into mid-vitreous region.</td>
<td>Anesthetized throughout the experiment — i.v. ketamine/xylocaine.</td>
<td>Acyclovir and ganciclovir dosed as 200-µg, 100-µl intravitreal injection, examined the vitreal disposition of the drugs.</td>
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<td>Adachi et al. [54]</td>
<td>CMA-10, 0.5 mm diameter, 3 mm length, polycarbonate, 20 kD mwco; NaCl, KCl, MgSO(_4), K(_2)HPO(_4), NaHCO(_3) buffer perfused at 2 ml/min.</td>
<td>Cats received microdialysis probe tip firmly attached to the retina with entire membrane perpendicular placed. Advanced through lumen of 15-ga needle penetrating pars plana and attached to retina in area centralis using micromanipulator; sealed with silicone boot.</td>
<td>Anesthetized with i.m. ketamine (25 mg/kg) and atropine (0.05 mg/kg) following loading dose of carboxylic acid ethyl ester (200 mg/kg, i.v.) during surgery and experiments.</td>
<td>Monitored glutamate release from in vivo cat retina during and after pressure-induced ischemia using a microdialysis, study the effects of MK-801 (dizocilpine), (\text{N}^\text{N})-NAME (N omega-nitro-L-arginine methyl ester), and (\text{N}^\text{N})-NAME (N omega-nitro-L-arginine methyl ester) on the histological changes in the rat retina induced by ischemia or intravitreal injection of NMDA.</td>
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<tr>
<td>Adachi et al. [46,47].</td>
<td>Microdialysis probe 5-mm length for eye, 0.25 mm o.d., 10 (\mu)m thickness membrane acetate, Ringer solution for perfusate; perfused at 2 (\mu)l/min; type not described although appears to be a concentric probe design based on figure.</td>
<td>Pigeons-tip of guide cannula for probe placed at dural surface of the sclera of left eye; guide fixed to the skull with dental cement. A few days post recovery, probe was inserted.</td>
<td>Sodium pentobarbital anesthesia; not clear whether conscious or under anesthesia during experiments (probably conscious).</td>
<td>Examined diurnal fluctuations in pineal gland versus vitreal concentrations of melatonin under light-dark cycles; also looked at dopamine concentrations; examined phase relationships between melatonin and dopamine.</td>
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<tr>
<td>Ebihara et al. [67]</td>
<td>10 (\mu)m thickness membrane 5000 kD mwco cellulose acetate, Ringer solution for perfusate; perfused at 2 (\mu)l/min; type not described although appears to be a concentric probe design based on figure.</td>
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Impact on intraocular drug distribution characteristics. Due to these regulatory mechanisms, the eye is sensitive to any intrusions to the anterior segment. Prior techniques such as paracentesis sampling resulted in disruption of this delicate regulatory balance that could precipitate appreciable alterations to ocular pharmacokinetics or pharmacodynamics of topically administered drugs. A major component in this disruption is compromise to the blood–aqueous barrier [15]. Other issues involved the effects of the natural cascade of events in aqueous humor, such as fibrin formation and increased aqueous humor protein influx that may impact on available substrate concentration in aqueous humor [15].

Recently, drug disposition in the anterior segment has been explored using microdialysis [55–60]. Fukuda et al. [55] and Sato et al. [56] were the first to examine the utility of microdialysis sampling of anterior chamber aqueous humor. In their studies, probes inserted into the temporal cornea through the anterior chamber and exteriorized out of the nasal cornea (a tube-per-se design) were used to examine intraocular disposition of fluoroquinolones in the anesthetized rabbit. In later experiments, Ohtori et al.
[57] examined the ocular pharmacokinetics of timolol and carteolol in rabbits shortly after recovery from anesthesia. Rittenhouse et al. [15,58–60] examined propranolol aqueous humor pharmacokinetics in anesthetized dogs and New Zealand white rabbits. The inter-species differences (dogs vs. rabbits) in the intraocular pharmacokinetics of propranolol were elucidated. These investigators later refined the sampling technique and developed a viable conscious animal model (functional probes of > 45 days [15,59]. Using propranolol as a beta-blocker probe and model highly protein-bound substrate, experiments were designed to assess the effects of recovery period (protein binding) and anesthesia on the aqueous humor pharmacokinetics of propranolol [15]. The uptake kinetics of endogenous aqueous humor ascorbate is a topic of considerable interest and has been examined for many years; conflicting results have been reported with poor in vitro to in vivo correlation [61–65]. Microdialysis probes were placed into the anterior and posterior chambers of rabbits in order to estimate the rate of ascorbate secretion into the posterior chamber from the ciliary body pigmented epithelium and clearance.

Fig. 4. Aqueous humor steady-state ascorbate concentrations in each eye of a conscious rabbit measured over ~9 h. >From Rittenhouse K. et al., Curr. Eye Res. 2000; 20:351–360. Used by permission of Swets and Zeitlinger Publishers.

Fig. 5. Aqueous humor ascorbate concentration versus time profiles following three 1500-μg doses of propranolol in three individual rabbits (A,B,C). Line represents the nonlinear least squares regression fit for each profile. From K. Rittenhouse et al., Exp. Eye Res. 2000;70:429–439. Used by permission of Elsevier Sciences, Inc.
from the anterior chamber [59]. Complex compartmental modeling was used to characterize the pharmacokinetics of exogenously administered ascorbate in blood, anterior aqueous humor and posterior chamber aqueous humor. For the first time, circadian fluctuations in steady-state aqueous humor ascorbate in both eyes of a conscious rabbit were elucidated by the microdialysis technique (Fig. 4; [59]). In later experiments, microdialysis simultaneous sampling of both pharmacologic agent (propranolol) and endogenous surrogate marker of aqueous humor turnover (ascorbate) [60] facilitated the examination of the pharmacodynamics of beta-blocker modulation of aqueous humor production. Nonlinear least-squares regression analysis of the aqueous humor time course of endogenous ascorbate provided estimates of aqueous humor flow; the inhibitory effects of propranolol on both aqueous humor and ascorbate secretion was examined in this model (Fig. 5; [60]). Summaries of all published studies using microdialysis sampling of the aqueous humor are presented in Table 2.

5. Conclusion

Microdialysis is a powerful tool for the examination of in vivo drug disposition. Moreover, microdialysis is amenable to the assessment of in vivo homeostasis of endogenous substrates in numerous sites of the body. This technique facilitates the simultaneous examination of the pharmacological agent and effects on endogenous substrate disposition precipitated by the agent, thus providing an opportunity to mechanistically examine difficult pharmacokinetic–pharmacodynamic problems in vivo. The eye is an exciting region for exploring the utility of this technique; important and interesting questions have been explored and elucidated. Microdialysis provides an important advance in the assessment of intraocular disposition of drugs; substantially reduced populations are required for examination and realistic pharmacokinetic parameters devoid of confounding effects of anesthesia and protein binding have been obtained. Regions of the

<table>
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<tr>
<th>Study</th>
<th>Ocular studies using microdialysis: aqueous humor</th>
<th>Examination of pharmacokinetics in aqueous humor of fluoroquinolones in rabbit aqueous humor; 1998 examination of beta-blockers, timolol and carteolol; timolol new formulation (gel forming).</th>
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<tr>
<td>Fukuda et al. [55]; Sato et al. [56]; Ohotiri et al. [57].</td>
<td>Tube-per-se type; 5 mm cellulose probe. 0.2 mm o.d., 50 Kd mwco with fused silica, 23 g tubing; in vitro recovery 16–20% for norfloxacin/lemfloxacin.</td>
<td>Pigmented rabbits (1.5–3.0 kg); stitched the nictitating membrane of one eye to immobile; 23-ga needle attached to one end of the probe was inserted in to ear-side cornea through anterior chamber and out of nose-side; glued puncture sites with epoxy resin; taped PE tubing to rabbit face; gave i.v. heparin before procedure.</td>
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<td>Rittenhouse et al. [15,58–60]</td>
<td>CMA/20 custom 4 mm polycarbonate membrane, 20 kD mwco, concentric probe design with 90 degree bend; In vivo recovery of propranolol ~20–50%; endogenous ascorbate ~10%. Probe perfusate – phosphate buffer or saline (2 µl/min).</td>
<td>New Zealand white rabbits (2.3–5.0 kg); following limbal-based conjunctival flap superior nasally or temporally ~3 mm from limbus, conjunctival pocket prepared 10–12 mm; probe inlet/outlets exteriorized to top of head. 20-ga needle created opening ~2–3 mm from limbus into anterior chamber and removed. Probe placed into opening; anchor of probe sutured to sclera and covered with conjunctiva.</td>
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<td>Anesthetized during surgery with ketamine/xylazine –Conscious experimentation from 5 to 45 days post probe implantation.</td>
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<td>Development of a conscious animal model for assessment of intraocular pharmacokinetics of anti-glaucoma drugs (propranolol as a model beta-blocker). Examination of beta-blocker anterior chamber disposition following topical administration. Examination pharmacodynamics of beta-blockers on aqueous humor turnover via ascorbate as a surrogate marker.</td>
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eye that have proven to be challenging for characterizing drug pharmacokinetics (vitreous humor) have been successfully examined using this technique. Exploration of the dynamic environment of the ocular anterior segment was facilitated with this approach.

References


